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Habitat fragmentation and its effects on groundwater populations

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ABSTRACT

The subterranean unsaturated zone of the karstic areas is highly fragmented. The maze of more or less connected voids harbors simple communities of mixed surface and subterranean species, especially crustaceans. Hypogean species are dominant in almost all communities of the seven stations sampled monthly in a cave of the north-western Romania. Water stable isotopes and drip rates were used to understand the morphological and spatial organization of the subterranean habitats in the unsaturated zone. The structure of the cave communities, the species synchrony and the spatial structure and variation of communities at different geographical levels are discussed in conservation terms. Copyright © 2011 John Wiley & Sons, Ltd.

KEY WORDS cave Crustacea; metacommunity; habitat fragmentation; drip water; stable isotopes

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INTRODUCTION

The broad definition of metapopulation is that of any group of populations that interact via dispersal (Levins, 1969; Hanski, 1999). These populations have to be spatially discrete, may differ in size, demography and carrying capacity, may be subject to extinction and recolonization and may interact via dispersal and gene flow (Bohonak and Jenkins, 2003). The fusion between metapopulation theory and community ecology generated the metacommunity theory where a metacommunity comprises a set of local communities on distinct habitat patches, linked by dispersal of one or more of its constituent species (Wilson, 1992; Holt, 1997). Though each of these two theories has different subjects of study—populations versus communities—some studies have considered the effects of interspecific interactions on metapopulation dynamics of a focal species and vice versa (Hanski, 1999). In a metapopulation there are internal and external drivers of dispersal for animals and plants (Ims and Andreassen, 2005) and dispersal is the parsimonious cause of population synchrony (Schwartz *et al*., 2002) and is density dependent. In metapopulations and metacommunities, dispersal prevents extinction, maintains species diversity and contributes to novel genetic variation (Bohonak and Jenkins, 2003).

Studying the dispersal in the unsaturated zone of the karstic areas is a real challenge because of the lack of direct access. However, studying subterranean communities is of great interest because they are perfect subjects for ecological and evolutionary studies, as inhabitants of the few continental habitats with discrete boundaries. For the groundwater communities of the unsaturated karst, such habitats are below surface, in carbonate soluble rocks (limestones and dolomites) and are represented by all types of karst voids (intergranular voids, pores, joints, cracks, fractures, fissures, conduits and caves) which offer conditions for survival and reproduction. In the underground, the maze of karst voids has more or less connectivity and harbor very simple communities, with stable temporal composition and without nowadays permanent colonization. It is one of the few continental habitats that can be used as simple models in ecological studies.

The unsaturated karst zone is represented by habitat patches in a surrounding karst matrix. It is a fragmented landscape that separates surface (epigean) communities from subterranean (hypogean) communities by the epikarst ecotone, inhabited by mixed surface-subterranean communities. For the underground communities, dispersal is possible only between habitats with favorable environmental features (lack of light, constant microclimate, presence of water) and there is no migration from these fragments to the surface. The only dispersal is gravitational, by using meteoric water as a vector that carries organic matter and epigean animals underground. Some of the surface or soil animals are able to survive for longer periods together with permanent, hypogean inhabitants. The matrix can influence species abundance or composition in the embedded patches, as mentioned for other habitats (Davies *et al*., 2001). The degree of competition posed by the epigean species, as temporary subterranean inhabitants, to their hypogean counterparts may directly or indirectly affect hypogean

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diversity. This study aims to show how habitat fragmentation shapes the subterranean metacommunity and the implication of connectivity in dispersal, both at local and regional levels. Water drip rate and stable isotopes were used for a better understanding of the patchiness of the subterranean landscape, metacommunity composition and spatial distribution. The significance of results for conservation is also discussed.

MATERIALS AND METHODS

Ciur Izbuc Cave (N 46°51′10″ E 22°24′00″), located in the Western Carpathians of Transylvania (Romania) was chosen for this study. It is developed in Middle Jurassic limestone at 395 m a.s.l. on two levels; an upper, 605 m long hydraulically inactive one and another lower, 425 m long, temporarily active (Figure 1). In order to test our working hypothesis, a simple experimental set-up has been established in two locations inside the cave, about 300 m apart, downstream and respectively upstream the subterranean river (Figure 1). Each of these two locations had four monitoring stations (1–8).

Fauna from the trickles was sampled in all the eight stations on a monthly basis from April 2008 to April 2009. The water from trickles was directed through a funnel in a container with the sides covered by a 100 µm planktonic net. Animals collected monthly were fixed in 98% alcohol and sorted to group level. Crustaceans were identified at species level (Meleg *et al.*, 2011).

