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Assembly processes of invertebrate communities in springs across different spatial scales

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Abstract

This work is aimed at analysing the processes underlying the patterns of species coexistence of aquatic invertebrates in springs, distinguishing between deterministic and stochastic dynamics. Springs are complex ecosystems and several characteristics (e.g. the insularity, the thermal stability, the "mosaic" ecotonal structure, the high occurrence of rare and endemic species and the high taxa diversity) make them natural laboratories for the study of ecological processes, among them assembly dynamics. Although their ecological relevance, springs are still habitats neglected by research and conservation efforts.

Since a multi-scale approach is necessary to study assembly processes, three spatial scales were taken into account. At local scale, one-year seasonal sampling was carried out on Mount Prinzera, an ophiolitic outcrop near Parma, in seven springs (four temporary and three permanent). In this area the effectiveness and the environmental impact of different sampling methods were evaluated, and the ecological drivers affecting community structure were analysed. At larger scale, 15 springs in the Emilia Romagna region were sampled twice in order to identify the role of dispersal and the presence of a niche-filtering dynamic. At continental scale, information on springs of the Western Palearctic area were collected from literature, and the presence of biogeographical patterns and the influence of climatic factors were investigated. Different invertebrate taxa (macroinvertebrates, ostracods, water mites, and copepods) were investigated, choosing the most suitable for explaining each process for their ecological characteristics and for the taxonomic resolution available.

Biological sampling in springs implies several methodological problems and moreover can cause disturbance. In this work different methods were compared: the net with proportional multi-habitat approach and the combined use of traps and the washing of vegetation sample. The use of the net provides more accurate and complete information, but also significant impacts on the biotic and abiotic components of springs. For these reasons, this method is only recommended for biodiversity inventories. On the other hand, traps and vegetation washing are still reliable methods with less negative effects on spring ecosystems, thus they are more suitable for ecological studies focused on the analysis of the community structure.

Niche-based processes resulted to be important in shaping invertebrate communities in springs, and environmental drivers explain a relevant percentage of community variation. Indeed, invertebrate communities of Mount Prinzera are affected by water chemistry, composition or heterogeneity of in-springs habitat, hydroperiod and discharge fluctuations. The permanent springs show seasonal changes in the concentration of main ions, whereas conductivity, pH and water temperature are more stable. The thermal stability probably determines the lack of seasonal changes in structure of macroinvertebrate communities of permanent springs. The niche filtering of springs on ostracod assemblages were studied by the analysis of functional diversity of communities from Emilia Romagna. Springs result to sustain almost the 50% of the regional

species pool, and several species are found exclusively in crenic habitats. This is the first study addressing the topic of functional diversity in ostracods and so a list of functional traits was compiled. Analysing the regional species pool, functional diversity in springs does not result significantly different than that expected by chance. Springs do not to limit functional similarity between co-existing ostracod species. These findings lead to conclude that, given the satisfaction of niche requirements of ostracod species, assembly processes in springs are probably affected by stochastic drivers as dispersal, speciation and local extinctions.

At the same time, spatial patterns are detected in all the studied communities, revealing a limitation to dispersal among springs for some taxa. The characteristic isolation of springs limits dispersal of taxa in the sites of the studied area, influencing more passive than active dispersers. Anyway, in Emilia Romagna communities, spatial factors explain only a low percentage of the total variation, and all invertebrate communities result mainly affected by environmental variables. Therefore, environmental control is prevalent if compared to spatial factors. These results show that, although stochastic dynamics are important in structuring all the studied communities, deterministic processes are prevalent at this spatial scale.

At continental scale, stochastic processes become more influential in arid climates. Indeed, the amount of variation explained by environmental factors decreases with the increasing of climate aridity. Frequent disturbances may provoke local extinctions followed by colonisations from nearby sites, leading to a source-sink dynamics. Early recolonisations after disturbance produce random assemblages, reducing the match between organisms and their environmental requirements, and therefore a low amount of variation is explained by environmental variables. In conclusion, stochastic and deterministic processes are not mutually exclusive, but they contribute together to shape invertebrate communities in springs.

Finally, at continental scale, spring ostracod communities show clear biogeographic patterns and they are organised along environmental gradients. Species composition varies along a geographic gradient, and the observed variation is mainly explained by altitude, latitude, water temperature, and conductivity. Also spring typologies (helocrene, rheocrene, and limnocrene) are influential factors in ostracod assemblage composition. The presence of endemic and rare species characterises specific regions.

Riassunto

Il presente lavoro ha lo scopo di comprendere i processi sottesi ai pattern di coesistenza tra le specie di invertebrati sorgentizi, distinguendo tra dinamiche stocastiche e deterministiche. Le sorgenti sono ecosistemi complessi e alcune loro caratteristiche (ad esempio l'insularità, la stabilità termica, la struttura ecotonale "a mosaico", la frequente presenza di specie rare ed endemiche, o l'elevata diversità in taxa) le rendono laboratori naturali utili allo studio dei processi ecologici, tra cui i processi di assembly.

Al fine di studiare queste dinamiche è necessario un approccio multi-scala, per questo motivi sono state prese in considerazione tre scale spaziali. A scala locale è stato compiuto un campionamento stagionale su sette sorgenti (quattro temporanee e tre permanenti) del Monte Prinzera, un affioramento ofiolitico vicino alla città di Parma. In questa area sono stati valutati l'efficacia e l'impatto ambientale di diversi metodi di campionamento e sono stati analizzati i drivers ecologici che influenzano le comunità. A scala più ampia sono state campionate per due volte 15 sorgenti della regione Emilia Romagna, al fine di identificare il ruolo della dispersione e la possibile presenza di un effetto di niche-filtering. A scala continentale sono state raccolte informazioni di letteratura riguardanti sorgenti dell'area Paleartica occidentale, e sono stati studiati i pattern biogeografici e l'influenza dei fattori climatici sulle comunità. Sono stati presi in considerazione differenti taxa di invertebrati (macroinvertebrati, ostracodi, acari acquatici e copepodi), scegliendo tra quelli che si prestavano meglio allo studio dei diversi processi in base alle loro caratteristiche biologiche e all'approfondimento tassonomico raggiungibile.

I campionamenti biologici in sorgente sono caratterizzati da diversi problemi metodologici e possono causare impatti sugli ambienti. In questo lavoro sono stati paragonati due diversi metodi: l'utilizzo del retino con un approccio multi-habitat proporzionale e l'uso combinato di trappole e lavaggio di campioni di vegetazione. Il retino fornisce dati più accurati e completi, ma anche significativi disturbi sulle componenti biotiche e abiotiche delle sorgenti. Questo metodo è quindi raccomandato solo se il campionamento ha come scopo un'approfondita analisi della biodiversità. D'altra parte l'uso delle trappole e il lavaggio della vegetazione sono metodi affidabili che presentano minori impatti sull'ecosistema, quindi sono adatti a studi ecologici finalizzati all'analisi della struttura delle comunità.

Questo lavoro ha confermato che i processi niche-based sono determinanti nello strutturare le comunità di ambienti sorgentizi, e che i driver ambientali spiegano una rilevante percentuale della variabilità delle comunità. Infatti le comunità di invertebrati del Monte Prinzera sono influenzate da fattori legati al chimismo delle acque, alla composizione e eterogeneità dell'habitat, all'idroperiodo e alle fluttuazioni della portata. Le sorgenti permanenti mostrano variazioni stagionali per quanto riguarda le concentrazioni dei principali ioni, mentre la conduttività, il pH e la temperatura dell'acqua sono più stabili. È probabile che sia la stabilità

termica di questi ambienti a spiegare l'assenza di variazioni stagionali nella struttura delle comunità di macroinvertebrati.

L'azione di niche-filtering delle sorgenti è stata analizzata tramite lo studio della diversità funzionale delle comunità di ostracodi dell'Emilia-Romagna. Le sorgenti ospitano più del 50% del pool di specie regionale, e numerose specie sono state rinvenute esclusivamente in questi habitat. Questo è il primo studio che analizza la diversità funzionale degli ostracodi, è stato quindi necessario stilare una lista di tratti funzionali.
Analizzando il pool di specie regionale, la diversità funzionale nelle sorgenti non è significativamente diversa da quella misurata in comunità assemblate in maniera casuale. Le sorgenti non limitano quindi la diversità funzionale tra specie coesistenti, ma si può concludere che, data la soddisfazione delle esigenze ecologiche delle diverse specie, i processi di assembly in sorgente potrebbero essere influenzati da fattori stocastici come la dispersione, la speciazione e le estinzioni locali. In aggiunta, tutte le comunità studiate presentano pattern spaziali riconoscibili, rivelando una limitazione della dispersione tra le sorgenti, almeno per alcuni taxa. Il caratteristico isolamento delle sorgenti potrebbe essere la causa di questa limitazione, influenzando maggiormente i taxa a dispersione passiva rispetto a quelli a dispersione attiva. In ogni caso nelle comunità emiliano-romagnole i fattori spaziali spiegano solo una ridotta percentuale della variabilità biologica totale, mentre tutte le comunità risultano influenzate maggiormente dalle variabili ambientali. Il controllo ambientale è quindi prevalente rispetto a quello attuato dai fattori spaziali. Questo risultato dimostra che, nonostante le dinamiche stocastiche siano importanti in tutte le comunità studiate, a questa scala spaziale i fattori deterministici ricoprono un ruolo prevalente.

I processi stocastici diventano più influenti invece nei climi aridi, dove il disturbo collegato ai frequenti eventi di disseccamento delle sorgenti provoca una dinamica source-sink tra le diverse comunità. Si è infatti notato che la variabilità spiegata dai fattori ambientali diminuisce all'aumentare dell'aridità del clima. Disturbi frequenti potrebbero provocare estinzioni locali seguite da ricolonizzazioni di specie provenienti dai siti vicini, riducendo la corrispondenza tra gli organismi e le loro richieste ambientali e quindi diminuendo la quantità di variabilità spiegata dai fattori ambientali.

Si può quindi concludere che processi deterministici e stocastici non si escludono mutualmente ma contribuiscono contemporaneamente a strutturare le comunità di invertebrati sorgentizi.

Infine, a scala continentale, le comunità di ostracodi sorgentizi mostrano chiari pattern biogeografici e sono organizzate lungo gradienti ambientali principalmente collegati altitudine, latitudine, temperatura dell'acqua e conducibilità. Anche la tipologia di sorgente (elocrena, reocrena o limnocrena) è influente sulla composizione delle comunità. La presenza di specie rare ed endemiche inoltre caratterizza specifiche regioni geografiche.

Chapter 1

General introduction

1.1 Conceptual framework of the thesis

My work is aimed at understanding the processes underlying the species coexistence patterns of aquatic invertebrate in springs. Identifying the processes causing the patterns of species coexistence is one of the main aims of community ecology. These processes are usually divided into three main groups:

1. Deterministic processes, which are niche based-mechanisms such as environmental filtering and interspecific interactions (Poff 1997).
2. Stochastic processes, which are related to ecological drift, dispersal limitation and colonization/extinction dynamics (Hubbel 2001).
3. Historical processes, which depend on geological or evolutionary events that could be both stochastic and deterministic (Ricklefs 1987).

Ecological determinism and stochasticity imply different assembly rules. Through deterministic processes, the physical habitat selects species by their ecological niches (Poff 1997). In this view, communities are rather predictable because similar habitats host similar communities. On the other hand, stochastic processes assemble unpredictable communities, not linked to species ecological requirements but assembled by random dynamics such as for example dispersal limitation, local extinctions, and ecological drift (Hubbel 2001). A wide debate arose about the relative importance of deterministic or stochastic processes in structuring biological communities (e.g. Chase 2007; Lepori & Malmqvist 2009).

Metacommunity ecology helps to identify the main processes acting in community assembly. It describes community structure considering local and spatial processes (Leibold et al. 2004). A metacommunity is defined as a set of local communities that are linked by dispersal of multiple potentially interacting species (Wilson 1992). Four metacommunity paradigms were proposed: patch dynamic, species sorting, mass effects, and neutral (Leibold et al. 2004). Anyway, according to Winegardner and colleagues (2012) the focus of metacommunity studies should be on the relative role of spatial and environmental control, in other words on deterministic and stochastic processes, rather than on the discrimination among the four paradigms.

Springs are complex ecosystems and several characteristics make them natural laboratories for the study of ecological processes, among them assembly dynamics. They are multiple ecotones that link aquatic (ground, surface, and interstitial waters) and terrestrial ecosystems (Scarsbrook et al. 2007; Barquín & Scarsbrook 2008). Despite their small size, they often show a "mosaic" structure characterised by various microhabitats at a small spatial scale that may sustain high taxa richness for invertebrates, plants, algae, and bacteria (Cantonati et al. 2012a). Indeed springs are described as sites with high occurrence of rare and endemic species and high taxa diversity, and some authors defined them as hotspots of biodiversity (Cantonati et al. 2012a; Ilmonen et al. 2012). Furthermore, some species are found to have in springs their exclusive (crenobionts) or favourite (crenophiles) habitat (Cantonati et al. 2012a). Spring biotopes are also characterised by insularity (Cantonati et al. 2012a), being small aquatic isolated habitats surrounded by terrestrial environments, which are unsuitable for crenic organisms. Furthermore springs usually occur in clusters at the intersection between the aquifer and the ground, creating characteristic patterns of suitable habitats for crenophiles and crenobionts. Compared to other aquatic ecosystems, most permanent freshwater springs, and associated aquifers, have a greater thermal stability (Glazier 1991; Williams 1991), albeit these features vary among spring types (Scarsbrook et al. 2007). Furthermore, their aquifer may persist over geological time (Glazier et al. 1991; van der Kamp 1995). This stability makes springs suitable refugia and accounts for the presence of glacial relicts (i.e. organisms more widespread during cold periods) and Tertiary relicts (i.e. organisms which colonised parts of central Europe in warmer periods) in springs (Nielsen 1950; Fischer 1998).

Although this ecological relevance, springs are still habitats neglected by research and conservation efforts. Springs are increasingly threatened by several impacts (Barquín & Scarsbrook 2008) and the most influental are related to reduction of their water permanence (Barquín & Scarsbrook 2008; Cantonati et al. 2009).

1.2 The state of the art of springs studies

Investigations on springs have increased in the last years, also becoming subject of special issues on international journals (Cantonati et al. 2011; 2012b). Studies focus on faunistic and botanic fields, and encompass different topics, e.g. conservation (Barquín & Scarsbrook 2008; Cantonati et al. 2009; Ilmonen et al. 2013), inventories and drivers of biodiversity or community analysis (Cantonati et al. 2012c; Ilmonen et al. 2012; Rader et al. 2012), and population genetics (Sei et al. 2009). The study of local factor affecting macroinvertebrates represents a large fraction of published papers on springs (e.g. Hoffsten & Malmqvist 2000; Smith et al. 2003; Ilmonen and Paasivirita 2005; Gathmann & Williams 2006; Staudacher & Füreder 2007; Maiolini et al. 2011; Kubíková et al. 2012; Martin & Brunke 2012; von Fumetti & Nagel 2012; Horsák et al. 2015). Spring meiofauna is less investigated (Bottazzi et al. 2011; Stoch et al. 2011; Spitale et al. 2012) and many studies concern a single taxon or few taxa (e.g. Di Sabatino et al. 2003; Bottazzi et al. 2008; Zullini et al. 2011). Few investigations concern analysis of spatial patterns of communities (Rádková et al. 2014; Escrivá et al. 2015; Zhai et al. 2015) In Italy research has been carried out mainly on the Alps (Crema et al. 1996; Sambugar & Ruffo 1997; 1998; Gerecke et al. 1998; Cantonati 1998; Stoch 2003; Mezzanotte & Sambugar 2004; Sambugar et al. 2006; CRENODAT project - Spitale et al 2007; Stoch et al. 2008), but also in Northern and Central Apennines and in Southern Italy (Cianficconi et al. 1998; D'Ambrosio et al. 2003; Di Lorenzo et al. 2003; Rossetti et al. 2005; Di Sabatino et al. 2003; Bottazzi et al. 2011).

1.3 Hypotheses and thesis outline

Given the peculiarity of springs and the ecological characteristics of considered taxa, I formulated the following hypotheses:

1. In crenic communities the main environmental drivers may differ from those acting in other aquatic habitats.
2. Some characteristics of springs, such as low concentrations of nutrients or oxygen (van der Kamp 1995) may act as niche filter on the communities.
3. Given the isolation of springs, dispersal may be limited for some invertebrate taxa, leading to the presence of spatial patterns in communities.
4. Stochastic processes, such as local extinction and re-colonization dynamics, are supposed to be more relevant in arid climates, where the springs could be subjected to frequent desiccations.

5. The limitation to dispersal and the probable role of springs as refugia or speciation sites (Cantonati et al. 2012a), could cause the presence of local endemisms and of biogeographic gradients in springs community at continental scale.

Since a multi-scale approach is necessary to study these dynamics, this work took into account three spatial scales. At local scale, one-year seasonal sampling was carried out on Mount Prinzera, an ophiolitic outcrop near Parma. In this area the effectiveness and the environmental impact of different sampling methods were evaluated (chapter 2), and the ecological drivers for communities were analysed (chapter 3). At larger scale, 15 springs in the Emilia Romagna region were sampled twice in order to identify the role of dispersal (chapter 4) and the presence of a niche-filtering dynamic (chapter 5). At continental scale, information on springs of the Western Palearctic area were collected from literature, and I investigated the presence of biogeographical patterns (chapter 6) and the influence of climatic factors (chapter 7). Different invertebrate taxa (macroinvertebrates, ostracods, water mites and copepods) were investigated, choosing the most suitable for explaining each process for their ecological characteristics and for the taxonomic resolution available.

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Chapter 2

Is there an ideal protocol for sampling macroinvertebrates in springs?

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2.1 Introduction

In spite of the their small size, the spring "mosaic" ecotonal structure results in a number of microhabitats that sustain high species richness (Cantonati et al. 2012). Several studies have highlighted the high biodiversity of macroinvertebrates in springs and the presence of rare and endemic species (e.g. Takhteev et al. 2010; Maiolini et al. 2011; Kubíková et al. 2012; Martin & Brunke 2012; Spitale 2012; Spitale et al. 2012). The uses and limits of various standard methods for sampling aquatic benthic macroinvertebrates have been extensively discussed (e.g. Davies 2001). On the contrary, despite great interest in spring biodiversity, a standardised quantitative method for sampling crenic macroinvertebrate taxa has never been developed. Cantonati and colleagues (2007) suggested effective methods for collecting spring invertebrates, but a variety of methods have been adopted in crenic investigations.

The technical difficulties of sampling in springs were well summarised by Gerecke and co-authors (2007): "The main dilemma of limnological studies in springs probably derives from the generally reduced dimensions and extreme heterogeneity of the habitat". Furthermore, many authors (Gerecke et al. 1998; Zollhöfer 1999; Myers & Resh 2002; Staudacher & Füreder 2007; Tichá et al. 2012) noted that some survey methods, which implied samplings in all microhabitats, could be destructive for the environment and the biota of these fragile ecosystems. Previous studies on macroinvertebrates in different aquatic ecosystem have shown that sampling methods affect the data precision, and the selection of sampling technique is among the most important decisions for freshwater studies (Carter & Resh 1993). Standardising the sampling procedure is thus necessary in order to obtain precise and comparable biological data for spring surveys and assessment.

The aim of this study was to summarize sampling methods in springs and to compare the effectiveness of some semi-quantitative sampling methods, taking into account their potential impacts on spring habitat and biota.

2.2 Methods

Three rheocrenic, permanent springs located between 474 and 589 m a.s.l. in the Mount Prinzera protected area (lat.: 44°37'N; long. 10°03'E), an ophiolitic outcrop in northern Italy, near Parma, were selected for the study. Samplings were carried out in two seasons (May-June and August-September 2014).

Macroinvertebrates were collected using three methods:

1. Multi-habitat proportional net: a hand net (frame dimensions: 10x10 cm; mesh size: 255 µm) was used for 10 replicas in each site. For each replica, a substrate area equivalent to the net frame was sampled for 15 seconds. The number of replicas for each microhabitat was proportional to its percentage cover in the spring, up to a total number of 10 replicas. For example, given a substrate composition of 50% of gravel, 30% of mosses and 20% of silt, 5 replicas were done for gravel, 3 for mosses and 2 for silt. All the 10 replicas were composited into a single sample.
2. Vegetation washing: about 250 ml volume of submerged vegetation was collected and washed in laboratory through a 255 µm sieve.
3. Traps: were built following Bottazzi et al. (2011). They were obtained from PVC centrifuge tubes (length 100 mm; diameter 28 mm), by cutting the conical end, drilling an opening (0.5 cm of diameter) in its apex, and inserting it, inverted, into one end of the tube. The other end of the tube was closed with a 50 µm net. These traps were filled with washed and sieved gravel (0.3-1.0 cm). Traps were baited with corned meat, placed at the sediment-water interface, and covered with stones to keep them in place for 7-8 days. Two pairs of traps were deployed in each spring: one pair at the source, and the other 2 m downstream. For each pair, one trap was placed with the opening in the flow direction and the other in the opposite direction.

Vegetation washing and macroinvertebrate trapping were performed two weeks after the sampling. In the laboratory, collected material were washed through a 255 µm sieve and fixed with 90% ethanol. Macroinvertebrates were identified with Plecoptera, Trichoptera, Ephemeroptera, Coleoptera, Diptera, Crustacea to the family, and Hirudinea, Gastropoda, Collembola, Hydrachnidiae, Odonata, Oligochaeta to coarser taxonomic level.

Differences in organism abundance between the three methods were tested with an Analysis of the Variance (ANOVA). Logarithmic transformation was used to obtain normal distribution and homogeneity of data, as determined by Shapiro and Bartlett tests (Legendre & Legendre 2012). Non-metric multidimensional scaling (NMDS, Legendre & Legendre 2012) was performed to evaluate possible differences in community structures determined by different methods. Centroids of methods were fitted on NMDS plots in order to identify these differences, then tested with PERMANOVA (Anderson & Walsh 2013). Differences between methods were assessed by considering both the three different methods (net, vegetation washing, and traps) and combining data from vegetation washing and traps. Differences were also tested for each of the most diverse insect orders (Trichoptera, Coleoptera, and Diptera).

Statistical analyses were performed using the R software, version 3.0.0 (R Development Core Team 2013), and vegan package version 2.0-7 (Oksanen et al. 2013).

2.3 Results

Ten different methods have been found; the use of hand or kick net is by far the most used protocol (table 2.1).

