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ESTIMATING BIODIVERSITY IN THE EPIKARSTIC ZONE OF A WEST VIRGINIA CAVE

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A total of 13 ceiling drips in Organ Cave, West Virginia, USA, were sampled for fauna for three consecutive 10 day intervals. A total of 444 individuals from 10 copepod genera were found. Incidence functions revealed that 90 percent of the genera were found in eight samples, and that estimates of total diversity indicated only one or two genera had yet to be found. The overall rate of false negatives for different drips was 0.39 and the overall rate for different time intervals was 0.31, also suggesting that the sampling scheme was sufficient. Compared to nearby pools which serve as collection points for epikarst water, the drip samples were significantly different and more diverse. In addition to copepods, a wide variety of other invertebrates were found in drips, including many terrestrial insects that serve as part of the food base for the cave community. Direct sampling of drips is the preferred method at present for sampling the epikarst fauna.

INTRODUCTION

The uppermost zone of karst, the skin of karst, 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. An extraordinarily complex and heterogeneous structure, it is home to a wide array of animals, mostly but not exclusively aquatic, that often rivals in diversity the rest of the karst aquifer (Pipan and Brancelj, 2004).

The position of the epikarst at the top of the karstic bedrock and the typical absence of enterable passages or voids have meant that sampling has had to be indirect. Water exiting from the epikarst appears in caves as drip and seeps from ceilings and walls. The exiting water often creates pools (often surrounded by rimstone) well above any streams that might be in the cave and well above the water table. The first collections of epikarst fauna were hand collections from these sites, and typically included macroscopic invertebrates, especially amphipods. More recently, drips have been collected directly, and special sampling devices have been designed to collect copepods and other microscopic invertebrates (Pipan, 2003; Brancelj, 2004). The use of these techniques has greatly increased the number of known epikarst species, especially from drips (Pipan and Brancelj, 2004).

In this paper we report on the differences in faunal composition between drips and pools. Collections from drips represent animals exiting from the epikarst, in the same sense that harpacticoid copepods collected by drift nets at springs by Rouch (1970) represented both losses from the system and a way to assess the dynamics and diversity of the system, data which Rouch used with great success in his multi-year study of the Baget karst basin in France. He called this phenomenon "auto-hémorragie." While the animals collected in drips may not be the entire epikarst fauna, it contains no other elements except for the possibility of surface-dwelling species being flushed through the system. By way of contrast, pools not only collect the animals from drips, there is also the possibility of interaction among these animals as well as the possibility of different levels of reproduction. In addition, there is the possibility that invertebrates living in other subterranean waters, especially streams, may colonize pools especially during times of flooding.

In particular, we have intensively studied the drip and pool fauna of a cave in southern West Virginia, USA, and compare both the copepod and non-copepod fauna in these habitats. We also look at measures of sampling completeness to estimate whether we have found all of the species present.

MATERIAL AND METHODS

A short-term field study was conducted in the Organ cave system (West Virginia) from May 17 to June 20, 2004. Rains are frequent in late spring and early summer and drips are usually more active than at other times of the year. The area receives an average of 95 cm of precipitation annually and during the sampling period precipitation was 24 cm. Jones (1997) provides more details on climate in the area. We chose three sampling sites of the Organ cave system: the Lipps, Sively No. 2, and Sively No. 3 sections of the cave (see Stevens 1988 for

a detailed map and description). In the Lipps section we collected from five drips within 4 m of each other. In the Sively 2 and Sively 3 sections we found and sampled four drips in each, separated by distances of up to 100 m. In the first case of Sively 2 the distance between the second and the fourth drip was around 7 m, and the distance between the second and the fourth drip of Sively 3 was 1 m. Thus we had four clusters of distances – sets of drips less then 1 m apart, sets less then 10 m apart, sets less then 100 m apart, and sets of drips less then 1500 m apart.