Drip rates were measured in each of the upstream stations (1–4) using Stalagmate drip loggers (A–D in Figure 1) (Collister and Mattey, 2008). These record the acoustically generated electrical impulse of drops of water falling onto the lid of a water-tight box (65 mm × 60 mm × 40 mm tall). Drop events were recorded every 2 min for 13 months during the study interval. Daily and monthly averages were used for the interpretation of the flow regime and fauna dynamics, respectively. Precipitation amount was measured on a daily basis at a nearby location, the Vadu-Crişului hydrometeorological station, 9 km NE from the Ciur Izbuc Cave. The precision of measurements is better than 0·1 mm.

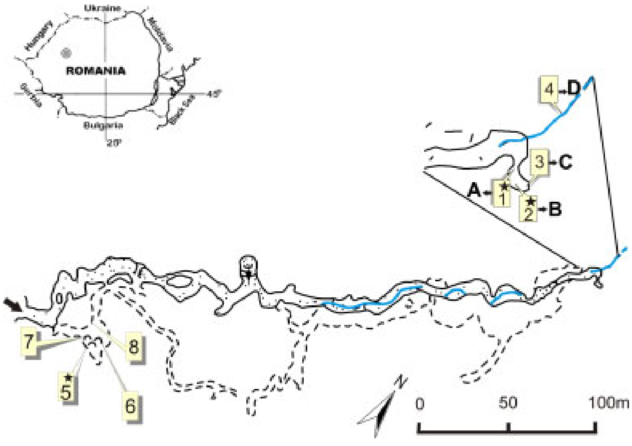

Figure 1. Map of the Ciur Izbuc Cave, Romania [after Rusu (1988), modified]: 1–8, stations, *, stations for isotopic sampling; A–D, drip loggers.

Precipitation and drip water for stable isotopes analyses were collected on a monthly basis, between July and December 2008. Water was collected in 1-l HDPE (High Density Poly-Ethylene) containers through a funnel placed below the drip logger, or below the close-by trickles. The water for stable isotopes analyses was collected outside the cave, and inside the cave for stations 1, 2 and 5. To prevent evaporation, the containers were coated with linseed oil. Stable isotope measurements were performed at the University of South Florida (USA) Stable Isotope Laboratory, on a Finnigan Delta V Plus IRMS, coupled to a Gasbench II extraction line, using the CO_2 equilibration method (Epstein and Mayeda, 1953) for oxygen, and H_2 equilibration method for hydrogen (Prosser and Scrimgeour, 1995). Results are reported in δ values versus VSMOW. The analytical precision (2σ) is better than 0·1‰for δ^{18}O, and 1‰for δ^{2}H, respectively.

Principal component analysis (PCA), spatial and temporal fauna distribution and time series obtained from the drip rate monitoring were analyzed with the XLSTAT ver.2010 and PAST ver. 1·97 (Hammer *et al.*, 2001) software. Cross-correlation is a standard method of estimating the degree to which two series are correlated and was used for each two pairs of data. Fourier smoothing method was applied for cross-correlations because of the typical rapid transitions from fast dripping to periods of droughts for the water percolating through the unsaturated zone.

RESULTS

Of the total of 11 taxonomical groups identified in the monthly samples of the Ciur Izbuc Cave, only 8 are aquatic: Oligochaeta, Nematoda, Cyclopoida, Harpacticoida, Ostracoda, Amphipoda, Gastropoda, Insecta larvae. Three groups: Acari, Isopoda, Collembola are semi-terrestrial animals. The four groups of Crustaceans represent more than 68% of the total number of sampled individuals and will be considered in further analyses. Seven Crustacean species were identified: one cyclopoid (*Acanthocyclops* n. sp), four harpacticoids [*Bryocamptus* (*Rheocamptus*) *caucasicus, Maraenobiotus brucei carpathicus, Parastenocaris* n. sp., *Spelaeocamptus spelaeus*], one amphipod (*Niphargus andropus*) and one ostracod (*Candona neglecta*). *B. caucasicus* and *C. neglecta* are epigean species. All the other crustaceans are typical hypogeans.

