

## Spatial and seasonal distribution of invertebrates in Northern Apennine rheocrene springs

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### ABSTRACT

Four perennial rheocrene springs located between 919 and 1252 m a.s.l. on substrata characterized by different lithologies were studied. Water samples and invertebrates were collected seasonally for one year. The crenic fauna was collected using three sampling techniques: moss washing, drift tubes and benthic traps. Each sampling technique was particularly efficient for collecting specific taxa typical of the different habitats (crenophilous crustaceans and crenoxenic benthic insects were dominant in benthic traps and moss; crenophilic, stygophilic and stygobiotic crustaceans in drift tubes). A total of 3,284 invertebrates belonging to 54 taxa were collected. Ostracoda, Harpacticoida, and Diptera were the most abundant taxa. Species assemblages collected at each spring, in each season, in traps and mosses, differed among springs, and, based on invertebrate assemblages, the ordination of the investigated springs did not correspond to that based on environmental parameters. Of the environmental variables only pH and temperature explained the diversity pattern. Assemblages collected from different habitats also differed: benthic traps collected mainly Chironomidae, Ostracoda, other Diptera, crenophilous Harpacticoida, and Gastropoda; in moss assemblages, the fauna was mostly represented by crenophilic Harpacticoida, Ostracoda, Plecoptera, Chironomidae. Finally, the groundwater assemblages, collected with drift tubes, were dominated by crenophilous Harpacticoida, Chironomidae and Plecoptera. Variation in number of taxa over time was observed in traps and moss samples, whereas drift tubes showed no seasonality. Meiofauna (i.e., permanent meiofauna, represented by Nematoda, Copepoda, Ostracoda, and Hydrachnidia, and temporary meiofauna, represented by early instars of insect larvae) dominated all habitats, probably because of constant flow and favourable habitats such as moss. The presence of mosses was a factor that increased the species diversity of the investigated springs; drift tubes allowed most of the stygobiotic taxa to be collected, although this technique did not necessarily increase the total number of taxa collected. In addition to the array of habitats, other factors, such as geology, might influence the structure of invertebrate communities. The diversity of the investigated springs was strictly dependent on the presence of different microhabitats and local environmental conditions.

*Key words:* crenobiology, microcrustaceans, meiofauna, macroinvertebrates, ecological specialization, habitat preferences, seasonal dynamics, sampling methods

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### 1. INTRODUCTION

Compared to higher-order streams, most springs have greater physical and chemical stability, smaller and more isolated habitat areas, and fewer large predators (Glazier 1991). At temperate latitudes, thermal stability has been thought to be one of the main characteristics of springs and the reason for the presence of cold-stenothermic animals (Illies 1952; Erman & Erman 1995). Springs are characterised by distinctive species assemblages, and marked heterogeneity of environmental conditions and communities (e.g., Bonettini & Cantonati 1998). Several studies have shown how geographical factors (Williams 1991; Williams & Williams 1998), permanent flow (Gooch & Glazier 1991; Erman & Erman 1995), flow variability (Smith *et al.* 2001, 2003; Smith & Wood 2002; Meyer *et al.* 2003), organic matter (Chafiq & Gibert 1993; Smith *et al.* 2003), water chemistry (Glazier 1991; Orendt 2000), water velocity

(Ilmonen & Paasivirta 2005; Ilmonen *et al.* 2009; von Fumetti *et al.* 2006), substratum composition (Glazier & Gooch 1987; Hahn 2000), altitude (Barquín & Death 2006) and local habitat features (Ilmonen & Paasivirta 2005) have a substantial effect on the spring faunal assemblages. However, within-spring habitat heterogeneity has been shown to be of particular importance in determining crenic macroinvertebrate community composition (e.g., Glazier & Gooch 1987; Lindegaard 1995; Lindegaard *et al.* 1998; Hahn 2000).

Springs sustain high levels of biodiversity: for instance, Illies (1978) reported the presence of about 1500 species in European springs, of which about 31% are crenobiotic or crenophilic. Springs represent the interface between two distinct ecosystems (groundwater and surface water), and can be considered as "hotspots" of aquatic biodiversity (Cantonati *et al.* 2006; Scarsbrook *et al.* 2007; Staudacher & Füreder 2007), due to their distinct "mosaic" and multiple ecotonal structure

**Tab. 1.** Characteristics of the springs considered in this study. Geographical coordinates are indicated in the UTM (WGS 84) system. Habitat type: A: altered; N: natural. Sampling methods: MO: moss, TR: traps, TU: drift tubes. Granulometric categories: boulders: 240-960 mm; cobbles: 63-240 mm; gravel, pebbles: 2-63 mm; clay, silt, sand: <2 mm.

Sampling name and code	Elevation (m a.s.l.)	Coordinates	Lithology	Organic substrate		Inorganic substrate		Estimated average discharge (L s <sup>-1</sup> )	Habitat Type	Sampling methods
				Total %	Composition	Total %	Composition			
Biam (BIA)	919	587772.24 E 921004.54 N	Marly, marly-silt unit	50	80% leaves 20% wood debris, roots	50	50% gravel, pebble; 40% clay, silt, sand; 10% cobble	15	A	MO, TR, TU
Cirone (CIR)	1124	580516.93 E 922501.23 N	Shales and limestone	10	95% bryophytes 5% algae	90	80% clay, silt, sand; 20% gravel, pebble	2	N	MO, TR
Lagdei (LGD)	1252	580311.77 E 918259.08 N	Sandstone; moraine deposits	70	50% leaves 50% wood debris, roots	30	90% clay, silt, sand; 10% boulder	5	N	MO, TR
Vezzosa (VEZ)	1150	580831.99 E 919000.25 N	Sandstone; alluvial deposits	80	80% bryophytes 10% leaves 10% wood debris, roots	20	20% cobble, 80% boulder	20	N	MO, TR, TU

(between aquatic-terrestrial (micro)habitats, groundwater-surface water, and crenal-rhithral), which results in a high number of microhabitats (Weigand 1998; Di Sabatino *et al.* 2003). These features, together with the abundant presence of suitable substrata for colonization and the availability of food sources, are among the main reasons for the high number of species and individuals often found in springs compared to other freshwater habitats (e.g., Stanford *et al.* 1994).

Springs host specialized and often endemic or rare taxa (Di Sabatino *et al.* 2003; Cantonati *et al.* 2006; Collier & Smith 2006), which can be locally threatened (Ilmonen & Paasivirta 2005; Ilmonen *et al.* 2009). The typical crenic fauna is composed of three groups of organisms (see Cantonati *et al.* 2006 for a detailed explanation): crenobionts (found only in crenic environments), crenophiles (mainly distributed in springs, but also occurring in habitats with similar environmental conditions, generally benthic) and crenoxenes (taxa colonizing different types of freshwaters, and only occasionally occurring in springs). Stygobiotic taxa (i.e., truly subterranean, reaching the spring from groundwater) are often present, usually at the mouth of the spring or in interstitial habitats. Crenic assemblages are influenced by temporal dynamics, but there is a general lack of studies involving seasonal variations (Gooch & Glazier 1991).

Springs can be considered insular biotopes (Mac Arthur & Wilson 1963, 1967) since their distinctive organisms (mostly crenobionts and crenophiles) are restricted to these environments because of unfavourable conditions in the surrounding habitats.

Several studies on the biotic assemblages of mountain springs have been carried out over recent years in Italy, mainly in the north-eastern Alpine and pre-Alpine area, and in the central and southern Apennines (see Bottazzi *et al.* 2008 for review). Information on northern Apennine springs is still poor, limited to the saline springs of Poiano in the Upper Secchia Valley (Stoch *et al.* 2008, 2009), and to a recent contribution on copepods and ostracods from northern Apennine springs, including those analysed in the present paper (Bottazzi *et al.* 2008).