The water from a drip was directed 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 three times at approximately 10-day intervals. Pools within 5 m of the drips were sampled by aspiration of the water filtered through the collecting container described above. In Lipps and Sively #2, there was one pool within the area and in Sively #3 there were two pools. Each pool was in an area of rimstone with many subdivisions and intercalations. Each of these four pools was sampled at the beginning and at the end of the study. The volume of water aspirated ranged from 0.2 L to 10 L, depending on the volume of water in pools.

Non-copepod specimens were usually identified to order but occasionally to species. Copepods, which are largely undescribed from North American caves (but see Reid [2004] for cave *Diacyclops* from Indiana), were identified to genus using Williamson and Reid (2001).

Patterns of similarity between faunas were assessed using log-likelihood contingency tables, using JMP™ (SAS Institute, Cary, NC, USA). Genera incidence curves were determined using the Mao-Tau function of Colwell *et al.* (2004), using EstimateS (Colwell, 2004). In addition, we calculated the total expected number of genera based on Chao's function S_{Chao1} and Smith and van Belle's jack-knife procedure, S_{boot}, where

$$S_{Chao1} = S_{obs} + \frac{F_1^2}{2(F_2+1)} - \frac{F_1 F_2}{2(F_2+1)^2} \quad (1)$$

$$S_{boot} = S_{obs} + \sum_{k=1}^{S_{obs}} (1 - p_k)^m \quad (2)$$

(Colwell, 2004) where F_i is the number of genera that have exactly i individuals when all samples are pooled, S_{obs} is the observed number of genera, p_k is the proportion of samples that contain genus k, and m is the total number of samples.

RESULTS

A total of 11 genera of copepods were collected, and a total of 14 morphological species were identified. Identification to species and description of new species requires further taxonomic work. We have taken the conservative approach and analyzed only generic diversity. It appears that for most genera, only one species is present (Table 1). Their morphology would indicate that at least seven species in five genera are adapted to and likely limited to subterranean habitats (see Reid and Strayer, 1994). A total of 10 genera were collected from drips and seven were collected from pools.

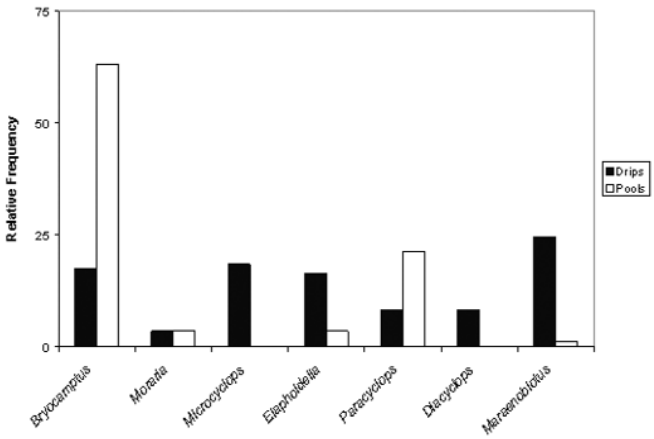

Figure 1. Relative abundance of different copepod genera in drips and in pools. Only the seven most common genera are shown, ones common enough for statistical analysis (see Table 1). Frequencies in each habitat sum to 100%.

A total of 444 copepods were collected in drips and 84 in pools. The difference in abundance is a reflection of the relatively few opportunities for collection from pools. Pools were thoroughly aspirated on the first sampling trip and subsequent samples were much reduced in abundance. The relative abundance of the seven most common genera is shown in Figure 1. In pools, the harpacticoid *Bryocamptus* was the most abundant genus, representing nearly two-thirds of the individuals collected. The only other common genus was the cyclopoid *Paracyclops*. In drips, the harpacticoids *Maraenobiotus*, *Elaphoidella*, and *Bryocamptus* and the cyclopoid *Microcyclops* were all common, each accounting for between 16 and 25 percent of individuals collected. The cyclopoid *Ectocyclops*, of which only three total specimens were found, was the only genus not represented in drips. By contrast, several common genera were missing from pools, including *Microcyclops* and *Diacyclops*; the abundant *Maraenobiotus* was only represented by a single specimen from pools. *Parastenocaris* (20 individuals), *Eucyclops* (2 individuals), and *Epactophanes* (two individuals) were found only in drips. The differences in relative abundance of the more common genera are quite evident in Figure 1 and Table 2. Among the seven most common genera, there was a large, statistically significant difference in relative proportions between the two habitats (log-likelihood $G = 136.7$, $df = 6$, $p < 0.001$).

