Spatial and seasonal distribution of invertebrates in Northern Apenninic rheocrene springs

Elisa BOTTAZZI[1,*)], Maria Cristina BRUNO[2)], Valentina PIERI[1)], Antonio DI SABATINO[3)], Luana SILVERI[2)], Mauro CAROLLI[2)] and Giampaolo ROSSETTI[1)]

[1)] Department of Environmental Sciences, University of Parma, Viale G.P. Usberti 33A, I-43100 Parma, Italy

[2)] Edmund Mach Foundation, IASMA Research and Innovation Centre – Environment and Natural Resources Area, Via E. Mach 1, I-38010 S. Michele all'Adige (TN), Italy

[3)] Department of Environmental Sciences, University of L'Aquila, Via Vetoio, I-67100 Coppito (AQ), Italy

*Corresponding author: elisa.bottazzi@nemo.unipr.it

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3 Tables, 7 figures

ABSTRACT

Four perennial, rheocrene springs located between 919 and 1252 m a.s.l. on substrata characterized by different lithology were studied. Water samples and invertebrates were collected seasonally for one year. Spring fauna was collected with three sampling techniques: moss washing, drift tubes and benthic traps. Each sampling technique showed to be particularly efficient in collecting specific taxa, typical of 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 3284 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 the ordination of the investigated spring based on the invertebrate assemblages did not correspond that based on the environmental parameters, because only pH and temperature among the environmental parameters explained the diversity pattern. Assemblages collected in different habitats differed as well: benthic traps mainly collected Chironomidae, Ostracoda, Diptera, crenophilous Harpacticoida, and Gastropoda; in moss assemblages, the fauna was mostly represented by crenophilic Harpacticoida, Ostracoda; Plecoptera, Chironimodae. Finally, the groundwater assemblages, as collected with drift tubes,were dominated by crenophilous Harpacticoida, Chironomidae, Plecoptera. Variations in number of taxa over time were present in traps and moss samples, whereas drift tubes showed no seasonality. Permanent meiofauna (Copepoda, Ostracoda, and Hydrachnidia) was dominant in all habitats, probably as a consequence of constant flow, and of favourable habitats such as moss. The presence of mosses was a factor increasing the species diversity of the investigated springs; an drift tubes allowed collecting most of the stygobiotic taxa, although sampling the groundwater habitat in addition to benthic and moss ones did not necessarily increase the number of taxa collected. In addition to the presence of an array of habitats, other factors such as the geological setting might influence the structure of invertebrate communities. The investigated springs appear to be “insular

biotopes", whose diversity is strictly dependent from the presence of different microhabitats and from the local environmental conditions. The "insularity" character of the investigated springs is also due to the dominance of taxa with low dispersal abilities (i.e. meiofauna), which increase the differences in faunistic composition even over a small geographical scale.

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). In 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 biotopes characterised by a high degree of zoocoenoses distinctiveness, and marked heterogeneity of environmental conditions and communities (e.g. Bonettini & Cantonati 1996; 1998). Several studies showed how geographical factors (Williams 1991; Williams & Williams 1998), flow permanence (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), substrate composition (Glazier & Gooch 1987; Hahn 2000), altitude (Barquín & Death 2006) and local habitat features (Ilmonen & Paasivirta 2005) play a substantial role on the spring faunal assemblages. However, within-spring habitat heterogeneity has been shown to be an important factor in determining the composition of spring macroinvertebrate communities (e.g. Glazier & Gooch 1987; Lindegaard 1995; Lindegaard *et al.* 1998; Hahn 2000).

Springs sustain high levels of biodiversity: for instance, Illies (1978) reports the presence of about 1500 species in European springs, of which about 31% is represented by crenobiotic or crenophilic organisms. Springs represent the interface between two distinct ecosystems (ground-water and surface water), and can be considered as ‘‘hot spots’’ for aquatic biodiversity (Cantonati *et al.* 2006; Scarsbrook *et al.* 2007; Staudacher & Fureder 2007), due to their distinct ‘mosaic’ and multiple ecotonal structures (between aquatic-terrestrial, ground-surface water, and crenal-rhithral), which result in a high number of microhabitats (Weigand 1998; Di Sabatino *et al.* 2003). These features, together with the abundant presence of substrata favouring 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); typical spring fauna is composed by three groups of organisms (see Cantonati *et al*. 2006, for a detailed explanation): crenobionts (found only in spring environments), crenophiles (mainly distributed in springs, but can also be found in habitats with similar environmental conditions, generally benthic ones) 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 the interstitial spaces.

Springs can be considered insular biotopes (biotopes surrounded by unfavourable habitats to the biological groups they host (Mac Arthur & Wilson 1963, 1967) since their distinctive organisms (crenobionts and crenophiles, mostly) are bound within these environments because of the unfavourable surrounding habitats.

Several studies on biotic assemblages of mountain springs have been carried out in Europe during the last years (e.g. Von Fumetti *et al*. 2006, 2007; Hahn 2000; Barquín & Death 2009); in Italy, spring fauna has been investigated mainly in the north-eastern Alpine and pre-Alpine area and in the central and southern Apennines (see Bottazzi *et al.* 2008 for a review). Information on northern Apenninic springs is still poor and refers to the brackish springs of Poiano in the Upper Secchia Valley (Stoch *et al*. 2008, 2009), and to a recent contribution on copepods and ostracods from northern Apenninic springs which also included the springs analysed in the present paper (Bottazzi *et al.* 2008).

The present research aims to fill some of these gaps in knowledge, by defining the faunistic component of four perennial Apenninic springs, thus assessing distributional patterns of invertebrate fauna among springs and among habitats.

2. METHODS

2.1 Study springs

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 catchment of the Parma River at an altitudinal range of 919-1252 m a.s.l. (Tab. 1). Three of them, i.e. Cirone (CIR), Lagdei (LGD), and Vezzosa (VEZ) springs, are located on the left orographic side of the Parma River (Fig. 1). Biam spring (BIA) is located on the right orographic side of the Bratica Stream, a right tributary of the Parma River. LGD and VEZ are very close to each other (about 1 km apart), and CIR is located at 4.3 and 3.4 km from them, respectively; these three springs are still in pristine condition. BIA is at a distance range of 7.2-7.8 km from the remaining three springs, and it has been partially modified through insertion of pipes (see below).

