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How to protect a diverse, poorly known, inaccessible fauna: identification and protection of source and sink habitats in the epikarst

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ABSTRACT

1. Aquatic subterranean species are often geographically and numerically scarce. Many of these species are denizens of epikarst, the uppermost zone of karst with semi-isolated solutional openings and channels, and are only known from drip pools in caves where they accumulate as a result of animals dripping out of the epikarst.
2. The question of whether these pool communities adequately reflected the epikarst community was addressed by directly collecting animals from drips in a continuous collecting device.
3. The study area was six caves in Slovenia, where a total of 35 drips and associated pools were sampled for copepods for a period of approximately one year. A total of 37 copepod species were found, 25 of them stygobionts and 16 epikarst specialists.
4. Overall, the frequency of stygobionts was 1.5 times higher in drips compared with pools and the frequency of epikarst specialists was three times higher in drips compared with pools, and the frequency of immature individuals was higher in drips compared with pools, with the exception of one artificially enlarged pool in Škocjanske jame. The cause of this difference is probably increased juvenile mortality in pools and reduced reproduction, indicating that pools are not source populations.
5. The results of this research suggest that epikarst *per se,* not just the sampling sites (including pools) in caves, needs to be the focus of conservation planning.

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INTRODUCTION

Worldwide, there are nearly 4000 described species of obligate aquatic cave dwelling species (usually referred to as stygobionts), and probably several times that number of undescribed species (Culver and Holsinger, 1992; Gibert and Culver, 2009). The species share a convergent morphology of reduced or absent eyes and pigment, appendage lengthening, and an elaboration of extra-optic sensory structures (Culver and Pipan, 2009). Nearly all stygobionts have highly restricted ranges. For example, Trontelj *et al.* (2009), on the basis of mt DNA sequence data, show that no European stygobiotic species with ranges of more than 200 km are extremely rare. Most species have ranges considerably less than that. In the USA, Culver *et al.* (2000) report that 54% of the 971 troglobionts (terrestrial obligate cave dwelling species) and stygobionts were known from a single county (average county size is approximately 2500 km^2). Ninety-five per cent of these species are listed by The Nature Conservancy as vulnerable or imperilled and these species represent 50% of all vulnerable or imperilled species in the USA (Culver *et al.*, 2000).

Stygobionts pose several problems both for conservation managers and biologists trying to understand the basic biology of these species. Among aquatic cave habitats are streams, groundwater (phreatic) ponds and lakes, and drip pools isolated from groundwater (Culver and Pipan, 2009). The first two have an obvious analogy with surface streams and ponds, and species in these habitats, except for predators, are

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usually numerically common, at least relative to other stygobionts. Pools above cave streams and even pools in caves without streams are common. Some of these pools are remnants of flood waters but many are fed primarily or exclusively by water dripping from epikarst. Epikarst, the skin of karst, is the uppermost layer of karst (typically occurring in carbonate rock such as limestone) with numerous cracks and crevices, as well as semi-isolated solution pockets (Bakalowicz, 2003; Williams, 2008).

The fauna of isolated drip pools show very different features, typically being both numerically and geographically rare. Epikarst is biologically important because it harbours a diverse specialized fauna, distinct from that of cave streams, flood pools and phreatic lakes (Brancelj and Culver, 2005; Pipan, 2005; Camacho *et al*., 2006). The fauna of epikarst is often very rich in species, rivalling or exceeding that of other aquatic cave habitats (Culver and Pipan, 2009). Likewise, microbial diversity is also high (Shabarova and Pernthaler, 2010).

Of 33 stygobionts known from West Virginia caves (Fong *et al*., 2007), seven species are known from fewer than 10 specimens, and five of these were from drip pools (Culver, unpublished data). For example, the amphipod *Stygobromus cooperi* is only known from two specimens collected in drip pools in Silers Cave (Holsinger, 1967). The number of such epikarst species is large. Of the 56 described species of *Stygobromus* from the eastern USA, 28 are found in epikarst (Culver *et al*., 2010). In other regions, epikarst species are also both numerically rare and have highly restricted ranges (Fišer and Zagmajster, 2009).