In addition to copepods, other invertebrates were found in drips and pools. A total of 454 other invertebrates (and one salamander) was found in drips and 100 other invertebrates

Table 1. List of genera found in drips and drip pools in Organ Cave. The number of morphologically distinct species and the number of troglomorphic species are also shown.

Genera found in Organ Cave	Number of morphospecies	Number of troglomorphs
Cyclopoida		
Diacyclops	1	1
Ectocyclops	1	0
Eucyclops	1	0
Microcyclops	1	0
Paracyclops	2	0
Harpacticoida		
Bryocamptus	1	1
Elaphoidella	2	2
Epactophanes	1	0
Maraenobiotus	1	0
Moraria	1	1
Parastenocaris	2	2

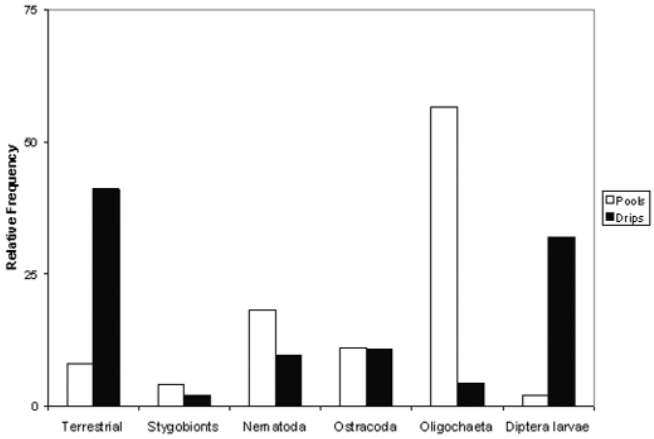

Figure 2. Relative abundance of non-copepods in drips and in pools.

were found in pools. They belonged to a wide variety of groups and many of them were not identifiable below class or order (Figure 2). Taken together, they rival the copepods in abundance. Included among the terrestrial species (presumably ones washed in through the epikarst (see Gibert, 1986) were Acarina, Collembola, Coleoptera and Diplopoda. The small but interesting category of stygobionts included the amphipod *Stygobromus*, the isopod *Caecidotea holsingeri*, a bathynellid and the archiannelid *Troglochaetus*. In pools, oligochaetes and nematodes predominated, probably because these benthic dwellers can find suitable microhabitat in the sediment of drip pools. Given the previous work of the French biologists Delay (1968) and Gibert (1986), it is not surprising that terrestrial accidentals are well represented. More unexpected was the large number of Diptera larvae. However, 95 of the 137 specimens collected were taken from a single drip and a single sampling interval. All groups were found in drips but some terrestrial accidentals (millipedes) and some rare stygobionts (archiannelids and bathynellids) were missing from pools.

In general, drips provided a more complete sample than pools, in the case of both copepods and other invertebrates. This is indicated primarily by the greater generic richness in drip samples, but also by the significant differences in composition between drips and pools (Tables 2 and 3). Drips provide a more or less unbiased sample except for any mortality that occurs in the sampling bottle during the 10 days of the sample. By contrast, pools are clearly biased samples. Groups are missing, and some groups are overrepresented. We do not have sufficient understanding of these creatures to know why particular copepods are proportionately more common in pools, but it is certainly true that pools are subject to environmental fluctuations (especially drying), that there are some microhabitats that may be quite abundant in pools, and that the relatively simple physical structure of pools may make predation more important.