The present research aims to fill some of the gaps in knowledge, by:

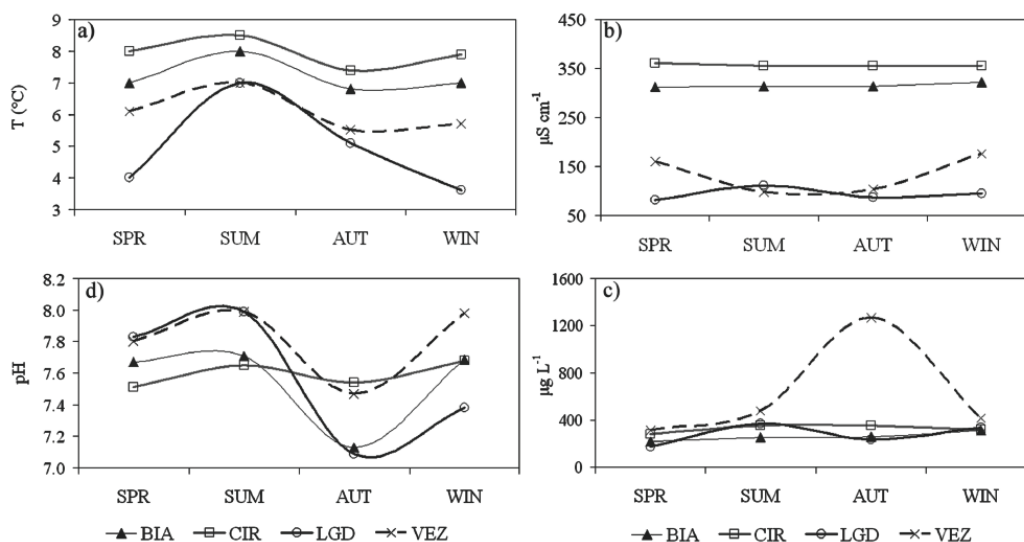
- defining the faunistic component of four perennial Apennine springs,
- assessing distributional patterns of the invertebrate fauna among springs and microhabitats;
- analysing the seasonal dynamics of crenic assemblages.

## 2. METHODS

### 2.1. Study sites

We studied four rheocrene springs characterized by perennial and relatively constant flow, all situated in a protected area ("Cento Laghi" Regional Park) of the north-western Apennines, in the Parma River catchment, 919-1252 m a.s.l. (Tab. 1). Three springs, namely Cirone (CIR), Lagdei (LGD), and Vezzosa (VEZ), are located on the left orographic side of the Parma River (Fig. 1). The Biam spring (BIA) is located on the right orographic side of the Bratica Stream, a right-side tributary of the Parma River. LGD and VEZ are very close to each other (about 1 km apart), and CIR is about 4.3 and 3.4 km from them, respectively; these three springs are still in pristine condition. BIA is 7.2-7.8 km from the remaining three springs, and has been partially modified by the insertion of pipes (see below).

The geology of the area is characterized by sedimentary layers of different origin: marly-arenaceous terrains in facies of flysch deposited from the upper Cretaceous to the lower Eocene (90-45 MYA); turbidites which were deposited from the Oligocene to the end of the Miocene (30-24 MYA) and produced sandstone strata 100 m thick; glacial deposits from the Pleistocene ice-ages (during the Würmian glaciation, glaciers covered the entire Northern Apennine mountain range, and extended into the valleys in question). The glacier running down the Parma Valley, with an estimated surface area of 25 km<sup>2</sup> (Federici & Tellini 1983), was the largest in the Northern Apennines. BIA is located on limestone, CIR on shales and limestone, LGD and VEZ on terrigenous flysch (moraine deposits on a sandstone bed and alluvial deposits on a sandstone bed, respectively).



**Fig. 1.** Seasonal variation of: **a)** water temperature, **b)** specific conductivity, **c)** nitrate concentration, **d)** pH, at each of the investigated springs.

BIA is characterized by the presence of two pipes; part of the spring water flows through them, albeit intermittently, and some emerges freely, forming a small springbrook. This spring is surrounded by meadows and chestnut trees, and is thus partially shadowed by tree canopies during the vegetative season. Disturbance is essentially due to the passage of hikers in summertime. CIR is situated in a hay field; water emerges freely from several discharge points that merge into a springbrook. The only noticeable impacts are due to hay cutting and ungulate treading (wild boars, roe deer). LGD is situated in a mixed (spruce and beech) forest. The substratum, characterized by fine sediment, is covered by various amounts of organic detritus; the presence of tall trees results in relatively constant shading throughout the year. VEZ, surrounded by a spruce forest, is permanently shaded and forms a small springbrook that, a few metres downstream, joins a fast-flowing torrent.

## 2.2. Sampling methods

Altitude and UTM coordinates (Tab. 1) of the four springs were measured using GPS. Lithology (Tab. 1) was determined from the geological map (scale 1:10000) of the Geological, Seismic and Soil Survey of the Emilia Romagna Region Geological Service ([www.regione.emilia-romagna.it](http://www.regione.emilia-romagna.it)) and from detailed geomorphological maps of the study area (Federici & Tellini 1983; Chielli & Tellini 2002). Approximate discharge was estimated by measuring the cross section and the water velocity from the spring outflow. Percentages of organic and inorganic substrata, and their relative compositions, were assessed visually for the first five metres from the spring outlet.

Four seasonal surveys were carried out on the following dates: spring: 04-24-2007 and 04-30-2007; summer: 08-23-2007 and 08-30-2007; autumn: 11-22-

2008 and 12-12-2008; winter: 01-30-2008 and 02-20-2008. Traps and drift tubes (see below) were left *in situ* for 7-14 days.

Water temperature, pH and specific conductivity were measured *in situ* on each sampling occasion using a XS-cond-6 portable conductivity and temperature meter, and a XS-pH-6 pH meter (Eutech Instruments). Specific conductivity was automatically corrected to the standard temperature of 20 °C. Soluble reactive phosphorus (SRP),  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$  concentrations were measured in the laboratory using standard methods (Valderrama 1977; Rodier 1978; A.P.H.A. *et al.* 1981).

Spring invertebrates were sampled in the eucrenal to minimize impact on the environment and to allow collecting from different habitats: traps to collect actively moving macro- and meiofauna from benthic and interstitial habitats; drift tubes to catch stygobionts transported passively by the water outflow from the groundwater aquifer; moss washes to sample semi-terrestrial and muscicolous invertebrates.

Traps (derived from PASCALIS research project, Malard *et al.* 2002) were built from PVC centrifuge tubes (length 100 mm; diameter 28 mm), by cutting the conical end, drilling a small opening in its apex, and inserting it, inverted, into one end of the tube, closing the other with a 50 µm net. These traps were filled with previously washed and sieved gravel (0.3-1 cm) collected from the area. Traps were baited with ham, and placed at the sediment-water interface, covered with stones to keep them in place and left *in situ* for one week. Two to eight traps were placed in each spring, according to its size and flow. In the laboratory, gravel from the traps was rinsed and the water was filtered through a 50 µm net. Drift tubes (length 160 mm; diameter 48 or 80 mm) were built by attaching a 50 µm net to one opening of a PVC pipe. These were placed at well-defined discharge points, which were only present

at BIA and VEZ, and left *in situ* for one week. The net was subsequently removed and rinsed, and the material obtained filtered through a 50 µm net. When present, clumps of mosses at the spring outlet were removed in variable quantities depending on their coverage. In the laboratory, mosses were carefully washed to remove animals; water was then filtered through a 50 µm net.

Traps were used in each crenon and season, although two springs did not have definite discharge points at which to place tubes, and bryophytes were not always present (no moss was present at CIR and VEZ in spring, or at LGD in spring and autumn). All the samples were fixed in 95% ethanol immediately after collection.