Because most of what is known about epikarst fauna comes from information about the fauna of drip pools, efforts at protection have often focused on these pools. For example, Piddling Pit in West Virginia, a cave protected by The Nature Conservancy, has two vulnerable and imperilled species — the amphipods *Stygobromus parvus* and *S. nanus*, both epikarst species found in drip pools. Drip pools *per se* may not be important for species protection because they are not part of the primary epikarst habitat, and may not even be able to sustain viable populations.

In this paper, drip pool habitats are investigated with regard to whether they are source or sink habitats (Pulliam, 1988), and whether they are important only as collecting sites. To do this, the extensive data set on epikarst drip and drip pool copepods developed by Pipan (2003, 2005) is used. For a series of sites in Slovenian caves, contemporaneous samples from dripping water and from pools were taken. If drip pools are source habitats, then there should be evidence that the proportion of the stygobiotic component of the drip pool fauna is high, because stygobionts are subterranean specialists, and there should be evidence for reproduction in pools.

The proportion of immature individuals relative to the number of adults in pools and associated drips was used as a measure of reproduction. Immature individuals include the six naupliar and five copepodid pre-adult stages of copepod development (Dahms, 1993). If the proportion of immature individuals is lower in pools, this indicates that the sum of survival of immature individuals entering from drips (the only source of new colonists) plus reproduction in pools was less than reproduction in the epikarst itself. Evidence of a lower proportion of immature copepods in pools suggests that *in situ* reproduction is low or that predation on immatures is higher. Either of these possibilities indicates that pools are a suboptimum habitat, perhaps even a sink habitat.

METHODS AND MATERIALS

Because of the physical structure of epikarst, it is not possible to sample it directly. The closest approach to direct sampling is that of Pipan (2005) who devised a special sampling net and container that allowed for continuous filtering of dripping water. Aside from the work of Pipan and her colleagues (Pipan, 2005; Pipan and Culver, 2005; Moldovan *et al*., 2007; Pipan *et al*., 2008), all collections of the epikarst fauna are indirect — from drip pools, usually as part of general fauna inventories.

Data obtained from a study of six caves in south-west Slovenia were utilized; Črna jama, Dimnice, Pivka jama, Postojnska jama, Škocjanske jame, and Županova jama. In these caves, Pipan (2003) continuously sampled drips over a 6–12 month period from mid-2000 to mid-2001. In each cave except Postojnska jama, five drips were sampled continuously for fauna using a flow-through fine-mesh (60 μm) and collecting bottle (Figure 1). In Postojnska jama, 10 drips were sampled. The exact starting date varied from cave to cave (Pipan, 2003). Samples were taken each month to minimize mortality and predation.

In addition, at 3-month intervals, pools were sampled directly below the sampled drips (Figure 2). The resulting volume ranged from 0.1 L to a maximum of 50 L, draining many but not all pools. Since only a small number of the total number of pools were sampled in this way and because the fauna was quickly replenished by animals coming in via drips, sampling had little overall impact. In order to compare drips and pools, samples were paired according to location (which drip) and which sampling interval. To do this, monthly drip

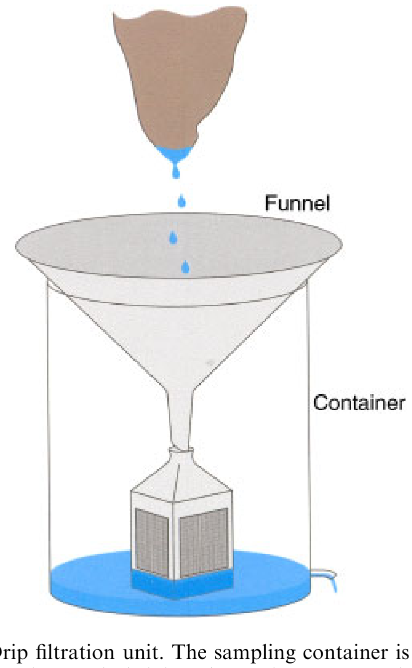

Figure 1. Drip filtration unit. The sampling container is about 10 cm in diameter and 30 cm in height. The mesh screens on the sides of the filtration bottle allow water through and retain all organisms entering via the drips.