Overall 32 taxa were collected in our survey (table 2.2). Insect orders with the highest number of families were Diptera (9), Trichoptera (7) and Coleoptera (6). Chironomidae was the most abundant taxon collected with the net (1029 specimens) and vegetation washing (60), whereas traps collected the highest number of Niphargidae (293). Lepidostomatidae, Chironomidae, Ceratopogonidae, Hirudinea, and Gastropoda were found in all samples collected by the net. The maximum number of taxa collected in one sampling session was eight using the net, and 11 combining traps and vegetation washing. Thirteen taxa were collected by all methods; net and the traps samplings shared seven taxa, whereas net and vegetation washing shared eight taxa. Finally, Hydropsychidae, Limnephilidae, and Hydrophilidae were only found in net samples, and Empididae were exclusively collected with traps (table 2.2).

Table 2.1: Spring sampling methods and relative references.

Methods	References
Surber sampler	Erman & Erman 1995; Erman 1998; Zollhöfer 1999; Smith et al. 2003; Von Fumetti et al. 2006; Barquín & Death 2008; Gerecke et al. 2011.
Hand or kick net	Williams 1991; Gerecke & Cantonati 1998; Hahn HJ. 2000; Myers & Resh 2002; Mezzanotte & Sambugar 2004; Mori & Brancelj 2006; Lencioni 2007; Staudacher & Füreder 2007; Ilmonen et al. 2012; Kubíková et al. 2012; Martin & Brunke 2012; Rader et al. 2012; Spitale 2012; Tichá et al. 2012.
Collection at sight	Williams 1991; Bonettini & Cantonati, 1998; Gerecke & Cantonati 1998; Myers & Resh 2002; Gerecke & Di Sabatino 2007.
Bou Rouch pump	Crema et al. 1996.
Sweep net	Crema et al. 1996; Sambugar et al. 2006.
Core sampler/ sediment sample	Gooch et al. 1991; Myers & Resh 2002; Dumnicka et al. 2007; Staudacher & Füreder L. 2007; Worthington Wilmer et al. 2008; Takhteev et al. 2010; Koperski et al. 2011; Spitale 2012.
Traps	Bottazzi et al. 2011.
Drift tube or net	Stoch et al. 2008; Bottazzi. 2010.
Squeezing mosses or washing vegetation	Bottazzi et al. 2011; Gerecke et al. 2011; Spitale 2012.
Emergence traps	Erman & Erman 1995; Erman 1998; Gathmann & Williams 2009.

Table 2.2: List of taxa and their abundances collected using different methods.

Taxa	Net	Traps	Vegetation washing
Leuctridae	6	2	1
Lepidostomatidae	29	4	36
Philopotamidae	10	4	4
Sericostomatidae	104	154	3
Chironomidae	1029	41	60
Stratiomyidae	18	1	3
Psychodidae	26	6	33
Limoniidae	6	10	1
Dixidae	22	14	12
Scirtidae	511	16	23
Hydraenidae	73	1	2
Niphargidae	172	293	2
Gastropoda	581	220	6
Collembola	19	3	3
Veliidae	1	2	
Ceratopogonidae	50	1	
Haliplidae	2	1	
Dytiscidae (adults and larva)	8	2	
Hirudinea	32	38	
Hydrachnidiae	2	1	
Nemouridae	9		8
Heptageniidae	4		1
Polycentropodidae	4		3
Beraeidae	7		1
Ptychopteridae	15		9
Simuliidae	1		1
Odonata	4		1
Hydropsychidae	2		
Limnephilidae	1		
Hydrophilidae	1		
Tipulidae	4		
Empididae		2	

Differences in taxa abundance between methods resulted significant, both considering the three distinct methods (fig. 2.1; F = 16.180; $p < 0.001$), and merging the traps and the vegetation washing (fig. 2.2; F = 9.464; p = 0.012).

Sampled communities formed three distinct groups near their centroids in the NMDS plot (stress = 0.15 - fig. 2.3). This indicates differences in macroinvertebrate assemblages according to the methods, as confirmed by the PERMANOVA test (R2 = 0.223; p = 0.010). Stress was 0.13 in the plot of NMDS ordination obtained merging data collected with traps and vegetation washing (fig. 2.4). The groups of the two different methods (net and traps plus washing vegetation) were less detectable. PERMANOVA test (R2 = 0.136; p = 0.134) indicated that there was not a significant difference between communities sampled with these two methods. Net sampling and combined traps and vegetation samples showed differences for Coleoptera (R2 = 0.219; p = 0.030) and Diptera (R2 = 0.250; p = 0.005), but not for Trichoptera (R2 = 0.056; p = 0.826) (fig. 2.5).

2.4 Discussion

The lack of a standardised sampling protocol for springs has led to the use of a wide variety of methodologies. Standard Surber samplers (sampling area: 0.09-0.1m2) have been rarely used (Smith et al 2003; Barquín & Death 2008). More frequently, smaller samplers were preferred (Erman & Erman 1995; Erman 1998; Zollhöfer 1999; von Fumetti et al. 2006; Gerecke et al. 2011). The mesh size of Surber, kick or hand nets varies from 100 µm to 1 mm. Although Gerecke et al. (2007) recommended sampling different microhabitats in springs, at their relative microhabitat proportion, few studies have used a proportional multi-habitat approach (Crema et al. 1996; Zollhöfer 1999; Martin & Brunke 2012) or sampled all available substrates (Bonettini & Cantonati 1998; Mezzanotte & Sambugar 2004; Ilmonen et al. 2012). In addition, combined methods have been frequently used in the same study (Williams 199; Erman & Erman 1995; Crema et al. 1996; Bonettini & Cantonati 1998; Erman 1998; Myers & Resh 2002; Sambugar et al. 2006; Staudacher & Füreder 2007; Bottazzi et al. 2011; Gerecke et al. 2011, Spitale 2012). Each method has advantages and disadvantages that may be dependent on the specific aims of the study. Methods such as the use of sweep nets or emergence traps sample only organisms with aerial imagoes, whereas drift tubes/nets underestimate taxa not exposed to drift for behavioural or niche characteristics. Also methods that require collection by sight could be biased against small, less-mobile and less-visible organisms. Surber net, Bou-Ruch pump, and core-sampler may allow the collection of quantitative data, but the Surber net is usually too large to be used in springs (see Gerecke et al. 2007), and the Bou-Rouch pump and the core sampler only collect sediment and interstitial samples.

Our results showed that macroinvertebrate community structure estimated by the combination of traps and washing vegetation can be considered comparable to those obtained with net. The four taxa exclusively collected by the net, Hydropsychidae, Limnephilidae, Tipulidae, and Hydrophilidae, have body sizes larger than the opening of the traps (Tachet et al. 2000). Furthermore Hydropsychidae, Limnephilidae, and Tipulidae rarely inhabit aquatic vegetation, and Hydrophilidae organisms are very mobile and could escape during vegetation collection (Tachet et al. 2000). Although similar communities were collected by both net sampling and combined vegetation washing and trap sampling, there were some differences. The two methods produced different results for Diptera and Coleoptera. This result was probably related to issues with single-habitat protocols. For example, traps and washing vegetation probably underestimated the presence of taxa not associated with vegetation or not attracted by meat. The abundances of organisms collected by traps and vegetation washing were significantly lower than those collected by net. Therefore the impact of these protocols on spring fauna would be expected to be lower, at least on some taxa. In addition, net sampling requires brushing, scraping, digging, and squeezing of different microhabitats and substrata, which cause disturbance of springs habitats and unknown recovering times. The use of traps is more time-consuming than other meth-

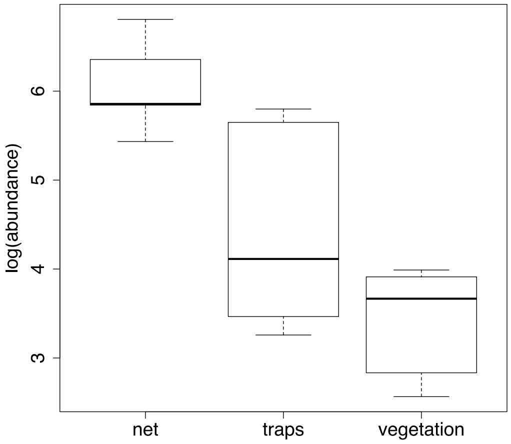

Figure 2.1: Logarithm of taxa abundances for the three distinct methods.

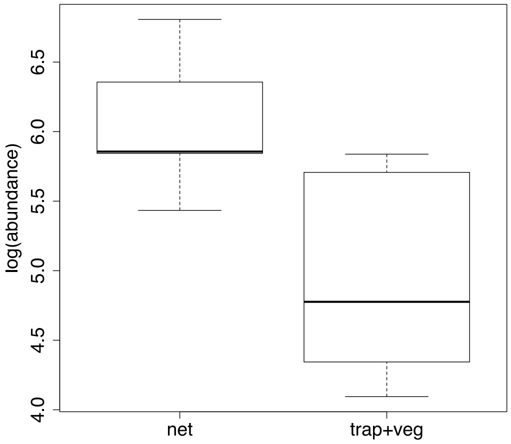

Figure 2.2: Logarithm of taxa abundances using net sampling and combing traps and vegetation washing.

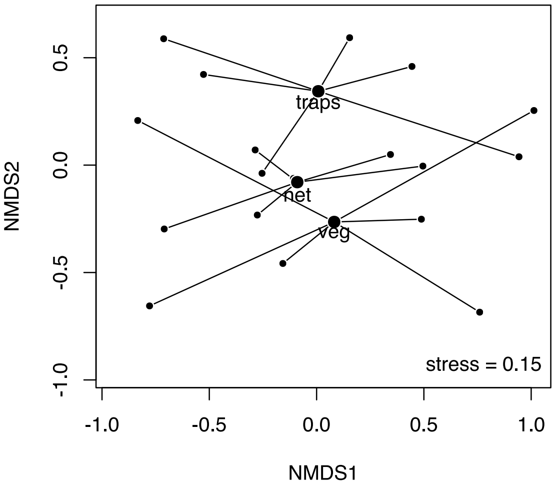

Figure 2.3: NMDS ordination of the three distinct methods (stress = 0.15). Black points are the centroids of methods (veg = vegetation washing). Grey points are sampled communities

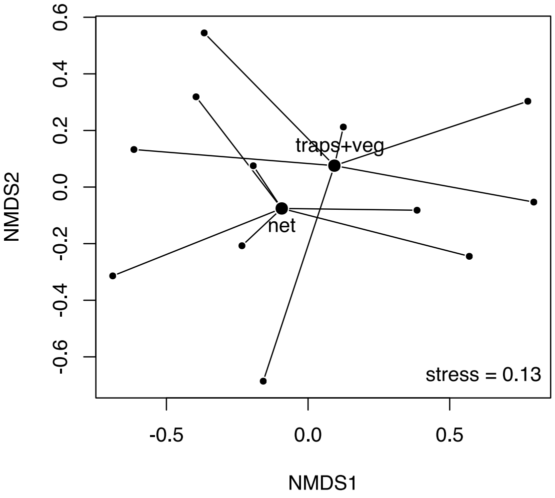

Figure 2.4: NMDS ordination of the net and the traps plus vegetation washing (stress = 0.13). Black points are the centroids of methods (traps+veg = traps plus vegetation washing).

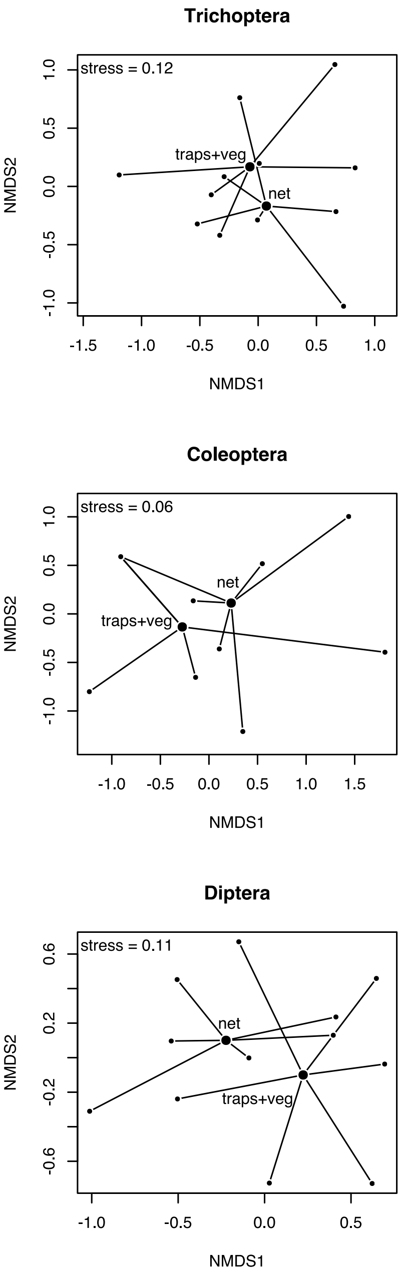

Figure 2.5: NMDS ordination of the net and the traps+vegetation washing for Trichoptera, Coleoptera, and Diptera. Black points are the centroids of methods (traps+veg = traps plus vegetation washing). Grey points are sampled communities.

ods, because they require an additional visit to the springs to be removed. Finally, some sampling methods cannot be suitable in peculiar habitat morphologies: for example, some springs lack any kind of vegetation, and traps cannot be placed in hygropetric springs, where the sediment layer is too thin, or in helocrene springs, that often are too deep.

Since spring fauna shows an evident habitat-preferences (von Fumetti et al. 2006), single microhabitat protocols should be used only to survey specific target taxa or habitats. As a consequence, a multi-habitat methodology allows a better estimation of the overall biodiversity. In order to obtain more comparable results, Gerecke et al. (2007) recommended sampling available habitats, using proportional sampling time for each substratum and including transitional zones among different substrata since they may host specialised taxa. In addition, the multi-habitat proportional sampling is considered by the Water Framework Directive (Directive 2000/60/EC) as the best approach for assessing macroinvertebrate diversity.

Proposed methods could be improved in order to be more effective. Traps with different opening dimensions may allow to collect the whole range of organism body sizes; the volume (or replicas) of vegetation-washing samples can be increased whenever possible.

In conclusion, the net and the vegetation washing with traps show different features and effectiveness, even though both protocols give very similar qualitative results. Indeed, the use of the net, with a multi-habitat proportional approach, provides more accurate and complete information, but also significant impacts on the biotic and abiotic components of springs. For these reasons, this method is only recommended for biodiversity inventories. On the other hand, traps and vegetation washing are still reliable methods with less negative effects on springs ecosystems, thus they are more suitable for ecological studies focused on the analysis of the community structure.

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Chapter 3

Local drivers of invertebrate communities in springs: the case of Mount Prinzera

3.1 Introduction

Investigations on springs have increased in the last years, also becoming subject of special issues on international journals (Cantonati et al. 2011; 2012a). Studies focus on faunistic and botanic fields, and encompass different topics, e.g. conservation, inventories and drivers of biodiversity, community analysis, population genetics. In this context the study of local factor affecting macroinvertebrates represents a large fraction of published papers on springs (e.g. Hoffsten & Malmqvist 2000; Smith et al. 2003; Ilmonen and Paasivirita 2005; Gathmann & Williams 2006; Staudacher & Füreder 2007; Maiolini et al. 2011; Kubíková et al. 2012; Martin & Brunke 2012; von Fumetti & Nagel 2012; Horsák et al. 2015). Spring meiofauna is less investigated (Bottazzi et al. 2011; Stoch et al. 2011; Spitale et al. 2012) and many studies concern a single taxon or few taxa (e.g. Di Sabatino et al. 2003; Bottazzi et al. 2008; Zullini et al. 2011).

In Italy research has been carried out mainly on the Alps (Crema et al. 1996; Sambugar & Ruffo 1997; 1998; Gerecke et al. 1998; Cantonati 1998; Stoch 2003; Mezzanotte & Sambugar 2004; Sambugar et al. 2006; CRENODAT project - Spitale et al 2007; Stoch et al. 2008), but also in Northern and Central Apennines and in Southern Italy (Cianficconi et al. 1998; D'Ambrosio et al. 2003; Di Lorenzo et al. 2003; Rossetti et al. 2005; Di Sabatino et al. 2003; Bottazzi et al. 2011). The study of environmental filtering and other niche-based processes contributes to the understanding of the patterns of taxa composition and distribution. Analysis of local drivers for biological assemblages is the starting point for research at regional and biogeographical scale, and leads to improve knowledge on the ecosystem processes. Consequently, the awareness of specific ecological requirements of communities allows efficiency conservation actions. Springs are important component of freshwater biodiversity (Cantonati et al. 2012b; Ilmonen et al. 2012), but conservation efforts for these environments are still not sufficient (Barquín & Scarsbrook 2008). The study of local factors that affect assemblages is pivotal to improve management guidelines for these vulnerable habitats.

The focus of the present study was identifying chemical, physical, seasonal, and habitat features affecting the composition and structure of invertebrate communities and their biodiversity in the Mount Prinzera. The study area of this work had several strengths. A small and homogeneous area was optimal to provide information on habitat requirements without the interferences of regional gradients and different lithology. The chosen area was protected and the near-pristine conditions of sites guaranteed reference results for further management indications. Mount Prinzera was deeply investigated from a hydrogeological point of view (Chelli et al. 2015; Segadelli 2014), thus geomorphology features and aquifer dynamics were well known.

3.2 Methods

Seven rheocrenic springs located between 474 and 618 m a.s.l. in the "Mount Prinzera" protected area (lat: 44°37'N; long. 10°03'E), an ophiolitic outcrop in Northern Italy, near Parma, were selected for the study. Three springs were permanent (P02, P03, P07), four temporary (P00, P06, P08, P11). Sampling was carried out seasonally for one year (2014).

Invertebrates were collected with a hand net (frame dimensions: 10x10 cm; mesh size: 50µm). Ten replicas were collected for each site. Substrate was sampled for an area equal to the net frame for 15 seconds for each replica. A multi-habitat proportional approach was used, every microhabitat being sampled for a number of replicas proportional to its percentage cover in the spring. Following the approach of Bottazzi (2010), only submerged microhabitats were taken into account (clay, silt, and sand; gravel; pebbles; boulders; rock; bryophytes; algae; other macrophytes; leaves; wood). In the laboratory, meiofauna and macroinvertebrates were separated through two sieves with different mesh size (50µm and 255µm, respectively). Meiofauna was analysed only in four springs (P06, P07, P08, P11) characterised by different hydroperiod and micro-habitat composition. Specimens were sorted under a stereomicroscope and preserved in 90% ethanol. Macroinvertebrates were identified as follows: to family level for Plecoptera, Trichoptera, Ephemeroptera, Coleoptera, Diptera, Odonata, and Gasteropoda; to order or coarser taxonomic level for Hirudinea, Collembola, and Oligocheta. Copepods and water mites were sent to experts for species identification, respectively to Maria Cristina Bruno (Edmund Mach Foundation, Italy) and Antonio Di Sabatino (University of L'Aquila, Italy). Water mites were considered in the meiofaunal fraction, even though their dimensions could be larger than 1 mm in some species.

The following environmental variables were measured in the field: water temperature, conductivity, pH (with Eutech Instrument PC 300 multi-parameter probe), number and percentage composition of microhabitats in the spring, altitude, and geographical coordinates (with Garmin GPS 12; datum: WGS84). Concentrations of NO_3^-, NO_2^-, NH_4^+, and SRP (Soluble Reactive Phosphorus) were measured in laboratory (APHA 2005). Concentrations of Ca^{2+}, Mg^{2+}, Na^+, K^+, Cl^-, SO_4^{2-}, HCO_3^- were determined by an external laboratory (Consorzio per lo sviluppo industriale della valle del Biferno, Italy) using Italian standard methods (APAT et al. 2003).

Data were analysed as follows:

1. The main patterns of spring chemical compositions were analysed by Principal Component Analysis (PCA; Legendre & Legendre 2012) of standardised ion concentrations.

2. Meiofauna and macroinvertebrate data were analysed separately. For both groups, taxa richness (number of taxa in every spring or sampling) and taxa occurrence (number of sites where each taxon was found) were calculated.

3. PCAs were performed also on biological data to compare sites according to the community structures.

4. Redundancy Analysis (RDA; Legendre & Legendre 2012) related community data to environmental variables. The overall significance of the model was assessed with 999-permutation test. Forward selection by permutation test evaluated the significance of the contribution of each explanatory variable to the RDA model (999 permutations, 0.05% significance level). In macroinvertebrate analysis SRP were prior log-transformed, because outliers were detected with graphic techniques. Concentrations of anions and cations (expressed as mEq/L) were summed and used instead of single ions in the analysis of copepod communities to reduce the number of variables. HCO_3^- and Cl^- concentrations were eliminated in the macroinvertebrate analysis because resulted correlated ($|r| > 0.8$).

5. PCA of macroinvertebrate data of the three permanent springs was performed to study seasonal changes in communities. In order to reveal differences in communities of permanent and temporary sites, the same analysis was carried out on macroinvertebrate data of the winter and spring samplings, when all the springs were active. These differences were shown by fitting centroids of seasons and permanence on PCA plot, and tested by Permutational Multivariate Analysis of Variance (PERMANOVA - Anderson & Walsh 2013).

6. Multivariate Analysis of Variance (MANOVA; Legendre & Legendre 2012) tested the seasonal differences in ion concentrations and the other physical-chemical variables (water temperature, conductivity, and pH) of the permanent springs.

7. I calculated the abundances of stigoxene and not-stigoxene (stigophile+stygobiont) copepod species. A Poisson General Additive Model (Poisson GAM with cubic regression splins - Zuur et al. 2009) was used to detect the relationship between these two abundances and the environmental variables (temperature, conductivity, pH, anion and cation concentrations). Since overdispersion was detected, the standard errors were corrected using a quasi-GLM model where the variance is given by the dispersion parameter multiplied for the mean ("quasipoisson" option of gam function in mgcv package of R).

8. A binomial Generalised Linear Model (binomial GLM) was built to establish which variables affect the presence of ostracods in springs. The model took into account environmental variables of springs inhabited by ostracods in the same region (see chapter 4) and Mount Prinzera variables. Variance inflation factors (VIF) were used to assess which explanatory variables were collinear. Variables with VIF >5 were eliminated before the analysis.