Between the eight stations located in two different parts of the cave, the differences are in fauna composition and crustacean species. All stations are dominated by hypogean copepods. Communities in the upstream stations (1–4) are dominated by cyclopoids and amphipods and less by harpacticoids, while the downstream stations (5–7) have more harpacticoids, as the tiny *Parastenocaris* sp. and *S. spelaeus* and the epigean ostracod *C. neglecta* (Table I and Figure 1). No crustaceans were

Table I. Matrix of Crustacea species identified in the stations of the Ciur Izbuc Cave, Romania (hypogean taxa in bold).

Month/genus	Stations 1–4						Stations 5–7					
	Bryocamptus	***Spelaeocamptus***	***Parastenocaris***	***Niphargus***	***Acanthocyclops***	*Candona*	***Maraenobiotus***	***Spelaeocamptus***	***Parastenocaris***	***Niphargus***	***Acanthocyclops***	*Candona*
April 2008	1	0	0	2	3	1	0	1	0	0	2	8
May 2008	6	0	1	0	8	0	8	3	16	0	3	4
June 2008	1	0	0	2	3	0	7	3	1	0	2	20
July 2008	0	1	0	0	0	0	1	1	0	0	0	4
August 2008	1	0	0	0	0	0	0	0	0	0	0	1
September 2008	0	1	1	0	0	0	0	0	0	0	0	0
October 2008	2	0	0	0	0	0	0	0	0	0	0	0
November 2008	3	0	0	3	5	0	0	2	0	0	0	0
December 2008	0	0	0	4	18	0	0	0	1	0	1	0
January 2009	1	0	0	1	4	0	0	11	5	1	1	1
February 2009	2	2	0	1	5	0	0	10	12	0	1	1
March 2009	1	3	0	6	1	0	0	6	4	0	3	0
April 2009	0	0	0	2	0	0	3	0	3	0	0	0

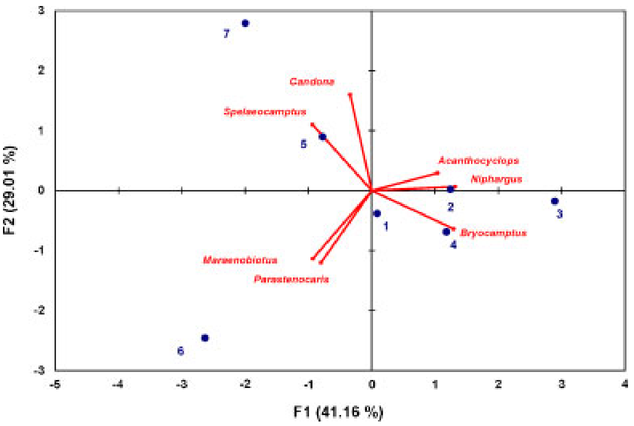

Figure 2. PCA of stations given by the presence of Crustacea species in the Ciur Izbuc Cave.

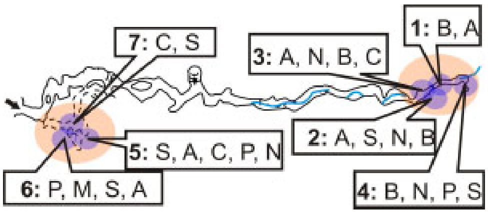

Figure 3. Distribution of taxa in the seven stations (1–7) of the Ciur Izbuc Cave: A, *Acanthocyclops* sp.; B, *B. caucasicus*; C, *C. neglecta*; N, *N. andropus*; P, *Parastenocaris* sp.; S, *S. spelaeus*. Species are represented in abundance order, in each station.

recorded in station 8. On the PCA analysis (Figure 2), the separation of the sampling stations by the number of individuals of each crustacean species makes visible the isolation of upstream and downstream stations along the first axis (41·61%). Upstream stations are grouped along the first axis and defined by the number of *Acanthocyclops* sp., *N. andropus* and *B. caucasicus*. Downstream stations are well-separated by the second axis (29·01%) with stations 5 and 7 dominated by *C. neglecta* and *S. spelaeus*, and both separated from station 6, with numerous *M. brucei* and *Parastenocaris* n. sp. Different fauna composition and numerical variations were also observed between neighboring stations (Figure 3). Mainly, downstream stations are very different in species composition. Species can be common for one or more stations, or can appear in different seasons even in stations very close to each other.