Figure 2. Apparatus for aspirating water in pools. After aspiration, the water and sediment mixture in the container is passed through a filtering bottle for preservation.

samples were combined into 3-month samples that correspond to the interval between pool samples.

Samples were preserved in 4% formalin for identification. All adult copepods were identified to species, and numbers of nauplii and immature individuals were recorded but could not be identified to species. Each species was categorized as stygobiotic (found only in subterranean habitats) or not, and as an epikarst specialist (found only in epikarst habitats) or not, based on the analysis of Pipan (2005) and Culver *et al.* (2009).

For each cave, epikarst specialists, stygobionts, and other copepods were listed. The frequency of epikarst specialists and stygobionts for drips and pools for each 3-month interval for each cave was calculated. Given that many samples, especially drips, had very few individuals (sometimes none), cases where a species from each cave was only found in one habitat, but with more than 10 individuals collected, were enumerated.

Conditional independence between habitat and life stage was tested, controlling for cave, using the Cochran–Mantel–Haenszel test. Given the high level of spatial and temporal heterogeneity of copepod abundance, especially among drips (Pipan and Culver, 2007b), such a spatial and temporal pairing is necessary. The Cochran–Mantel–Haenszel test can be used to test for an association between two binary variables (life stage and habitat), controlling for a third qualitative variable (Agresti, 2002), in this case the particular drip/pool and cave. The same test was used to test for conditional independence between habitat and ecological status (stygobiont, epikarst specialist, or stygophile), controlling for the particular drip/pool and cave. Analyses were done with SAS© 9.2 (SAS Institute Inc.).

RESULTS

Based on species accumulation curves and estimates of numbers of missing species, Pipan and Culver (2007a) estimated that nearly all species present are likely to have been found in most caves studied, with the exception of Postojnska jama where the numbers of individuals collected in drips was much lower than in the other caves (Pipan, 2005). The list of species found in each cave is given in Table 1. Overall, 37 species of copepods were collected, 25 were stygobionts, and 16 were found primarily in epikarst and associated drip pools. The 12 non-stygobiont species include species often found in epikarst, such as *Bryocamptus dacicus* and *B. zschokkei*, and accidentals, such as *Diacyclops languidus* and *Atheyella crassa*. Harpacticoida predominated, accounting for 31 of the species.

Stygobionts and epikarst specialists were more likely to be found in drips than in pools in all six caves (Table 2). The frequency of stygobionts among all copepod species found in drips ranged from 0.73 in Pivka jama to 1 in Črna jama and Dimnice. The frequency of stygobionts in pools ranged from 0.46 in Škocjanske jame to 0.88 in Dimnice. None of the differences in individual drip/pool pairs was statistically significant (Fisher's exact test), but the Cochran–Mantel–Haenszel statistic was significant ($\chi^2 = 8.81$, df = 1, $P = 0.0030$), indicating that there is an association between water source (drip/pool) and whether a species is stygobiotic or not, adjusting for cave. The probability of finding a stygobiont copepod was 47% higher in drips than in pools, with a 95% confidence interval of 14–88%. The actual number of stygobionts was higher in drips than in pools in all but Postojnska jama. Since only 11 specimens were collected in drips compared with more than 500 in pools in Postojnska jama (Pipan, 2005), it is likely that the drip fauna was incompletely sampled, a conclusion supported by the lack of an asympotote of the curve of number of species versus number of samples (Pipan and Culver, 2007a). The difference in the frequency of epikarst specialists in drips compared with pools was even more striking. Frequency of epikarst specialists in drips ranged from 0.63 in Dimnice to 0.31 in Županova jama; frequency in pools ranged from 0.25 in Dimnice to 0 in Črna jama (Table 2). None of the differences in individual drip/pool pairs was statistically significant (Fisher's Exact Test), but the Cochran–Mantel–Haenszel statistic was significant ($\chi^2 = 10.43$, df = 1, $P = 0.0012$), indicating an association between epikarst specialists and water source, controlling for cave. The probability of a drip copepod being an epikarst specialist was 2.98 times higher than that of a pool copepod being an epikarst specialist, with a 95% confidence interval of 1.46 to 6.07. In all caves, even Postojnska jama, the number of epikarst specialist species in drips exceeded the number in pools. Only Škocjanske jame had more than two epikarst species in drip pools (Table 2).