False negatives are cases where a species was initially found, but was not recollected in subsequent samples (Tyre *et al.* 2003). When individual drips and individual 10-day sampling intervals were considered, overall rates of false negatives were quite high, averaging 0.71 (Table 4). However, rates of false negatives were much lower for the entire time course of a single drip, averaging 0.39. If all four (or five in the case of Lipps) drips were combined for one 10-day interval, rates were also lower, averaging 0.31. As a rule of thumb (Tyre *et al.,* 2003), rates higher than 0.50 indicated repeat sampling was advisable. By way of contrast, Schneider (pers. comm.) found false negative rates higher than 0.50 for collections of macroscopic terrestrial and aquatic cave fauna. Rates in Sively #2 were highest, presumably because of the lower number of animals found at this site. Overall, the results indicate that sufficient sampling intensity was employed in Organ Cave, and in fact false negatives reached an acceptable level after 10 days of sampling.

A better sense of the thoroughness of sampling can be gained from the genera accumulation curve shown in Figure 3. Based on Colwell *et al.* (2004) sample-based rarefaction, the accumulation curve together with 95% confidence intervals is shown. It rapidly begins to reach an asymptote, reaching 75 percent of the maximum after four samples, 90 percent of the maximum after eight samples, and 95 percent of the maximum after 10 of 13 samples. This asymptotic relationship is nearly unprecedented: examples of failure of sampling in caves to reach an asymptote abound (Culver *et al.*, 2004; Schneider and Culver, 2004). The number of genera unique to a single drip within the study sites also declined, indicating that sampling was thorough.

An alternative approach to sampling completeness is to use estimates of the total number of genera, based not on accumulation curves, but rather on the internal structure of species abundance, especially the number of genera in one (singleton)

Table 2. Comparison of observed and expected numbers of individuals of different copepod genera in drips and in pools. Overall there were highly significant differences (log–likelihood test, G = 136.7, df = 6, p < 0.001).

Genera found in Organ Cave	Pools		Drips	
	Observed	Expected	Observed	Expected
Bryocamptus	53	20.0	78	110.99
Diacyclops	0	5.7	37	31.40
Elaphoidella	3	11.6	73	64.40
Maraenobiotus	1	16.8	109	93.20
Microcyclops	0	12.5	82	69.50
Moraria	3	2.9	16	16.10
Paracyclops	18	8.4	37	46.60

Table 3. Comparison of observed and expected numbers of individuals of different non–copepod groups in drips and in pools. Overall there were highly significant differences (log–likelihood test, G = 192.99, df = 5, p <0.001).

Non–copepod groups found in Organ Cave	Pools		Drips	
	Observed	Expected	Observed	Expected
Diptera larvae	2	26.11	137	112.89
Nematoda	18	11.08	41	47.92
Oligochaeta	56	14.09	19	60.91
Ostracoda	46	46.29	11	10.71
Stygobionts[a]	9	10.56	4	2.44
Terrrestrials[b]	8	34.57	176	149.44

[a]Includes *Stygobromus pollostus* (Amphipoda), *Caecidotea holsingeri* (Isopoda), *Troglochaetus* (Archiannelida), and Bathynellacea.
[b]Includes mostly non–troglobitic Acarina, Coleoptera, Collembola, and Diptera.

or two (doubleton) drips (Colwell and Coddington, 1994). We looked at this at the scale of individual drips and sites as well as overall combined sites since there are differences in generic composition among sites and drips (Culver *et al.* 2005). At all scales there was good agreement between the number of observed and the total number of predicted genera (Table 5). Because of the particularities of the bootstrap formula, S_4, predicted number of genera can actually be less than the observed number of genera if there are doubletons, but no singletons, and this happened in several cases. For the three sites, only Sively #2 appeared to be under-sampled, with seven observed genera and 11 predicted genera. This is in accord with the overall smaller sample size in Sively #2 (Table 5). For Organ Cave taken as a whole, there was good agreement between observed and expected numbers of genera (10 vs. 12).