In the laboratory, macrofauna and meiofauna in each sample were separated using two sieves with 255 and 50 µm mesh, respectively: the first sieve retains the macrofauna, the material passing through it is filtered through the second sieve to retain the meiofauna. Meiofaunal taxa were classified as permanent meiofauna (i.e. invertebrates between 63 and 500 µm throughout their whole life cycle, i.e. small enough to be considered meiofauna even as adults), or temporary meiofauna, in the same size range as permanent meiofauna but including larvae and juvenile stages of macroinvertebrate taxa (Mare 1942; Palmer & Strayer 1996). Invertebrate specimens were sorted in the laboratory under a dissecting microscope. Copepoda, Ostracoda and Hydrachnidia were classified to species level following: Dussart (1967, 1969), Stoch (1998a), and Karaytug (1999) for Copepoda; Meisch (1984) for the genus *Potamocyparis*; Baltanás *et al.* (1993) for *Psychrodromus*, and Meisch (2000) for the remaining Ostracoda; Gerecke (1996), Davids *et al.* (2007) and various specialised keys for Hydrachnidia. Only one species of Trichoptera was represented by late larval stages, and tentatively identified to species level following Lechthaler & Stockinger (2005). All other taxa were identified to the lowest possible level following Campaioli *et al.* (1994, 1999), and Fochetti *et al.* (2009). Some specimens were not classified at the rank adopted for the rest of their taxonomic groups because of their early stage of development. As far as possible, taxa were classified as crenoxene, crenophile, crenobiont, and stygoxene, stygophile, stygobiont, based on a review of published specialist literature and recent data on the distribution of the Italian fauna (Stoch 2000-2006).

### 2.3. Statistical analysis

The springs were first classified based on the physico-chemical variables. The environmental data matrix was standardized prior to running the analysis. Only temperature, pH, conductivity, and nitrate concentration were considered in the analyses; the remaining nutrients were not included because most of the measured concentrations were below the detection threshold of the analytical methods.

We performed a one-way MANOVA for all physico-chemical variables, and a Tukey's Honestly Significance Difference (HSD) post-hoc test (Sokal & Rohlf 1995) to identify which variables were significantly different between pairs of springs. Ordination was carried out on the environmental data matrix by running a Principal Component Analysis (PCA). We ran a multiple correlation on a matrix including the abundance of each taxon and the physical-chemical variables of each sample to examine the relationships among biotic and environmental factors.

Because of the different sampling methods designed to collect from different habitats, we tested for significant differences in species assemblages between habitats by running a one-way ANOSIM (Analysis of Similarities, Clarke 1993) on a Bray-Curtis similarity matrix, which was then used to run an ordination (Non-metric Multi-Dimensional Scaling, NMDS). The analysis was applied to a root-transformed matrix obtained by calculating the mean number of individuals of each taxon for each sampling method at each station, on each sampling occasion, resulting in 34 samples (15 traps, 6 drift tubes, 13 mosses) and 54 taxa. We also ran a one-way ANOSIM for moss and trap data separately, to assess differences among springs in these assemblages.

Due to the different number of samples and/or methods used to collect at each spring each season, we averaged the trap and moss data, and calculated the mean number of individuals of each taxon for each spring on each sampling occasion, giving a faunistic matrix of 16 samples (four springs sampled in four seasons) and 54 taxa. Because of the low number of replicates and sites, data from "drift tubes" were not included in the analysis and are only presented qualitatively. The faunistic matrix was root-transformed to down-weight the influence of abundant taxa (Clarke & Warwick 2001), and ordination (NMDS) was carried out following calculation of Bray-Curtis similarities. One-way ANOSIM was used to investigate differences between seasons and springs. The Euclidian distances of the standardized values of temperature, conductivity and pH were used to run a RELATE procedure (Clarke & Gorley 2006) to test the hypothesis that there was no relationship between the multivariate pattern from two sets of samples – in our case, the invertebrate and environmental resemblance matrices. The BEST procedure was then used to test which environmental variables best explained the observed invertebrate community pattern found in each spring over time. All analyses were performed using PRIMER-E® (v6) (Clarke & Gorley 2006) and Statistica ver. 8.1 (StatSoft Inc. 2008).

## 3. RESULTS

### 3.1. Physico-chemical characteristics of waters

Springs differed in their physico-chemical variables (one-way MANOVA, factor: SPRING,  $F = 9.81$ ,  $p < 0.001$ ); temperature and specific conductivity in par-

particular were significantly different in some springs (temperature: Tukey test  $p = 0.02$  for BIA-LGD,  $p = 0.002$  for CIR-LGD; specific conductivity:  $p < 0.001$  for BIA-LGD, BIA-VEZ, CIR-LGD, CIR-VEZ). Each spring was characterized by relatively stable physical and chemical water parameters (Fig. 1). The highest water temperature (8.5 °C) was recorded at CIR, and the larger seasonal amplitude at LGD (3.6 °C). The lowest pH values were observed in autumn, except at CIR where they remained almost constant throughout the sampling period. Specific conductivity was the most stable parameter throughout the seasons in all springs, although values were significantly higher at CIR and BIA than at LGD and VEZ. All springs were oligotrophic: SRP concentration was not detectable in 10 of 16 samples; in the remaining six samples it ranged between 5 and 17  $\mu\text{g L}^{-1}$ ;  $\text{NO}_3^-$  concentrations varied between 173 and 480  $\mu\text{g L}^{-1}$ , with an isolated autumn peak (1266  $\mu\text{g L}^{-1}$ ) at VEZ (Fig. 1).

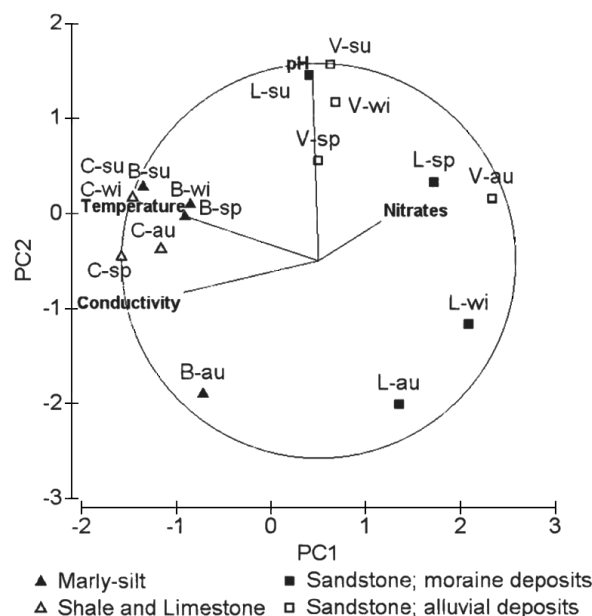
The first two axes of the PCA plot explained 73.8% of the total variance. The gradient on the first axis was explained primarily by temperature (eigenvector: -0.656) and conductivity (eigenvector: -0.684), which increase from right to left, and secondarily by nitrates (eigenvector: 0.317), which increase from left to right. The gradient on the second axis was explained by pH (eigenvector: 0.942) increasing with the Y axis. Samples formed two main groups in the PCA plot (Fig. 2), one of samples collected from limestone substrata at BIA and CIR, characterized by higher temperature and conductivity and very similar to each other, and the second group with the samples collected from sandstone at LGD and VEZ, which were more scattered over the biplot (i.e., more variable), with higher pH values. The winter and autumn samples collected at LGD, and the autumn sample collected at VEZ were more isolated, due to the low water temperature, pH and specific conductivity recorded at the former, and the higher nitrate concentration at the latter. This nitrate load could be attributed to the increased input of dissolved organic matter after heavy rainfall events.