The geology of the area is characterized by sedimentary layers of different origin: 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 gave origin to sandstone strata 100 m thick; glacial deposits from the Pleistocenic ice-ages (during the Würmian glaciation, glaciers covered all the Northern Apennine mountain range, and extended into the studied valleys). The glacier running down the Parma Valley, with an estimated surface of 25 km^2 (Federici & Tellini 1983), was the largest in the Northern Apennines. BIA is situated 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).

BIA is characterized by the presence of two pipes; part of the spring water flows, although intermittently, through them, and part of water emerges freely, forming a small springbrook. This spring is surrounded by fields and chestnut trees, thus during the vegetative season it is partially shadowed by tree canopies. Disturbance is essentially related to passage of hikers during summertime. CIR is situated on a hay field; water emerges freely from several discharge points which eventually join together 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 relevant amounts of organic detritus; the presence of high trees determines relatively constant shading throughout the seasons. VEZ, surrounded by a spruce forest, is permanently shadowed and forms a small springbrook that, a few meters downstream, joins a fast flowing torrent.

2.2 Sampling methods

Altitude and geographic coordinates (Tab. 1) of the four investigated springs were determined with a GPS. Lithology (Tab. 1) was determined using 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) and 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.

Four seasonal surveys were carried out in the following time periods: spring: April, 24th-June, 20th, 2007; summer: August, 23rd-30th, 2007; autumn: November, 22nd-December, 12th, 2007; winter: January, 30th-February, 20th, 2008. Water temperature, pH and specific conductivity were measured *in situ* at 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), NH_4^+, NO_3^-, 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 with methods proved to create a minimal impact on the environment and which allowed collecting from different habitats: traps to collect macro- and meiofauna actively moving from benthic and interstitial habitats; drift tubes to retain stygobionts passively transported by the water outflow from the groundwater system; moss washing 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 in one end of the tube, closing the other with a 50 µm net. These traps were filled with gravel (0.3-1 cm) collected in the area, which was previously washed and sieved. Traps were baited with ham, and placed at the interface between sediment and water, 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 traps was accurately rinsed and water was filtered through a 50 µm net. Drift tubes (length 160 mm; diameter 48 or 80 mm) were built attaching a 50 µm net to one opening of a PVC pipe; they were placed in well-defined discharge points which were present only at stations BIA and VEZ and left *in situ* for one week. The net was subsequently removed and rinsed, and the material obtained in this way was filtered through a 50 µm net. When present, clumps of mosses from the spring outlet were removed in variable quantities depending on their coverage area; some stations at some sampling dates had no moss (CIR and VEZ in spring; LGD in spring and autumn). In the laboratory, mosses were carefully washed to remove animals; water was subsequently filtered through a 50 µm net. Traps were overall the most extensively sampling method used (in each spring and season); in fact, two springs did not show a well definite point of discharge to place tubes, while bryophytes were not present in every season. All the samples were fixed in 95% ethanol immediately after collection. Invertebrate specimens were sorted in the laboratory under a dissecting microscope.

Copepoda, Ostracoda and Hydrachnidia were classified to the species level following Dussart (1967, 1969), Stoch (1998a), and Karaytug (1999) for Copepoda; Meisch (1984) for the genus *Potamocypris*, Baltanás *et al*. (1993) for the genus *Psychrodromus*, and Meisch (2000) for the remaining Ostracoda; Gerecke (1996), Davids *et al.* (2007) and scattered special keys for Hydrachnidia. Only one species of Trichoptera was represented by late larval stages, and was identified to the 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 highest rank adopted for the rest of their taxonomic groups, because of their early stage of development. All identified taxa were classified as crenoxene, crenophile, crenobiont, and stygoxene, stygophile, stygobiont, based on the review of published specialized literature and on recent data on the distribution of 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. The springs were ordered based by applying a non-metric multidimensional scaling (NMDS) to a resemblance matrix based on the Euclidean distance, calculated using the values of water temperature, pH, conductivity and nitrate concentration data; the remaining nutrient concentrations were not included into the analysis because mostly under the threshold limit of the analytical methods.

We performed a one-way MANOVA over all physico-chemical variables, and a Tukey's Honestrly 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 environmental data matrix by running a Principal Component Analysis (PCA). We run a multiple correlation between each taxon and the physical-chemical variables of each sample.

Because the different sampling methods were designed to collect from different habitats, we tested for significant differences in species assemblages between habitats by running a one-way ANOSIM analyses (Analysis of Similarities, Clarke 1993) on a Bray-Curtis similarity matrix, which was 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, at each sampling occasion, eventually resulting in 34 samples (15 traps, 6 drift tubes, 13 mosses) and 54 taxa. We also run a one-way ANOSIM for moss and trap data, separately, to assess for differences among spring in those assemblages.

Due to the different number of samples and /or methods used to collect at each spring for each season, we averaged the traps and moss data and calculated the mean number of individuals of each taxon for each spring, at each sampling occasion, eventually resulting in 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" method were not included in the analysis and 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 the calculation of Bray-Curtis similarities. One-way ANOSIM was used to investigate differences between season and spring, while Indicator Species Analyses (ISA; Dufrêne & Legendre 1997) were run, with a Monte Carlo randomization, to investigate more specifically which particular taxa were characteristic of each spring, each season, and each habitat. The Euclidian distances of the standardized values of temperature, conductivity, pH ad was used to run a RELATE procedure, 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

Springs differed in their physico-chemical variables (one-way MANOVA, factor: SPRING, $F = 9.81$, $p < 0.001$); in particular, temperature and specific conductivity 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 a relative stability of physical and chemical water parameters (Fig. 1). The highest water temperature (8.5 °C) was recorded at CIR, and the highest seasonal variation in 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 parameter found most stable throughout the seasons in all the springs, although with values significantly higher at CIR and BIA than at LGD and VEZ. All the investigated springs were oligotrophic: SRP concentration was not detectable for 10 out of 16 samples; in the remaining six samples it ranged between 5 and 17 $\mu g\ l^{-1}$; NO_3^- concentrations varied between 173 and 480 $\mu g\ l^{-1}$, with an isolated peak (1266 $\mu g\ l^{-1}$) recorded in autumn at VEZ (Fig. 1).