DISCUSSION

The easiest way to sample the epikarstic fauna of a cave is to sample pools. The sampling can be easily done in a single visit and it is relatively easy and quick to collect the samples. Unfortunately, pool sampling is far from an unbiased sample of the epikarst, and indeed common species may be completely missing from pools. For example, in our study *Diacyclops* and *Microcyclops* were completely missing from pools and *Maraenobiotus* was nearly so (Fig. 1, Table 1). Of course, many genera of copepods were found in drip pools, but unless drips are sampled directly, there are likely to be gaps in faunal lists. The same is true for non-copepod species although we had less detailed information on these. One non-copepod component of epikarst communities that is under-represented in pools is the terrestrial arthropods entering the cave through drips. They included Collembola, Diplopoda, Acarina, and Coleoptera, with Collembola being numerically dominant. The assemblage is interesting both because the animals are food input into the cave and because it is a sample of terrestrial epikarst fauna. We know virtually nothing about this fauna, although it is likely similar to the non-karstic MSS, "milieu souterrain superficial" (Juberthie *et al.*, 1980).

We only saw one genus unique to pools in Organ Cave—*Ectocyclops*, only three of which were found. In her study of the Postojna-Planina Cave System (PPCS), Pipan (2003) sampled both drips and pools in a similar way. She found a total of 23 species of copepods; of these 10 were found in both drips and pools, five were found only in drips, and eight were found only in pools. The major difference between the two studies was the presence of several species in pools that were not found in drips. The reasons for the difference are not clear, but in general the drips in Pipan's study yielded fewer individuals than in the Organ Cave study. In PPCS she found only 505

Table 4. Rates of false negatives.

Section	Type	Frequency	N
Lipps	by drip by time	0.71	24
Sively #2	by drip by time	0.92	12
Sively #3	by drip by time	0.65	23
Total	by drip by time	0.71	55
Lipps	by drip	0.16	19
Sively #2	by drip	0.60	10
Sively #3	by drip	0.53	15
Total	by drip	0.39	44
Lipps	by time	0.10	11
Sively #2	by time	0.67	9
Sively #3	by time	0.25	16
Total	by time	0.31	36

individuals in 20 drips sampled for five months and 1574 individuals taken in two collections in pools. This contrasts with the current study where 444 individuals were taken in 13 drips and 84 individuals were taken in pools in 30 days. We have no information about drip rates in the Slovenian sites, so total volume of dripping water cannot be compared. The difference in abundance in pools is probably mostly a reflection of the amount of volume or surface area of pools collected, but the differences between drips may reflect real differences between the "bleeding" from the epikarst in the two caves or it may reflect more short-term temporal differences. The total generic richness of copepods in the two cave systems was not that different. PPCS had a total of 14 copepod genera compared to 11 in Organ Cave; there were eight genera from PPCS in drips compared to 10 in Organ Cave; there were 12 genera in pools in PPCS and only seven in Organ Cave.

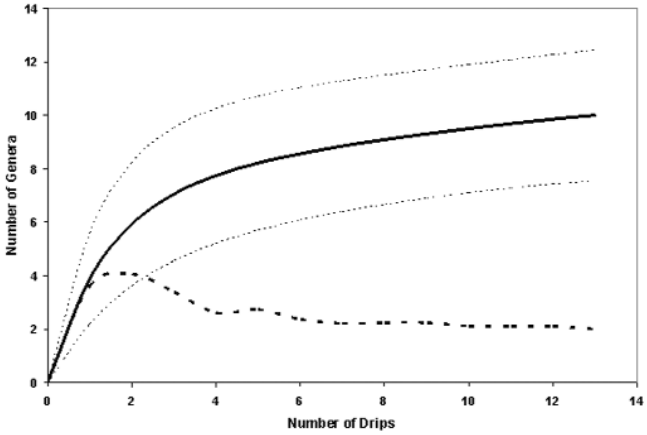

Figure 3. Copepod genera accumulation curve based on Mao-Tau procedure of Colwell *et al.* (2004). The solid line is the accumulation curve and the two dotted lines are the 95% confidence intervals. The heavy dashed line is the number of genera unique to a single drip.