Biological data were Hellinger-transformed prior to PCAs and RDAs, as suggested by Legendre and Gallagher (2001). All the analyses were performed with R software (R Core Team 2013), using the packages vegan (Oksanen et al. 2013), packfor (Dray et al. 2011), and mgcv (Wood 2006)

3.3 Results

The complete list of physical and chemical values is shown in Appendix 3.1. NO_2^- and NH_4^+ concentrations were below the analytic thresholds for the majority of the samples, so they were removed from further analysis. Table 3.1 describes the arithmetic mean of percentage microhabitat coverage of each spring. Fig. 3.1 shows the biplot for the PCA of chemical variables. The first two axes explained the 72.2% of the total variation. All the samples from the permanent springs P02 and P03 were separated from the others and were characterised by high concentrations of Na^+, K^+ and Cl^-, and by low concentrations of NO_3^- and SRP. P06 and the winter sample from P11 were plotted at the opposite side, with high concentrations of NO_3^- and SRP. The permanent spring P07 was grouped with the temporary springs.

3.3.1 Macroinvertebrates

A total of 5476 organisms were collected, belonging to 36 taxa (see Appendix 3.2). The highest abundance was recorded in the summer samples from P03. The list of collected taxa is in Appendix 3.2. Some water mite specimens were found also in the macroinvertebrate fraction, but they were analysed with the meiofauna. Taxonomic richness varied from 2 to 20 in each sample and from four to 25 in each spring. The three permanent springs hosted the most diverse assemblages. Brachycentridae, Haliplidae, Limnebiidae, Helophoridae, Velidae, Lymnaeidae, and Oligocheta occurred in just one sample; whereas Simulidae and Cordulegasteridae were found only in one spring but on several occasions. Collembola and Chironomidae were collected in all the sites. Further information on taxa occurrence can be found in fig. 3.2.

Fig. 3.2 shows the PCA biplot of macroinvertebrate data. The first two axes accounted for 61.15% of the total variation. Samples collected in the different seasons from the same site were grouped in

Table 3.1: Means of percentage microhabitat compositions

	Clay, silt, sand	Gravel	Pebbles	Boulders	Rock	Bryophytes	Algae	Other macrophytes	Leaves	Wood (roots and branches)	Vegetal detritus (leaves+wood)
P11	0.0	50.0	17.0	7.0	0.0	0.0	0.0	0.0	16.0	10.0	26.0
P03	27.5	27.5	2.5	7.5	0.0	0.0	0.0	0.0	20 .0	15.0	35.0
P02	5.0	50.0	15.0	0.0	0.0	0.0	0.0	0.0	12.5	17.5	30.0
P07	20.0	30.0	5.0	0.0	0.0	2.5	0.0	42.5	0.0	0.0	0.0
P08	0.0	30.0	30.0	25.0	0.0	0.0	0.0	0.0	15.0	0.0	15.0
P06	7.5	5.0	0.0	0.0	57.5	0.0	22.5	2.5	5.0	0.0	5.0
P00	20.0	35.0	0.0	15.0	20.0	0.0	0.0	0.0	10.0	0.0	10.0

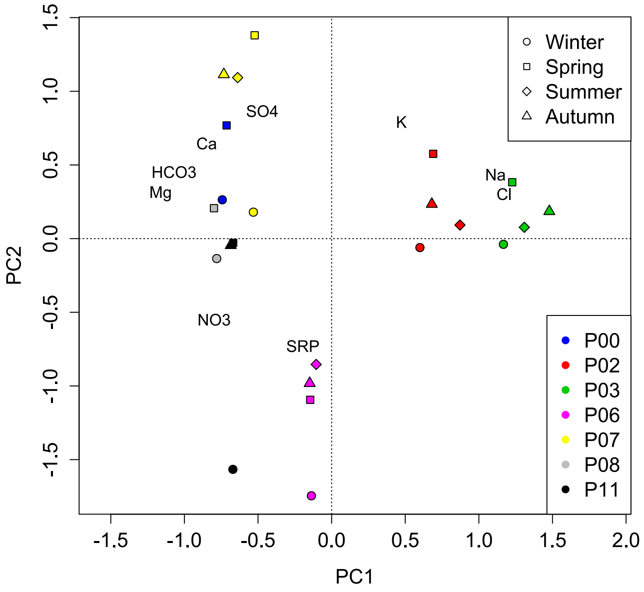

Figure 3.1: PCA of chemical variables.

clusters, with the exception of the winter sample from P08. The plot shows three different groups of springs. The first group is composed by P02 and P03. As in the PCA of chemical variables, these springs are separated from the others and dominated by Hydrobiidae, Scirtidae, and Chironimidae. P00, P06, P07, and P11 form the second group. The permanent spring (P07) is located in the upper part of the graph, near to P02 and P03, and it is characterised by the presence of Hydraenidae. Niphargidae were frequent in temporary springs P00, P06, and P11. P08 and the winter sample from P06 form the third group, separated from the other sites by the rare occurrence of Nemouridae.

The RDA of macroinvertebrate and environmental data was significant (p=0.005) and the explanatory variables accounted for 65.5% of the variation of biological data. The following variables resulted significant after forward selection: Na^+ (p=0.0001), NO_3^- (p=0.0097), Ca^{2+} (p=0.0073), SO_4^- (p=0.0002), pH (p=0.0006), altitude (p=0.0064), and conductivity (p=0.0120). RDA of the significant variables explained the 64.9% value. The RDA triplot with significant variables (fig. 3.3) shows the same three groups found in the previous PCA. The communities of P02 and P03 were affected by high concentration of Na^+ and values of pH; P00, P06, P07, and P11 by high conductivity and Ca^{2+}; P08 and the winter sample from P06 by SO_4^- and NO_3^-.

PERMANOVA of winter and springs samples revealed that permanent and temporary springs host different communities (p=0.005). In the PCA (fig. 3.4) permanent and temporary sites were grouped together near the season centroids, but P07 was located nearer temporary springs. Communities of permanent springs were characterised by the presence of Lepidostomatidae, Scirtidae, Chironomidae, and Hydrobiidae. Temporary sites were characterised by Nemouridae, Hydraenidae, Collembola, and Niphargida.

In the PCA biplot of the three permanent springs (fig. 3.5), the seasonal centroids were clustered together, and no seasonal differences in communities were found. PERMANOVA assessed no seasonal changes in communities (p=0.970). MANOVA detected seasonal differences in ion concentrations of permanent springs (p=0.020), but no significant differences in conductivity, pH and water temperature (p=0.417).

3.3.2 Meiofauna

Early instars of macroinvertebrates (namely Chironomidae, Ceratopogonidae, Psychodidae, Oligocheta and Collembola) were found in the meiofaunal fraction, for a total of 228 organisms. In addition, I found 8766 meiofaunal organisms belonging to Nematoda, Copepoda, Tardigrada, Hydracarina, and Ostracoda. Nematoda was the most abundant taxon (7527 organisms), followed by Copepoda (1133). Only larval stage of Ostracoda have been found, therefore species identification was impossible.

Only pH resulted significant in the binomial GLM of adult ostracod presence (p = 0.026). The model accounts for 17.6%. Water mites were found in 6 samples, whereas terrestrial mites (excluded by further analysis) in 8 samples. Hydracarina occurred in 4 springs (P02, P03, P06, and P07) with 4 taxa: *Atractides* sp., *Partnunia* sp., *Soldanellonyx monardi*, and *Sperchon thienemanni*. Species richness varied from 1 to 3. Further details of species occurrences are shown in Appendix 3.2.

Seven copepod species were found: *Bryocamptus tatrensis*, *Bryocamptus echinatus*, *Bryocamptus pygmaeues*, *Moraria varica*, *Elaphoidella pseudophreatica*, *Paracyclops imminutus*, *Eucyclops serratulus serratulus*, *Paracyclops imminutus*. Two organisms were identified at genus level (*Diacyclops* sp.). See Appendix 3.2 for species details. Species richness varied from one to five. The permanent spring P07 had the highest organisms abundance and species richness. Only *Bryocamptus pygmaeues* occurred in all the four springs, whereas *Bryocamptus echinatus*, *Diacyclops* sp., *Eucyclops serratulus serratulus*, and *Paracyclops imminutus* occurred only in one site. Five species were stygoxene, one stygophile, and two stygobiont. Species belonging to genera *Diacyclops* can be either stigoxene and stygophile, so *Diacyclops* sp was eliminated from this analysis. Stygophiles and stygobiontics (797 organisms) represented the 75.3% of the total copepod abundance, Stygoxenes (259) the

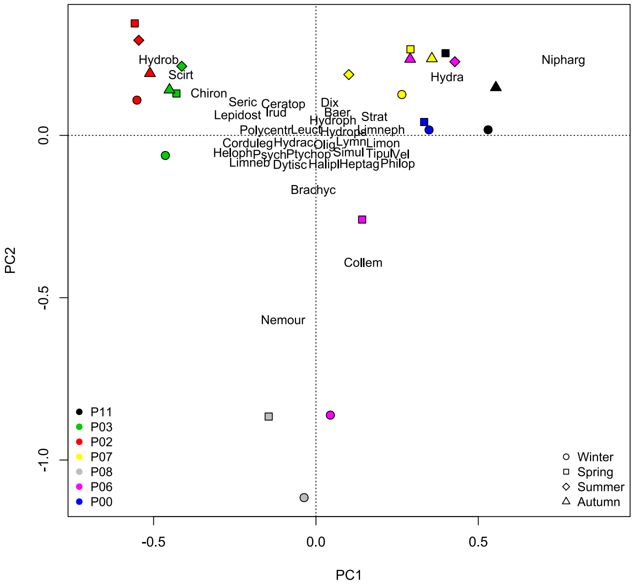

Figure 3.2: PCA of macroinvertebrate data. For taxa abbreviations see Appendix 3.2.

24.5%. As resulted from the Poisson GAM, abundance of stygoxene was related to pH ($p = 0.18$), cation concentration ($p = 0.019$), and water temperature ($p = 0.043$). The model explained the 82.5% of the deviance. In the GAM of stygobionts and stygophiles, none of the environmental variables were statistically significant.

Fig. 3.6 shows the PCA biplot of copepod data. The first two axes explained the 92.8% of total variation. The RDA model (fig. 3.7) accounted for 38.1% of the total variation. After forward selection the number of substrates ($p = 0.003$) and the anion concentrations ($p = 0.028$) resulted significant.

3.4 Discussion

3.4.1 Macroinvertebrates

In the present study Chironomidae was the most abundant taxon. The dominance of chironomids in springs was pointed out also by previous investigations (Takhteev et al. 2010; Horsák et al. 2015). This prevalence was probably due to their adaptation to low oxygen concentration (Tachet et al. 2000), as found in groundwater, and to the ability to utilise freshwater habitats prone to extreme hydrological variability (Smith et al. 2003; Fenoglio et al. 2007).

Macroinvertebrate community structures of Mount Prinzera springs were affected by the concentrations of Na^+, NO_3^-, Ca^{2+}, SO_4^-, pH, altitude, and conductivity. Chemical variables were indicated as important factors only in few studies (Virtanen et al. 2009; Koperski et al. 2011; Pokorny et al. 2012; Spitale et al. 2012). On the contrary, other studies were unable to detect relationship between chemical variables and community structures in springs (Smith et al. 2003; von Fumetti et al. 2006; Staudacher & Füreder 2007). Sometimes that relationship was excluded based on the analysis of only the main nutrients and not taking into account the concentration of more types ions (Martin & Brunke 2012). The variation in dissolved oxygen may also be an

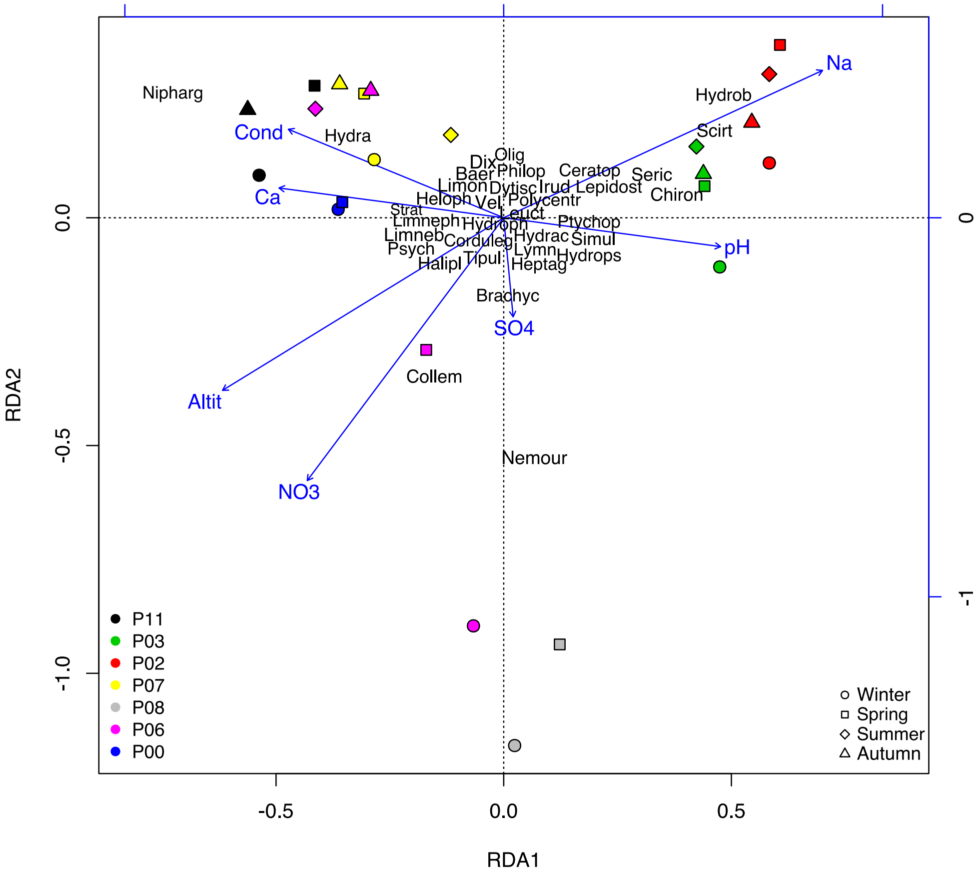

Figure 3.3: RDA of macroinvertebrate data and significant environmental variables. For taxa abbreviations see Appendix 3.2.

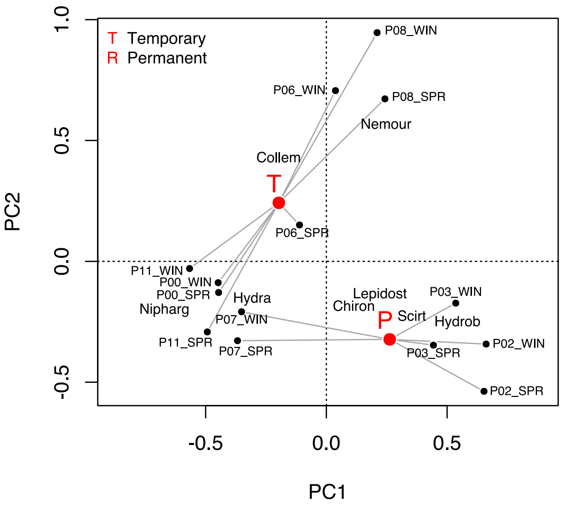

Figure 3.4: PCA of macroinvertebrate data of winter (WIN) and spring samplings (SPR). Black dots are samplings and red ones are centroids of temporary (T) and permanent (P) springs. Only selected taxa are shown. For taxa abbreviations see Appendix 3.2.

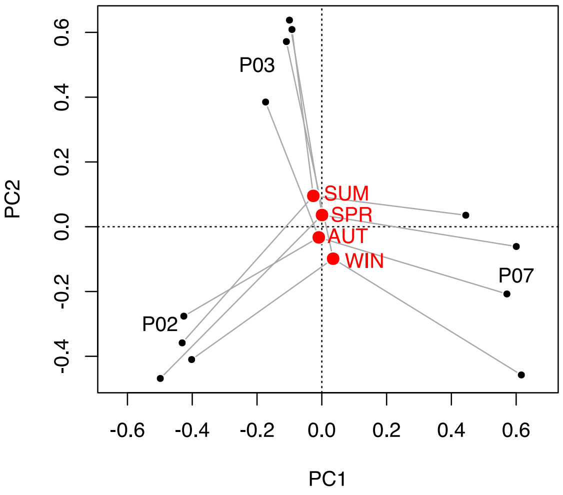

Figure 3.5: PCA of macroinvertebrate data of permanent springs (P02, P03, P07). Black dots are samplings and red ones are centroids of seasons (WIN = winter, SPR = spring, SUM = summer, AUT = autumn).

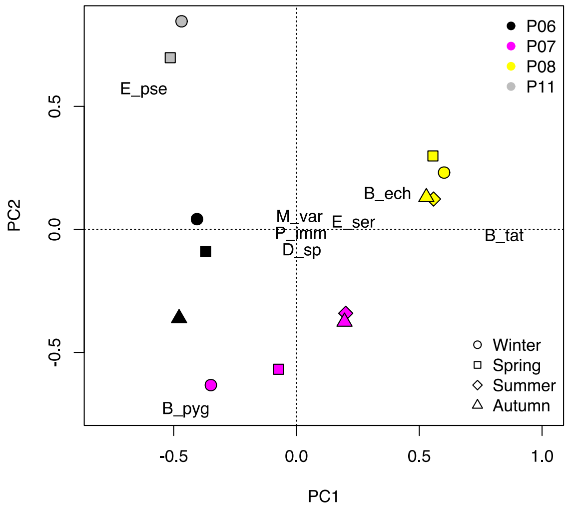

Figure 3.6: PCA of copepod data. Species abbreviations are shown in Appendix 3.2.

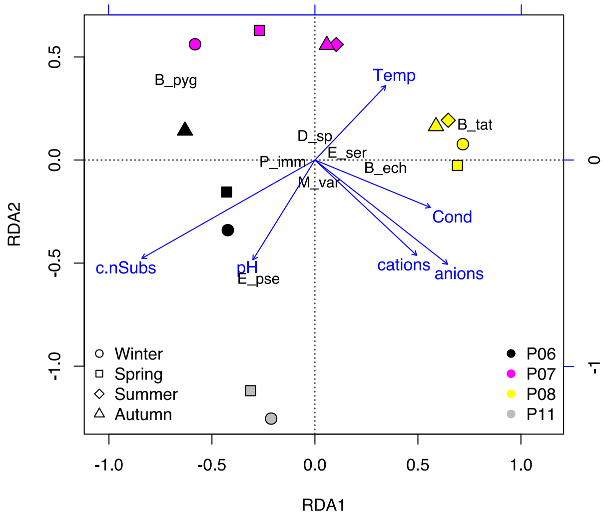

Figure 3.7: RDA of copepod data and environmental variables. c.nSubs = number of substrates; Temp = water temperature; Cond = conductivity. Species abbreviations are shown in Appendix 3.2.

important driver for invertebrate spring communities, according to Pokorny and colleagues (2012). Indeed Prinzera communities in the ordination methods were separated by the presence of taxa with different oxygen requirements: Chironomidae (adapted to low oxygen concentrations) characterised P02 and P03, and Nemouridae (requesting more oxygenated waters) were found in P08 and the winter sample from P06.

Altitude and pH were often identified as important drivers for spring assemblages (Gathmann & Williams 2006; Mori and Brancelj 2006; Barquín & Death 2008; Bottazzi et al. 2011; Pokorny et al. 2012). High values of pH characterised P02 and P03, the latter spring reached a peak of 10.9. Ophiolitic lithology can cause those rare pH anomalies (Segadelli 2014). The significance of altitude was surprising because it was quite homogeneous in the present study area. On Mount Prinzera, Segadelli (2014) observed differences between basal and higher springs in terms of discharge, water temperature, pH, and chemistry. Therefore the influence of altitude on communities could be due to various hydrological, chemical and physical features of springs rather than altitude per se.

Chemical variables of springs P02 and P03 were different from the other sites. These differences in water chemistry determined peculiar communities, indeed light differences were detected between communities of P02 and P03, whereas they were markedly different from the other assemblages. Differences could be also due to hydrogeological characteristics. P02 and P03 had a higher minimum discharge and less flow fluctuations during the year (Segadelli 2014). Rather than physical-chemical variables, many studies assessed that hydrological regime affects macroinvertebrate communities in springs, directly or by influencing habitat and substrate characteristics (von Fumetti et al. 2006). In particular discharge (Hoffsten & Malmqvist 2000; Maiolini et al. 2011), water velocity (Ilmonen & Paasivirta 2005; Maiolini et al. 2011), and discharge variability (Smith et al. 2003; von Fumetti & Nagel 2011) were the variable most affecting assemblages. The results of this work confirm the significant difference between temporary and permanent springs well known in freshwater ecosystems (Rüegg & Robinson 2004).

The only stigophilous taxon found in macroinvertebrate community of the present study was Niphargus sp., which characterised temporary springs of Mount Prinzera. The association between Niphargidae and temporary springs was probably due to the drift from groundwater during the peak of discharge following raining periods.

Although statistical differences between temporary and permanent springs were detected, the community of permanent site P07 was most similar to temporary ones. P07 had marked discharge fluctuations, comparable to fluctuations of temporary springs (Segadelli 2014). Therefore distinction between temporary and permanent springs is not sufficient to explain community differences, but discharge fluctuations should be evaluated too.

Finally, substrate composition (Hahn 2000; Dumnicka et al. 2007; Maiolini et al. 2011; von Fumetti & Nagel 2011; Martin & Brunke 2012) and heterogeneity (Staudacher & Füreder 2007; Kubíková et al. 2012;) were shown to be two of the most influent variables structuring macroinvertebrate communities in springs. However in the present study the number of substrates did not affect the assemblages, even though the peculiar communities in P02 and P03 could be also explained by the larger amount of decomposing organic matter found in those springs. The exclusive presence of Scirtidae in these two sites seems to validate this hypothesis, since Scirtidae larvae are known to prefer habitat rich in decomposing plant material (Campaioli et al. 1994). Although springs were traditionally considered stable environments (Glazier 1991), this issue should be deeper analysed. The permanent springs under study showed seasonal changes in the concentration of main ions, whereas conductivity, pH and water temperature were more stable. The thermal stability probably determined the lack of seasonal changes in structure of macroinvertebrate communities of permanent springs, contrary to what is common observed in other freshwater ecosystems macroinvertebrates (Cummins 1974).

3.4.2 Meiofauna:

The absence of adult ostracods in Mount Prinzera was surprising, because these organisms usually inhabit springs, being sometimes the most abundant meiofaunal taxon (Bottazzi et al. 2011). Probably ostracods were not totally absent in the studied springs, as shown by the presence of larvae and valvas, but their abundance was too low to be detected. When compared to springs of the same region, pH was the variables that affected the absence (or the low abundance) of ostracods in Prinzera springs. The importance of pH in shaping ostracod communities of springs has been yet pointed out by Rosati and colleagues (2014) over a large geographical scale. Differences in pH between Prinzera and other springs were due to the ophiolitic composition of Prinzera mentioned above.