The first two axes of the PCA plot explained 73.8 % of the total variance, with the gradient on the first axis explained by temperature (eigenvector: -0.656) and conductivity (eigenvector: -0.684); and the gradient on the second axis by pH (eigenvector: 0.942). Samples formed two main groups in the PCA plot (Fig. 2), one with the samples collected at BIA and CIR characterized by higher temperature and conductivity and very similar to each other, and the second one with those collected at LGD and VEZ with higher pH, and more scattered on the biplot. The winter and autumn samples collected at LGD, and the autumn sample collected at VEZ were more isolated, due to the low values of water temperature, pH and specific conductivity recorded at the former, and the higher nitrate concentration at the latter. Such nitrate load could be attributed to an increased input of dissolved organic matter caused by heavy rainfall events. Samples collected at

BIA and CIR, on calcareous substrates were less variable than those collected from the sandstone substrate.

A total 3284 individuals were collected during this study and were 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 to the highest taxonomic detail, LGD had the highest richness, hosting 31 taxa; it was followed by BIA and CIR (29) and VEZ (19); in the latter, no ostracods were collected (Tab. 2). LGD had the most distinct community, with approximately 48% exclusive taxa, while their percentage in BIA, CIR and VEZ was, respectively, 24%, 12%, and 16% (Tab. 2).

The NMDS ordination of the total invertebrate assemblages collected at each spring, in each season, in traps and mosses (Fig 3), showed a clear spatial trend in changes of the community 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 grouped closer on the NMDS plot, and so did LGD and VEZ: the average Bray-Curtis dissimilarity calculated between these groups were the lowest of all the possible pair comparisons (BIA and CIR average dissimilarity: 63.64; LGD and VEZ: 62.59). Seasonal variations were significant (ANOSIM, factor SEASON, global $R = 0.30$, $p < 0.01$); CIR and LGD were respectively the less and the most variable springs over time (Fig. 3).

The ISA indicated that separation of samples collected at each station mainly reflected the presence of *Bryocamptus pygmaeus* at VEZ, *Acanthocyclops* sp., *Megacyclops viridis*, and *Potamocypris pallida* at LGD, *Psychrodromus olivaceus* at CIR. No indicator species were identified for BIA. For the season, Chironomidae were indicators for spring, Plecoptera for summer, Oligochaeta for autumn, other Diptera for winter.

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 out of the four environmental variables included (T, conductivity, pH, nitrates), only temperature and pH best explained the observed community patterns (Spearman's rho = 0.55, $p < 0.01$). Taxa which were significantly correlated with temperature were (negative correlation) *B. tatrensis*, *M. alpina*, *M. poppei*, *C. neglecta*, *C.* cf. *lindneri*, *C. vavrai*, *P. fontinalis*, *P. olivaceus*, *Lebertia* sp., Bivalvia, Oligochaeta; with pH (negative correlation) for Tipulidae; with conductivity (positive correlation) for *E. richardi*, *P. olivaceus*, with nitrates (positive) for *Parastenocaris* sp., *P. olivaceus*.

Species assemblages at each habitat tested to be highly significantly different (one-way ANOSIM, factor: METHOD, Global R = 0.41, $p = 0.001$) and traps and moss 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), whereas the few drift tube samples were quite different (Bray-Curtis similarity index: 9) and were also scattered in 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 pair of stations except BIA and VEZ; differences were not significant for moss. The dissimilarity between samples collected from different habitats was very high: the Bray-Curtis dissimilarity index was highest when the groundwater assemblages were compared to benthic (drift tubes and traps average dissimilarity: 88.76) and moss (drift tubes and moss average dissimilarity: 89.19) assemblages; the average dissimilarity between traps and moss assemblages scored 83.15. Traps collected more taxa than the other methods except at BIA, where more species were present in moss (Fig. 5B, D); more taxa were collected in drift tubes at BIA than at CIR (Fig. 5C). Variations in number of taxa over time were present in traps and moss samples, whereas drift tubes showed no seasonality (Fig. 6).

Permanent meiofauna was represented in this study by Copepoda, Ostracoda, and Hydrachnidia. They were dominant in all habitats representing 62%, 80%, and 51% respectively in traps, drift tubes, moss, whereas macrofauna (Insecta and other invertebrates) respectively represented 38%, 20%, 37% (Tab. 3). Permanent meiofauna was most abundant at CIR (83% of the total, due to the high abundance of ostracods), followed by LGD and VEZ (68% for both springs), and was least abundant at BIA (36% of the total). The most abundant meiofaunal taxa were the harpacticoids *B. zschokkei* (mean number of individuals per sample scored 2 for traps and 14 for moss) and *B. pygmaeus* (1 ind/sample for traps and 5 for moss) and the ostracod *P. olivaceus* (20 ind/sample for traps and 17 for moss). Chironomidae was the most abundant insect taxon, with a mean number of individuals per sample scoring 7 and 6, respectively for traps and moss. Insecta showed the same seasonal trend in traps and mosses, decreasing from a maximum values in spring to minimum values in winter (Fig. 7). Meiofauna, on the other hand, peaked in abundance in summer in traps and had lower values in the remaining seasons, whereas had a minimum in autumn and higher values in the other season in mosses (Fig. 7).

The traps collected mainly Chironomidae, Ostracoda, Diptera, crenophilous Harpacticoida, and Gastropoda. Stygobiotic Harpacticoida were not found in traps. In the 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). The ISA indicated that the taxa discriminating between assemblages collected in different habitats were *Megacyclops viridis* for benthic habitats (traps), *Elaphoidella pseudophreatica* and *Moraria stankovitchi* for groundwater (drift tubes), and *Hygrobates norvegicus* and *Bryocamptus tatrensis* for mosses.