Temporal correlation was observed for some pair of species, at different levels of significance (Figure 4): e.g. positively between *S. spelaeus* and *N. andropus*, or negatively between *Parastenocaris* sp. and *Acanthocyclops* sp. for stations 1–4. The presence of the epigean *B. caucasicus* in these stations is correlated with a reduced number of the hypogean *Acanthocyclops* sp. Hypogean crustaceans have seasonal numerical variations in both locations inside the cave, a rise in spring and winter months and drastic reduction during summer–autumn. Monthly dynamics for common crustaceans in both locations is roughly similar but not completely correlated, but this can be caused by different abundances at the two different sites (Table I). *Parastenocaris* sp. has significant

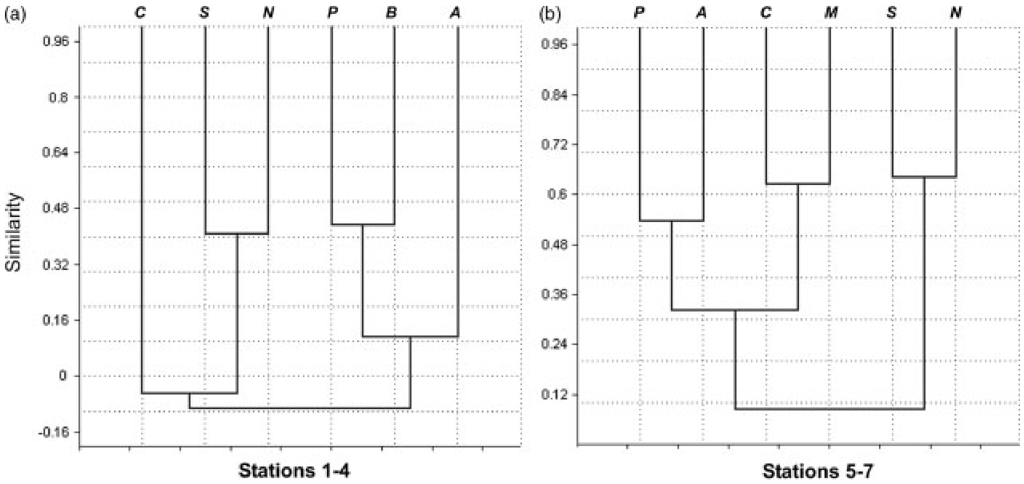

Figure 4. Some of the Crustacea species have similar dynamics both upstream (a) and downstream (b) inside the Ciur Izbuc Cave.

population growth during spring only in the downstream stations. A lag between the *S. spelaeus* population maximum appears at the two locations. Only *Acanthocyclops* has relatively similar seasonal trend, but different abundance in the upstream and downstream stations.

Time series analysis of the dripping rates in the four monitored stations shows similar seasonal trends, but different daily and monthly behaviors. Time series are non-significantly correlated at 95% confidence level and the lag is varying from 0 to 30 days (Figure 5). Although weakly significant, the time series that show best correlation are between each A and C and B and C, indicating a common origin of water, but different flow paths. Time series of the upper stations, A, B, C are not correlated to D, the station at the lower level. The time lag from the upper stations to the lower level station (D) is from less than 5 days (for A) to 30 days (for B and C), for only ~5 m of rock thickness.

Monthly averages of the drip rates were compared with the abundances of hypogean and epigean crustaceans for each station. There is no correlation, on short time interval, between the rate of precipitation and the drip rate inside the cave; precipitation water accumulates in the voids and it is discontinuously released into the cave. Moreover, the increase of the number of individuals is generally not correlated with the higher drip rates at the same station (Figure 6). A significant correlation was obtained only for station 1 and seems rather circumstantial because the Crustacea community is composed only by the epigean *B. caucasicus* and the hypogean *Acanthocyclops* sp., both in small number. The hydrological variations do not seem to influence directly the fauna composition or the number of individuals per drip.

Results on stable isotope analysis are shown in Table II. The variations are maximum outside the cave (8·9‰for δ^{18}O, and 75‰for δ^{2}H, respectively), and decrease to less than 1‰for both δ^{18}O and δ^{2}H inside the cave. Variation in isotopic composition of drip water in station 5 follows that of precipitation, suggesting a relatively fast transfer of water inside the cave, with less than 4 weeks residence time (the minimum sampling interval) in the unsaturated zone above the cave. Drip waters in stations 1 and 2 display a more reduced variation of their isotopic composition, suggesting a longer than 5 months (the total length of sampling interval) residence time of the meteoric water in the epikarst.

DISCUSSION

The habitat can shape the composition of communities and their interspecific interactions are influencing the spatial and temporal dynamics of populations, including dispersal in time and space. Therefore, for the karst unsaturated zone a combination between biotic and abiotic constraints must be considered for explaining the composition and distribution of a metacommunity. Although subterranean communities are simple, the extreme heterogeneous habitat and micro-habitats and competition from epigean species (though on short-term) shape mosaic-like assemblages of species.