### 3.2. Spatial patterns in invertebrate assemblages

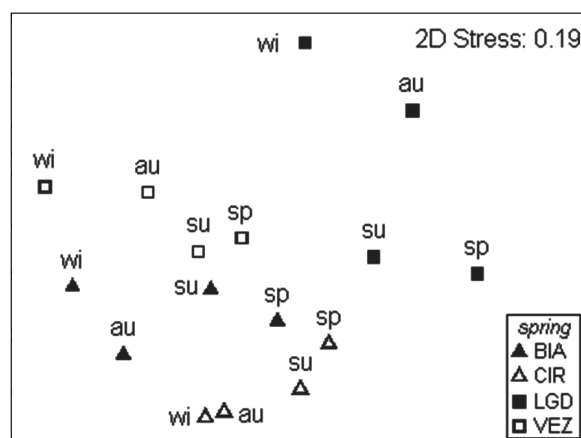
A total of 3284 individuals were collected during this study and assigned to 54 taxa (Tab. 2). Ostracoda represented the most abundant taxon (49.0% of the total individuals), followed by Harpacticoida (20.9%) and Diptera (17.3%). Considering all taxa in the highest taxonomic detail, LGD had the highest richness, 31 taxa, followed by BIA and CIR (29) and VEZ (19). No ostracods were found at VEZ (Tab. 2). LGD had the most distinctive community, with approximately 48% exclusive taxa, while BIA, CIR and VEZ had 24%, 12%, and 16% exclusive taxa, respectively (Tab. 2).

The NMDS ordination of the entire invertebrate assemblage from each spring, in each season, in traps and mosses (Fig. 3), showed a clear spatial trend in

community changes, with samples from each spring forming relatively discrete groups on the plot and differing significantly (ANOSIM, factor SPRING, global  $R = 0.52$ ,  $p < 0.01$ ). However, BIA and CIR were closer on the NMDS plot, as were LGD and VEZ: the average Bray-Curtis dissimilarity between these groups were the lowest of all possible pair-wise comparisons (BIA and CIR average dissimilarity: 63.64; LGD and VEZ: 62.59). Seasonal variations were significant (ANOSIM, factor SEASON, global  $R = 0.30$ ,  $p < 0.01$ ); over time CIR and LGD were the least and the most variable springs, respectively (Fig. 3).



**Fig. 2.** PCA of the different samples based on physical-chemical variables (water temperature, pH, specific conductivity, nitrate concentration). Samples categorized by dominant lithology. au: autumn; wi: winter; sp: spring; su: summer.



**Fig. 3.** Non-metric multidimensional scaling of faunal assemblages in trap and moss samples; abundances calculated as mean value for each season: au: autumn; wi: winter; sp: spring; su: summer.

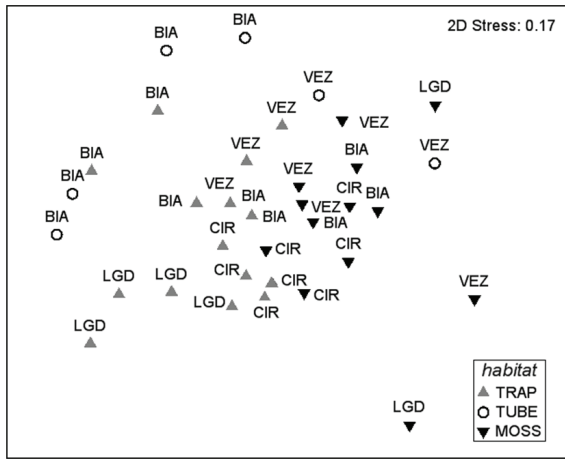
**Tab. 2.** Percentages of each taxon for each method and spring, and mean abundance for each method at each spring; specialization level to crenal habitat, and, when possible, to groundwater habitat; number of exclusive taxa collected with the different methods and at the different stations and with each method at each spring; the same taxa are underlined in the relative column. L: larvae. Crx: crenoxene; crp: crenophile; crb: crenobiotic; stb: stygobiotic; stp: stygophile; stx: stygoxene; bt: benthic; n.d.: not determinable. GW: groundwater, MO: moss; TR: trap; TU: drift tube.

	Habitat specialization		Sampling method				Springs				Sampling method x spring								
	Springs	GW	% Traps	% Drift tubes	% Mosses	% BIA	% CIR	% LGD	% VE Z	BIA-MO	BIA-TR	BIA-TU	CIR-MO	CIR-TR	LGD-MO	LGD-TR	VEZ-MO	VEZ-TR	VEZ-TU
<b>NEMATODA</b>	crx, bt		<u>0.03</u>			0.03							<u>0.04</u>						
<b>MOLLUSCA</b>	crx, bt		<u>0.21</u>					<u>0.62</u>							<u>0.46</u>				
Bivalvia	crx, bt		1.08		1.85	4.96	0.39	1.66		3.75	0.23		0.33	0.38	1.00				
Gastropoda																			
<b>ANNELIDA</b>	crx, bt		2.27	0.12	0.12	2.03	0.64	0.65	0.59	<u>0.25</u>	1.23		<u>0.33</u>	1.00	0.31		0.63		
Hirudinea	crx, bt		0.77			0.15	0.15	1.04	0.74						0.31		0.63		
Oligochaeta																			
<b>ACARI, HYDRACHNIDIA</b>	crb		0.06	6.71	4.81	5.73	2.18			4.00			12.67	0.04	2.00	0.08			
<i>Hygrobates norvegicus</i> (Thor, 1897)	crb		<u>0.03</u>			0.3	0.1								<u>0.08</u>				
<i>Lebertia</i> sp.	crb		0.07	0.12	0.3	2.1	0.06			<u>0.25</u>									
<i>Parmunia aprutina</i> Gerecke, 1993	crb			0.81	1.75														
<i>Sperchon thienemanni</i> Koenike, 1907	crb																		
<b>CRUSTACEA</b>																			
<b>COPEPODA HARPACTICOIDA</b>																			
<i>Attheyella</i> ( <i>Attheyella</i> ) <i>crassa</i> (G.O. Sars, 1863)	crx, bt	stp	0.09	0.26											<u>0.08</u>				<u>0.50</u>
<i>Bryocamptus</i> ( <i>Areticocamptus</i> ) <i>cuspidatus</i> (Schmeil, 1893)	crp	stx	0.02	1.24			0.02		0.3					<u>0.04</u>					<u>0.13</u>
<i>B.</i> ( <i>A.</i> ) <i>rhaeticus</i> (Schmeil, 1893)	crp	stx	0.04		0			0.07											<u>0.50</u>
<i>B.</i> ( <i>A.</i> ) <i>vandouweii</i> (Kessler, 1914)	crp	stx	<u>0.17</u>					<u>0.3</u>											
<i>B.</i> ( <i>Bryocamptus</i> ) <i>pygmaeus</i> (G.O. Sars, 1863)	crx, bt	stx	2.13	64.6	6.82	5.41	3.18	4.16	22.53	4.25	0.08		7.00	0.04	4.00	3.25	3.63	26.00	
<i>B.</i> ( <i>Echinocamptus</i> ) <i>echinatus</i> (Mrázek, 1893)	crp	stp	1.09		4.86	1.5	0.9	11.96	2.95	1.25			1.67	0.21	11.00	0.15	2.50		
<i>B.</i> ( <i>Rheocamptus</i> ) <i>tatrensis</i> (Minkiewicz, 1916)	crp	stp	0.34			1.35	0.3												
<i>B.</i> ( <i>R.</i> ) <i>typhlops</i> (Mrázek, 1893)	crp	stp	0.17	5.9		18.48	5.98	9.2	33.46	13.75	0.62		10.33	2.29	2.77	25.00	4.00	2.00	
<i>B.</i> ( <i>R.</i> ) <i>zschokkei</i> (Schmeil, 1893)	crp	stx	6.98	6.83	21.5	0.45													
<i>Elaphoidella pseudophireatica</i> (Chappuis, 1928)	crp	stb	1.86			0.3													
<i>Elaphoidella richardi</i> Mrázek, 1894	crx, bt	stp	<u>1.24</u>																
<i>Hypocamptus brehmi</i> (Van Douwe, 1922)	crp	stp	3																
<i>Morarina</i> ( <i>Morarina</i> ) <i>alpina</i> Stoch, 1998	crp	stp		<u>0.46</u>				<u>2.08</u>	<u>5.17</u>						<u>2.00</u>				<u>8.50</u>
<i>M.</i> ( <i>M.</i> ) <i>poppei</i> (Mrázek, 1893)	crp	stp		<u>1.62</u>				5.72	0.89						5.50		0.75		
<i>M.</i> ( <i>M.</i> ) <i>stankovitchi</i> Chappuis, 1924	crp	stp		<u>1.86</u>		<u>0.45</u>													
<i>M.</i> ( <i>M.</i> ) <i>varica</i> (Graeter, 1910)	crp	stx		<u>0.62</u>		<u>0.15</u>													
<i>Parastenocaris</i> sp.	crx	stb	<u>3.73</u>																
<i>Bryocamptus</i> copepodids unid.	unid		1.56	0.62	0.58	0.83	0.02		0.89		0.23	<u>0.20</u>	0.04		<u>1.25</u>	3.38			<u>1.50</u>