Copepoda were represented by 763 individuals. All Harpacticoida belong to the family Canthocamptidae, with the exception of *Parastenocaris* sp. (family Parastenocarididae), and all Cyclopoida to the family Cyclopidae. Crenoxenous and crenophilous species characterised the assemblages (Tab. 2). Crenoxenes were benthic, stygophilous and stygobiotic species. Crenophilic species were the stygobitic *E. pseudophreatica*, collected from drift tubes, the stygophilic and stygoxenic taxa.

A total of 1518 adult ostracods were identified, belonging to eleven taxa in six genera (Tab. 2). Four different families were represented (Candonidae, Ilyocyprididae, Cyprididae and

Loxoconchidae), with the most divesified ostracod fauna recorded in LGD where nine taxa were recorded, 5 of which exclusive of this spring (*Candona neglecta, C.* gr. *neglecta*, *Cryptocandona vavrai*, *Psychrodromus fontinalis* and *Potamocypris pallida*). Seven taxa were collected at CIR, two of which (i.e. *Ilyocypris bradyi* and *Pseudolimnocythere* cf. *hypogea*) were collected exclusively there. Only three species were collected at BIA*,* none of which was exclusive of this spring. Ostracoda were not collected at VEZ. All taxa collected are crenophilic, with the remarkable exception of the potentially stygobiont *Pseudolimnocythere* cf. *hypogea*. All taxa were collected from traps, with the exception of *Psychrodromus betharrami, P.* cf. *betharrami* and *Ilyocypris bradyi*, collected also (the first two species) or exclusively (the latter) in moss.

Water mites (Hydrachnidia) represent one of the most diverse and specialized taxa living in springs (Di Sabatino *et al.* 2000, 2003). However, in the present study only four species and 79 individuals of water mites were sampled. Mosses represented the preferred habitat type, few individuals were also collected in benthic traps and no water mites were present in drift tubes. All the species collected are true crenobionts: *Partnunia aprutina*, collected in mosses in spring BIA, is a rare crenobiont mite previously known only from spring systems of Central Apennines (Gran Sasso National Park, Abruzzo) (Gerecke 1996; Cicolani *et al.* 1996; Di Sabatino *et al.* 2009b); *Sperchon thienemanni* and *Hygrobates norvegicus* are more widely distributed and colonize mainly rheocrene springs in Central Europe, Alps and Central and Southern Apennines (Di Sabatino *et al.* 2003). It was impossible to identify to species level the deutonymph of the genus *Lebertia*, however, the presence of some characters allowed the attribution to the former subgenus *Hexalebertia,* represented almost exclusively by species bound to spring habitats. Three species were collected in BIAM, two species in CIR and LGD and, very remarkably, no water mites were recorded in VEZ.

4. DISCUSSION

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

The grouping of the investigated spring based on the invertebrate assemblages did correspond quite well to the grouping obtained from the environmental parameters, based on the fact that pH and temperature were the variables explaining the diversity. The same environmental parameters were those better explaining the large-scale spatial patterns in insect richness and abundance in 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 of Finland (Ilmonen *et al*. 2009). In our study, the presence of several species of Copepoda, Ostracoda, and Hydrachnidia, taxa which include almost all the crenophilous 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-values than rhithrobiontic ones are (Hahn 2000). pH was also relevant in determining 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.

Other environmental factors which might have been important in structuring the invertebrate communities are the relative distance of the springs, geological features, organic matter, and substrate granulometry. Relative distance apparently was not an important factor, given that the springs which were more similar in species assemblages were not those geographically closer to each other, as a consequence of the dominance of low-vagility meiofaunal taxa in the assemblages. Some of the differences in taxa composition in our springs might have been accounted by the position of one spring (BIA) in a different watershed, which enhances isolation. Organic matter, geological features, and substrate composition might have also contributed to explain 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 substrate, and such substrate was mainly represented by fine particles. Therefore, the available habitat for indwelling taxa (i.e. copepods) was reduced, and epibenthic meiofauna (i.e. ostracods) dominated the assemblage. Biotic factors, such as competition, predation, complex host-parasite interactions might have been important in structuring the assemblages composition, but due to the lack of taxonomic identification to the species level for most of the macrofauna, it was not possible to investigate them. The importance of habitat-related parameters besides the physico-chemical characteristics of water in structuring the diversity of spring habitat has been investigated recently, and stressed the overall importance of substrate, altitude, habitat structure and complexity, microclimate, food and competition (Fischer 1996; von Fumetti *et al*. 2006; Staudacher & Füreder 2007; Di Sabatino *et al.* 2009b) in fostering high species diversity and abundance in springs. The importance of local habitat factors is shown in our study, where the faunal assemblages of each spring appeared to differ from each other, and sampling for different habitats determined the faunal assemblages and the diversity level: the benthic, moss and groundwater habitats did host specific invertebrate assemblages which differed among springs.

The physical stability (thermal and flow constancy) and small habitat area of springs may favour non-emergent life-styles (i.e. those lacking aerial adults), and the environmental stability and paucity of large predators in springs may promote high population densities of macroinvertebrates (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 defaunating spates or droughts in constant-flow springs (Glazier 1991; Lingle-Gillis & Hamilton 1991). In fact, the macrofauna of 23 springs in central Sweden was dominated by short-lived, vagile insects, due to spring instability with respect to flow (Hoffsten & Malmqvist 2000). In our springs, the permanence of a constant flow probably favours the increase of non-emergent taxa, which were the dominant component. Moreover, such taxa belonged to meiofauna, and their reduced size would allow dwelling into the sediment in order to find a refuge from adverse surface conditions, and to exploit moss habitats. The dominance of meiofauna over macrofauna, as recorded in our samples, is not commonly reported, mainly because the meiofaunal component has been often neglected in stream ecology studies (Hakenkamp & Morin 2000). However, the few available records indicate that they contribute 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 whole 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. In our study, the Indicator Species Analysis listed as characterizing each habitat, meiofaunal species which have been reported from literature as typical of that habitat: the copepod *Megacyclops viridis* for epibenthic habitats, the harpacticoids *Elaphoidella pseudophreatica* and *Moraria stankovitchi* for groundwater habitats, the harpacticoid *Bryocamptus tatrensis* and the water mite *Hygrobates norvegicus* for mosses.