Copepod and water mites are usually an important component of spring fauna (Stoch et al. 2011) in terms of abundance and diversity. Overall, the number of copepod and water mite species found in Mount Prinzera was lower compared to other studies (Bottazzi et al. 2011; Stoch et al. 2011), probably due to the small extension of the investigated area, and to the consequent small range of ecological and biogeographical factors. Taking into account species richness and abundance of each spring could be more informative of the amount of biodiversity host in the studied springs. Indeed, copepods species richness for each spring was comparable to other studies (Sambugar et al. 2006; Stoch et al. 2011). On the other hand in the same region Bottazzi (2010) found springs with 12 species, that is more than the double of Prinzera richness. The differences is probably due to the small size and to the low discharge of Prinzera springs. Water mite abundance and species richness per spring in Mount Prinzera were comparable to those found by Bottazzi (2010). On the other hand, diversity and abundance were markedly lower than in Alpine springs (Sambugar et al. 2006; Goldschmidt & Melzer 2010; Stoch et al. 2011). Since water flow was identified as one of the drivers for water mite communities (Stoch et al. 2011), the lower discharge of Apennine springs could affect the diversity of water mites. According to previous studies (Sarkka et al. 1997; Stoch et al. 2011), water chemistry resulted an important factor also for copepod community composition and for the abundance of stigoxene. This study confirm the role of substrate in shaping copepod assemblages, as pointed out by Fiasca and colleagues (2014).

In conclusion, both macroinvertebrate and meiofauna assemblages of Mount Prinzera were affected by water chemistry, which explained also the absence of adult ostracods and the abundance of stigoxene copepods. Composition and heterogeneity of in-spring habitats resulted important factors in shaping macroinvertebrate and copepod communities. Finally, hydroperiod and water flow regime influence the biodiversity of the studied assemblages. These results should be took into account for a proper management of the protected area of Mount Prinzera. For example water abstraction could have serious consequences on crenic communities, both for the direct influence on hydrological regime and for the consequent reduction of the number of submerged microhabitats.

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Appendix 3.1: Physical and chemical values

	Latitude (°)	Long (°)	Altit (m a.s.l.)	Temperature (°C)	pH	Cond (µS/cm)	Ca^{2+} (mg/L)	Mg^{2+} (mg/L)	Na^{+} (mg/L)	K^{+} (mg/L)	Cl^{-} (mg/L)	NO_3^{-} (mg/L)	NO_2^{-} (mg/L)	NH_3^{+} (mg/L)	SO_4^{2-} (mg/L)	HCO_3^{-} (mg/L)	S (mg/L)	SRP (mg/L)
P11_WIN	44.64142	10.08761	542	12.2	8.05	281.2	28.3	29.0	4.2	0.12	2.1	3.7	<0.10	<0.10	6.8	186	<0.10	0.201
P03_WIN	44.64697	10.07956	474	10.0	10.88	156.5	12.0	8.2	19.9	0.31	18.8	<1.0	<0.10	<0.10	12.1	31	<0.10	0.015
P02_WIN	44.64696	10.07951	477	9.4	7.81	161.5	12.8	11.3	17.6	0.29	10.6	2.1	<0.10	<0.10	16.1	107	<0.10	0.018
P07_WIN	44.63923	10.08556	589	12.2	7.74	318.5	31.8	32.4	6.2	0.21	3.5	2.9	<0.10	<0.10	16.2	192	<0.10	0.006
P08_WIN	44.63988	10.0849	612	11.7	8.00	234.5	24.4	27.8	1.6	0.13	3.0	5.4	<0.10	<0.10	34.4	159	<0.10	0.029
P06_WIN	44.64455	10.08156	618	6.6	7.91	84.2	<10.0	14.3	<1.0	<0.10	1.7	5.7	<0.10	<0.10	5.2	67	<0.10	0.055
P06b_WIN	44.64455	10.08159	619	6.8	7.94	84.6	<10.0	14.1	2.1	0.12	1.7	5.9	<0.10	<0.10	5.0	73	<0.10	0.017
P00_WIN	44.64233	10.08802	545	11.0	8.04	271.2	42.6	25.5	4.0	0.30	2.8	5.2	<0.10	<0.10	19.7	189	<0.10	<0.004
P11_SPR	44.64142	10.08761	542	13.3	8.15	367.0	31.4	34.5	5.2	0.19	2.2	3.0	<0.10	<0.10	7.7	244	<0.10	0.006
P03_SPR	44.64697	10.07956	474	11.6	9.70	203.0	11.4	7.4	24.2	0.41	20.5	<1.0	<0.10	<0.10	12.5	92	<0.10	<0.004
P02_SPR	44.64696	10.07951	477	11.3	7.88	228.0	14.0	13.3	19.3	0.56	10.9	1.9	<0.10	<0.10	18.1	122	<0.10	0.014
P07_SPR	44.63923	10.08556	589	13.9	7.82	419.0	35.3	38.6	7.9	0.63	4.1	2.0	< 0.10	<0.10	22.5	250	<0.10	0.009
P08_SPR	44.63988	10.0849	612	13.9	8.10	361.0	26.4	33.1	2.0	0.11	3.0	3.9	<0.10	<0.10	33.5	183	<0.10	0.010
P06_SPR	44.64455	10.08156	618	17.5	8.27	266.0	<10.0	21.5	1.3	0.13	1.5	3.8	<0.10	<0.10	6.5	104	<0.10	0.015
P06b_SPR	44.64455	10.08159	619	14.4	8.05	161.6	<10.0	21.6	1.3	0.17	1.7	4.4	<0.10	<0.10	6.4	104	<0.10	0.017
P00_SPR	44.64233	10.08802	545	13.5	8.01	390.0	36.7	32.3	6.0	0.46	2.9	4.4	<0.10	<0.10	23.2	226	<0.10	<0.004
P03_SUM	44.64697	10.07956	474	13.4	9.94	181.0	17.7	5.1	22.2	0.32	20.8	<1.0	<0.10	<0.10	11.9	<3.0	<0.10	0.010
P02_SUM	44.64696	10.07951	477	14.7	8.23	203.0	10.4	9.8	18.0	0.28	16.1	<1.0	<0.10	<0.10	17.3	98	<0.10	0.018
P07_SUM	44.63923	10.08556	589	15.5	7.88	505.0	36.3	34.2	7.0	0.22	5.2	< 1.0	<0.10	<0.10	33.5	244	<0.10	0.011
P06_SUM	44.64455	10.08156	618	18.7	7.66	328.0	2.0	24.5	1.2	<0.10	3.8	2.0	<0.10	<0.10	8.8	177	<0.10	0.020
P11_AUT	44.64142	10.08761	542	13.0	7.20	365.0	37.2	35.1	5.7	<0.10	2.9	3.3	<0.10	<0.10	13.0	183	<0.10	0.005
P03_AUT	44.64697	10.07956	474	13.5	10.36	192.0	15.5	<5.0	30.1	0.36	20.2	<1.0	<0.10	<0.10	11.3	<3	<0.10	0.013
P02_AUT	44.64696	10.07951	477	13.2	7.84	253.0	13.9	15.3	22.1	0.24	13.1	<1.0	<0.10	<0.10	18.6	122	<0.10	0.018
P07_AUT	44.63923	10.08556	589	13.9	7.16	417.0	36.4	40.6	8.6	0.16	5.0	<1.0	<0.10	<0.10	35.0	238	<0.10	0.009
P06_AUT	44.64455	10.08156	618	17.0	7.01	128.0	<10.0	25.0	1.3	0.10	2.4	2.6	<0.10	<0.10	7.1	122	<0.10	0.025
P06b_AUT	44.64455	10.08159	619	15.7	7.43	182.0	<10.0	24.7	1.2	0.23	2.2	2.3	<0.10	<0.10	6.7	122	<0.10	0.057

Appendix 3.2: Biological data
Macroinvertebrates

	Abbreviation	P11_WIN	P03_WIN	P02_WIN	P07_WIN	P08_WIN	P06_WIN	P06b_WIN	P00_WIN	P11_SPR	P03_SPR	P02_SPR	P07_SPR	P08_SPR	P06_SPR	P06b_SPR	P00_SPR	P03_SUM	P02_SUM	P07_SUM	P06_SUM	P11_AUT	P03_AUT	P02_AUT	P07_AUT	P06_AUT	P06b_AUT	Occurrence
Leuctridae	Leuct	0	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	5	0	0	0	1	15	0	0	0	6
Nemouridae	Nemour	0	52	6	0	4	12	0	0	0	3	0	0	3	5	0	0	0	2	4	0	0	3	4	0	0	0	11
Plecoptera spp	Plec	0	0	0	0	0	0	0	0	0	17	0	0	4	0	0	0	0	2	0	0	0	0	0	0	0	0	3
Hydropsychidae	Hydrops	0	3	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	3
Polycentropodidae	Polycentr	0	6	2	0	0	0	0	0	0	2	1	0	0	0	0	0	1	0	0	0	0	6	2	0	0	0	7
Lepidostomatidae	Lepidost	0	69	33	0	0	0	0	0	0	7	16	1	0	0	1	0	1	2	2	0	0	9	11	0	0	0	11
Philopotamidae	Philop	0	9	4	0	0	0	0	0	0	2	0	0	0	0	0	0	5	2	1	0	0	3	3	0	0	0	8
Sericostomatidae	Seric	0	9	7	0	0	0	0	0	0	13	2	2	0	0	0	0	7	62	0	0	0	26	46	0	0	0	9
Baeridae	Baer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	1	7	3	1	5
Limnephilidae	Limneph	2	0	0	2	0	0	0	2	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	5
Brachycentridae	Brachyc	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Trichoptera spp	Tric	0	0	0	0	0	2	0	0	0	0	9	0	0	0	0	0	3	7	3	1	0	1	0	0	0	1	8
Heptagenidae	Heptag	0	3	5	0	0	0	0	0	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
Scirtidae	Scirt	0	18	57	6	0	0	0	0	0	17	82	0	0	0	0	0	42	338	32	0	0	38	96	5	0	0	11
Haliplidae	Halipl	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Dytiscidae	Dytisc	0	0	0	0	0	0	0	0	0	0	4	0	0	1	0	0	2	2	0	0	1	2	1	1	0	0	8
Limnebiidae	Limneb	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Helophoridae	Heloph	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Hydrophoridae	Hydroph	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2
Hydraenidae	Hydra	1	0	0	28	0	0	0	4	27	0	0	34	0	1	3	6	1	2	36	1	4	1	2	12	0	0	16
Tipulidae	Tipul	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	3	0	0	0	0	0	0	0	3
Empididae	Empid	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Chironomidae	Chiron	0	33	26	35	0	4	8	4	14	248	2	6	2	3	2	3	471	17	123	0	0	452	71	6	2	1	22
Limonidae	Limon	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	4	0	0	1	1	2	1	1	7
Stratyonomidae	Strat	0	0	0	8	0	0	0	0	0	0	0	15	0	1	0	0	0	0	3	1	0	0	0	3	0	0	6
Ceratopogonidae	Ceratop	0	4	1	0	0	0	0	0	0	29	8	4	0	0	0	0	4	1	4	0	0	15	7	2	0	0	11
Psychodidae	Psych	0	3	3	13	0	0	0	0	0	3	0	4	0	3	0	1	1	4	14	0	0	1	1	0	0	0	12
Ptychopteridae	Ptychop	0	8	0	0	0	0	0	0	0	3	0	0	0	0	0	0	11	1	0	0	0	3	0	0	0	0	5
Dixidae	Dix	0	3	0	14	0	0	0	0	1	1	0	4	0	0	0	1	1	2	14	0	0	1	7	3	0	0	12
Simulidae	Simul	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	3
Diptera spp	Dit	0	0	0	0	0	0	0	0	0	4	0	1	0	1	0	0	14	0	0	1	0	29	0	0	0	0	6
Velidae	Vel	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Heteroptera spp	Het	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
Cordulagasteridae	Corduleg	0	1	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Odonata spp	Odon	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1
Hirudinea	Irud	0	1	8	1	0	0	1	0	0	1	9	3	0	0	0	0	8	1	1	0	0	6	2	0	0	0	12
Lymnaeidae	Lymn	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Hydrobioidea	Hydrob	0	11	76	0	0	0	0	0	0	6	179	0	0	0	0	0	18	362	0	0	0	19	354	0	0	0	8
Gasteropoda spp	Gaster	0	0	0	0	0	0	0	0	0	0	1	3	0	0	0	0	1	0	7	0	0	0	0	0	1	0	5
Collembola	Collem	3	14	5	24	2	2	3	4	1	0	0	5	2	0	0	3	0	2	12	0	1	3	19	6	0	5	19
Oligocheta	Olig	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	1
Niphargida	Nipharg	19	0	0	51	0	2	4	8	39	0	0	89	0	3	3	4	0	0	83	1	18	0	0	45	7	3	16
	Taxa richness	4	19	13	11	2	5	5	7	7	18	15	16	5	8	4	7	19	18	19	6	4	21	19	13	5	6	

Copepods

	P11_WIN	P07_WIN	P08_WIN	P06_WIN	P11_SPR	P07_SPR	P08_SPR	P06_SPR	P07_SUM	P06_SUM	P11_AUT	P07_AUT	P06_AUT	Occurrence
Bryocamptus (Rheocamptus) tatrensis	5	187	0	0	6	256	0	3	12	7	0	46	19	9
Bryocamptus (Echinocamptus) echinatus	0	33	0	0	0	54	0	0	19	0	0	1	0	4
Bryocamptus (Bryocamptus) pygmaeus	51	0	0	2	56	0	1	14	2	5	15	1	15	10
Moraria (Moraria) varica	5	2	0	0	2	2	0	0	0	0	0	1	0	5
Elaphoidella pseudophreatica	45	0	17	0	29	3	35	0	0	0	2	0	0	6
Diacyclops sp.	0	0	0	0	0	0	0	0	0	2	0	0	0	1
Paracyclops imminutus	0	0	0	0	0	2	0	0	0	0	0	0	0	1
Eucyclops serrulatus serrulatus	0	0	0	0	0	0	0	0	0	0	0	2	0	1
Cyclopoid: young copepodid	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Harpacticoid: young copepodid	1	0	0	0	2	0	1	0	0	0	0	0	0	3
Species richness	5	3	1	1	5	5	3	3	3	3	2	5	2	

Water mites

	P11_WIN	P03_WIN	P02_WIN	P07_WIN	P08_WIN	P06_WIN	P06b_WIN	P00_WIN	P11_SPR	P03_SPR	P02_SPR	P07_SPR	P08_SPR	P06_SPR	P06b_SPR	P00_SPR	P03_SUM	P02_SUM	P07_SUM	P06_SUM	P11_AUT	P03_AUT	P02_AUT	P07_AUT	P06_AUT	P06b_AUT	Occurrence
Atractides sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
Partnunia sp.	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Soldanellonyx monardi	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Sperchon thienemanni	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	6	0	0	0	2
Species richness	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	1	1	0	0	0	

Chapter 4

Quantifying the effect of local, regional, and spatial factors in determining invertebrate assembly in springs

An oral presentation at 9th Symposium of Freshwater Ecosystem, Geneva (Switzerland), 5-10 July 2015 was based on this chapter: “Quantifying the effect of environmental and spatial factors in determining macroinvertebrate community assembly in springs” by Rosati M., Cantonati M., Fenoglio S., Celico F., Segadelli S, Petrella E, Levati G1, Rossetti G.

4.1 Introduction

In the previous chapter I identified local factors shaping invertebrate communities of springs. Separating the influences of regional and local factors is important to understand the reasons of variation in local assemblages, due to natural or anthropogenic causes. Indeed local community composition could be affected by the spatial patterns of taxa distribution at larger spatial scale. Broad-scale spatial patterns could be the result of several processes, such as historical effects, climatic constraints, dispersal limitation, or spatially structured local environmental variables (Cottenie 2005). The study of the relative importance of local, climatic and spatial drivers allows the identification of the main processes shaping communities. The prevalence of spatial or environmental (both local and regional) factors depends on the dispersal abilities of the organisms and the connectivity of sites but also on the spatial scale analysed (Martiny et al. 2006, Landeiro et al. 2012). In fact at small spatial scale dispersal could be more effective, due to the lack of barriers, and environmental filtering and local biotic interaction could prevail.

The aim of this work is to identify the role of local, climatic and spatial drivers on macroinvertebrate, water mites, and ostracod communities of springs. Springs are discrete habitats that often occur in clusters at the interface between aquifer and ground surface. Dispersal limitation may thus influence crenic communities. Dispersal ability and community spatial structure were deeply studied on macroinvertebrates (e.g. Rádková et al. 2014; de Mendoza et al. 2015). On the contrary, the spatial structure of ostracod and water mite communities was rarely investigated (Escrivà et al. in press; Zhai et al. 2015), even though these taxa are important constituent of fauna in many freshwater ecosystems (Di Sabatino et al. 2008; Martens et al. 2008). Three different scenarios can result from this study, depending on the factor that explain most of the community variation:

1. Spatial: the role of dispersal will be determinant in shaping communities.
2. Climatic: regional factors affect the community

structure.

3. Local: local niche-based processes prevail on regional or dispersal factors.

4.2 Methods

The study area consists of 15 permanent springs located in Emilia Romagna, Italy (Fig. 4.1 for geographical location). The sites range from 31 to 1614 m a.s.l. The springs have been sampled twice: in August - September 2013 and in May 2014.

Invertebrates were collected with a hand net (frame dimensions: 10x10 cm; mesh size: 255 µm). Ten replicas were collected for each site. Substrate was sampled for an area equal to the net frame for each replica. In 2013 the replicas were of 1 minute each, in 2014 of 15 seconds, but a preliminary ANOVA test detected no differences in the number of specimens found in each spring between 2013 and 2014. A multi-habitat proportional approach was used: every microhabitat was sampled for a number of replicas proportional to its percentage cover in the spring. Following the approach of Bottazzi et al. (2008), only submerged microhabitats were taken into account (clay, silt, and sand; gravel; pebbles; boulders; rock; bryophytes; algae; other macrophytes; leaves; wood). Organisms were sorted under a stereomicroscope and preserved in 90% ethanol. Invertebrates were identified at family level with the exception of Hirudinea (not deeper recognised), Ostracoda (at species level), and Hydracarina (identified at species level by Antonio Di Sabatino, University of L'Aquila, Italy). Ostracods and water mites were analysed together, but macroinvertebrate were kept separated because of the different taxonomical resolution. The following environmental variables were measured in field: pH, conductivity, water temperature (with Eutech Instrument PC 300 multi-parameter probe), number of microhabitats, altitude, and geographical coordinates (with Garmin GPS 12; datum: WGS84). Concentrations of NO_3^-, NO_2^-, NH_4^+, DRSi (Dissolved Reactive Silica) and SRP (Soluble Reactive Phosphorus) were measured in laboratory (APHA 2005). Concentrations of Ca^{2+}, Mg^{2+}, Na^+, K^+, Cl^-, and SO_4^{2-}, were analysed by the Department of Chemistry, University of Parma. Climatic variables were extracted from spatial database with a resolution of about 1 Km^2 (Hijmans et al. 2005). The spatial structure of data was represented by eigenvectors derived from Principal Coordinates of Neighbour Matrices (PCNM) analysis (Borcard & Legendre 2002). Only positive eigenvectors with positive spatial correlation (test by Moran's I) were retained.

Biological abundance data were Hellinger-transformed in order to be suitable for the chosen multivariate analysis, as suggested by Legendre and Gallagher (2001). NO_3^-, NH_4^+, Ca^{2+}, Na^+, K^+, Cl^-, SO_4^{2-}, and conductivity were log-transformed, because outliers were detected with graphic techniques. Explanatory variables were tested for collinearity, and only variables with $r \leq |0.8|$ were retained. The ratio $(Mg^{2+}+Ca^{2+})/(Na^+ + Cl^-)$ of mEq/L concentrations was used instead of ion concentrations in order to avoid collinearity. Four Redundancy Analyses (RDA - Legendre & Legendre 2012) were performed with different explanatory variables:

1. Climatic variables: annual mean temperature, annual precipitation and precipitation seasonality (as standard deviation of the weekly precipitation, expressed as % of the mean).

2. Local variables: NO_3^-, NH_4^+, DRSi, $(Mg^{2+}+Ca^{2+})/(Na^+ + Cl^-)$, water temperature, pH, and conductivity.

3. Total environmental variables: local and climatic variables.

4. Spatial PCNM variables

The overall significances of the models were assessed with a 999-permutation test. Variation explained by the model was assessed using the R2 adjusted for multivariate analysis (Legendre and Legendre 2012). Forward selection by permutation test evaluated the significance of the contribution of each explanatory variable to the RDA models (999 permutations, 0.05% significance level). Significant variables were retained and used for the variation partitioning analysis on the RDAs. The first variation

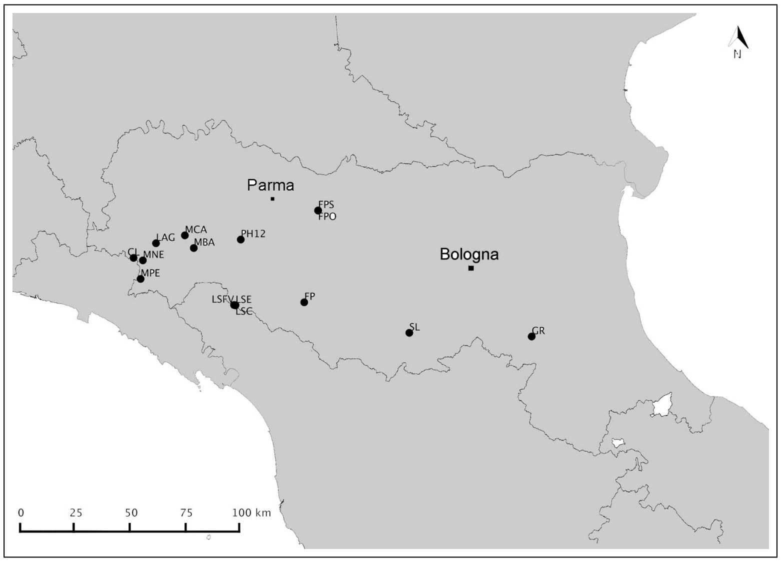

Figure 4.1: Map of studied area.

partitioning was performed on the RDAs of total environmental variables and spatial variables, the second variation partitioning on three RDAs, relative to climatic, local and spatial variables. Variation partitioning separated the percentage of community variation in different fractions: pure environmental (environmental without the spatial fraction), pure spatial (spatial without the environmental fraction), residual (unexplained), and the variation shared by spatial and environmental variables (Borcard et al. 1992). The significance of the pure fractions was evaluated by 100 permutations of the original data (Borcard et al. 1992).