When we examined the results of drip collections more thoroughly, it was clear from a variety of perspectives that the cave was well sampled for epikarstic copepods. This is especially the case for the generic accumulation curve (Fig. 3). Not only did it reach 90% of the total after eight of 13 samples, but the number of single drip genera declined to two after seven samples. It is likely that few if any genera are limited to a single drip within the set of study sites, but we simply do not know enough about the distribution of animals in the epikarst to state this with any certainty. The reaching of an asymptote is not a trivial point. It is rare to find a thoroughly sampled cave or cave region for macro-invertebrates. Culver *et al.* (2004) showed that even in the relatively well-studied Classic

Table 5. Estimates of genera richness based on Chao's S_1 and Burnham and Overton's S_4. See text for details.

Cave	Drip No.	Total S (S_{obs})	Total N (n_3)	Singletons (n_1)	Doubletons (n_2)	S_{Chao1} (Chao's)	S_{boot} (bootstrap)
Lipps	1	1	1	1	0	–	0.0
Lipps	2	6	97	0	2	6.0	4.0
Lipps	3	5	21	0	0		4.9
Lipps	4	4	38	1	0	–	5.9
Lipps	5	7	65	0	2	7.0	5.1
Lipps	Combined	7	223	0	1	7.0	6.0
Sively #2	1	1	1	1	0		0.0
Sively #2	2	2	5	1	0		3.4
Sively #2	3	2	2	2	0		4.5
Sively #2	4	5	11	2	1	7.0	8.0
Sively #2	Combined	7	19	3	2	9.2	11.0
Sively #3	1	1	1	1	0		0.0
Sively #3	2	6	52	1	1	6.5	7.0
Sively #3	3	8	138	0	1	8.0	7.0
Sively #3	4	3	11	1	0		4.7
Sively #3	Combined	9	202	1	0		10.0
Organ Cave	Combined	10	444	2	0		12.0

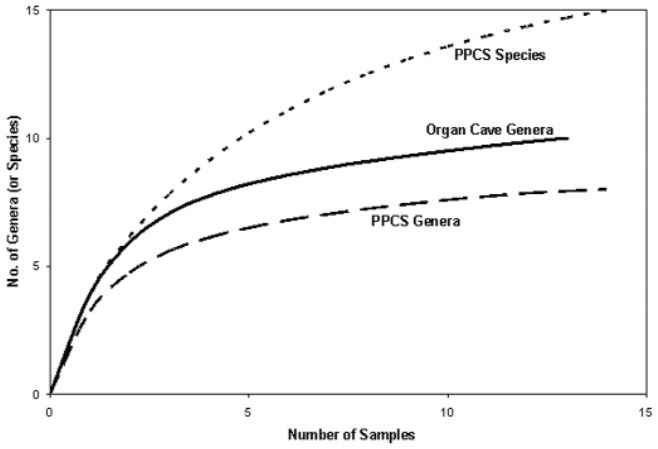

Figure 4. Comparison of accumulation curves of genera and species in Postojna Planina Cave System and genera in Organ Cave, based on the Mao-Tau procedure of Colwell *et al.* (2004).

Karst of Slovenia that the species accumulation curves for 100 km² hexagons were not reaching an asymptote. Stoch (pers. comm.) reported that it took more than ten samples of a single cave (Antro di Bagnoli near Trieste, Italy) for the species accumulation curve to reach an asymptote. In Schneider and Culver's (2004) study of more than 60 caves in a 15 km² area in West Virginia, USA, the species accumulation curve never reached an asymptote. In the present study, the number of copepod genera begins to reach an asymptote (90 percent saturation) after eight samples.