The presence of emergent mosses was a factor increasing the species diversity of the investigated springs. The importance of sampling aquatic, semi-aquatic and semi-terrestrial habitats in order to account for the biodiversity patterns of spring habitats has been underlined in several research (e.g., Barquín & Death 2009), and indeed Ilmonen & Paasivirta (2005) reported how the studied macroinvertebrates were most abundant in the moss carpet sites and 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 environmental conditions varying horizontally from edges to the inner parts of moss carpets, and also vertically from dry to submerged conditions through a madicolous transition zone (Lindegaard *et al.* 1975; Thorup & Lindegaard 1977). Mosses are therefore well known as sources of high invertebrate diversity, and are also particularly important for emerging adults of aquatic insects and the related water mites, and colonization of the submerged bryophyte mats in aquatic ecosystems by enormous numbers of invertebrates is well known (Thorup & Lindegaard 1977; Fischer 1993; Lindegaard 1995; Gerecke & Di Sabatino 1996; Staudacher & Füreder 2007; Lindegaard *et al.* 1975; Maurer & Brusven 1983; Brusven *et al.* 1990; Suren 1991; Tada & Satake 1994). The bryophyte mats retain coarse (CPOM), fine (FPOM) and ultra fine (UFPOM) benthic particulate organic matter as a food source for the detritial benthic consumers (Habdija *et al*. 2004), and stream mosses serve as a refuge from the flow (Madaliński 1961; Elliot 1967; Gurtz & Wallace 1984; Suren 1992; Glime 1994). A direct association with organic and mineral matter (30 µm-1 mm) trapped within the moss and Nematoda and Harpacticoida (and nauplii) was recorded by Linhart *et al*. (2001-2002), as a result of its use as food, habitat or transport particles.

Springs are ecotones linking surface with groundwater, and indeed, we collected most of the stygobiotic taxa with drift tubes. Sampling the groundwater habitat in addition to benthic and moss ones, did not necessarily increase the number of taxa collected; in fact, 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 contrary, VEZ which is very close to LGD and is 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 ostracod taxa and crenobiont Hydrachnidia. A possible explanation may be that this spring has a high discharge rate and a very steep slope at the emerging points, i.e.

conditions that could limit the availability of suitable habitats for some invertebrates species or groups.

Many permanent meiofauna taxa are characterized by short life cycles (Schmid-Araya 1998), resulting in species turnover through the year. In contrast, most macrofaunal taxa such as Plecoptera, Trichoptera and some Chironomidae, generally have one or fewer generations a year, and take longer to develop to adults (Stead *et al.* 2003). Therefore, changes in the composition of the macrofauna assemblage are mainly because of seasonal changes in abundance taking place 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 due to the massive emergence of adults occurring in temperate streams during summer (López-Rodríguez *et al.* 2008), they decrease to minimum values in winter. This occurred in both benthic and moss samples. Meiofauna is composed by taxa which spend all their life cycle in the aquatic habitat, and have shorter life cycles than macrobenthos. For instance, cyclopoid copepods tend to have life strategies of rapid recruitment (Robertson 2002), and copepods and ostracods have more rapid development if compared, for instance, 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. The crenophilic species are either reported from literature as stygobionts, or as species with strong affinity for groundwater habitats (Stoch 2003; Pipan & Brancelj 2004), or stygophilic, and were collected from benthic traps and moss; the latter group of species have been previously reported as moss-living in Italian springs (Stoch 1993; 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. The only potential stygobiont was *Pseudolimnocythere* cf. *hypogea*, collected with one adult female in CIR. It is one of the few recent freshwater loxoconchids described so far (Savatenalinton & Martens 2009). Individuals (only males) belonging to this genus and possibly to the same species which were collected in 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 species being true crenobionts. In our samples, we found *Partnunia aprutina*, a crenobiont parasitic mite previously known only from the type locality in Abruzzo, Central Italy (Gerecke 1996). All known *Partnunia* larvae are terrestrial (semiaquatic) and search their hosts (mainly Plecoptera) by active moving and jumping above mosses and mineral substrata. Well shadowed rheocrene springs at high altitude represent the preferred habitat-type. *Sperchon thienemanni* is a crenobiont species with no clear preference for spring typology. It was frequently sampled in rheocrene springs, but also occurs in helocrene and spring with intermediate typology (rheohelocrenes). Larvae are truly aquatic and attach their hosts (Chironomidae) beneath water. Females lay eggs in clutches, often in moss mats (Di Sabatino, unpublished). The species is widely distributed in Central Europe and colonizes a large number of Italian springs, from the Alps to Southern Apennines, including Sicily and Sardinia (Di Sabatino *et al.* 2003). In the Central Apennines, it was often sampled in springs with mineral substrata and mosses (Cicolani *et al.* 1996; Di Sabatino *et al.* 2009b); in the present study it was more abundant in mosses than in benthic traps.

Hygrobates norvegicus is the only representative of the subgenus *Hygrobates* in Italy. Small rheocrenes and rheohelocrenes, often with abundant moss cover and coarse mineral substrates, are the preferred spring-type in the Alpine and Apennine area (Di Sabatino *et al.* 2009b). In the present investigation, this species was more abundant in mosses, also collected with few individuals in benthic traps. The species is distributed in Central-East Europe and colonizes a large number of springs in the whole Alpine area. In Italy, it was also found in the Central Apennines at an altitudinal range between 800 and 2300 m a.s.l., with temperatures ranging between 5 and 13 °C.

It was impossible to identify to species level individuals of the genus *Lebertia* because the only specimen collected was one deutonymph, collected in a benthic trap at LGD. However, the presence of some characters allowed the attribution to the former subgenus *Hexalebertia,* represented almost exclusively by species bound to spring habitats. For this reason, *Lebertia* sp. was classified as crenobiont.