All the analyses were run in R (R Core Team 2013), using the packages vegan (Oksanen et al. 2013), packfor (Dray et al. 2011), and PCNM (Legendre et al. 2012).

4.3 Results

Geographical, climatic, physical, and chemical data were reported in Appendix 4.1. NO_2^- concentration was below the analytic thresholds for the majority of the samples, so it was removed from further analysis.

Overall 9686 macroinvertebrate specimens were found, belonging to 55 taxa. Chironomidae was the most frequent macroinvertebrate taxon, occurring in 86.7% of samples and in 12 springs out of 15. After Chironomidae, the five most frequent taxa in samples were insect families (namely Nemouridae, Baetidae, Leuctridae, Ceratopogonidae, Limnephilidae), followed by Niphargidae with the 30% of occurrence. Chironomidae were also the most abundant taxon, with 4528 organisms, followed by Asellidae (1135) and Gammaridae (804). Taxa richness per spring varied from 2 to 24.

Ostracods were found in 8 springs, with 919 specimens (see Appendix 4.2). They belong to 15 taxa: 14 species and one organism assigned to *Candona neglecta* group. *Herpetocypris reptans* and *Cypria ophtalmica* were the most frequent species, occurring in the 30.8% of the samples. *Cypria ophtalmica* was also the most abundant species, with 369 individuals. *Cypria ophtalmica*, *Potamocypris pallida*, *Herpetocypris reptans* occurred in two springs, and the other taxa only in one site.

Seventy-five water mites were found in 13 springs, belonging to 11 taxa: *Atractides brendle*, *Hydrovolzia placophora, Lebertia* sp., *L. glabra, L. maglioi,* *Partnunia aprutina, Piona disparilis, Rivobates psammocrenicus, Sperchon resupinus, S. thienemanni* (see Appendix 4.2). *Sperchon thienemanni* was the most occurring species, found in 5 springs and 7 samples. *Partnunia aprutina* was the most abundant species in the studied sites, with 26 specimens, followed by *Sperchon thienemanni* (19). No ostracods and no water mites were found in two sites (CL and LSC).

Both macroinvertebrate and ostracod-mite communities showed spatial structures: the RDAs of the PCNM variables were significant and explained respectively 28.3% and 13.2% of the variance. Fig. 4.2 and fig. 4.3 show the scores for each site of the two significant axes of spatial RDAs. Two spatial PCNM variables resulted significant in both the models: the first and the third PCNM eigenvalues for macroinvertebrates and the first and the second for ostracods and water mites.

Fig. 4.4 shows the biplot of the RDA of macroinvertebrate and the total environmental variables. The model was significant and accounted for the 47.3% of the total variance. Annual mean temperature, precipitation seasonality, conductivity, and pH were forward-selected. The first axis of RDA separated the sites based on the annual mean temperature and the conductivity: springs located in colder area (often at higher altitude) were in the left side of the graph, whereas springs of warmer area with higher conductivity were on the right. The second axis organised the sites along a gradient of precipitation seasonality; the presence of Niphargidae was related to higher variability of precipitation. The samples of three springs located in exploited agriculture regions were plotted together, characterised by high concentration of NO_3^- and the presence of Gammaridae. RDA of ostracod-water mite data and environmental variables accounted for the 29.1% of variation (fig.4.5). Water temperature, conductivity, annual precipitation, and NO_3^- concentration were significant. The first axis organised the springs in a gradients of water temperature and NO_3^- concentration. The second axis showed a gradient of increasing precipitation and decreasing conductivity from the bottom to the top of the plot.

RDAs of the macroinvertebrate data with local variables and climatic variables were both significant and respectively accounted for 40.7% and 34.5%. NO_3^- concentration, conductivity, and pH were significant in the first analysis, annual mean temperature and precipitation seasonality in the second one. Also RDAs of ostracod and water mite data were significant. Local variables explained the 25.0% of the community structure variability, and climatic variables model accounted for the 16.5%. Water temperature, conductivity and NO_3^- concentration were the significant variables in the first model, annual mean temperature and annual precipitation in the second one.

Results of variation partitioning of spatial and environmental variables are shown in fig. 4.6 for macroinvertebrates and in fig. 4.7 for ostracod and water mites. Fig. 4.8 and 4.9 display the results of variation partitioning of local, climatic and spatial variables for macroinvertebrate and ostracod and water mite assemblages. All the fractions were significant with the exception of the pure climatic fraction of fig. 4.9. R2 adjusted for some fractions resulted to be negative, corresponding to explanatory variables that explain less than would be expected by chance, and have to be interpreted as 0% (Legendre & Legendre 2012).

4.4 Discussion

Invertebrate communities of studied springs were spatially structured. Only a fraction of this spatial pattern was explained by the geographical distribution of environmental variables. Part of the variability in spatial distribution of taxa remained unexplained, and could be due to dispersal limitation or biogeographical and historical reasons. Anyway, in this study spatial processes resulted significant but less relevant than the environmental control. This result agrees with Cottenie (2005), which stated a prevalence of niche-based processes in structuring communities in freshwater ecosystems.

The prevalence of environmental or spatial variables depends on 1) the extent of the study area, 2) the connectivity between sites, and 3) the dispersal

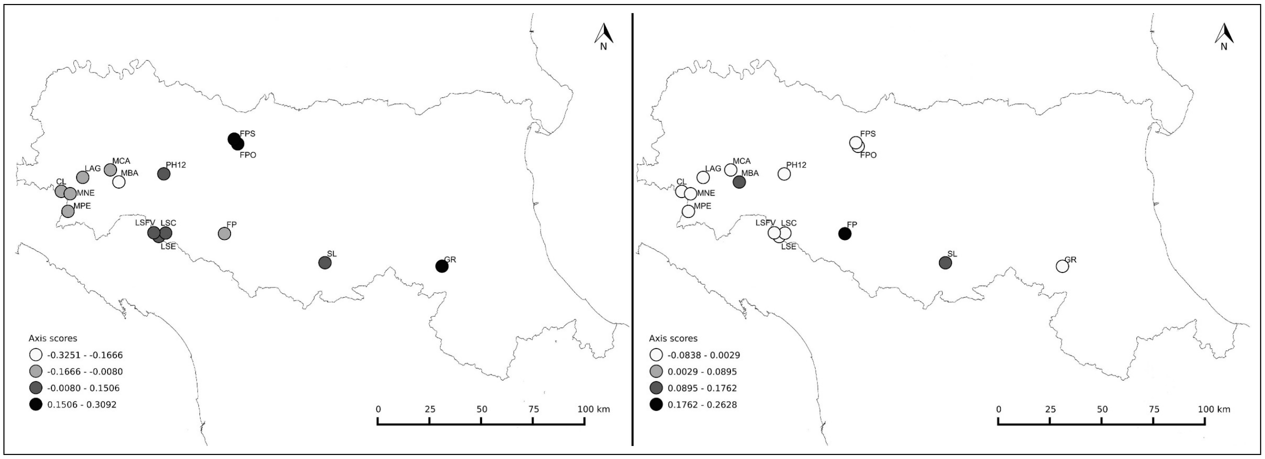

Figure 4.2: Map of scores of the first (on the right) and the second (on the left) axes of spatial RDA of macroinvertebrate community.

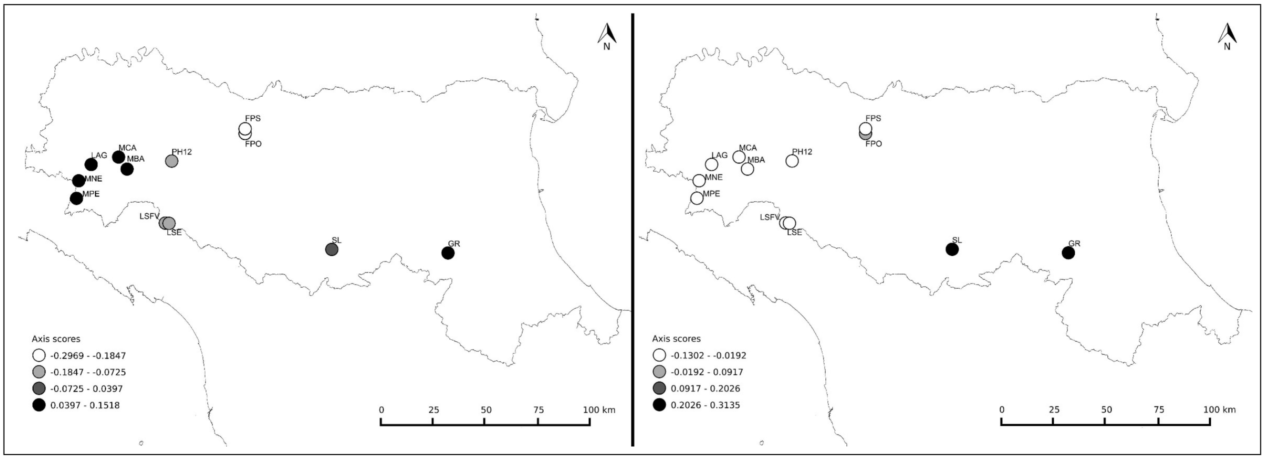

Figure 4.3: Map of scores of the first (on the right) and the second (on the left) axes of spatial RDA of ostracod and water mite community.

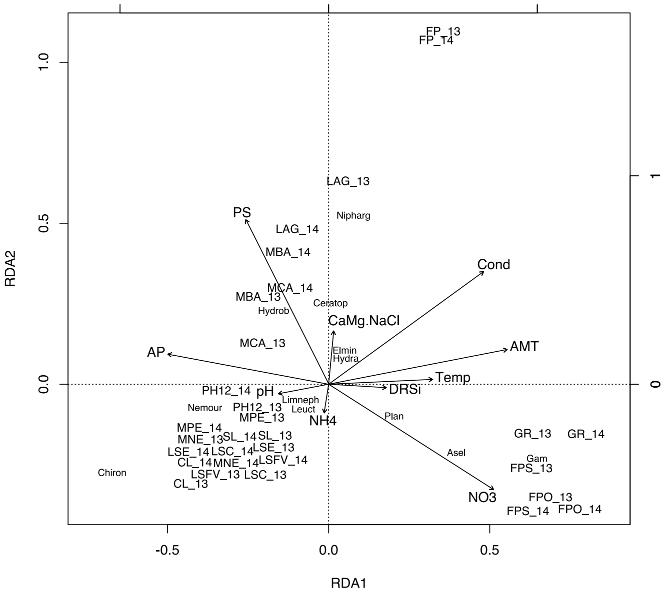

Figure 4.4: RDA of macroinvertebrate data and environmental variables. Only selected taxa are shown.

ability of organisms (Grönroos et al. 2013; Heino et al. 2015). Dispersal ability of organisms affects the relative importance of spatial and environmental control: in the same area, strong dispersers are more under environmental control and less affected by spatial processes compared to weak dispersers (Heino et al, 2013). Indeed, actively dispersing species are better able to reach all the sites in the area than passively dispersers, following the environmental heterogeneity. (Grönroos et al. 2013). In this study, the amount of variance explained by the pure spatial effect was higher for ostracods and water mites than for macroinvertebrates, even though spatial and environmental variables together explained almost the same amount of variance. Dispersal of ostracods occurs through passive transport by animals, humans and probably the wind (McKenzie & Moroni 1986; Meisch 2000; Lopez et al. 2005). Also water mites are passive dispersers, even though little is known about their dispersal dynamics (Bilton et al. 2010). On the contrary, macroinvertebrates include several taxa with active dispersal abilities in their larval or adult stages (Tachet et al. 2000). These differences in dispersal modalities are probably the causes of the lack of significant spatial patterns in freshwater macroinvertebrate communities in several studies (Heino & Mykrä 2008; Grönroos et al. 2013), whereas for example ostracods are more frequently found to be spatially structured (Escrivà et al. 2015; Zhai et al. 2015). This is consistent also with my results: in the studied area, the amount of variance explained by pure spatial factors in macroinvertebrates and ostracod and water mites was affected by the different dispersal abilities among taxa. This result could be affected by the differences in taxonomical resolution between the two communities. Dolédec et al. (1998) found slight differences in functional traits, included dispersal, between macroinvertebrate species and concluded that genus and even families level is sufficient to describe functional diversity in macroinvertebrates. Thus the family level is expected to provide a reliable description of macroinvertebrate dispersal abilities.

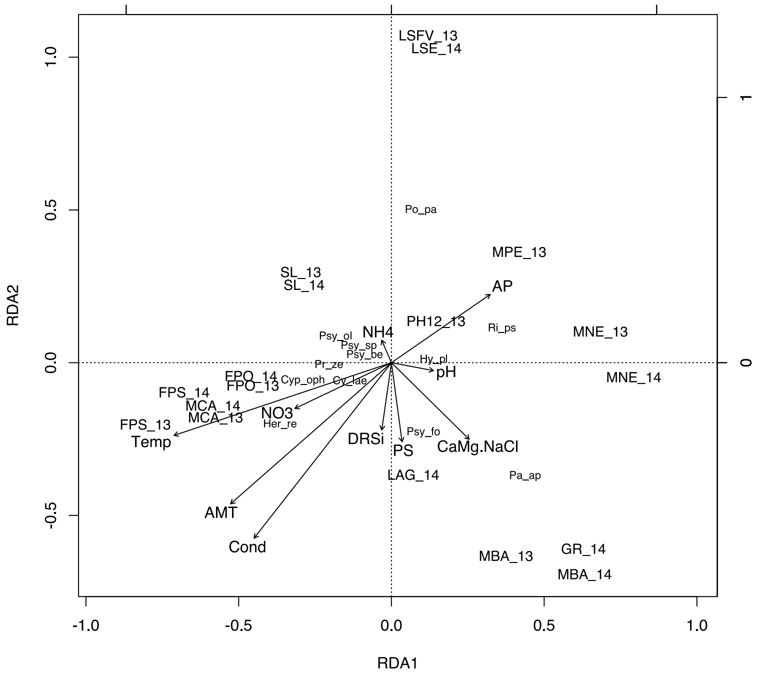

Figure 4.5: RDA of ostracod and water mite data and environmental variables. Only the selected species are shown.

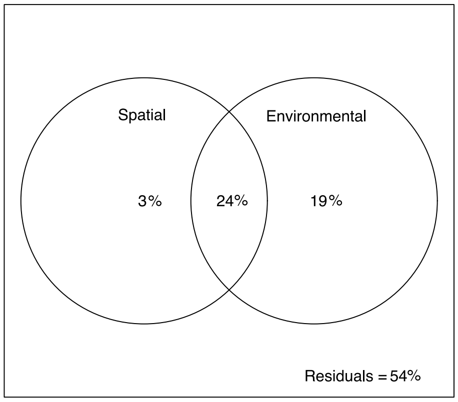

Figure 4.6: Variation partitioning of spatial and total environmental variables for macroinvertebrate communities.

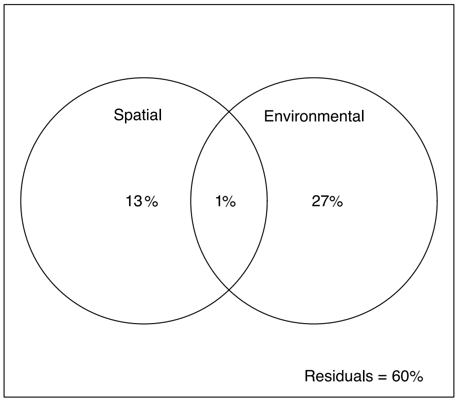

Figure 4.7: Variation partitioning of spatial and total environmental variables for ostracod and mite communities.

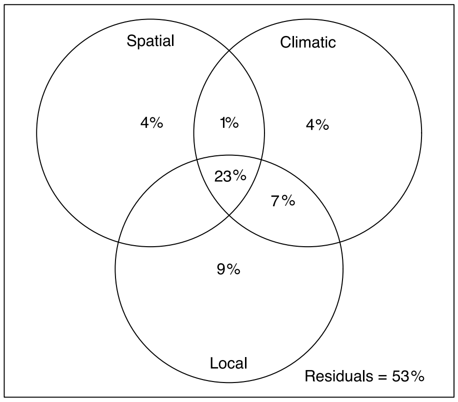

Figure 4.8: Variation partitioning of spatial, local, and climatic variables for macroinvertebrate communities. Only values > 0 are shown.

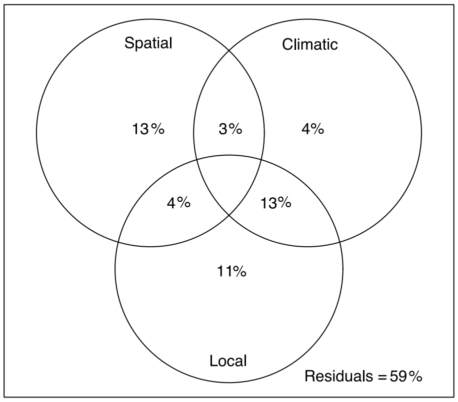

Figure 4.9: Variation partitioning of spatial, local, and climatic variables for ostracod and mite communities. Only values > 0 are shown.

Given a fixed extent of the studied area, the presence of spatial patterns depends on dispersal ability but also on the degree of connectivity of sites. Springs are discrete habitats, with high degree of isolation. This characteristic explained the detection of a spatial control also on strong dispersers, found in this study and also pointed out by Rádková and colleagues (2014) in permanent spring habitats. Conversely, several study in streams assessed a lack of spatial pattern in the macroinvertebrate (Heino & Mykrä 2008; Heino et al. 2012). The role of climatic variables as community drivers is rarely investigated, even though precipitation and air temperature resulted influent factors for macroinvertebrates and ostracods in springs (Rádková et al. 2014; Zhai et al. 2015). In the studied area regional climatic variables affect communities less than local factors, and for ostracods and water mites their effect was negligible. For these taxa the regional variables influence the local factors, as suggested by the high fraction of biological variability explained by the shared effects of climatic and local factors. Rather than regional drivers, invertebrate assemblages were influenced by local factors related to water chemistry.

In conclusions the characteristic isolation of springs affects the ability of taxa to reach all the sites of the studied area, influencing more passive than active dispersers. Anyway, spatial factors explained only a few percentage of the total variation, and invertebrate communities resulted mainly affected by environmental variables. This resulted confirm the importance of the environmental filtering of springs on invertebrate communities, compared to the role of dispersal and the regional factors.

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Appendix 4.1: Physical and chemical values

AMT = annual mean temperature (°C), mT = minimum temperature of coldest month (°C), MT = maximum temperature of hottest month (°C), MDR = mean diurnal range (subtraction between maximum and minimum monthly mean temperature, °C), AP = annual precipitation (mm), PWM = precipitation of the wettest month (mm), PS = precipitation seasonality (standard deviation of the weekly precipitation, expressed as % of the mean)

	Latitude (°)	Longitude (°)	Altit (m a.s.l.)	Temperature (°C)	pH	Cond (µS/cm)	Type	Number of substrates	AMT (°C)	MDR (°C)	MT (°C)	mT (°C)	AP (mm)	PWM (mm)	PDM (mm)	PS (mm)
LSE_13	44.37819	10.04919	1525	12.9	7.54	34.3	H	3	5.6	5.2	17.9	-4.3	898	107	56	21
LSC_13	44.37788	10.04835	1557	13.1	7.23	32.6	L	3	5.6	5.2	17.9	-4.3	898	107	56	21
LSFV_13	44.3792	10.04085	1614	6.5	7.78	51.3	R	3	5.2	5.1	17.4	-4.5	901	107	57	20
CL_13	44.57053	9.46733	1383	7.3	8.27	81.3	R	4	5.6	5.6	18.1	-4.4	1002	119	64	21
MNE_13	44.56072	9.52039	1510	6.6	7.96	128.2	R	6	5.7	5.6	18.2	-4.4	991	118	63	21
MPE_13	44.4849	9.5081	1337	5.3	7.34	71.4	R	5	6.5	5.7	19.2	-3.7	979	119	58	22
FP_13	44.38949	10.4403	433	10.97	7.08	13010	R	6	11.6	7.9	26.5	-0.8	899	109	42	24
SL_13	44.26038	11.03624	619	15.66	7.9	537	R	5	10.2	6.9	24.6	-1.5	937	117	47	22
MCA_13	44.66388	9.75981	757	12.5	7.81	415	R	3	9.3	7	23.6	-2.7	912	115	53	24
FPO_13	44.7642	10.52253	33	13.8	7.1	921	L	5	12.9	9.1	29.2	-1.2	837	97	45	20
FPS_13	44.76482	10.5228	31	14.1	7.42	920	L	5	12.9	9.1	29.2	-1.2	837	97	45	20
LAG_13	44.63111	9.59489	946	18.4	7.3	225.9	H	3	8	6.5	21.6	-3.2	960	120	57	23
PH12_13	44.64697	10.07956	473	12.7	10.57	227.6	R	5	10.7	7.7	25.8	-2	867	107	49	23
MBA_13	44.61219	9.811	878	8.2	7.72	219.4	R	4	9	6.8	23	-2.8	911	114	53	24
GR_13	44.23602	11.73062	150	12.14	7.61	2135	R	3	13.1	8.6	28.6	-0.1	753	91	48	19
LSE_14	44.37819	10.04919	1525	5.6	7.27	37.9	H	3	5.6	5.2	17.9	-4.3	898	107	56	21
LSC_14	44.37788	10.04835	1557	4	7.46	21.4	L	3	5.6	5.2	17.9	-4.3	898	107	56	21
LSFV_14	44.3792	10.04085	1614	14.5	8.08	45.9	R	4	5.2	5.1	17.4	-4.5	901	107	57	20
CL_14	44.57053	9.46733	1383	7.2	7.25	149.7	R	3	5.6	5.6	18.1	-4.4	1002	119	64	21
MNE_14	44.56072	9.52039	1510	5.1	6.83	112.9	R	7	5.7	5.6	18.2	-4.4	991	118	63	21
MPE_14	44.4849	9.5081	1337	4.6	6.81	61.6	R	4	6.5	5.7	19.2	-3.7	979	119	58	22
FP_14	44.38949	10.4403	433	10.2	7.45	16290	R	7	11.6	7.9	26.5	-0.8	899	109	42	24
SL_14	44.26038	11.03624	619	13.5	8.21	529	R	7	10.2	6.9	24.6	-1.5	937	117	47	22
MCA_14	44.66388	9.75981	757	10.7	7.43	420	R	4	9.3	7	23.6	-2.7	912	115	53	24
FPO_14	44.7642	10.52253	33	14.5	7.16	880	L	5	12.9	9.1	29.2	-1.2	837	97	45	20
FPS_14	44.76482	10.5228	31	17.2	7.22	854	L	2	12.9	9.1	29.2	-1.2	837	97	45	20
LAG_14	44.63111	9.59489	946	9.82	7.45	211	H	3	8	6.5	21.6	-3.2	960	120	57	23
PH12_14	44.64697	10.07956	473	11.6	9.7	203	R	4	10.7	7.7	25.8	-2	867	107	49	23
MBA_14	44.61219	9.811	878	7.59	8.5	304	R	5	9	6.8	23	-2.8	911	114	53	24
GR_14	44.23603	11.73062	150	11.8	7.92	1892	R	3	13.1	8.6	28.6	-0.1	753	91	48	19