The eight dripping points sampled in the Ciur Izbuc Cave are highly heterogeneous in biological community composition and species temporal dynamics. Distant stations or even close stations showed both different composition and abundance of species. Heterogeneity of the fauna from the unsaturated zone has been discussed by other authors (Rouch and Danielopol, 1987; Sket *et al*., 2004; Moldovan *et al*., 2007) and is a characteristic of any groundwater habitat as a consequence of biotic and abiotic interactions that took place from microscale to megascale during historical times (Gibert *et al*., 2009). Between the two distant locations inside the cave, the differences are more important than between close stations, mainly in dominant species, in number of individuals of each species and in community composition. Among the identified crustaceans, only two taxa are site-specific for one of the two distant locations, *B. caucasicus* and *M. brucei carpathicus*, and only two species have populations in temporal synchrony between the two distant locations, *Acanthocyclops* n. sp. and *S. spelaeus*. As Liebhold *et al*. (2004) have shown, the

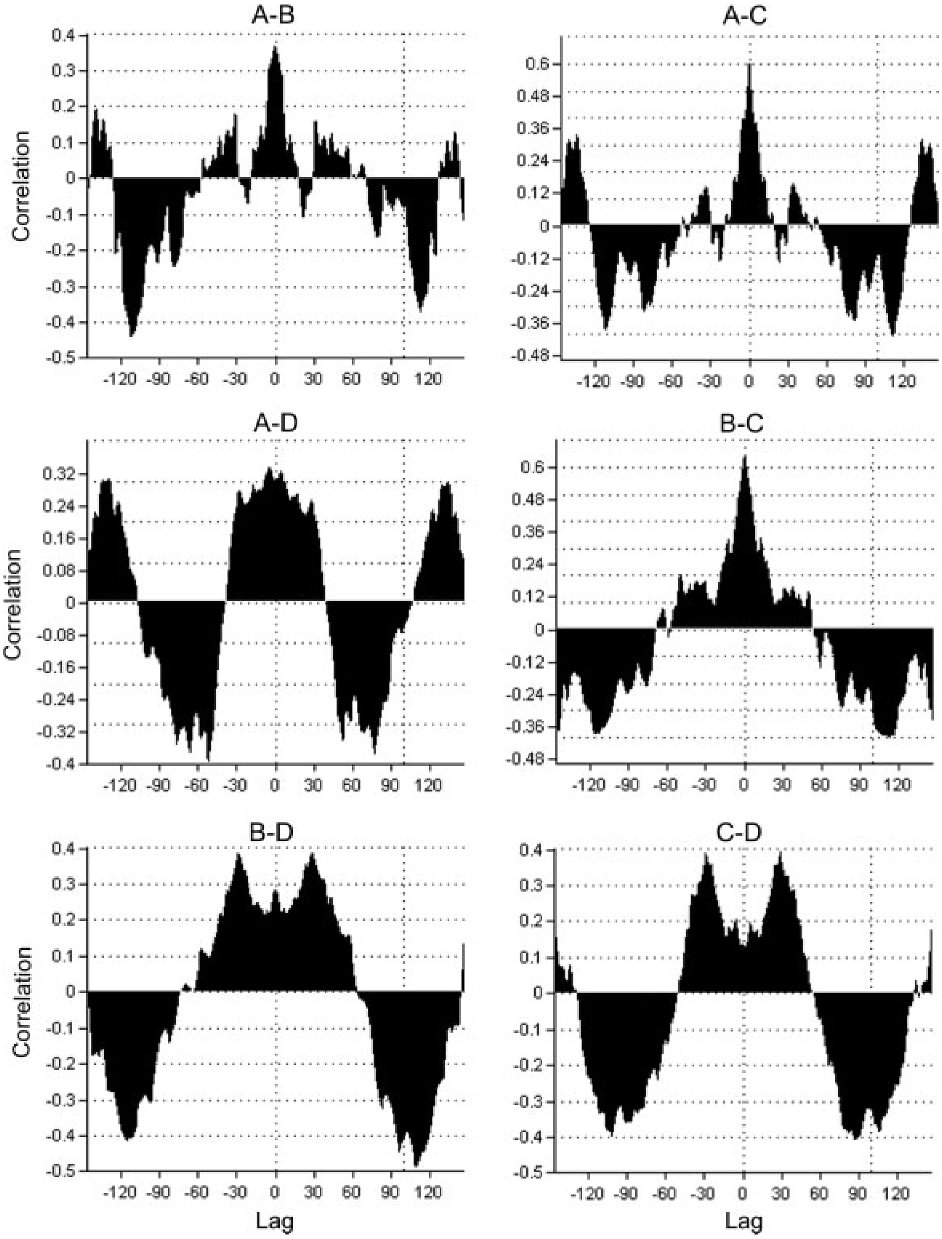

Figure 5. Cross-correlations on the daily time series of the drip rates in the Ciur Izbuc Cave: A–D, data loggers.