The investigated springs appeared to 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 Apenninic springs have been carried out so far 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 Apenninic 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 Apenninic springs were not collected in the present study; on the other hand, here we report seven species of copepods and one species of ostracod which are new to these spring habitats. A comparison with taxa richness of other Italian springs is possible only in few cases, because the sampling methods and taxonomic accuracy 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 water mites, which are their parasites), with Diptera (represented mainly by Chironomidae) being the most diverse group; in the same study, copepod were dominant in captured springs, and mainly represented by species related to groundwater habitats. For those groups which were identified at the same taxonomic detail as in our study (i.e. copepods, ostracods and water mites), the 12 rheocrene natural springs investigated by Sambugar *et al.* (2006) hosted a total of 31 species (12 taxa of Copepoda, 6 Ostracoda, 13 Hydrachnidia) whereas our springs hosted a total of 35 species (20 taxa of Copepoda, 11 Ostracoda, 4 Hydrachnidia) with 18% of the species in common (39% species in common for

Copepoda, 21% for Ostracoda, 6% for Hydrachnidia) . These observations further indicate a strong affinity of copepod taxocoenosis of the investigated springs with those recorded in Alpine and pre-Alpine systems, with low numbers of stygobiotic and/or endemic taxa compared to fauna of the Central Apennines, as already recorded by Bottazzi *et al.* (2008). For Ostracoda and Hydrachnidia, the low number of taxa collected does not allow a faunistic comparison with spring assemblages of other Alpine and Apenninic areas. Remarkably, we recorded a low number of species of water mites, compared to the high diversity recorded in the Alps, Central and Southern Apennines, where Di Sabatino *et al.* (2003) report for natural rheocrenes, a number of 8-10 water mite species per spring. On the other hand, the few taxa collected were all highly specialized for spring habitats (i.e. crenobionts). The low diversity recorded in the present study, is probably related to the life cycle of these organisms (parasitic larvae and predatory adults and nymphs), and to the relatively low abundance of their preferred host, i.e. insects.

Bottazzi *et al.* (2008) investigated the copepod and ostracod fauna of 19 Apenninic 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 Apenninic springs were not collected in the present study; on the other hand, here we report six species of copepods and two species of ostracod which are new to these springs.

These faunistic data further confirm the differences in faunistic composition over a small geographical scale for most axa with low dispersal abilities. In synthesis, the investigated springs appear to be “insular biotopes”, whose diversity strictly depends from the presence of different microhabitats and from the local environmental conditions. Gathmann *et al.* (2009) remarked how each individual coldwater springs can represent a “habitat island” for a high proportion of their inhabitant species, which are well-adapted to their environmental conditions, and with no way of getting 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 to colonize other springs, enhancing the uniqueness of each spring. Hoffsten & Malmqvist (2000), based on the analysis of macroinvertebrate fauna of 23 springs in central Sweden, observed that dispersal between springs is infrequent, and that obligate springs species such as crenobionts are likely to be restricted to permanent springs.

In conclusion, this study highlights the relevance of springs as biodiversity hotspots; although their small dimensions, they host invertebrate communities which are complex and diverse. Habitat destruction may determine the loss of spring communities, which may be delayed in time (Tilman *et al.* 2002), and thus not promptly perceived; this "extinction debt" is particularly relevant in patchy environments such as springs. Springs are endangered by direct and indirect human activities, such as water abstraction, cattle watering, pollution or climate change; once altered, their full recovery is unlikely to occur (Zollhöfer *et al.* 2000). The awareness of their vulnerability should therefore help to enhance the efforts for an effective management and conservation of these important biodiversity hotspots, which represent unique habitats for ecological and biogeographical studies.

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FIGURES

Figure 1. Seasonal variations of: a) water temperature, b) conductivity, c) nitrate concentration, d) pH, at each of the investigated springs.

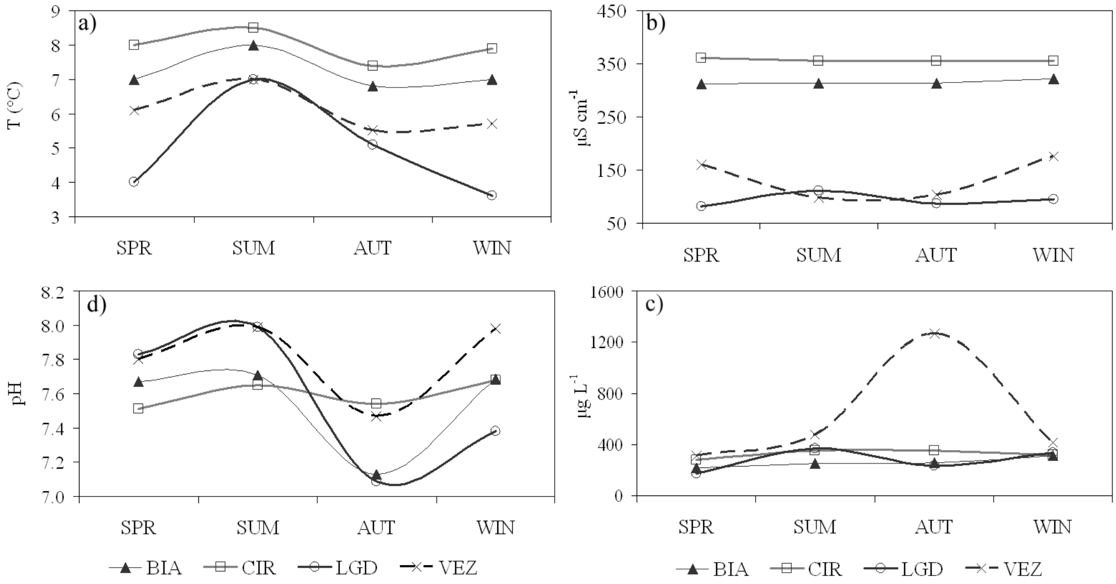

Figure 2. PCA of the different samples based on physical-chemical variable (water temperature, pH, conductivity, nitrate concentration). Samples categorized by dominant lithology. A: autumn; W: winter; sp: spring; su: summer.