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	Ca^{2+} (mg/L)	Mg^{2+} (mg/L)	Na^{+} (mg/L)	K^{+} (mg/L)	Cl^{-} (mg/L)	NO_3^{-} (mg/L)	NO_2^{-} (mg/L)	NH_3^{+} (mg/L)	SO_4^{2-} (mg/L)	SRP (mg/L)	DRSi (mg/L)
LSE_13	13.2	0.63	1.95	0.67	4.7	0.1952	<0.003	0.2684	3.2	0.0129	0.379
LSC_13	4.67	0.51	1.19	0.59	1.8	0.2201	0.004	0.3565	<2.0	0.0131	0.262
LSFV_13	12.3	0.63	1.71	0.59	1.7	0.2855	<0.003	0.1658	<2.0	0.0065	0.373
CL_13	10.97	1.61	1.31	0.61	4.7	0.3341	0.003	0.4924	4.8	0.0073	3.949
MNE_13	1.22	14.3	1.03	0.6	4.6	0.2796	0.005	0.2239	<2.0	0.0066	9.871
MPE_13	8.52	1.27	1.21	0.59	4.5	0.8507	0.008	1.0168	3.7	0.0053	2.724
FP_13	1171.8	19.7	1941.9	30	3024	0.159	0.004	0.1309	1608	0.011	0.638
SL_13	56.7	16.5	4	1.1	8.1	0.4418	<0.003	0.0832	20.9	0.0157	7.484
MCA_13	73.3	13.1	3.46	1.44	4.2	0.1174	0.003	0.2789	36.1	0.0125	3.475
FPO_13	97.3	20.14	14.04	2.76	29	8.8051	<0.003	0.1152	51.5	0.0138	5.788
FPS_13	118.8	23.8	16.4	3.14	28	7.4783	0.06	0.3771	47.4	0.0141	5.739
LAG_13	5.47	27.1	1.45	0.66	1.6	0.1411	<0.003	0.2789	3.4	0.0184	12.673
PH12_13	6.58	1.19	14.8	0.79	17.8	0.3629	0.007	0.2608	11.1	0.0127	0.622
MBA_13	38	13.2	4.64	1.59	4.8	0.2866	<0.003	0.1404	22.5	0.0082	2.525
GR_13	771.7	24.5	12.1	3.35	10.3	3.0975	<0.003	0.2305	1364	0.0121	6.951
LSE_14	6.05	0.35	1.08	0.62	1.5	0.1137	<0.003	0.0035	<2.0	0.0056	0.219
LSC_14	1.86	0.19	0.8	0.56	1.6	0.1263	<0.003	0.001	<2.0	0.004	0.12
LSFV_14	7.8	0.4	1.16	0.61	1.6	0.1102	<0.003	0.0034	<2.0	0.0145	0.483
CL_14	11.21	1.79	1.35	0.65	4.6	0.1329	<0.003	0.0187	4.5	0.0072	2.375
MNE_14	1.37	11.72	0.92	0.59	4.3	0.1812	<0.003	0.0151	<2.0	0.0051	3.259
MPE_14	6.9	1.04	1.05	0.6	4.6	0.2937	<0.003	0.0081	3.4	0.0046	1.255
FP_14	1257.2	20.9	2969	47.6	3129	0.0941	<0.003	0.001	1774	0.0102	0.635
SL_14	75.2	22.2	4.05	1.28	7.4	0.4572	<0.003	0.0247	20.1	0.0135	3.773
MCA_14	69.9	12.2	3.1	1.38	4.6	0.0557	<0.003	0.0154	29.2	0.0107	1.787
FPO_14	127.6	27	17.6	3.66	29.2	9.2658	0.024	0.0046	48.9	0.0145	1.763
FPS_14	102.3	21.3	15.8	2.61	31.2	7.2969	0.028	0.0167	50.2	0.0172	4.69
LAG_14	3.55	17.8	2.53	0.61	2	0.0834	<0.003	0.0095	3.3	0.0098	4.32
PH12_14	11.4	7.4	24.2	0.41	20.5	0.2967	<0.003	0.02	12.5	0.0116	2.561
MBA_14	33.3	11.5	4.34	1.53	4.7	0.2672	<0.003	0.0098	22.5	0.0076	1.612
GR_14	903.1	22.1	9.46	2.79	10.4	2.428	<0.003	0.0223	1254	0.0118	5.165

Appendix 4.2: Biological data
Macroinvertebrates

	Abbreviation	LSE_13	LSC_13	LSFV_13	CL_13	MNE_13	MPE_13	FP_13	SL_13	MCA_13	FPO_13	FPS_13	LAG_13	PH12_13	MBA_13	GR_13	LSE_14	LSC_14	LSFV_14	CL_14	MNE_14	MPE_14	FP_14	SL_14	MCA_14	FPO_14	FPS_14	LAG_14	PH12_14	MBA_14	GR_14	Occurrence
Leuctridae	Leuct	19	1	8	0	24	3	0	3	0	0	0	1	29	0	0	13	2	4	4	1	2	0	0	0	0	0	0	0	0	0	14
Nemouridae	Nemour	31	2	3	3	99	26	0	16	51	0	0	0	72	66	14	45	4	1	58	53	3	0	14	67	0	0	12	3	7	0	22
Perlodidae	Perlod	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2
Chloroperlidae	Chloroper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Hydropsychidae	Hydrops	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	1	0	0	0	3
Polycentropodidae	Polycentr	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2
Rhyachophilidae	Rhyac	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	0	1	5	3	0	0	5	0	0	0	0	1	0	8
Lepidostomatidae	Lepidost	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	3	0	0	5	7	0	0	4
Philopotamidae	Philop	0	2	0	0	8	0	0	1	0	0	0	0	1	1	0	6	0	0	0	0	0	0	0	0	0	0	3	2	0	0	8
Sericostomatidae	Seric	0	0	0	0	0	0	0	2	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	3	0	4
Beraeidae	Baer	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Limnephilidae	Limneph	0	0	124	0	2	4	0	0	0	0	0	0	0	2	0	3	5	28	0	2	1	0	36	2	0	0	3	0	1	0	13
Brachycentridae	Brachyc	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3
Baetidae	Baetidae	1	0	0	14	12	1	0	11	78	0	2	0	0	1	0	6	0	1	1	9	4	0	1	2	0	0	0	0	0	0	15
Heptagenidae	Heptag	2	1	2	1	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	7
Leptophlebiidae	Lept	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1
Scirtidae	Scir	0	0	0	0	0	0	0	0	0	0	0	0	475	2	0	0	0	0	0	0	0	0	0	2	0	0	1	17	0	0	5
Haliplidae	Halipl	0	0	0	0	0	0	0	0	0	5	19	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	0	0	0	0	4
Dytiscidae	Dytisc	2	0	0	0	0	0	0	0	0	0	6	0	3	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	0	7
Limnebiidae	Limneb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2
Helophoridae	Heloph	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	2	22	0	0	0	0	3
Hydrophilidae	Hydroph	0	0	0	0	0	0	0	0	16	1	3	1	1	0	3	0	0	0	0	0	0	0	0	2	0	14	0	0	0	0	8
Hydraenidae	Hydra	0	0	0	0	0	0	0	0	8	0	0	5	15	0	0	0	0	0	0	0	0	0	1	0	0	4	0	0	0	0	5
Gyrinidae	Gyrin	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Elmintidae	Elmint	0	0	0	0	0	1	0	1	21	0	0	1	0	1	0	0	0	0	0	0	0	0	0	16	0	1	0	0	2	0	8
Tipulidae	Tipul	0	0	0	0	1	3	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0	1	0	6
Empididiae	Empid	0	0	0	0	0	0	0	0	0	0	0	0	16	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Chironomidi	Chiron	24	188	188	36	126	7	0	218	334	34	9	4	837	233	0	53	3	31	141	64	159	0	88	29	2	129	36	248	38	0	26
Limoniidae	Limon	0	0	0	3	0	0	0	2	0	0	0	0	2	9	0	3	0	0	0	0	0	0	0	0	0	1	0	0	0	0	6
Strationomidae	Strat	0	0	0	0	0	0	0	2	43	1	1	0	0	0	0	0	0	0	0	0	0	0	18	26	0	4	0	0	0	0	7
Ceratopogonidae	Ceratop	0	0	0	0	3	0	0	1	5	1	3	3	17	0	0	2	1	0	0	0	0	2	0	0	0	1	35	29	0	0	13
Psychodidae	Psych	0	0	0	11	0	0	0	5	0	1	0	0	1	0	0	19	0	0	14	0	0	0	3	1	0	0	0	3	0	0	9
Ptychopteridae	Ptychop	0	0	0	0	0	0	0	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	2
Dixidae	Dix	0	1	0	0	0	0	0	0	0	0	0	0	12	9	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	5
Simulidae	Simul	0	0	0	1	0	0	0	0	23	0	0	0	18	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	4
Tabanidae	Taban	0	0	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Athericidae	Ather	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Muscidae	Musc	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Culicidae	Culic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1
Velidae	Vel	0	0	0	0	1	0	0	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	5
Notonectidae	Noton	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	0	0	0	0	4
Nepidae	Nep	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2
Pleidae	Pleid	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

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	Abbreviation	LSE_13	LSC_13	LSFV_13	CL_13	MNE_13	MPE_13	FP_13	SL_13	MCA_13	FPO_13	FPS_13	LAG_13	PH12_13	MBA_13	GR_13	LSE_14	LSC_14	LSFV_14	CL_14	MNE_14	MPE_14	FP_14	SL_14	MCA_14	FPO_14	FPS_14	LAG_14	PH12_14	MBA_14	GR_14	Occurrence
Naucoridae	Nauc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	0	0	0	0	1
Gerridae	Gerr	0	0	0	0	0	0	0	0	0	6	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	3
Cordulagasteridae	Cordulag	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1
Coenagrionidae	Coenagr	0	0	0	0	0	0	0	0	0	2	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	3
Libellulidae	Libel	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Hirudinea	Hiru	0	0	0	0	0	0	0	0	2	2	2	0	2	0	0	0	0	0	0	0	0	0	2	3	0	2	0	1	0	0	8
Lymnaeidae	Lymn	0	0	0	0	0	0	0	58	0	2	4	2	0	0	0	0	0	0	0	0	0	0	3	3	0	14	2	0	0	0	8
Hydrobiidae	Hydrob	0	0	0	0	0	0	0	0	21	0	0	2	3	83	0	0	0	0	0	0	0	0	0	21	0	0	47	6	26	0	8
Planorbidae	Plan	0	0	0	0	0	0	0	0	0	0	94	0	0	0	0	0	0	0	0	0	0	0	0	0	0	198	0	0	0	0	2
Niphargidae	Nipharg	0	0	0	0	0	0	2	0	11	0	0	2	0	44	0	0	0	0	0	0	3	1	0	5	0	0	3	0	14	0	9
Gammaridae	Gam	0	0	0	0	0	0	0	0	0	155	27	0	0	0	57	0	0	0	0	0	0	0	0	0	378	184	0	0	0	3	6
Asellidae	Asel	0	0	0	0	0	0	0	0	0	421	79	0	0	0	0	0	0	0	0	0	0	0	0	0	471	164	0	0	0	0	4
Taxa richness		7	8	5	8	12	9	1	15	16	14	17	10	20	13	4	14	6	5	7	7	7	2	13	16	8	20	13	16	11	2	

Ostracods

	Abbreviation	LSE_13	LSC_13	LSFV_13	CL_13	MNE_13	MPE_13	FP_13	SL_13	MCA_13	FPO_13	FPS_13	LAG_13	PH12_13	MBA_13	GR_13	LSE_14	LSC_14	LSFV_14	CL_14	MNE_14	MPE_14	FP_14	SL_14	MCA_14	FPO_14	FPS_14	LAG_14	PH12_14	MBA_14	GR_14	Occurrence
Candona gr. *neglecta*	C_gr_neg	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Candona neglecta	C_neg	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Pseudocandona lobipes	Ps_lo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Candonopsis kingsleii	Can_kin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Cypria ophtalmica	Cyp_oph	0	0	0	0	0	0	0	0	0	335	1	0	0	0	0	0	0	0	0	0	0	0	0	0	29	4	0	0	0	0	4
Cyclocypris ovum	Cy_ovu	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	1
Cyclocypris laevis	Cy_lae	0	0	0	0	0	0	0	0	0	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	0	0	0	0	0	2
Notodromas persica	No_pe	0	0	0	0	0	0	0	0	0	62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Herpetocypris reptans	Her_re	0	0	0	0	0	0	0	0	12	0	11	0	0	0	0	0	0	0	0	0	0	0	0	8	0	6	0	0	0	0	4
Prionocypris zenkeri	Pr_ze	0	0	0	0	0	0	0	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0	0	0	3
Psychrodromus sp.	Psy_sp	0	0	0	0	0	0	0	71	0	0	0	0	0	0	0	0	0	0	0	0	0	0	47	0	0	0	0	0	0	0	2
Psychrodromus olivaceus	Psy_ol	0	0	0	0	0	0	0	129	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33	0	0	0	0	0	0	0	2
Psychrodromus fontinalis	Psy_fo	0	0	0	0	0	0	0	0	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2
Psychrodromus betharrami	Psy_be	0	0	0	0	0	0	0	54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	2
Potamocypris pallida	Po_pa	0	0	16	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Species richness		0	0	1	0	1	0	0	4	1	4	3	0	0	1	0	1	0	0	0	0	0	0	3	1	4	4	0	0	1	0	13

Water mites

	Abbreviation	LSE_13	LSC_13	LSFV_13	CL_13	MNE_13	MPE_13	FP_13	SL_13	MCA_13	FPO_13	FPS_13	LAG_13	PH12_13	MBA_13	GR_13	LSE_14	LSC_14	LSFV_14	CL_14	MNE_14	MPE_14	FP_14	SL_14	MCA_14	FPO_14	FPS_14	LAG_14	PH12_14	MBA_14	GR_14	Occurrence
Arrenurus sp.	Ar_sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Atractides brendle	At_br	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Hydrovolzia placophora	Hy_pl	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2
Lebertia sp.	Le_sp	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Lebertia glabra	Le_gl	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Lebertia maglioi	Le_ma	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Partnunia aprutina	Pa_ap	0	0	0	0	1	0	0	0	0	0	0	0	0	5	0	0	0	0	0	2	0	0	0	0	0	0	0	0	17	1	5
Piona disparilis	Pi_di	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Rivobates psammocrenicus	Ri_ps	0	0	0	0	8	2	0	0	0	0	0	0	1	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	4
Sperchon resupinus	Sp_re	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Sperchon thienemanni	Sp_th	0	0	0	0	1	0	0	2	2	0	0	0	11	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	7
Species richness		0	0	0	0	5	3	0	1	1	0	0	0	4	2	0	2	0	0	0	3	0	0	1	1	1	0	0	0	1	1	

Chapter 5

Using ostracod functional traits to assess the role of springs as environmental filters

5.1 Introduction

Springs have been considered peculiar environments, characterised by temperature stability, persistence over geological times, isolation and low concentration of oxygen and nutrients (Glazier et al. 1991; van der Kamp 1995). Springs were described as sites with high occurrence of rare and endemic species and high taxa diversity for bacteria, algae, plants, and invertebrates, and some authors defined them as hotspots of biodiversity (Cantonati et al. 2012; Ilmonen et al. 2012). Some species were found to have in springs their exclusive (crenobionts) or favourite (crenophiles) habitat (Cantonati et al. 2012). Despite these statements, no studies investigated the role of springs as a filter able to select species with particular adaptations to crenic environments. The study of functional traits and functional diversity allow to identify the role of niche filtering in structuring communities. Functional diversity is the facet of biodiversity encompassing the values of "those organismal traits that influence one or more aspect of the functioning of an ecosystem" (Tilman 2001). Those traits were called "functional traits" and defined as "measurable aspect(s) of an organism which impacts its interaction with the environment, its capacity to find and acquire resources, and which therefore affects the fitness of a species" (Flynn et al. 2009). In other words, the differences in functional traits among species in a community are the measure of functional diversity of the community, and reflect some aspects of ecosystem functioning (Petchey & Gaston 2002). Different functional trait distribution may imply the acting of different assembly processes (Petchey et al. 2007). If coexisting species have more similar functional traits than expected by chance (low functional diversity), it means that environmental conditions act as a filter, selecting the presence of species with specific traits (environmental filtering process). On the other hand, competitive exclusion only permits the coexistence of species with different functional traits and a consequent higher functional diversity.

This work is focused on ostracod communities. Ostracods are a relevant part of biodiversity of freshwater ecosystems (Martens et al. 2008) and in particular of springs (Roca & Baltanás 1993; Külköylüoğlu & Vinyard 2000; Bottazzi et al. 2011). Despite many studies analysed ostracod biogeography and biodiversity (e.g. Danielopol et al 1994; Martens et al. 2008), little is known about their functional diversity. A first attempt of identify functional traits of ostracod was made by Marmonier and colleagues (1994), that found a relationship between the use of a particular habitat and a set of species trait modalities. Given the Marmonier et al. (1994) results and the particular environmental characteristics of springs, two hypothesis could be formulated about the assembly processes of ostracods in springs: 1) ostracods may show a lower functional diversity than expected by chance, therefore communities may be subject to environmental filtering process, 2) ostracod functional diversity may be the same that would be expected by chance, therefore springs may not select any particular adaptations.

This work is aimed to:

1. identify functional traits for ostracod species, consistent with their role in the ecosystem,
2. indicate functional groups at regional scale (Emilia Romagna region),
3. understand the assembly processes acting in springs,
4. evaluate the relevance of springs in supporting regional biodiversity.

5.2 Methods

5.2.1 Ostracod community data

The list of species inhabiting different freshwater ecosystems of Emilia Romagna region was based on the Italian checklist of ostracods (Pieri et al. 2015), with recent updates (Pieri, pers. comm.), for a total of 247 sites (rivers, headwaters, lakes, ponds, ditches, peat bogs, and springs). Spring community data were collected in Bottazzi et al. (2011), in this thesis (see chapter 4) with a supplementary survey of the same springs carried on in 2011 by Marco Cantonati and Stefano Segadelli (unpublished data), and from 4 sites sampled in 2006 by Valentina Pieri (Marchiani & Venturelli, 2006), for a total of 25 helocrenic, limnocrenic and rheocrenic springs. Lowland springs (called "fontanili" in the Po lowland) where excluded from the analysis because of their peculiar morphological, ecological and biological characteristics. Species data found in the same spring in different surveys were integrated and all the data were transformed in species presence-absence. When species identification was uncertain (specific name preceded by "cf.": e.g., *Cyclocypris* cf. *helocrenica*), the record was considered as the reference species (e.g., *C. helocrenica*).

5.2.2 Selection of functional traits and modalities

The analyses were focused on biological traits related to characteristics of life cycle, resistance or resilience potential, physiological traits, and behavioural aspects of reproduction and nutrition (Usseglio-Polatera et al. 2000). Significant ostracod traits from Marmonier et al. (1994) were also taken into account. Homogeneous traits (no differences among species) or traits related to poorly known aspects of ostracod biology were eliminated from further analysis. Table 5.1 shows the considered traits. Information on ostracod morphology and biology were collected from Ghetti and McKenzie (1981), Baltanás et al. (1993), Meisch (1993), Rossi et al. (1996), Külköylüoğlu (1998), Meisc (2000), Karanovic and Pesce (2001), Özuluğ (2005), and Díaz and Lopretto (2011). Traits were described with a fuzzy coding procedure (Chevenet et al. 1994). Variables describing traits were separated into 2 or more modalities. A score was assigned to each species describing its affinity to each modality of each trait: "3" when the species has high affinity for that modality, "2" with medium affinity, "1" with low affinity, "0" with no affinity. The fuzzy coding procedure avoids the assignment of a species to a single category for each trait, which could lead to a wrong characterization of species (Chevenet et al. 1994). Missing information about a trait of a species was coded as "0" for all the modalities, and the following analysis weighted as zero this trait for this particular species (Chevenet et al. 1994).

5.2.3 Analysis of functional traits and measure of functional diversity

A fuzzy Correspondence Analysis (FCA, Chevenet et al. 1994) ordered species in a multivariate space based on their trait similarities. This analysis resembles a correspondence analysis of species and

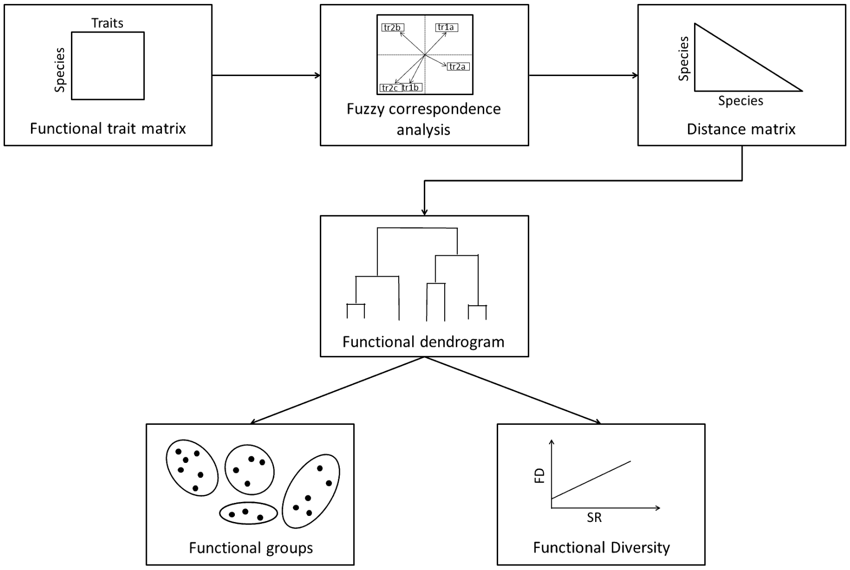

Figure 5.1: Procedure of data analysis.

modalities, where the affinities of taxa for modalities are indicated as frequencies (for a given taxa with three modalities the original code 2/1/0 becomes 0.666/0.333/0 - Usseglio-Polatera et al. 2000). Dissimilarities among species in trait space were calculated as the Euclidean distances of species scores of the first and second axes of FCA. A dendrogram was built based on this dissimilarity matrix, using a cluster analysis with Ward's minimum variance method (Legendre & Legendre 2012). The FCA and the dendrogram allowed the graphical identification of functional groups, namely clusters of species with similar functional trait modalities. Functional Diversity (FD) was measured as the total branch length of the functional dendrogram that link the species of each community, according to Petchey and Gaston (2002). Monospecific communities were eliminated by the analysis because FD cannot be calculated, therefore 13 springs were analysed. The FD observed in springs was compared to FD of ostracod assemblages generated by a null model. Null model was built by random sampling species from the regional pool. The SR of null model was constraint to range from 2 to the maximum SR of the observed communities. Differences in FD between data and null model were detected with a linear model of FD as a function of a factor (null/data) with SR as a covariate. Given that FD is affected by the species richness (SR) (Petchey & Gaston 2002), relationship between FD and SR was explored with a linear regression. Graphical methods were used for model validation (Zuur et al. 2009). Fig. 5.1 shows the diagram of the procedure of functional analysis.