Another way to look at the faunal differences between drips and pools is to enumerate species known from 10 or more individuals exclusively in either drips or pools, in each cave. Because the number of individuals collected was often very low (Pipan, 2003, 2005), this reduces the noise in the data. There were five such cases in drips and 10 in pools (Table 3). For the drips, all five were stygobionts and three were epikarst specialists. Especially noteworthy is an undescribed species of *Parastenocaris,* which was common in drips in Pivka jama and Županova jama. Of the 10 cases in pools, only four were stygobionts and only one was an epikarst specialist, an undescribed species of *Bryocamptus* from Škocjanske jame.

The proportion of immature (juveniles plus nauplii) individuals in drips and the associated pools for each of the three sampling periods was also compared. Because the same drip pools were sampled up to three times, whether the sampling was intensive enough to result in diminished numbers in later samples was investigated. The most likely cases where

Table 1. List of copepod species found in drips and/or drip pools in six Slovenian caves

Species	Stygobiont	Epikarst specialist	Postojnska jama	Pivka jama	Črna jama	Škocjanske jame	Dimnice	Županova jama
CYCLOPOIDA								
Acanthocyclops kieferi	●		●					
Diacyclops languidoides	●		●					●
Diacyclops languidus						●		
Megacyclops viridis						●		
Paracyclops fimbriatus			●			●		
Speocyclops infernus	●		●	●	●	●	●	●
HARPACTICOIDA								
Attheyella crassa						●		
Bryocamptus balcanicus	●		●	●	●		●	●
Bryocamptus borus	●	●					●	
Bryocamptus dacicus			●	●	●			
Bryocamptus pygmaeus							●	
Bryocamptus pyrenaicus	●			●			●	●
Bryocamptus typhlops			●	●		●		●
Bryocamptus zschokkei						●		
Bryocamptus sp.	●	●		●		●		
Elaphoidella cvetkae	●			●	●	●		●
Elaphoidella kieferi	●	●				●		
Elaphoidella stammeri	●	●						●
Elaphoidella millennii	●							●
Elaphoidella tarmani	●	●		●				
Epactophanes richardi				●				
Maraenobiotus cf. *brucei*	●	●		●				
Moraria poppei			●	●	●	●		
Moraria stankovitchi	●	●						●
Moraria varica			●	●				
Moraria sp. A	●	●			●	●		
Moraria sp. B	●	●						●
Morariopsis dumonti	●	●						●
Morariopsis scotenophila	●		●		●	●	●	
Nitocrella sp.	●	●	●				●	
Parastenocaris nolli alpina	●			●	●	●	●	●
Parastenocaris cf. *andreji*	●	●					●	●
Parastenocaris sp. 1	●	●			●		●	
Parastenocaris sp. 2	●	●	●	●	●	●	●	●
Parastenocaris sp. 3	●	●				●		●
Phyllognathopus viguieri			●					●
cf. Stygepactophanes sp.	●	●			●	●	●	

Stygobionts are exclusviely found in subterranean waters and epikarst specialists are species primarily known from epikarst associated habitats. Data from Pipan (2005) with some corrections.