(continued)

Tab. 2. Continuation.

	Habitat specialization		Sampling method		Springs			Sampling method x springs											
	Springs	GW	% Traps	% Drift tubes	% Mosses	% BIA	% CIR	% LGD	% VEZ	BIA-MO	BIA-TR	BIA-TU	CIR-MO	CIR-TR	LGD-MO	LGD-TR	VEZ-MO	VEZ-TR	VEZ-TU
<b>COPEPODA CYCLOPOIDA</b>																			
<i>Acanthocyclops</i> sp.																			
crx	stb		2.34			0.15	7.1					0.20				2.38			
crp	stp		0.09	0.62			0.26									0.08			
crx, bt	stx		0.03				0.1									0.08			
crx, bt	stx		1.71				0.19	4.52						0.29		1.69			
crp	stp		0.66		0.23	0.9	0.22	0.73		0.50	0.08			0.33		0.31			
<i>Paracyclops imminutus</i> (Fischer, 1853)																			
<b>OSTRACODA</b>																			
<i>Candona</i> cf. <i>lindheri</i> Petkovski, 1969																			
crp	stp		0.03				0.03	0.73								0.08			
crp	stp		0.14				0.42									0.31			
crp	stp		0.27				0.1						0.04			0.31			
crp	stp		0.03				0.1									0.08			
crp	stx				0.12		0.15						0.33						
crp	stp		6.82				20.7									6.54			
crp	stb		0.02				0.02							0.04					
crp	stp		2.14		0.23	0.6	0.23	5.72		0.50				0.33		1.85			
crp	stp		0.27		0.12	0.3	0.12	0.42		0.25				0.17		0.31			
crp	stp		0.07				0.21									0.15			
crp	stp		36.95		25.55	2.1	64.57			1.50	0.08		71.67	48.04					
n.d.	stp		2.35	1.24		0.3	1.64	1.46				0.20		3.29		0.92			
<i>Ostracoda</i> larvae unid.																			
<b>MALACOSTRACA AMPHIPODA</b>																			
<i>Niphargus</i> sp. aff. <i>puteanus</i>																			
crp	stb		0.04		0.12	0.08	0.15	0			0.08								
<b>INSECTA</b>																			
<b>PLECOPTERA L</b>																			
<b>DIPTERA L</b>																			
Chironomidae																			
crx, bt	crp		2.7	3.73	10.75	16.08	4.44	0.52	8.2	12.25	0.69		8.67	0.79	0.00	0.15	4.50	1.75	1.50
crx, bt	crp		21.92	3.73	9.71	29.75	4.91	13.88	16.69	12.00	4.77	0.60	2.33	7.88		8.31	7.25	6.88	
crx, bt	crp		0.03	1.24	0.23	0.6	0.18			0.25		0.20	0.33	0.04					
crx, bt	crp			1.24	0.46	0.3	0.45	0.3	0.3	0.25			1.00						0.50
crx, bt	crp		0.34		4.05	3.61	2.71	2.07	2.07	2.50	0.15		6.00			1.75			
crx, bt	crp				0.12	0.3				0.25									
crx, bt	crp		0.11		2.08	0.9	1.98	0.74	0.74	0.75			4.33	0.04		0.50	0.13		
Diptera unid. identified early L																			
<b>TRICHOPTERA</b>																			
<i>Crunoecia</i> cf. <i>kempnyi</i>																			
crb	n.d.		0.17		0.69	0.9	0.71	0.26	0.37	0.75			1.00	0.42		0.15			
crb	n.d.		0.6													0.08			0.38
Trichoptera L. stygophile																			
<b>PERCENTAGE OVER TOTAL</b>																			
<b>NUMBER OF TAXA</b>																			
<b>NUMBER OF EXCLUSIVE TAXA</b>																			
			38.15	5.27	56.58	21.77	43.51	12.58	22.14	20	11	11	16	24	5	28	9	13	6
			43	16	26	29	29	31	19	20	11	10	3	5	1	12	1	4	5
			19	5	5	6	3	12	4	6	1	10	3	5	1	12	1	4	5



**Fig. 4.** Non-metric multidimensional scaling of faunal assemblages in drift tubes, trap and moss samples, collected on each sampling occasion.

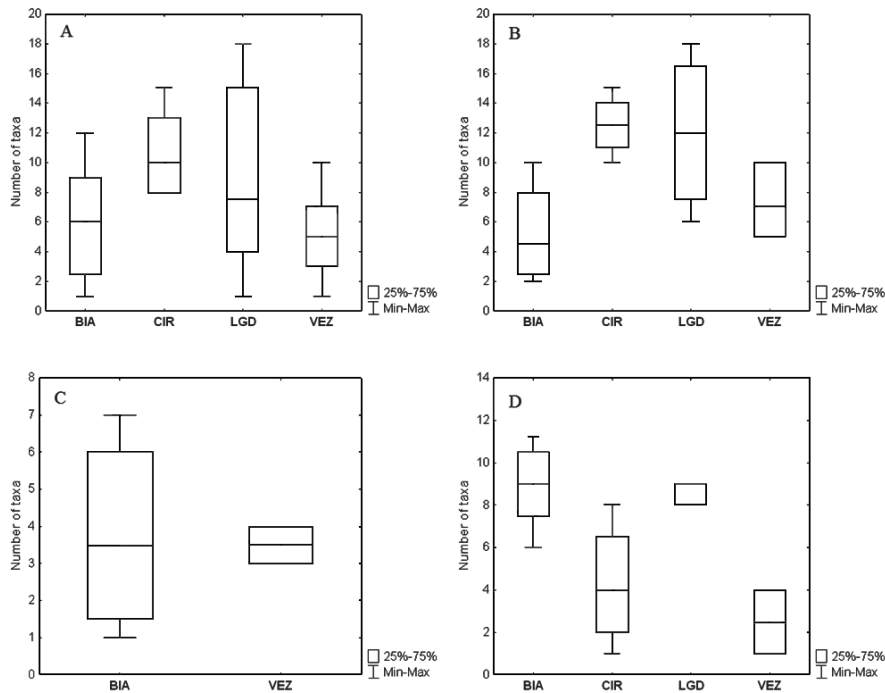
The RELATE procedure found the measured environmental data and biotic community data (mean value for each sampling occasion at each spring) to be significantly related (Spearman's  $\rho = 0.425$ ,  $p < 0.03$ ). The BEST procedure determined that among the four environmental variables (temperature, specific conductivity, pH, nitrates), only temperature and pH best explained the observed community patterns (Spearman's  $\rho = 0.55$ ,  $p < 0.01$ ). Significant taxon correlations were as follows: *Bryocamptus tatrensis*, *Moraria alpina*, *M. poppei*, *Candona neglecta*, *C. cf. lindneri*, *Cryptocandona vavrai*, *Psychrodromus fontinalis*, *P. olivaceus*, *Lebertia*

sp., *Bivalvia* and *Oligochaeta* were negatively correlated with temperature; Tipulidae negatively correlated with pH; *Epactophanes richardi* and *P. olivaceus* positively correlated with conductivity, and *Parastenocaris* sp. and *P. olivaceus* positively with nitrates.