All the analyses were performed with R software (R Core Team, 2013), using the packages vegan (Oksanen et al. 2013), ade4 (Dray & Dufour 2007), and mgcv (Wood 2006).

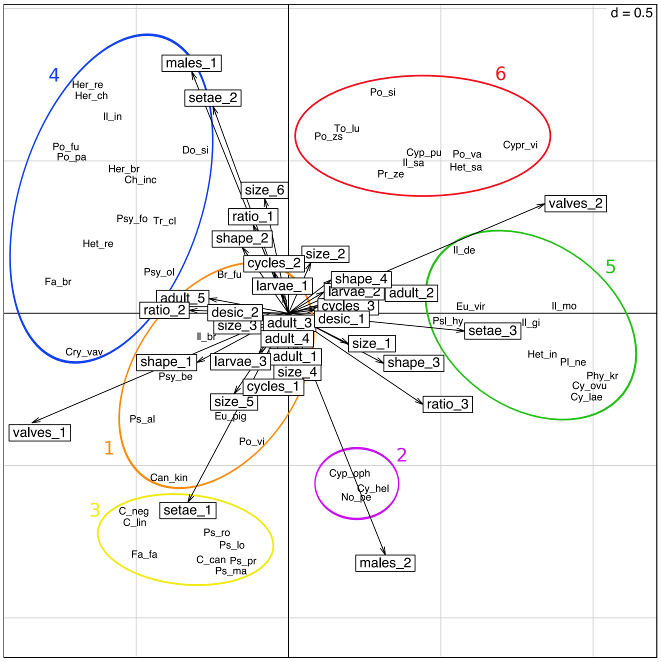

Figure 5.2: Fuzzy correspondence analysis. See tab. 5.1 for species abbreviations.

5.3 Results

Emilia Romagna region hosts 51 species of ostracods, and 25 of them were found in springs. In the region nine species (*Cryptocandona vavrai, Cyclocypris helocrenica, Fabaeformiscandona breuili, Psychrodromus betharrami, P. fontinalis, Potamocypris pallida, P. zschokkei, Pseudocandona rostrata,* and *Pseudolimnocythere hypogaea*) are exclusive of springs. FD measured on 23 species because *F. breuili* and *Pseudocandona albicans* were only present in monospecific communities. Ten functional traits were taken into account, shown in tab. 5.1.

Modalities range from 2 to 6 for each trait. The first and the second axes of FCA (fig. 5.2) account for the 40% of variability. First axis of FCA mainly ordered the species by the valve decoration trait, second axis by the presence of males. Fig. 5.3 shows the functional dendrogram. The six functional groups were indicated in Fig. 5.2 and 5.3 with coloured circles and rectangles respectively. Valve decoration trait splits functional groups in two clusters: decorated and smooth valves. Within the cluster of decorated valve species, the presence of males divided one functional group (group 6) with rare or absent male and two groups with males: one (group 5) characterised by occurrence of larvae in May and June, the other (group 2) by spherical shape, long natatory setae, and height/length ratio > 0.6. Within the cluster of smooth valves species, three functional groups were identified: the first (group 4) with rare or absent male, the second (group 3) with the presence of males, the third (group 1) with no characteristic shared traits. Shared traits within each functional group were shown in tab. 5.2.

The regression between observed SR and observed FD is significant ($p < 0.001$) and SR explained the 73.4% of observed FD. In the linear model of FD as a function of a factor (null/data) with SR as a covariate, the factor resulted not significant ($p = 0.057$ - Fig. 5.4).

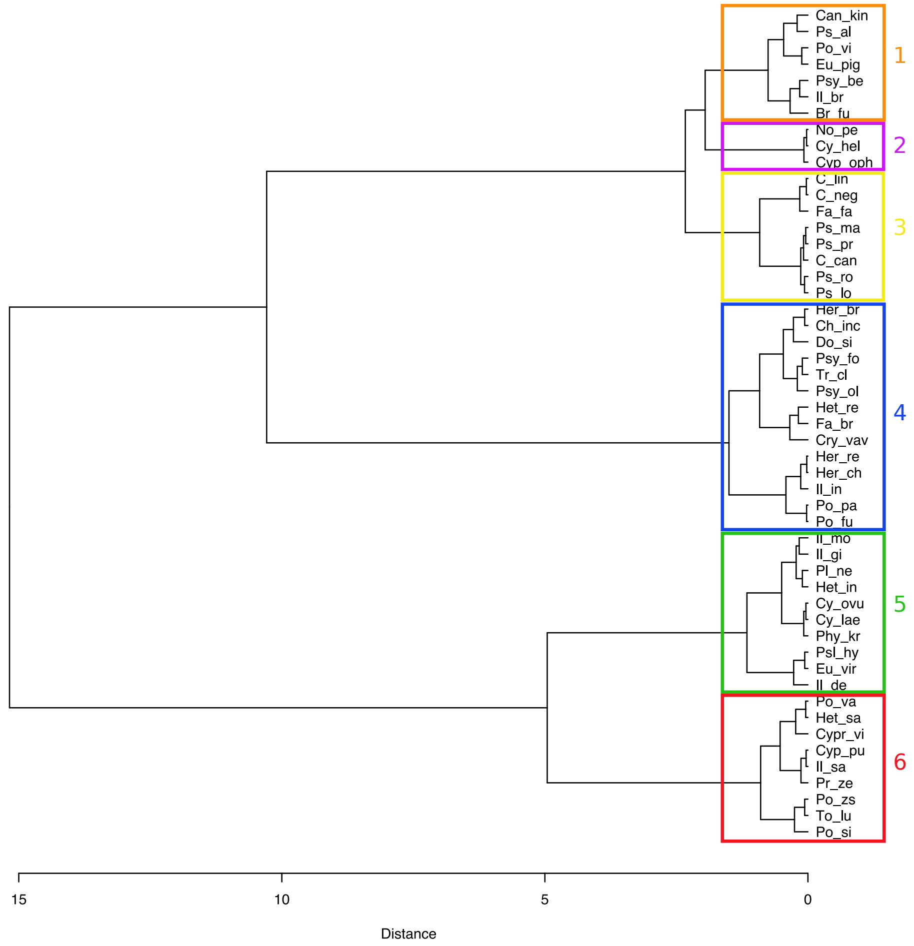

Figure 5.3: Functional dendrogram. See tab. 5.1 for species abbreviations.

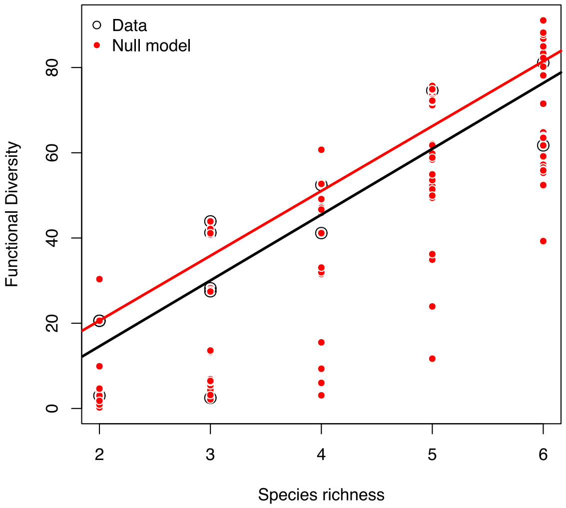

Figure 5.4: Comparisons between observed data and null model.

5.4 Discussion

Compiling a list of functional traits for ostracods is a hard task, mainly because some aspects of life history and behaviour are still unknown for many species. Among the traits proposed by Usseglio-Polatera and colleagues (2000) for aquatic macroinvertebrates, only "size" and "number of reproduction cycles per year" have been taken into account. Within traits analysed by Marmonier and colleagues (1994), "natatory setae length", "body shape", and "tolerance to desiccation" were included in this work. Some traits from both studies, such as "aquatic stages", "dispersal", "resistance forms", "respiration modalities", "locomotion and substrate relation", and "feeding habits" were excluded because all the species showed affinity for the same modality and therefore they were not informative. Marmonier et al. (1994) highlighted the importance of the occurrence of a brooding chamber in some species, which is an important functional traits, but not analysed in the present study because is exclusive of Darwinulidae. Other traits such as "life span", "number of descendants per reproductive cycle", "type of food", and "presence of eyes" (the last one not taken into account by previous studies) could be relevant for describing the functional role of ostracods in ecosystems, but information about these traits are sparse and known for only few species. Instead of focusing on periods of laying and hatching (Marmonier et al. 1994), periods of adults and larvae occurrence should be analysed because information on these traits are more easily found in literature (Meisch 2000). Describing ostracod reproduction with two modalities (sexual/parthenogenesis) is impossible for many species, because findings of population of exclusively parthenogenetic females or lack of males do not inevitably involve a fully parthenogenetic reproduction. I suggest the use of presence of males instead of reproductive modalities (Marmonier 1994) as more correct and significant trait. I introduced two new traits: "height/length ratio of carapace", as a possible indicator of habitat preference (Meisch 2000), and "valves decoration" probably related to environmental characteristics (Ruiz et al. 2013).

This study underline the importance of springs as spots of ostracod biodiversity, hosting in few sites almost the 50% of the regional species pool, and in the studied area several species were found exclusively in those habitat. Springs resulted not to limit functional similarity between co-existing species. This conclusion appears to contradict the assumption of environmental peculiarity of springs. This apparent contradiction could be due to the characteristics of the studied taxon. Given the similar modalities of several aspects of life history and ecology (e.g. life stages, dispersal, resting stages, respiration, locomotion and feeding habits), ostracods represent a high redundant taxon from a functional point of view. Probably, the range of ostracod functional traits is too narrow to make them subjected to spring environmental filtering. For example, several ostracod species show high affinity or tolerance to spring environmental characteristics as low oxygen and low nutrients concentrations (Meisch 2000). Since this is the first study on niche-filtering of springs, comparing different taxa is impossible, but analysis of more sensitive and functional-diverse taxa could lead to different results. Furthermore, linking ostracod functional groups to specific ecological characteristics is not yet possible, and for now they have only descriptive aim.

Species co-existing patterns were mainly determined by deterministic and stochastic processes. In the first ones the physical habitat selects species by their ecological niches (Poff 1997). In the second ones communities are assembled by random dynamics such as for example dispersal limitation, local extinctions, and ecological drift (Hubbel 2001). Several studies assessed the importance of environmental factors on ostracod assemblages in springs (e.g. Roca & Baltanás 1993; Mezquita et al. 1999; Van der Meeren et al. 2010) and the lack of environmental filtering does not contradict these results. My findings lead to conclude that, given the satisfaction of niche requirements of species, the co-existence of ostracod species in springs is probably affected by stochastic drivers as dispersal, speciation and local extinctions (Hubbell 2001). This imply also that the nearly exclusive presence of some species in springs or the peculiar species compositions of crenic communities (Cantonati et al. 2012; Rosati et al. 2015) may be due to biogeographical reasons rather then peculiar ecological characteristics of springs, although more studies at larger scale are needed.

Table 5.1: Functional traits and modalities of species from Emilia Romagna The length of natatory setae was described by three modalities: "none" for lack of setae, "short" when the setae reach the distal claws of antennae, or if it is shorter, and "long" when setae go beyond the terminal claws.

		Potentital size (female)						Potential number of reproductive cycles per year			Period of adult occurrence					Period of larvae appearance			Presence of males		A2 natatory setae length			Body shape				Height/lenght ratio			Valves decoration		Tolerance to dessication	
		1	2	3	4	5	6	1	2	3	1	2	3	4	5	1	2	3	1	2	1	2	3	1	2	3	4	1	2	3	1	2	1	2
		≤ 0.5 mm	> 0.5-0.7 mm	> 0.7-0.9 mm	> 0.9-1.1 mm	> 1.1-1.3 mm	> 1.3 mm	< 2	2	> 2	spring	summer	autumn	winter	All the year	Nov-Feb	Mar-Jun	Jul-Oct	No/rare	Yes	None	Short	Long	Streamlined	Elongated	Spherical	Geometric	≤ 0.4	> 0.4-0.6	>0.6	No	Yes	No	Yes
Candona candida	C_can					3		3							3		3			3	3			3						3	3			3
Candona neglecta	C_neg					3			3						3		2	2		3	3			3					3		3			3
Candona lindneri	C_lin					3									3		2	2		3	3			3					3		3			3
Fabaeformiscandona fabaeformis	Fa_fa				2	2		3			3							3		3	3			3					3		3			3
Fabaeformiscandona breuili	Fa_br		3												3		2	2	3		3			3					3		3			3
Pseudocandona lobipes	Ps_lo			3							2		2	2			2	2		3	3			3						3	3			
Pseudocandona pratensis	Ps_pr				2	2		3			2	1					3			3	3			3						3	3			3
Pseudocandona cf. *marchica*	Ps_ma				3			3			2	1					1	2		3	3			3						3	3			3
Pseudocandona rostrata	Ps_ro					3			3						3		3			3	3			3						3	3			3
Pseudocandona cf. *albicans*	Ps_al			3				3							3			3	2	1	3			3						3	3			3
Cryptocandona vavrai	Cry_vav			3					3						3				2	1	3			3					3		3			3
Candonopsis kingsleii	Can_kin				3				3						3					3	3			3				3			3			
Cypria ophthalmica	Cyp_oph		3						2	2					3	1	2	2		3			3			3				3	3			3
Physocypria kraepelini	Phy_kr		3												3		2	2		3			3			3				3		3	3	
Chlamydotheca incisa	Ch_inc						3												3			3		3						3	3			3
Cyclocypris helocrenica	Cy_hel	3																		3			3			3				3	3			
Cyclocypris laevis	Cy_lae	3						2	1						3		2	1		3			3			3				3		3		3
Cyclocypris ovum	Cy_ovu	3							2	2					3	1	2	2		3			3			3				3		3		3
Ilyocypris gibba	Il_gi				3				3		2	2	2				2	2		3			3	3			3	3				3		3
Ilyocypris monstrifica	Il_mo			3								3								3			3				3	3				3	3	
Ilyocypris decipiens	Il_de				3							3					2	2		3		3					3	3				3		3
Ilyocypris bradyi	Il_br			1	2				3						3					3		3					3	3			3			
Ilyocypris inermis	Il_in			3					3						3		2	2	3			3					3	3			3			
Ilyocypris salebrosa	Il_sa				3																	3					3	3				3		
Cypris pubera	Cyp_pu						3	2	1		2	2					3		2	1		3		3						3		3		3
Notodromas persica	No_pe				2	2			3		2	2	2				2	2		3			3			3				3	3			3
Eucypris pigra	Eu_pig			2	2			1	2						3		3			3		3		3						3	3			3
Eucypris virens	Eu_vir						3	2	1		3					2	2			3		3		3						3		3		3
Prionocypris zenkeri	Pr_ze					3			3						3		2	2	2	1		3		3						3		3		
Tonnacypris lutaria	To_lu						3	2	1		3						3		2	1		3		3					3			3		3
Trajancypris clavata	Tr_cl						3	2	1		2	2	1				3		3				3	3					3		3			3
Bradleystrandesia fuscata	Br_fu					1	2	3			3						3		3				3	3						3	3			3

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		Potentital size (female)						Potential number of reproductive cycles per year			Period of adult occurrence					Period of larvae appearance			Presence of males		A2 natatory setae length			Body shape				Height/lenght ratio			Valves decoration		Tolerance to dessication	
		1	2	3	4	5	6	1	2	3	1	2	3	4	5	1	2	3	1	2	1	2	3	1	2	3	4	1	2	3	1	2	1	2
		≤ 0.5 mm	> 0.5-0.7 mm	> 0.7-0.9 mm	> 0.9-1.1 mm	> 1.1-1.3 mm	> 1.3 mm	< 2	2	> 2	spring	summer	autumn	winter	All the year	Nov-Feb	Mar-Jun	Jul-Oct	No/rare	Yes	None	Short	Long	Streamlined	Elongated	Spherical	Geometric	≤ 0.4	> 0.4-0.6	>0.6	No	Yes	No	Yes
Herpetocypris brevicaudata	Her_br						3								3				2	1		3			3			3			3			
Herpetocypris chevreuxi	Her_ch						3								3				3			3			3			3			3			3
Herpetocypris reptans	Her_re						3		3						3		2	2	3			3			3			3			3			3
Psychrodromus betharrami	Psy_be					3														3		3		3				3			3			
Psychrodromus fontinalis	Psy_fo				1	3									3				2	1		3		3				3			3		3	
Psychrodromus olivaceus	Psy_ol				1	3			3						3		2	2	2	1		3		3						3	3			
Heterocypris incongruens	Het_in						3		2	1					3		3			3			3	3						3		3		3
Heterocypris reptans	Het_re				2	2			3								2	2	2	1		3		3					3		3			
Heterocypris salina	Het_sa				2	2				3					3		3		3				3	3						3		3		
Dolerocypris sinensis	Do_si						3		3			2	2				3		3				3		3			3			3			3
Cypridopsis vidua	Cypr_vi		3						3			3					2	1	3				3			3				3		3		3
Plesiocypridopsis newtoni	Pl_ne		2	2					3			3					3			3			3	3						3		3		
Potamocypris fulva	Po_fu		3												3	3			3			3		3					3		3			
Potamocypris pallida	Po_pa		3												3				3			3		3					3		3			
Potamocypris similis	Po_si		2	1															3			3		3						3		3		
Potamocypris variegata	Po_va		3					2	2								3		3				3	3						3		3		
Potamocypris villosa	Po_vi		2	1					3						3		2	2		3			3	3					3		3		3	
Potamocypris zschokkei	Po_zs		2	2											3	2	2	2	2	1		3		3					3			3		
Pseudolimnocythere hypogea	Psl_hy	3																		3							3		3			3		

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Chapter 6

Ostracod diversity in Western Palearctic springs

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6.1 Introduction

Springs are relevant environments for ecological studies. They are multiple ecotones that link aquatic (ground, surface, and interstitial waters) and terrestrial ecosystems (Scarsbrook et al. 2007; Cantonati et al. 2012), and they often show various microhabitats at a small spatial scale. This microhabitat-mosaic structure may sustain high species richness, and therefore springs are sometimes referred to as biodiversity hotspots (Cantonati et al. 2006, 2012). Furthermore, springs can be considered distributional islands due to their spatial isolation, where among-springs dispersal is expected to be limited, although with marked differences among taxa (Stutz et al. 2010).

Compared to other aquatic ecosystems, most permanent freshwater springs, and associated aquifers, have a greater physical and chemical stability (Glazier 1991; Williams 1991), albeit these features vary among spring types (Scarsbrook et al. 2007). Springs and their aquifers have persisted over geological times through significant climate fluctuations, acting as refugia for aquatic fauna and as potential speciation sites (Cantonati et al. 2012).

Classifying spring typologies is a rather difficult task, that should merge information from hydrogeology, ecology, and biology (Springer & Stevens 2009). Those springs the temperature of which approaches the mean annual air temperature (MAAT) in the drainage basin, have traditionally been called cold springs, but it was recommended that they be renamed ambient springs (sometimes also called non-thermal springs), because cold springs should be those that have temperatures below the MAAT (Glazier 2009; Cantonati et al. 2014). The traditional ecomorphological classification of Steinmann (1915) and Thienemann (1922) is based on flow velocity at the source: in limnocrenic springs water is almost still and creates a pond, helocrenic springs have weak currents and form a swampy zone, whereas in rheocrenic springs the water flows away with rapid currents (Cantonati et al. 2006). This classification is the most widespread, because it is easy to use and informative but, following in-depth analysis, most springs tend to belong to intermediate types (Cantonati et al. 2006).

Ostracods (Ostracoda, Crustacea) are commonly found in springs, although with different frequencies and abundances (e.g., Forester 1991; Roca & Baltanás 1993; Mezquita et al. 1999; Bottazzi et al. 2008; Külköylüoğlu et al. 2012a). They may even be the most abundant invertebrate taxon (Särkkä et al. 1997; Bottazzi et al. 2011). Previous investigations, aimed at analysing the ecological determinants of ostracod-assemblage composition in different spring types, have been performed at various spatial scales and in different biogeographic areas (e.g., Forester 1991; Mezquita et al. 1999; Külköylüoğlu & Vinyard 2000; Bottazzi et al. 2008). Many environmental constraints have been taken into account to understand their role in shaping ostracod communities: Among others, water chemistry, pH or conductivity (Roca & Baltanás 1993; Mezquita et al. 1999; Mezquita et al. 2000; Pieri et al. 2007; Van der Meeren et al. 2010; Peterson et al. 2013), water flow and temperature (Roca & Baltanás 1993; Mezquita et al. 1999; Van der Meeren et al. 2010; Stoch et al. 2011; Külköylüoğlu et al. 2012a, b), type and complexity of substrata and habitats (Hahn 2000; Staudacher & Füreder 2007; Bottazzi et al. 2011; Külköylüoğlu et al. 2012b, c).

In this study we reviewed the current knowledge on diversity and distribution of ostracod faunas associated with Palearctic ambient springs and on the ecological factors that have been reported to affect their occurrence. The work is aimed at identifying biogeographical patterns of ostracods at continental and regional scales, and revealing environmental gradients that shape ostracod communities in a multi-scale approach.