Table 2. Frequency of stygobiotic copepods in pools and drips in the six study caves

Cave	Habitat	Number of copepod species	Number of stygobionts	Number of epikarst specialists
Črna jama	drips	8	8	4
Črna jama	pools	7	5	0
Dimnice	drips	8	8	5
Dimnice	pools	8	7	2
Pivka jama	drips	11	8	4
Pivka jama	pools	10	5	2
Postojnska jama	drips	5	4	2
Postojnska jama	pools	12	6	1
Škocjanske jame	drips	9	8	5
Škocjanske jame	pools	13	6	3
Županova jama	drips	13	12	4
Županova jama	pools	9	7	2

this would happen are drip pools situated on the top of stalagmites (type A pools of Pipan, 2005). Two type A pools were sampled more than once, and both of these showed no reduction in numbers through time (Figure 3), indicating that the individual samples (see Figure 2) were not complete samples of pools. The rise in numbers in some pools through time is most likely the result of additions from unsampled drips.

Table 3. All copepod species known from 10 or more specimens exclusively in one habitat, for the cave indicated

Cave	Species	Habitat	Stygo-biont	Epikarst specialist
Pivka jama	*Elaphpoidella cvetkae*	Drip	Yes	No
Pivka jama	*Parastenocaris* sp. 2	Drip	Yes	Yes
Županova jama	*Elaphpoidella stammeri*	Drip	Yes	Yes
Županova jama	*Parastenocaris nolli alpina*	Drip	Yes	No
Županova jama	*Parastenocaris* sp. 2	Drip	Yes	Yes
Črna jama	*Moraria poppei*	Pool	No	No
Črna jama	*Morariopsis scotenophila*	Pool	Yes	No
Pivka jama	*Epactophanes richardi*	Pool	No	No
Škocjanske jame	*Megacycloops viridis*	Pool	No	No
Škocjanske jame	*Bryocamptus typhlops*	Pool	No	No
Škocjanske jame	*Bryocamptus zschokkei*	Pool	No	No
Škocjanske jame	*Bryocamptus* sp.	Pool	Yes	Yes
Škocjanske jame	*Morariopsis scotenophila*	Pool	Yes	No
Županova jama	*Bryocamptus typhlops*	Pool	No	No
Županova jama	*Speocyclops infernus*	Pool	Yes	No

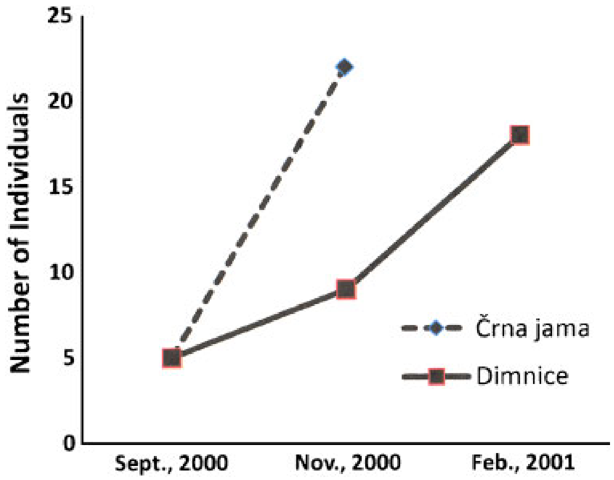

Figure 3. Numbers of copepods collected from drip pools in two caves located on the tops of stalagmites over the course of the study.