more spatially synchronous a metapopulation, the shorter the expected persistence time. Increased synchrony can decrease metapopulation and metacommunity persistence either by increasing extinction risks for all habitats or by reducing inter-habitats migration because of increasing isolation (Hudson and Cattadori, 1999).

Surface or soil invertebrates (*B. caucasicus* and *C. neglecta*) that represent the living karst matrix component that can be advectively transported inside the unsaturated zone, will not survive for long periods because of lack of food or competition/predation by hypogean species. The combination of species in the today communities of the unsaturated zone are more similar in resource use and less invasible by new species (such as the epigean potentially new colonizers). These species combinations are therefore more likely to resist colonization, as sustained by Leibold *et al.* (2004) for similar situations. However, epigean species 'spill-over' (Cook *et al.*, 2002) from the matrix can influence hypogean abundance or species composition in the embedded patches. Matrix species can contribute with 'noise' (Cook *et al.*, 2002) to the species richness data set, masking a more general pattern of species accumulation. Diffuse competition from 'spill-over' matrix species could depress the richness of species restricted to the patches. It is the case for the Ciur Izbuc Cave, where even though hypogeans are dominant components of communities, the relative abundance of *B. caucasicus* depressed the number of the other copepods in stations 1–4. The presence of the epigean competitor and the larger voids makes this part of the unsaturated zone less favorable for the development of the hypogean harpacticoid populations. Moreover, negatively correlated dynamics of *B. caucasicus* and *Acanthocyclops* sp. is pointing on the strong influence of the

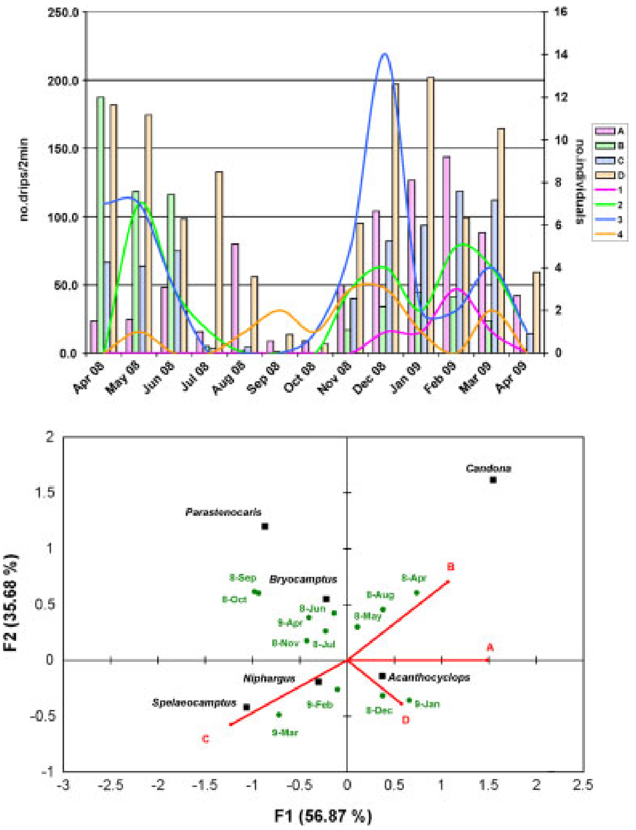

Figure 6. Temporal variation of the Crustacea number in relationship to the number of drips: 1–4, stations; A–D, drip loggers (above); CCA of the temporal variation of the total number of individuals for Crustacea species in the upstream stations: A, B, C, D, drip rates for the four data loggers (below).

epigean species on the subterranean communities. The dominance of hypogeans in Ciur Izbuc communities can be explained probably to constraints linked to a restrictive environment in terms of physico-chemical features. However, surface species can influence seasonal abundance of hypogeans, even if epigeans can be relatively rapidly eliminated from communities inhabiting deeper zones. Nevertheless, 'spill-over' from the matrix is rather beneficial as food source in such low energy systems.