We suspect that the ability to more or less completely sample the species in the epikarst by collecting from 10 or more drips is quite general. Pipan's data on PPCS can also be examined in this way, both for genera and species. The shapes of the accumulation curves for Organ Cave and PPCS are compared in Figure 4. The shapes of the accumulation curves for genera are quite similar, with 90% of the genera found after an average of eight samples. It took 10 of 14 samples to find 90% of the species.

Likewise, analysis of patterns of false negatives (Table 3) and predicted numbers of genera (Table 4) indicated sufficient sampling. Values of false negatives were less than 0.40 for individual drips or individual dates, indicating that a strategy of either a single time period with multiple drips or a single drip over a longer time period would keep false negatives below the critical value of 0.50 (Tyre *et al.*, 2003). Predicted and observed numbers of genera (Table 4) were remarkably close with only Sively #2 indicating that any additional sampling would likely yield more genera.

What generalities about the amount of drip sampling required can be gleaned from this study? The first is that given the heterogeneity of the fauna in individual seeps, sampling in multiple seeps would be advisable. For example, seeps in the Sively #2 site were generally less productive for reasons that cannot be adequately explained by differences in flow rate (Culver *et al.* 2005). Second, at least in Organ Cave, a ten day sample of 10–13 drips would probably have been sufficient (see Tables 3 and 4). Of course we do not know if the scale of heterogeneity we observed is typical of caves.

Finally, we note the extraordinary diversity of the epikarstic copepod assemblage in Organ Cave. Although many species await description, it appears that there are at least 14 species of copepods among the 11 genera, seven of which are troglomorphic and likely stygobitic. By contrast, the streams of Organ Cave, a well-studied system (Culver *et al.* 1994), harbor only a total of 6 stygobionts. The only copepods found in the stream (sampled with a modified Hess sampler) were two stygophilic species: *Brycamptus nivali* and *Bryocamptus* nr. *morrisoni* (Culver *et al.*, 1994). When the epikarst fauna in Organ Cave and elsewhere in North America is fully described (see Reid 2004), it may well dominate the lists of stygobitic fauna.

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REFERENCES

Brancelj, A., 2004, Biological sampling methods for epikarst water, p. 99–103. *in* Jones, W.K., Culver, D.C. & Herman, J.S., eds., Epikarst. Proceedings of the symposium held October 1 through 4, 2003, Sheperdstown, West Virginia, USA: Karst Waters Institute Special Publ. 9, Charles Town, W.Va., 200 p.

Colwell, R.K., 2004, EstimateS: Statistical estimation of species richness and shared species from samples Version 7. User's Guide and application published at http://purl.oclc.org/estimates.

Colwell, R.K., & Coddington, J.A., 1994, Estimating terrestrial biodiversity through extrapolation: Philosophical Transactions of the Royal Society (Series B), v. 345, p.101–118.

Colwell, R.K., Mao, C.X., & Chang, J., 2004, Interpolating, extrapolating, and comparing incidence–based species accumulation curves: Ecology, v. 85, p. 2717–2727.

Culver, D.C., Jones, W.K., Fong, D.W., & Kane, T.C., 1994, Organ Cave karst basin, p. 451–473. *in* Gibert, J., Danielopol, D., & Stanford, J., eds., Groundwater ecology: San Diego, Academic Press, 571 p.

Culver, D.C., Christman, M.C., Sket, B. & Trontelj, P., 2004, Sampling adequacy in an extreme environment: species richness patterns in Slovenian caves. Biodiversity and Conservation, v.13, p. 1209–1229.

Culver, D.C., Pipan, T. & Christman, M.C., 2005, Dynamics of epikarstic communities: microgeographic pattern and environmental determinants of epikarstic copepods in Organ Cave, West Virginia. American Midland Naturalist, in review.