Table 4. All cases where the total number of copepods collected in drips and in pools in a sampling period was greater than 10

Cave	Adult *N*	Immature *N*	Immature/Total
Dimnice pool 2-3	211	30	0.12
Dimnice drip 2-3	6	10	0.63
Pivka jama pool 1-1	57	5	0.08
Pivka jama drip 1-1	58	18	0.24
Pivka jama pool 2-2	918	18	0.02
Pivka jama drip 2-2	18	22	0.55
Škocjanske jame pool 3-3	10	3	0.23
Škocjanske jame drip 3-3	43	2	0.04
Škocjanske jame pool 4-2	42	6	0.13
Škocjanske jame drip 4-2	0	14	1.00
Škocjanske jame pool 4-3	25	6	0.19
Škocjanske jame drip 4-3	6	11	0.65
Škocjanske jame pool 5-1	12	20	0.63
Škocjanske jame drip 5-1	30	11	0.27
Škocjanske jame pool 5-2	101	17	0.14
Škocjanske jame drip 5-2	23	1	0.04

The first number following the cave name refers to the drip number and the second number to the sampling period (see Pipan, 2003).

Based on a Cochran–Mantel–Haenszel test, there was strong evidence of an association between copepod maturity and water source, after adjusting for cave ($\chi^2 = 75.76$, df = 1, $P < .0001$), for the 37 samples with non-zeros for drips and pools. The probability of a copepod in a drip being immature was 2.08 times higher than the probability of a pool copepod being immature, with a 95% confidence interval of 1.73 to 2.50. Table 4 lists all eight cases of paired drips and pools over a 60 day sampling period for which the total abundance of copepods (immature and mature) collected was greater than 10 in each habitat. In five of the eight individual cases, the frequency of immature individuals in drips was significantly greater (Fisher's exact test) than in pools, two were not significant, and only one anomalous case was found — that of pool 5 in Škocjanske jame, where the frequency of immature individuals in pools was higher than in the associated drips. The frequency of immature individuals in Škocjanske jame pool 5 was always higher than in the associated drip, the only pool for which this was the case. Unlike all of the other drip pools in the study caves, pool 5 had

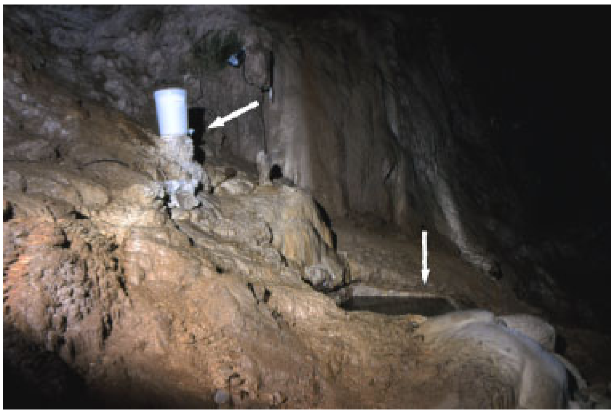

Figure 4. Drip pool 5 in Škocjanske jame modified to be a permanent water source. Arrows indicate the drip with a filtration unit underneath and the pool, offset from the drip.

been artificially deepened and lined with grout to establish an accessible water source (Figure 4), making it a very different and much larger habitat.

DISCUSSION

Are the differences between drips and pools artefacts?

The faunal differences between drips and pools are striking, and the obligate subterranean component of the drip fauna is much higher. Since drips are directly from epikarst, the composition of the animals in drips should be an approximation of the fauna of epikarst, but only an approximation. The fauna of drips is the result of dislodgement of copepods in the water column. Species are likely to differ in their susceptibility or propensity to enter the water column, if for no other reason than their differences in size (Pipan and Culver, 2007b).

The fauna of drip pools is also augmented by species, typically not stygobionts, that are either rare or not found in drips (Tables 1 and 3). The pools in this study are well above the 100 year flood levels and in the case of Županova jama there is no stream at all. We suspect that these species are rare colonists from the epikarst that have not been detected in drips, suggesting that while sampling of stygobionts from epikarst is more or less complete (Pipan and Culver, 2007a), it is less so for more generalist species.