The comparison of the stable isotopes of oxygen and hydrogen from surface and subterranean stations indicates a fast transfer of the water inside the cave and low retention times at downstream station 5, as proof for highly fractured or high secondary porosity limestone with high connectivity between habitats (voids). Downstream stations are inhabited by tiny harpacticoids (*S. spelaeus* and *Parastenocaris* sp.), and together with the relatively well-represented epigean species of *C. neglecta* are the proof for a fast transfer from the surface and small interconnected voids. Thus, the combined information given by isotopes and fauna is showing that above the downstream stations (5–7) smaller and well connected voids allow fast transfer of water and animals from the surface or between levels of the subsurface zones. In the upstream stations (1–4), a less fractured limestone or one with lower secondary porosity and lower connectivity may explain the observed stability in terms of isotope composition of drip water. In the upstream stations the cyclopoid *Acanthocyclops* sp. and especially the larger amphipod *N. andropus* are dominant which argues for the existence of larger volumes of water/spaces. The presence of the epigean *B. caucasicus* in the upstream community is rather controversial. The transfer of water is slow and this species should be eliminated from the hypogean communities through competition or predation. However, the voids are large and are not the kind of habitat inhabited by hypogean tiny harpacticoids, as potential competitors for *B. caucasicus*. Another possible cause is that the input of *B. caucasicus* from the surface is important in number of individuals and predation cannot eliminate all of them.

Water is the vector that connects underground patches and according to Danielopol *et al*. (2003), water controls the composition and functioning of aquatic subsurface ecosystems. The approach in understanding dispersal of underground animals implies studying the hydrological behavior of the water percolating through the unsaturated zone. Variations in time of water dripping in caves are not correlated with the number of individuals found in the cave. This points on the role of the water as a vector for migration and pleads against the theory of accidental downward drift of individuals when voids are flooded (Brancelj and Culver, 2005) and more for an active drift when water floods the voids (Moldovan *et al*., 2007). Variations of water dripping in caves and

Table II. Stable isotopic composition of precipitation and cave drip water, Ciur Izbuc Cave.

Station	Isotope	Date				
		August 2008	September 2008	October 2008	November 2008	December 2008
Surface	$\delta^{18}O$	−5·4	−3·2	−10·4	−13·1	−10·6
	$\delta^{2}H$	−31	−14	−70	−89	−74
1	$\delta^{18}O$	[a]	[a]	[a]	−9·8	−9·9
	$\delta^{2}H$	[a]	[a]	[a]	−67	−69
2	$\delta^{18}O$	[a]	−10	−9·8	−9·4	−9·6
	$\delta^{2}H$	[a]	−67	−67	−64	−66
5	$\delta^{18}O$	−6·2	−5·4	−7	−7·7	−8·3
	$\delta^{2}H$	−37	−31	−44	−52	−54

[a] Not enough water for the analysis.

water residence time in the unsaturated zone give an indirect measure of rock porosity as habitat available for animals. Meteoric water infiltrates underground, filling the empty voids and percolates to lower levels at different speeds. During periods of water input, voids become connected and aquatic animals can disperse actively, while during periods of drought the spaces are filled with air, which act as a barrier against dispersal. Different authors (Bottrell and Atkinson, 1992; Kogovšek and Šebela, 2004) affirm that the paths of the drips are not determined by proximity, but by the distribution of permeable conduits and fissures, the infilling with water, and the distribution and the precipitation amount. This is the simple explanation of the differences in trickles behavior and in community composition and number of individuals even between very close stations in the Ciur Izbuc Cave.

As it was generally shown by McGill *et al.* (2006), the unsaturated zone can be either spatially explicit during drought periods and metacommunity is a set of interconnected local communities or spatially implicit during rainy periods when local communities draw migrants from a separate pool represented by surface species. Local communities are represented by groups of species colonizing voids in different part of a cave or in different caves belonging to the same karstic area. At local level, as in Ciur Izbuc Cave, spatially structured communities are those of the two different locations inside the cave (upstream and downstream) and several sub-communities, at the same location. Drips with common surface origin follow different paths underground and cross different voids which harbor different sub-communities. Therefore, even spatially close subcommunities have different species composition in space and time, but share one or more species, such as *B. caucasicus* in stations 1–4 and *S. spelaeus* in stations 5–7, even if they are not dominant in the community.