### 3.3. Microhabitat distribution

Species assemblages of each habitat were significantly different (one-way ANOSIM, factor: METHOD, Global  $R = 0.41$ ,  $p = 0.001$ ). Traps and moss assemblages formed different groups on the NMDS ordination (Fig. 4). Traps and moss assemblages were quite similar among springs (Bray-Curtis similarity index between samples: 26.67 and 25.70, respectively for traps and moss), whereas the few drift tube samples were quite different (Bray-Curtis similarity index: 9) and were also scattered over the NMDS plot. Differences among springs tested significant for traps (one-way ANOSIM, factor: SPRING, Global  $R = 0.56$ ,  $p = 0.001$ ), with significant differences at  $p < 0.05$  for all pairs of sites except BIA and VEZ; differences were not significant for moss. Traps collected more taxa than the other methods except at BIA, where more species were present in moss (Fig. 5B, D) and more taxa were collected in drift tubes at BIA than at CIR (Fig. 5C). Variation in numbers of taxa in traps and moss samples over time was clear, whereas drift tubes showed no seasonality (Fig. 6).

The traps mainly collected Chironomidae, Ostracoda, Diptera, crenophilous Harpacticoida, and Gastropoda. Stygobiotic Harpacticoida were not found in



**Fig. 5.** Box-plot of taxon richness at each spring. Line: median value, box: 25%-75% values; whisker: minimum-maximum range. **A:** all methods; **B:** traps; **C:** drift tubes; **D:** moss.



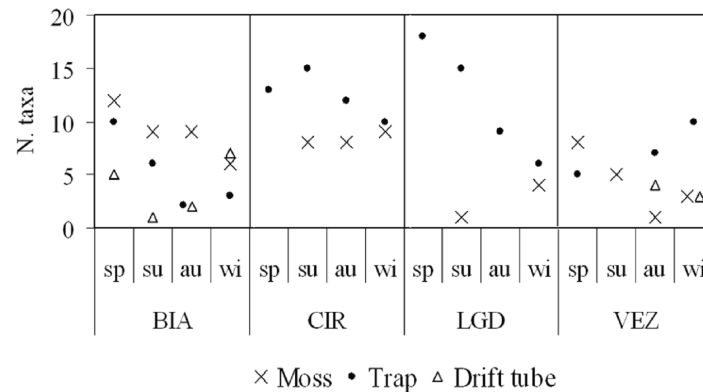


Fig. 6. Number of taxa collected from each habitat at each spring (BIA, VEZ, CIR, LGD), and for each season (SP: spring; SU: summer; AU: autumn; WI: winter).

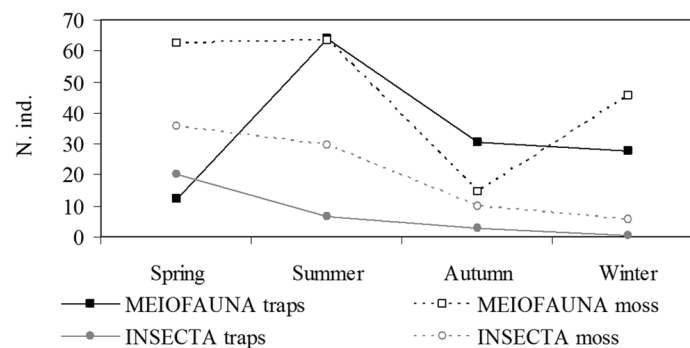


Fig. 7. Mean seasonal number of individuals collected from all springs in traps and moss. T meiof = temporary meiofauna (Chironomidae, Plecoptera, and Trichoptera larvae); P meiof = permanent meiofauna (Nematoda, Copepoda, Ostracoda, Hydrachnidia). Macrof = macrofauna (Dixidae, Psychodidae, Stratiomyidae, Tipulidae, *Crunoecia* cf. *kempnyi* larvae; Gastropoda, Bivalvia, Hirudinea, Oligochaeta, the amphipod *Niphargus* sp. aff. *puteanus*).

traps. In moss assemblages, the fauna was mostly represented by crenophilic Harpacticoida, Ostracoda, Plecoptera, and Chironomidae, while crenoxenic and stygobiotic Harpacticoida and Cyclopoida, and crenobiotic Trichoptera, were absent. Finally, the groundwater assemblages were dominated by crenophilous Harpacticoida, Chironomidae, Plecoptera (Tab. 3).

### 3.4. Meio- and macrofauna assemblages

Most of the invertebrates (95.2%) belonged to the meiofauna (i.e., invertebrates with dimensions between 63 (or 45) and 500  $\mu$ m; Mare 1942), while only 4.8% was represented by macroinvertebrates.

Permanent meiofauna (78.2% of total) was represented by Copepoda, Ostracoda, and Hydrachnidia; Nematoda were also present but will not be discussed further because of their low abundance. Microcrustaceans and water mites were dominant in all habitats, representing 62%, 80%, and 51% of the individuals in traps, drift tubes, and moss, respectively. These taxa were most abundant at CIR (83% of total abundances, due to high ostracod densities), followed by LGD and VEZ (68% for both springs), and least abundant at BIA (36% of the total). The most represented meiofaunal

taxa were the harpacticoids, *Bryocamptus zschokkei* (mean number of individuals per sample = 2 for traps, 14 for mosses) and *B. pygmaeus* (1 ind/sample for traps, 5 for mosses), and the ostracod *Psychrodromus olivaceus* (20 ind/sample for traps, 17 for mosses). Temporary meiofauna (21.8% of total) was represented by Chironomidae, Plecoptera, and Trichoptera larvae, and by unidentified dipteran larvae. Chironomidae was the most abundant taxon, with mean numbers of 7 and 6 individuals per sample for traps and mosses, respectively. Macroinvertebrate taxa were represented by several families of dipterans (Dixidae, Psychodidae, Stratiomyidae, Tipulidae), the caddisfly *Crunoecia* cf. *kempnyi*, molluscs (Gastropoda, Bivalvia), annelids (Hirudinea, Oligochaeta) and a few specimens of the amphipod *Niphargus* sp. aff. *puteanus* (Tab. 2).

Macrofauna decreased in abundance from a maximum in spring to minimum in winter in moss, whereas it remained almost constant in traps (Fig. 7). In trap samples, abundance of permanent meiofauna peaked during summer but was lower in other remaining seasons, whereas in moss it had a minimum in autumn and was higher in the other seasons (Fig. 7). Temporary meiofauna peaked in abundance in spring and decreased subsequently in both trap and moss samples (Fig. 7).



Tab. 3. Continuation.