Since drips and pools were sampled in different ways (Figures 1 and 2), it might be possible that the differences in frequency of immature individuals is a sampling artefact. However, we think this is highly unlikely for the following reason. The sampling of pools involves aspiration of the copepods, and based on Hjulstrom curves of hydrologists, copepods in the range of 0.3 to 0.6 mm, the size of immature individuals, are most likely to be aspirated into the sampling device (Pipan and Culver, 2007b). The size of most of the adult copepods in this study was at the upper end of this range or even larger (Culver *et al.*, 2009).

Protecting the epikarst fauna

Throughout the world in karst areas, the epikarst fauna is rich in species, many of which are geographically rare (Brancelj and

Culver, 2005). Ranges of no more than a few tens of kilometres in linear extent are common among epikarst species, and populations of many epikarst copepods appear to have a linear extent of only a few hundred metres (Pipan *et al.*, 2006). In addition, the epikarst itself is extremely vulnerable to some kinds of environmental degradation, especially toxic and other spills (Loop and White, 2001). Because epikarst species have typically been collected in drip pools, there has been a focus on the protection of caves and the pools they contain in order to protect epikarst species. For example, the purchase of Piddling Pit in West Virginia by The Nature Conservancy was motivated in large part because of the presence of two rare epikarst species — *Stygobromus parvus* and *S. nanus*. However, there is no reason to believe that epikarst populations do not occur where there is no cave nearby; it is just that at present there is no way to sample such habitats.

This study shows that epikarst species can be rare or entirely absent from pools. The most striking cases of this are low frequency or complete absence of epikarst specialists in pools (Tables 2 and 3). Furthermore, there is evidence that the drip pool populations of stygobionts in general, and epikarst specialists in particular, are derived entirely from dripping water. For example, in a general review of the copepod fauna of Škocjanske jame, Petkovski and Brancelj (1985) reported 23 species, which were collected in a variety of aquatic habitats, including some from drip pools. Yet, 12 of these species were not found by Pipan (2003) in her extensive survey of the epikarst fauna of the cave. An additional eight species not reported before were found. If Petkovski and Brancelj (1985) had not included drip pools in their general sampling, the differences would certainly have been greater. In Škocjanske jame, it is the epikarst that is the biodiversity hotspot and the focus of protection must include the epikarst.

Protection of epikarst habitats requires a shift of emphasis from protection of caves to protection of surface areas. Epikarst lies only a few metres below the surface so land-use changes can pose a threat to epikarst populations. Of course, the protection of caves is of value, but at least for the present study, that value lies primarily with the importance of caves as monitoring sites. This holds whether drip pools are sampled or drips themselves are sampled. A more complete protection strategy would be to include not only the cave for its value as a monitoring point, but also a significant area of karst with epikarst. A starting point for an appropriate sized area is Pipan and Culver's (2007b) finding that most epikarst copepod populations extend less than 1 km along a cave passage.

With the shift in emphasis comes the realization that the numerical rarity of many species known from drip pools is more apparent than real. At least for copepods, epikarst populations must be large in order to sustain the loss rates from dripping water, reaching upwards of one copepod per drip per day in Organ Cave, West Virginia (Pipan *et al.*, 2006). Other stygobiotic species in other habitats such as phreatic waters may also be more numerically common than thought because of sampling difficulties and inaccessible habitats. Such may be the case for North American cave crayfish species in the genus *Orconectes* (Buhay and Crandall, 2005).

Are drip pools an important habitat in their own right?