Some species of the Ciur Izbuc Cave communities are distributed along the entire mountain range of the Western Romanian Carpathians (*S. spelaeus*), other at basin level (*Acanthocyclops* sp.) and one is endemic for this cave (*Parastenocaris* sp.). At this point is rather unclear if this can be explained by biotic or abiotic constraints, or by historical or more recent causes, or by a combination of several of these causes. Historical constraints can be related to distribution of the surface ancestors, moment(s) of underground colonization, evolution of the subterranean hydrological network, etc. while more recent constraints are linked to maintenance of fragments on large scales, energetic variations of surface inputs etc. Spatial distribution is also a matter of rock internal geometry, of how much the voids are interconnected, no matter the water input. Economo and Keitt (2008) highlight the importance of the spatial structure on the biological parameters of the neutral ecological model in determining species diversity of a local community among spatially separated communities and on the scale of the entire metacommunity. If diversity is low compared to the number of patches, as for the underground fauna, the spatial effect is important for the regional diversity.

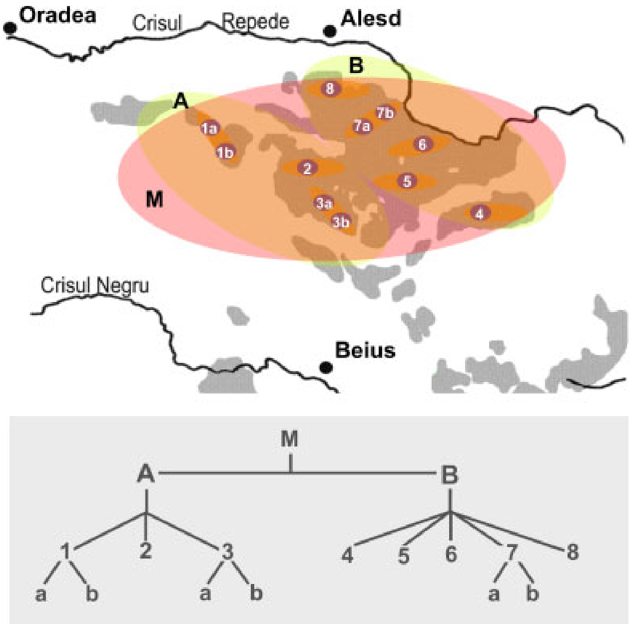

Figure 7. Schematic representation of the hierarchical distribution of a subterranean metacommunity in the Padurea Craiului Mountains: M, mountains; A–B, basins; 1–8, caves; a–b, cave populations; grey, limestone areas.

Hierarchically organization of metacommunities was proposed for freshwater invertebrates (Bohonak and Jenkins, 2003) and its significance emphasized by genetic studies is still ignored in ecology. In the very fragmented underground zone (Figure 7), habitats and micro-habitats are more likely to be more correlated on small spatial scales through hydrological linkages that decrease at upper scales. Habitat connectivity can have different effects on local and regional diversities when there is considerable heterogeneity among habitat patches (Forbes and Chase, 2002) and the roles played by spatial dispersal in balancing extinction on longer evolutionary time scales are largely unknown (Bohonak and Jenkins, 2003). However, habitat fragmentation is a potentially critical factor in determining community persistence (Davies *et al.*, 2001) and the hierarchical organization of the unsaturated zone is the possible explanation for the maintenance of gene flow in such a patchy environment. Otherwise how one could explain that species with extremely limited dispersal in a highly fragmented landscape have populated catchments and watersheds millions of years ago and have not speciate? at least at karstic massif or regional levels.

CONCLUSIONS

Groundwater ecosystems are extremely heterogeneous systems at micro- or macro-levels and protection should consider not only distinct groundwater bodies (aquifers) but also recharge and discharge areas (Danielopol *et al.*, 2004). Conservation of the unsaturated zone fauna is important because this zone acts as groundwater recharge area and hypogeans can be used as indicators for hydrology and water quality. Understanding how metacommunities function, their dispersal driving forces and internal synchronies and the hierarchical organization helps understanding local and regional hydrology and decisions for conservation and management of groundwater sources.

Hypogean species can be considered as focal species in establishing conservation measures if: (i) they are present on larger but discrete defined areas, (ii) appear in large number, (iii) are indicator of some of the habitat features (such as porosity of the unsaturated zone and underground interconnectivity) and (iv) are components of different communities as indication of their adaptability and persistence through time. The copepods *Acanthocyclops* sp. and *S. spelaeus* identified in the Ciur Izbuc Cave are such species and can be used as indicators for extinction risks of underground fragmented communities.

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