	TRAPS		TUBES		MOSSES	
	T.A.	%	T.A.	%	T.A.	%
<i>Bryocampius tatrensis</i>	2.00	1.01				
<i>P. sychrodromus</i> cf. <i>betharrami</i>	1.79	0.91			1.00	0.47
Stratiomyidae sp.	1.41	0.72			1.00	0.47
Diptera unidentified early L	1.12	0.56			1.00	0.47
Bivalvia	1.10	0.55			1.00	0.47
<i>Bryocampius typhlops</i>	1.00	0.51			1.00	0.47
<i>Bryocampius vandanwei</i>	1.00	0.51			1.00	0.47
<i>Crinoecia</i> cf. <i>kempnyi</i>	1.00	0.51			1.00	0.47
<i>Candona neglecta</i>	0.89	0.45			1.00	0.47
<i>Hygrobates norvegicus</i>	0.80	0.41			1.00	0.47
<i>Attheyella crassa</i>	0.71	0.36			1.00	0.47
<i>Diatylops copepodites</i>	0.71	0.36			1.00	0.47
<i>P. sychrodromus fontinalis</i>	0.63	0.32			1.00	0.47
<i>Sperchon thienemanni</i>	0.63	0.32			1.00	0.47
<i>Bryocampius rhaeticus</i>	0.50	0.25			1.00	0.47
Amphipoda	0.50	0.25			1.00	0.47
<i>Euicylops</i> sp.	0.45	0.23			1.00	0.47
<i>Candona</i> cf. <i>lindneri</i>	0.45	0.23			1.00	0.47
<i>Cryptocandona vavrai</i>	0.45	0.23			1.00	0.47
<i>Lebertia</i> sp.	0.45	0.23			1.00	0.47
Dixidae L.	0.45	0.23			1.00	0.47
Nematoda	0.45	0.23			1.00	0.47
<i>Bryocampius cuspidatus</i>	0.35	0.18			1.00	0.47
<i>Pseudolimnocythere</i> cf. <i>lypogea</i>	0.35	0.18			1.00	0.47
COPEPODA	62.22	31.50	23.58	76.74	83.62	39.18
OSTRACODA	58.35	29.54	1.00	3.25	24.42	11.44
HYDRACHNIDIA	1.88	0.95	0.00	0.00	1.00	0.47
INSECTA	51.16	25.90	6.15	20.00	71.54	33.52
OTHER INVERTEBRATES	23.92	12.11	0.00	0.00	7.87	3.69

## 4. DISCUSSION

### 4.1. Physico-chemical characteristics of springs

In our study, springs differed in physical-chemical characteristics probably due to lithology: the springs on sandstone substrata with moraine and alluvial deposits (VEZ, LGD) were very close to each other, but nevertheless differed more than those on limestone and limestone-shale substrata (BIA, LGD). The latter were relatively distant from the other two springs and from each other, but were more similar and less variable over time. This might be due to the higher permeability of sandstone relative to that of shale and carbonate sedimentary rocks, which would in turn increase the mixing of different water types (surface water, groundwater, and runoff). The organic matter content might also have been important in determining pH and nutrient concentration which were higher at LGD and VEZ, where the percentage of organic debris was also higher.

### 4.2. Spatial patterns in invertebrate assemblages

The grouping of the investigated springs based on the invertebrate assemblages corresponded quite well to the grouping obtained from the environmental parameters, with pH and temperature being the variables best explaining diversity. The same parameters better explained large-scale spatial patterns in insect richness and abundance of 27 coldwater springs in Canada (Gathmann *et al.* 2009). Large-scale geographical variation in temperature was the main factor related to the pattern of benthic invertebrates assemblages recorded for 153 springs in Finland (Ilmonen *et al.* 2009). In our study, the presence of several species of Copepoda, Ostracoda, and Hydrachnidia, which include almost all the crenophilic and crenobiotic species, correlated with lower temperatures, as expected for the cold-stenothermic spring fauna (Illies 1952; Erman & Erman 1995). Acidity influenced the community structure of rheocrene springs in Germany, and spring-dwelling species appeared to be more tolerant to low pH than rhithrobic ones (Hahn 2000). pH also determined the relative abundance of insect and non-insect taxa in a wide sample of North American springs (Glazier 1991), with a higher proportion of non-insect taxa in temperate limestone springs, in sharp contrast with the insect dominance of most temperate stream faunas.

Relative distance was not an important factor, since the springs that were more similar in species assemblages were not always geographically closer, as a consequence of the dominance of low-vagility meiofaunal taxa in the assemblages. Some of the differences in taxon composition might have been accounted for by the position of one spring (BIA) in a different watershed, which enhanced isolation. Organic matter, geological features, and substratum composition (Tab. 1) might have also contributed to local differences in community composition. For instance, the dominance of

meiofaunal taxa at CIR was due to the high abundance of ostracods. This spring had the highest percentage of inorganic substratum, which was mainly fine particles. Therefore, the available habitat for interstitial taxa (i.e., copepods) was reduced, and epibenthic meiofauna (i.e., ostracods) dominated the assemblage. Biotic factors, such as competition, predation and complex host-parasite interactions, might have been important in structuring the assemblage composition, but no further investigation was carried out due to the lack of taxonomic identification to species level for most of the macrofauna.

### 4.3. Microhabitat distribution

The importance of habitat-related parameters in structuring the diversity of spring habitats has been recently investigated, stressing the significance of substratum type, altitude, habitat structure and complexity, microclimate, food and competition in fostering high species diversity and abundance in springs (Fischer 1996; von Fumetti *et al.* 2006; Staudacher & Füreder 2007; Di Sabatino *et al.* 2009b). Local habitat factors were important in our study: the faunal assemblages of each spring differed from each other, and different habitats determined the faunal assemblages and diversity: benthic, moss and groundwater habitats hosted specific invertebrate assemblages, which differed among springs.

The presence of emergent mosses was a factor that increased species diversity of the springs. The importance of sampling aquatic, semi-aquatic and semi-terrestrial habitats to evaluate the biodiversity patterns of spring habitats has been underlined in several studies (e.g., Barquín & Death 2009), and Ilmonen & Paasivirta (2005) reported that macroinvertebrates were most abundant in the moss carpet microhabitat, least abundant in the pool sites, although not significantly. Mosses provide an ecotone between terrestrial and aquatic conditions by creating a wide array of microhabitats with horizontally variable environmental conditions from the edges to the centre of moss carpets, and vertically from dry to submerged conditions through a madicolous transition zone (Lindgaard *et al.* 1975; Thorup & Lindgaard 1977). Mosses are therefore well known sources of high invertebrate diversity and are also particularly important for emerging adults of aquatic insects and the related water mites. Colonization of submerged bryophyte mats in aquatic ecosystems by large numbers of invertebrates is well known (e.g., Thorup & Lindgaard 1977; Suren 1991, 1992; Gerecke & Di Sabatino 1996). The bryophyte mats retain coarse (CPOM), fine (FPOM) and ultra fine (UFPOM) particulate organic matter as a food source for the detrital benthic consumers (Habdija *et al.* 2004), and stream mosses serve as a refuge from flow (Madaliński 1961; Elliot 1967; Gurtz & Wallace 1984; Suren 1992; Glime 1994). A direct association between Nematoda and Harpacticoida (and nauplii) and organic and mineral matter trapped within

the moss was recorded by Linhart *et al.* (2001-2002), thanks to its use as food, habitat or transport particles.