The overall difference in the proportion of immature individuals in drips and pools is striking — the proportion of immature individuals in drip pools is less than half that in drips. When individual paired drips and pools are compared, the difference holds with the exception of the anomalous pool 5 in Škocjanske jame (Table 4 and Figure 4). How can this difference be accounted for? Two factors are probably at work. First, the mortality rate of juveniles relative to adults is likely to be higher in pools relative to drips. This may be due to increased predation on juveniles in pools both by adult copepods and other invertebrate predators such as Turbellaria, Isopoda, and Amphipoda in the study caves (Pipan, 2005). The relative structural simplicity of drip pools compared with epikarst makes this a reasonable hypothesis. Physico-chemical conditions in pools may be less suitable and result in higher mortality of immature individuals. In addition, pools are likely to have less dissolved organic carbon because the only carbon source is dripping water and this carbon is utilized by the animals in pools (Simon *et al.*, 2007). Second, some species fail to reproduce in pools. Some stygobionts, especially epikarst specialists, such as an undescribed species of *Moraria*, have not been found in drip pools even though they are present in the water dripping into the pools. Pipan (2005) found eight such species. Based on the relatively low frequency of immature individuals in drip pools, it is likely that other stygobiotic species either fail to reproduce or have very limited reproductive success. Pool 5 in Škocjanske jame (Figure 4) is informative in this regard. The only pool in the study with a higher frequency of immature individuals than the associated drip, indicating *in situ* reproduction is more likely, it has been highly modified, including enlarging and deepening. The end result is a quite different and larger habitat, and one that can apparently sustain populations. All in all, it would seem that for epikarst copepods, pools are generally sink habitats (Pulliam, 1988).

The other interesting feature of immature individuals is the relatively high frequency ($>40\%$) present in the copepod population found in drips. It is possible that this is a biased sample of the epikarst populations, although not of course a biased sample of the animals reaching pools. By analogy with the mobilization of an inert particle into a water current (Hjulstrom curves), copepods with sizes between 0.3 and 0.6 mm should be most easily mobilized into the current and hence washed out of the epikarst (Pipan and Culver, 2007b).

Even if all pools were sink habitats to all epikarst species, an extreme view that we are not advocating, pools would be important in any conservation strategy. The drips associated with these pools are the only means available at present to sample water in the epikarst, or more properly, water directly leaving the epikarst. Any successful conservation strategy must include much more than the caves that contain the accessible drips. Significant areas of land with epikarst must also be protected.

Is epikarst adequately protected?

The greatest threats to epikarst generally are toxic spills and illegal dumping, and the Slovenian caves studied are no exception. Among the specific threats are spills from road accidents, leading to underground storage tanks, and illegal dumping in dolines and sinkholes. Epikarst acts as both a collector of toxic waste because of its storage capacity and a dispersal path because of its vertical and horizontal connections (Loop and White, 2001).

The caves themselves are relatively well protected. Since Slovenia is in the European Union, one of the major legal protection tools is the European Commission Habitats Directive, which includes a habitat type 'caves not open to the public'. In fact, all of the caves in the Slovenian study are open to the public, although the definition of what is open to the public is open to interpretation. All of the caves studied except Dimnice have illuminated sections, and tours of Dimnice are available to tourists. Škocjanske jame has additional protection as both a Ramsar and UNESCO Natural Heritage site. All of the caves are protected by additional Slovenian national legislation, including the 1993 decree on protection of threatened animal species, including all species living in caves and subterranean waters, and a 1994 act on cave protection and restriction of collecting. Based on international treaties, resolutions, recommendations, state and local community regulations, a nature conservation act was also passed in 2004. This includes Articles 31–33 protecting species and habitat types in ecologically important areas and in sites in the Habitats Directive Natura 2000 network.

Rather than attempting to add epikarst and show caves to the list of habitats in the EC Habitats Directive, Michel *et al.* (2009) suggested that a network of small areas be protected, based on criteria of protection of maximum diversity. This allows the inclusion of habitats like epikarst, caves listed under the Habitats Directive, undiscovered caves, and other shallow subterranean habitats.

Epikarst communities are but one of several subterranean communities that are difficult or impossible to sample directly, and are potential subterranean biodiversity hotspots. These include small seeps of superficial groundwater, described and named the 'hypotelminorheic' by Meštrov (1962) and relatively large spaces in the soil and talus as a result of the spaces created by rocks, cracks, and fissures, the milieu souterrain superficiel or MSS (Gers, 1998). Species from these habitats are also sometimes found in caves but caves are unlikely to be a source habitat for this fauna.

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