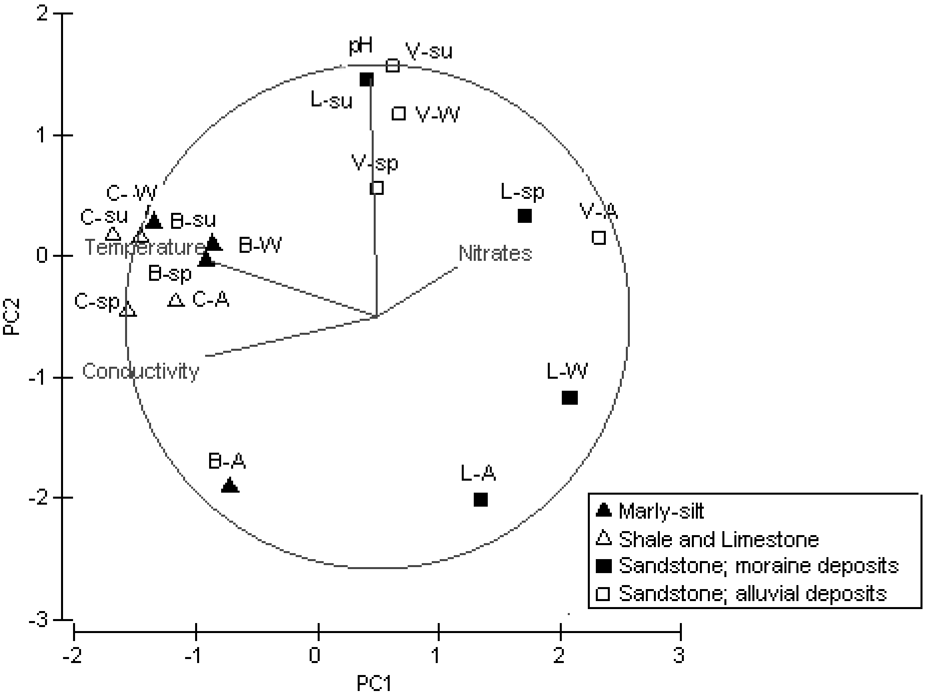

Figure 3. Non-metric Multidimensional Scaling of faunal assemblages in trap and moss samples; samples calculated as mean value for each season: au: autumn; wi: winter; sp: spring; su: summer.

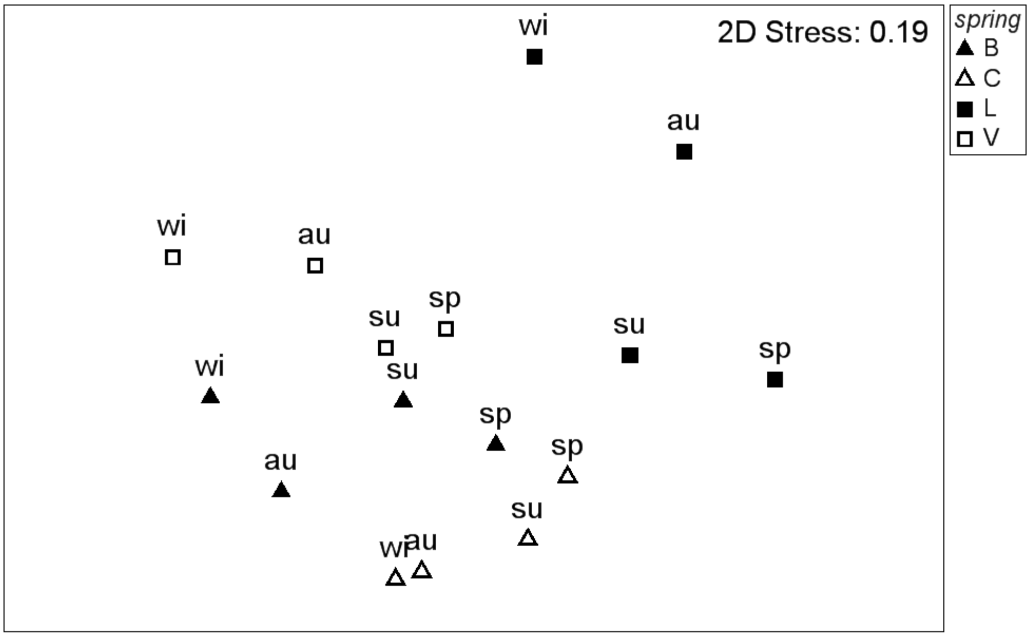

Figure 4. Non-metric Multidimensional Scaling of faunal assemblages in drift tubes, trap and moss samples, collected at each sampling occasion.

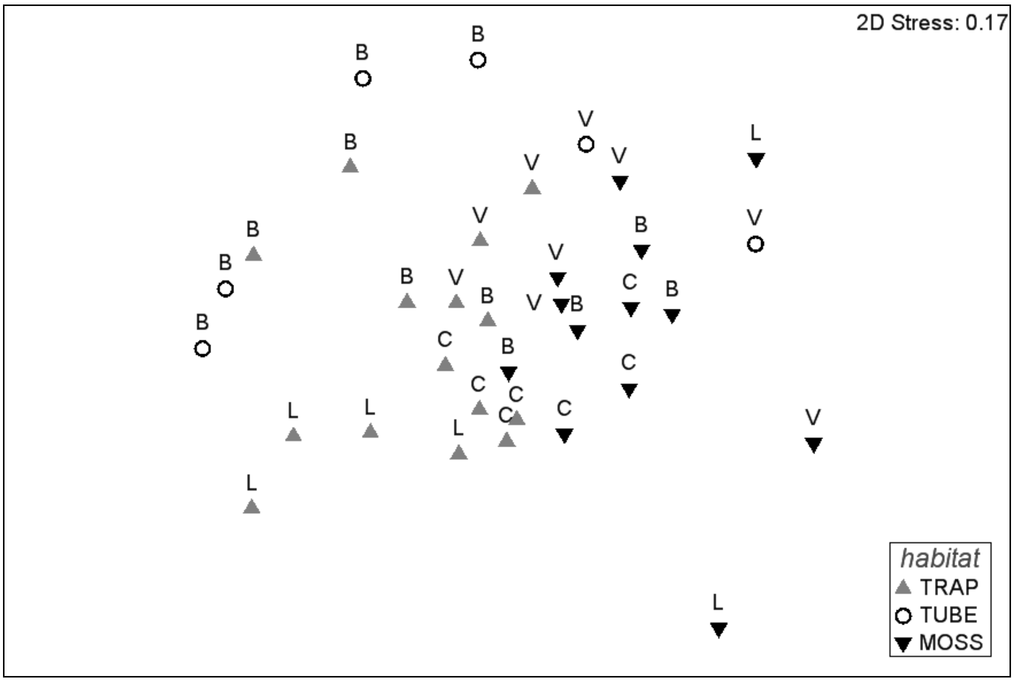

Figure. 5. Box-plot of taxa richness at each spring. Line: median value, box: 25%-75% values; whisker: minimum-maximum range. A: all methods; B: traps; C: drift tubes; D: moss.