Springs are ecotones that link surface and groundwater, and indeed, we collected most stygobiotic taxa in drift tubes. Sampling the groundwater in addition to the benthic and moss habitats, did not necessarily increase the number of taxa collected; LGD (where drift tubes were not set due to the absence of a well-defined point of discharge) had the highest number of taxa, and the highest percentage of exclusive ones. On the other hand, VEZ which is very close to LGD and located on similar lithological formations, had the lowest number of taxa, and very low percentage of exclusive ones, also taking into account the drift samples. The peculiarity of VEZ is also reflected in the absence of ostracods and crenobiotic Hydrachnidia. A possible explanation may be that this spring has a high discharge and very steep slope where it emerges, i.e. conditions that could limit the availability of suitable habitats for some invertebrate species or groups. Also, due to the lack of long-term data, we can not exclude the possibility that this spring is intermittent over the years. The physical stability (thermal and flow constancy) and restricted extension of springs may favour non-emergent life-styles (i.e., species lacking aerial adults), and the environmental stability and paucity of large predators in springs may promote high population densities of invertebrates (Glazier 1991). In springs, non-emergent taxa can take better advantage of the year-round opportunity for growth and reproduction and find a refuge from scouring spates or droughts in constant-flow springs (Glazier 1991; Lingle-Gillis & Hamilton 1991). In a study on 23 springs with unstable flow in central Sweden (Hoffsten & Malmqvist 2000), macrofauna was dominated by short-lived, vagile insects. The authors also observed that dispersal between springs is infrequent, and that obligate crenic species, such as crenobionts are likely to be restricted to permanent springs. In our springs, the high abundance of non-emergent taxa was probably due to permanent, constant flow, at least at the annual scale.

#### 4.4. Meiofauna/macrofauna assemblages

A higher abundance of meiofauna over macrofauna, as recorded in our springs, is not commonly reported, mainly because the meiofaunal component has often been neglected in stream ecology (Hakenkamp & Morin 2000). However, the few available records indicate that meiofauna contributes between 58 and 81% of the total number of species in stream systems (Robertson *et al.* 2000). Meiofauna was more numerous and diverse than the macrofauna in the metazoan community of an acidic, fishless stream in south-east England (Stead *et al.* 2003), and the two assemblages appeared to respond to different environmental factors.

Many permanent meiofaunal taxa are non-emergent, their small size allows them to dwell in the sediment and to exploit moss habitats, and they are characterized

by short life cycles (Schmid-Araya 1998), resulting in high species turnover through the year. In contrast, most macrofaunal taxa have one or fewer generations per year, and take longer to develop to adults (Stead *et al.* 2003). Therefore, changes in composition of the macrofaunal assemblages are mainly due to seasonal changes in abundances within one generation. The seasonal trends in diversity shown by benthic and moss samples in this study reflects the dominance of taxa typically adapted to each habitat. Insect larvae peaked in spring, and decreased to minimum values in winter. This occurred in both benthic and moss samples. Meiofauna is composed of taxa which spend all (or most of, i.e. mites) their life cycle in the aquatic habitat, and have shorter life cycles. For instance, cyclopoid copepods tend to have rapid recruitment life strategies (Robertson 2002), while copepods and ostracods develop more rapidly compared to insects, in most cases not exceeding few months (Dole-Olivier *et al.* 2000).

In our study, Copepoda were represented by families with the highest number of free-living taxa in freshwater habitats, and particularly in groundwater (Galassi 2001). Crenoxenic and crenophilic species characterised the assemblages; among the crenoxenes were benthic, stygophilic and stygobiotic species, among the crenophiles were the stygobiotic *Elaphoidella pseudophreatica*, collected from drift tubes, as well as stygophilic and stygoxenic taxa (Stoch 2003; Pipan & Brancelj 2004). Some of the stygophilic species which we collected in moss were previously reported from the same habitat in Italian springs by Stoch (1993) and Stoch *et al.* (2009).

All ostracod taxa collected in the present study are common representatives of the European fauna (Meisch 2000) and can be considered crenophilic. They typically occur in springs and waters connected to springs, but also in a broad range of different freshwater ecosystems, occasionally including interstitial and groundwater habitats, showing a wide ecological tolerance. In fact, with the exception of *Psychrodromus betharrami*, *P. cf. betharrami* (both also in mosses) and *Ilyocypris bradyi* (only in mosses), all taxa were collected from traps. The only potential stygobiont was *Pseudolimnocythere cf. hypogea*, one adult female from CIR, which is one of the few recently described freshwater loxoconchids (Savatenalinton & Martens 2009). Individuals (only males) of this genus, possibly the same species, were collected from the brackish springs of Poiano in the Upper Secchia Valley, in the northern Apennines (Stoch *et al.* 2009).

Water mites (Hydrachnidia) represent one of the most diverse and specialized taxa living in springs (Di Sabatino *et al.* 2000, 2003), with more than 50% of the spring-dwelling species being true crenobionts. However, in the present study only four species were collected, with low densities. In the investigated springs, mosses represented the preferred habitat type; few individuals were collected in benthic traps, and none were

present in drift tubes. All the species are true crenobionts. *Partnunia aprutina* was previously known only from the type locality in Abruzzo, Central Italy (Gerecke 1996). It was impossible to identify a deutonymph of the genus *Lebertia* to species level; however, some characters allowed its attribution to the former subgenus *Hexalebertia*, represented almost exclusively by species restricted to spring habitats.

#### 4.5. Overall taxonomic richness

The investigated springs host a rich fauna. Although several studies on mountain spring fauna have been conducted in Italy, mainly in the north-eastern Alpine and pre-Alpine area, the only faunistic studies in Apennine springs were so far carried out only in the Central and Southern Apennines (see literature review in Bottazzi *et al.* 2008). Bottazzi *et al.* (2008) investigated the copepod and ostracod fauna of 19 Apennine springs, including those considered in the present study and others in adjacent areas. Four species of Copepoda and four taxa of Ostracoda, previously identified in those Apennine springs were not collected in the present study; on the other hand, we report seven species of copepods and one species of ostracod which are new to spring habitats of the study area. A comparison with the taxon richness of other Italian springs is only possible for a few cases, because the sampling methods and taxonomic precision are not always equivalent. For instance, in a study on 63 springs of different typology of South Tyrol (Sambugar *et al.* 2006), the fauna of natural springs was dominated by insects (and as a consequence by their parasitic water mites), with Diptera (represented mainly by Chironomidae) being the most diverse group. In the same study, copepods were dominant, and mainly represented by species related to groundwater habitats. For those groups which were identified to the same taxonomic precision as in our study (i.e., copepods, ostracods and water mites), the 12 rheocrene natural springs investigated by Sambugar *et al.* (2006) hosted 31 species (12 taxa of Copepoda, 6 Ostracoda, 13 Hydrachnidia) whereas our springs hosted 35 species (20 taxa of Copepoda, 11 Ostracoda, 4 Hydrachnidia) with 18% of the species in common (39% species for Copepoda, 21% for Ostracoda, 6% for Hydrachnidia). These observations further indicate a strong affinity of the copepod taxocoenosis of the investigated springs with that recorded in Alpine and pre-Alpine systems, with low numbers of stygobiotic and/or endemic taxa compared to the Central Apennine fauna, as already pointed out by Bottazzi *et al.* (2008). For Ostracoda and Hydrachnidia, the low number of taxa collected does not allow a comparison with crenic assemblages from other areas. Remarkably, we recorded a low number of water mite species compared to the high diversity recorded in the Alps, Central and Southern Apennines, where Di Sabatino *et al.* (2003) report 8-10 water mite species per spring for natural rheocrenes.

On the other hand, the few taxa collected were all highly specialized for spring habitats (i.e., crenobionts). The low diversity in the present study is probably related to the life cycle of these organisms, which are parasites in the larval stages and predators as adults and nymphs. Their preferred hosts and prey are insects and their eggs, which were low in abundance.

The investigated springs appear to be "insular biotopes", whose diversity depends strictly on the presence of different microhabitats and local environmental conditions. Gathmann *et al.* (2009) remarked that individual coldwater springs can represent a "habitat island" for a high proportion of their inhabitants, which are well-adapted to their environmental conditions and have no way of moving from one spring to another without entering less benign surrounding. In the case of organisms without winged adults, the reduced dispersal abilities further limit the possibility of colonizing other springs, enhancing the uniqueness of each spring.

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