Delay, B., 1968, Recherches sur les eaux souterraines – 2 – Données sur le peuplement de la zone percolation temporaire: Annales de Spéléologie, v. 23, p. 705–716.

Gibert, J., 1986, Ecologie d'un systeme karstique Jurassien. Hydrogéologie, derive animale, transits de matières, dynamique de la population de Niphargus (Crustacé): Mèmoires de Biospéologie, v. 12, p. 1–376.

Jones, W.K., 1997, Karst hydrology atlas of West Virginia. Karst Waters Institute, Charles Town, W.Va. 111p.

Jones, W.K., Culver, D.C. & Herman, J.S., eds., 2004, Epikarst. Proceedings of the symposium held October 1 through 4, 2003, Sheperdstown, West Virginia, USA: Karst Waters Institute Special Publ. 9, Charles Town, W.Va., 200 p.

Juberthie, C., Delay, B., & Bouillon, M., 1980, Extension du milieu souterrain en zone non calcaire: description d'un nouveau milieu et de son peuplement par les Coléopterès troglobies. Mèmoires de Biospéologie, v. 7, p. 19–52.

Pipan, T., 2003, Ecology of copepods (Crustacea: Copepoda) in percolation water of the selected karst caves: Doctoral dissertation (in Slovene with English abstract and summary). Ljubljana, Slovenia: University of Ljubljana, Dept. of Biology.

Pipan, T., & Brancelj, A., 2004, Diversity and peculiarity of epikarst fauna: Case study from six caves in Slovenia (Europe), p. 119–126. *in* Jones, W.K., Culver, D.C. & Herman, J.S., eds., Epikarst. Proceedings of the symposium held October 1 through 4, 2003, Sheperdstown, West Virginia, USA: Karst Waters Institute Special Publ. 9, Charles Town, W.Va., 200 p.

Reid, J.W., 2004, New records and new species of the genus Diacyclops (Crustacea; Copepoda) from subterranean habitats in southern Indiana, USA: Jeffersonia, no. 12, p. 1–65.

Reid, J.W., and Strayer, D.L., 1994, Diacyclops dimorphus, a new species from Florida, with comments on morphology of interstitial cyclopine cyclopoids: J. North Amer. Benthological Soc., v. 13, p. 250–265.

Rouch, R., 1970, Recherches sur les eaux souterraines. 12. Le système karstique du Baget. I. Le phénomène d' "hémorragie" au niveau de l'exutoire principal: Annales de Spéléologie, v. 25, p. 665–709.

Schneider, K., & Culver, D.C., 2004, Estimating subterranean species richness using intensive sampling and rarefaction curves in a high density cave region in West Virginia: Journal of Cave and Karst Studies, v. 66, p. 39–45.

Sket, B., Trontelj, P., and Žagar, C., 2004, Speleobiological characterization of the epikarst and its hydrological neighborhood: its role in dispersion of biota, its ecology and vulnerability, p. 104–113. *in* Jones, W.K., Culver, D.C. & Herman, J.S., eds., Epikarst. Proceedings of the symposium held October 1 through 4, 2003, Sheperdstown, West Virginia, USA: Karst Waters Institute Special Publ. 9, Charles Town, W.Va., 200 p.

Stevens, P.J., 1988, Caves of the Organ Cave Plateau, Greenbrier County, West Virginia: West Virginia Speleological Survey, Bulletin 9, Barrackville, WV, 200 p.

Tyre, A.J., Tenhumberg, B., Field, S.A., Niejalke, D., Parris, K., & Possingham, H.P., 2003, Improving precision and reducing bias in biological surveys by estimating false negative error rates in presence–absence data: Ecological Applications, v. 13, p. 1790–1801.

Williamson, C.C., & Reid, J.W., 2001, Copepoda, p. 915–954. *in* Thorp, J.H., & Covich, A.P., eds. Ecology and classification of North American freshwater invertebrates: San Diego: Academic Press, 1056 p.