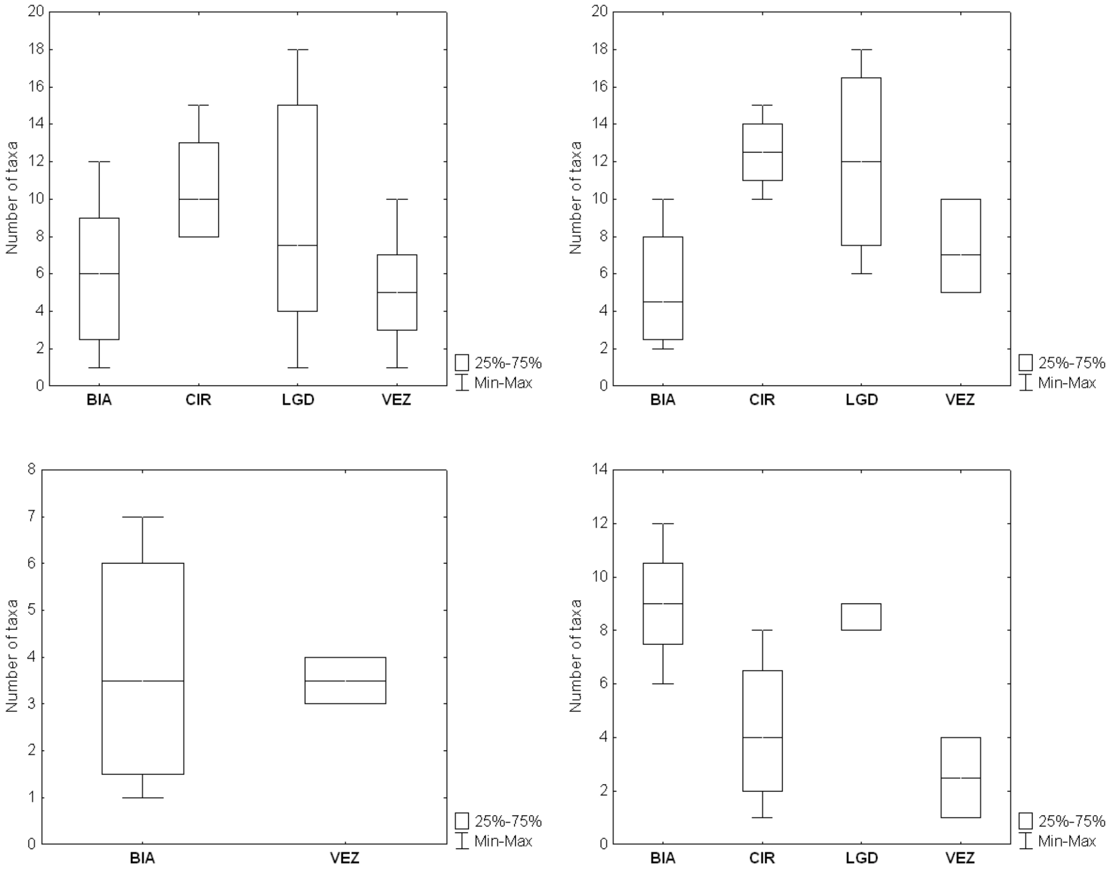

Figure 6. Number of taxa collected in each habitat at each spring (BIA, VEZ, CIR, LGD), and season (SP: spring; SU: summer; AU: autumn; WI: winter).

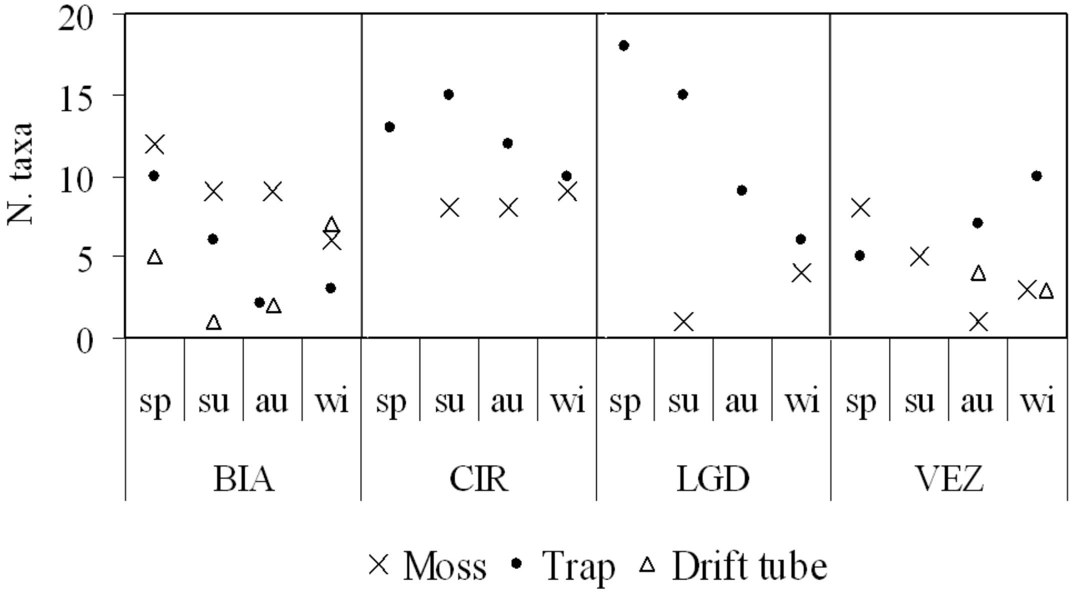

Figure 7. Mean monthly number of individuals collected from all springs in traps, and in moss.

Meiofauna: Copepoda, Ostracoda, Hydrachnidia.