6.2 Methods

We obtained data on ostracods in ambient springs located in the Western Palearctic (the study area is shown in fig. 6.1) from 37 studies (articles from scientific journals, published and unpublished reports, master and PhD theses; see Appendix 1 for references). More detailed information was available in 20 studies considering 302 springs from the Central-eastern Alps and Northern Apennines. Potential ecological gradients are less hidden by longitudinal and latitudinal gradients at relatively small spatial scales than at larger ones. For these reasons we conducted a more extensive analysis, hereafter mentioned as "focus study", based on this area.

Only occurrence data (i.e. presence-only) were used. Undetermined species or taxa identified at a supra-generic level were excluded from the analysis. Records of species groups were considered as an undetermined species of the same genus (for example, *Candona* gr. *neglecta* was included in the data base as *Candona* sp.). When species identification was uncertain (specific name preceded by "cf.": e.g., *Cyclocypris* cf. *helocrenica*), the record was kept only when the reference species (e.g., *Cyclocypris helocrenica*) was not found in the same spring. Species names were standardised following Martens and Savatenalinton (2011).

Environmental data were recorded whenever available. Units of measurement were standardised. Conductivity was compensated at 25 °C. When more than one sampling was performed in the same spring, the mean value of each variable was calculated. All values below instrumental thresholds were set to zero. When reported, geographic coordinates were recorded directly from the studies, whenever necessary they were obtained by processing provided maps with Quantum GIS software (Quantum GIS Development Team 2012).

Springs were assigned to freshwater ecoregions (Freshwater Ecoregions Of the World - FEOW) according to Abell et al. (2008). Unlike other classifications, e.g. Illies (1978), FEOW provide information on the entire study area. Furthermore, ecological and evolutionary processes are taken into account in the FEOW classification. Original FEOW names were used, except that the "Gulf of Venice Drainage" ecoregion is here referred to as "Northern Italy" ecoregion.

Statistical analyses were performed using the R software, version 3.0.0 (R Development Core Team 2013), and vegan package version 2.0-7 (Oksanen et al. 2013). We compared the species richness of limnocrenes, helocrenes, and rheocrenes with one-tailed or two-tailed t-tests. The main patterns in ostracod-community compositions were revealed using Correspondence Analysis (CA), an ordination method that applies to presence-absence data, and allows to extract gradients of community composition (Greenacre & Primicerio 2013). In order to relate the compositional variation to environmental gradients, a Canonical Correspondence Analysis (CCA) was performed.

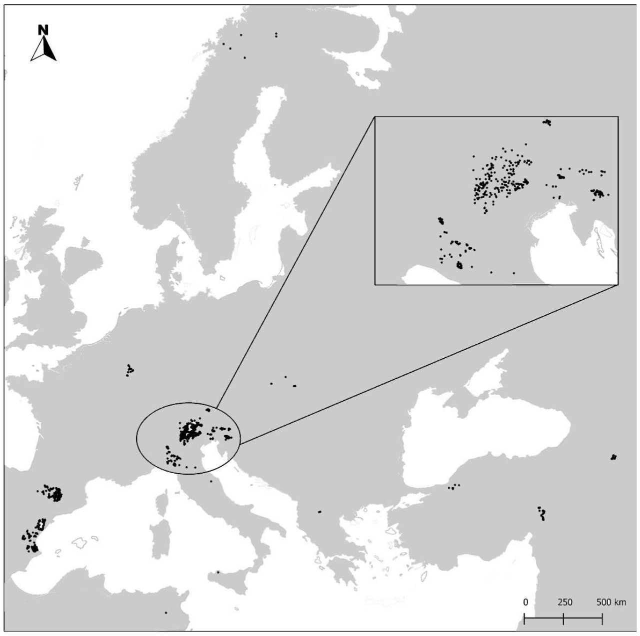

Figure 6.1: Map showing locations of the sites studied. Springs analysed in the focus study are shown in the square.

The CCA allowed to estimate the variation accounted for by selected environmental parameters, and to test for the significance of the association between environmental characteristics and community variation via permutation (Greenacre & Primicerio 2013). In particular, permutation tests (1000 permutations) were used to assess the statistical significance of the models and the explanatory variables (Legendre & Legendre 2012). Species forming only mono-specific assemblages and found in ≤ 3 sites across the study area were excluded from the CA and CCA analysis to reduce the influence of singleton species on the results. The CA was performed on the data from all the springs where ostracods were found, excluding those with only undetermined taxa. As suggested by Legendre and Legendre (2012), we used two procedures to set the limit to the axes that represent interesting variation of the data, namely the Kaiser-Guttman criterion and the Broken-Stick Model. The CCA analysed the following environmental factors: altitude, latitude, spring type, and water temperature. The CCA of the focus study included also pH and conductivity. Both CCAs were conducted on subsets of the database, including 178 and 145 springs respectively, since environmental data were not available for all sites. A preliminary test had shown that water temperature was significantly correlated to altitude and latitude. For this reason, we calculated the residuals of linear regressions of water temperature as a function of latitude and altitude, then we performed CCAs using these residuals instead of water temperature data. Spring type was analysed as a factor variable (Rheocrene, Limnocrene, Helocrene and intermediate types). In the CCA plots, dispersion ellipses were drawn around the class factor centroid, using standard deviation of point site scores (function ordiellipse of the R vegan package). Rheo-limnocrenic and Rheo-helocrenic springs were considered Rheocrenes in this case. Due to some features of the data set (presence-absence data, species-poor sites, and high compositional heterogeneity even at the regional scale) we expected a very high data variability. Therefore the possibility of accounting for a large proportion of variation using few axes was quite low. On the other hand, large-scale studies usually allow to interpret a relatively low fraction of the observed variability.

6.3 Results

The review yielded information on 743 springs of the Palearctic region, with ostracods occurring in 612 sites. After removing springs in which ostracods were found but species were undetermined, the number of springs was reduced to 598. Spring type was available for 333 of these. In detail: 111 springs were classified as helocrenic, 119 as rheocrenic, 76 as limnocrenic, 23 as rheo-helocrenic, and 4 as rheo-limnocrenic.

The 598 analysed springs (fig. 6.1) were included in 13 freshwater ecoregions (FEOW): Northern Italy (240 springs), Eastern Iberia (211), Lower Danube (54), Southern Anatolia (32), Central and Western Europe (15), Upper Danube (14), Lake Van (13), Northern Anatolia (10), Northern Baltic Drainage (3), Vardar (2), Barent Sea Drainage (2), Mediterranean Northwest Africa (1), Norwegian Sea Drainage (1).

Altogether, 106 ostracod taxa were identified: 91 species in 31 genera, and 15 taxa at the generic level (list of taxa and abbreviations used in the figures are in Appendix 2). Fifty-nine species were exclusive of the Palearctic region (Martens et al. 2008). Fig. 6.2 shows the species found in more than 20 sites, and their relative frequency. See Appendix 2 for the frequencies of all the species. The most common species was *Candona neglecta*, found in 99 springs, with higher occurrence in Eastern Iberia (46 springs) and Northern Italy (23 springs). *Cypria ophtalmica* occurred in 82 springs, located in five ecoregions (Northern Italy, Eastern Iberia, Upper Danube, Southern Anatolia, and Central and Western Europe). On the other hand, several species had very low frequencies: 26 species occurred in just one spring, 11 species in two springs. Considering the spring types, *C. neglecta* was the most frequent species in helocrenes (49 springs), *Cypria ophtalmica* in limnocrenic springs (55), and *Psychrodromus fontinalis* in rheocrenes (29). Species richness varied from 1 to 9. The mean species richness per spring was 2.3 ś 1.5. Rheo-limnocrenic and rheo-helocrenic springs had a mean species richness of 4.7 ś 2.7 and 3.3 ś 2.4, respectively. Helocrenes' (2.1 ś 1.2) and rheocrenes' (2.1 ś 1.4) mean species richness were not significantly different ($t = -0.095$, $df = 225.196$, $p = 0.920$); on the other hand, mean species richness was greater in limnocrenes (2.8 ś 1.4) than in both helocrenes and rheocrenes ($t = 3.237$, $df = 139.719$, $p < 0.001$ and $t = 2.997$, $df = 158.003$, $p = 0.002$, respectively).

The first axis of the CA accounted for 2.5% of the total variability but it was dominated by rare species hiding the biogeographical patterns. The second and the third axis accounted for 2.4% each. Both the Kaiser-Guttman criterion and the Broken-Stick Model assessed that the first three axes were interpretable. Fig. 6.3 shows the ordination of springs in the biplot of the second and third CA axes; spring ecoregions are represented by different symbols, and the position of the biogeographically most interesting species is also shown. Along the second axis (biplot in the upper panel of fig. 6.3) three distinct groups of springs are detectable: those belonging to Turkish ecoregions (Northern Anatolia, Southern Anatolia, Lake Van), Northern Italy, and Lower Danube (mainly located in Slovenia). In particular, Slovenian springs are characterised by the following species: *Mixtacandona chappuisi, M. laisi, M. lattingerae, Fabaeformis aemonae, Cypria bicolor, C. reptans, C. sketi,* and *Pseudocandona cavicola.* Along the third axis (biplot in the lower panel of fig. 6.3), a gradient from Central and Northern Europe (Barent Sea Drainage, Northern Baltic Drainage, Norwegian Sea Drainage, Upper Danube, Central and Western Europe), to Macedonia (Vardar ecoregion), through Eastern Iberia and Tunisia (Mediterranean Northwest Africa), is identifiable. This latter is characterised by *Candona peterseni, C. strumicae, Pseudocandona sarsi,* and *Psychrodromus peristericus.*

Data on altitude, water temperature, latitude, and spring type were available for 178 springs. Altitude varied from 28 to 2153 m a.s.l. (mean = 856 ś 671 m a.s.l.), and 47% of the springs were above 1000 m a.s.l. Water temperature range was 1.1-23.6 °C

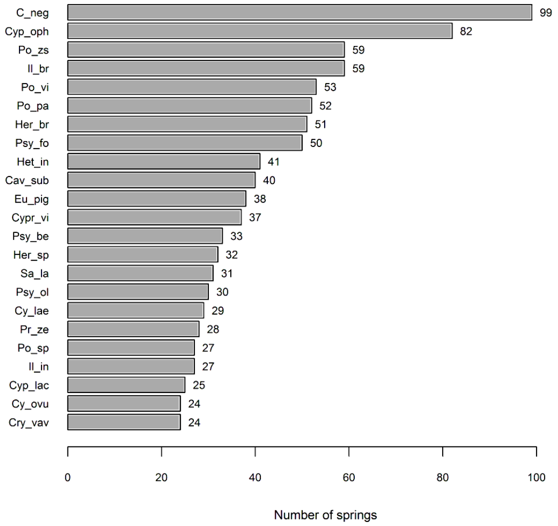

Figure 6.2: Frequency of the most represented species in the springs studied. The number of springs in which each ostracod species occur is indicated as well. (See Appendix 2 for taxa abbreviations).

(mean = 9.9 ± 4.6 °C). Figures 6.4 and 6.5 show the CCA biplots for species and sites, respectively. The model accounted for 11.5% of the total variance, and was statistically significant (F = 3.40; p = 0.001). The first CCA axis explained 4.7% and the second axis 2.5% of the variance. The model was dominated by altitude (F = 8.83; p = 0.005), and latitude (F = 5.20; p = 0.001). The CCA species biplot (Fig. 6.4) shows that *Cyclocypris* sp. and *C. helocrenica* were found at higher latitudes, and *Fabaeformiscandona tyrolensis* at higher altitudes. Furthermore, *Chlamydotheca incisa*, *Herpetocypris* sp., *H. reptans*, *Heterocypris reptans*, and *Pseudocandona* sp. were related to springs with the highest water temperatures, whereas *Pseudocandona rostrata*, and *P. marchica* preferred the coldest sites. In the focus study, ostracods were found in 224 out of 302 springs, while environmental data (altitude, water temperature, latitude, spring type, pH, and conductivity) were available for 145 springs. pH ranged from 3.07 to 11.20, (mean = 7.43 ś 0.48), and conductivity values (at 25 °C) varied from 11 to 2356 µS/cm (mean = 385 ś 382 µS/cm). Also the CCA model performed on this data set was significant (F = 2.55; p = 0.001) and accounted for 13.4% of the total variance. Figures 6.6 and 6.7 show the species and the spring biplots, respectively. The first CCA axis explained 4.9% and the second axis 1.9% of the variance. Tested by permutation, altitude (F = 7.17; p = 0.001) and water temperature (F = 2.39; p = 0.001) appear to be the most influential variables; among the tested variables, only pH was not significant (F = 1.97; p = 0.065), but pH was selected as explanatory variable by the forward selection. The CCA diagrams show relatively discrete groups of species arranged near the centroids of spring typologies. Species as *Psychrodromus fontinalis, P. betharrami,* and *Cryptocandona vavrai* are linked to rheocrenic springs, *Candona neglecta, C. lindneri,* and *Pseudolimnocythere hypogaea* to helocrenes, and *Ilyocypris inopinata* and *Cypridopsis vidua* to limnocrenes.

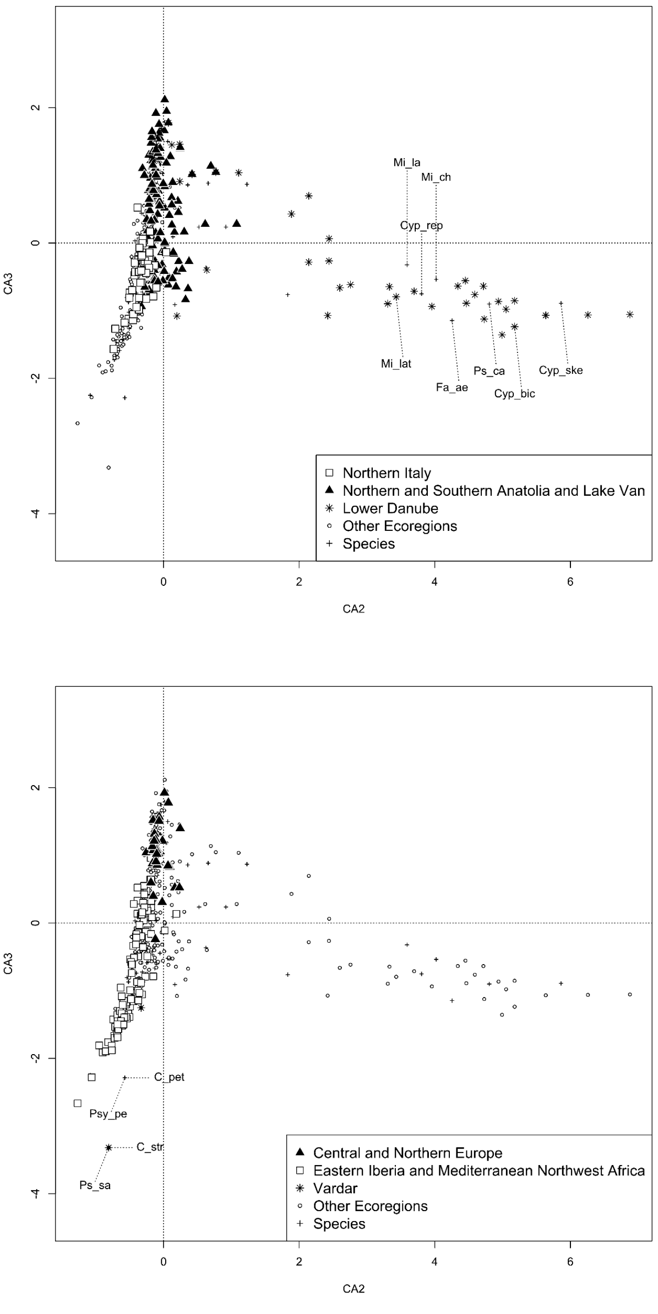

Figure 6.3: CA biplot of the second (eigenvalue = 0.852; explained variation = 2.4%) and third (0.844; 2.4%) axis. First axis (0.894; 2.5%) is not shown because rare species hamper the delineation of geographic patterns. For greater clarity, the biplot is given twice with different symbols indicating distinct ecogeographical regions. (See Appendix 2 for taxa abbreviations).

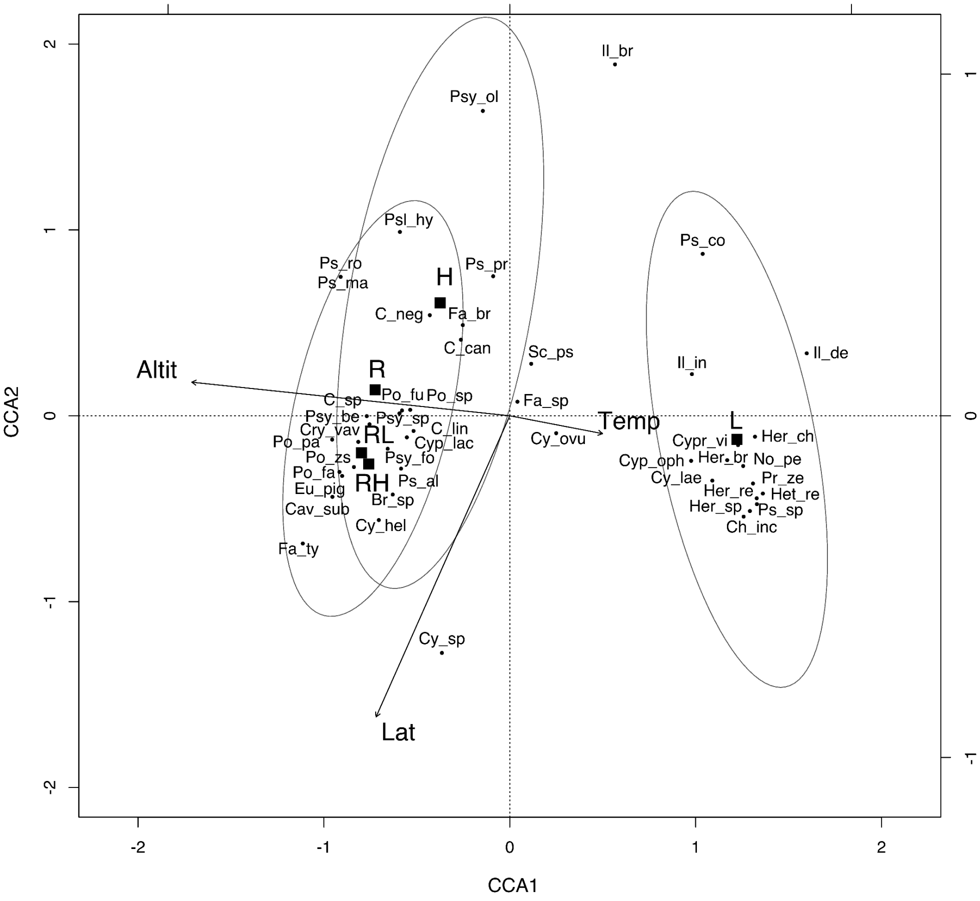

Figure 6.4: Biplot of the species scores and of the environmental variables of CCA on the entire database. CCA1: eigenvalue = 0.784; explained variation = 4.7%. CCA2: eigenvalue = 0.421; explained variation = 2.5%. Ellipses indicate groups of species arranged near the centroids of spring types. (Altit = altitude; Lat = latitude; Temp = water temperature; L = limnocrene; H = helocrene; R = rheocrene ; RL = rheo-limnocrene; RH = rheo-helocrene; see Appendix 2 for taxa abbreviations).

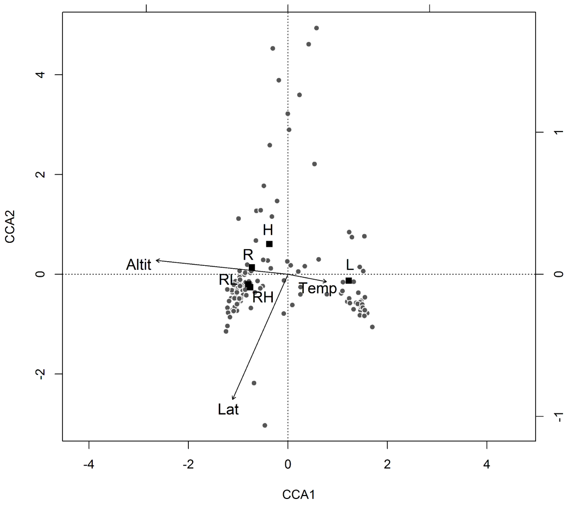

Figure 6.5: Biplot of the site scores and the environmental variables of the CCA on the entire database CCA1: eigenvalue = 0.784; explained variation = 4.7%. CCA2: eigenvalue = 0.421; explained variation = 2.5%. Ellipses indicate groups of springs arranged near the centroids of spring types. (Abbreviations are as in fig. 6.4).

6.4 Discussion

Candona neglecta and *Cypria ophtalmica* are the most commonly found species in Palearctic springs. These species show a widespread distribution in different freshwater ecosystems. *Candona neglecta* is very frequent in cold waters, while *Cypria ophtalmica* is remarkably tolerant to a wide range of environmental factors (Meisch 2000).

Several species occurred only in very few springs of the study area. Some of them can be considered as occasional records, for instance, *Metacypris cordata, Paralimnocythere psammophila*, and *Limnocythere inopinata* are usually inhabitants of lakes or lotic habitats (Meisch 2000). Some are truly stygobiontic or stygophilous species, as *Fabaeformiscandona brisiaca, F. latens,* and *Cryptocandona phreaticola* (Meisch 2000), rarely collected in surface waters. Other ostracod species with extremely low frequency (one or two springs) are considered rare within the sampled area. For example *Mixtacandona lattingerae* could be considered a Balkan endemism: It was found in one Slovenian spring, and previously it has been only reported from an alluvial aquifer in Croatia (Rogulj and Danielopol 1993). So far, *Psychrodromus tunisicus* is only known from its type locality, a spring in Northern Tunisia (Zaibi et al. 2013). The congeneric *P. peristericus*, recorded in two springs, is restricted to the Perister Mountains, Macedonia (Petkovski & Meisch 1995).

Other endemic ostracods are relatively common in the restricted geographic areas that they colonise. For example, *Fabaeformiscandona aemonae* is widely distributed in the karstic areas of South Slovenia, but it has never been recorded elsewhere (Mori et al. 2011), and *F. tyrolensis*, first described from Austria (Meisch 2000), has recently been found in several springs in the Italian Alps (Sambugar et al. 2006). A high frequency of endemic and rare species in springs is reported in many studies. Martin and Brunke (2012), for example, stated that the invertebrate fauna in most springs is dominated

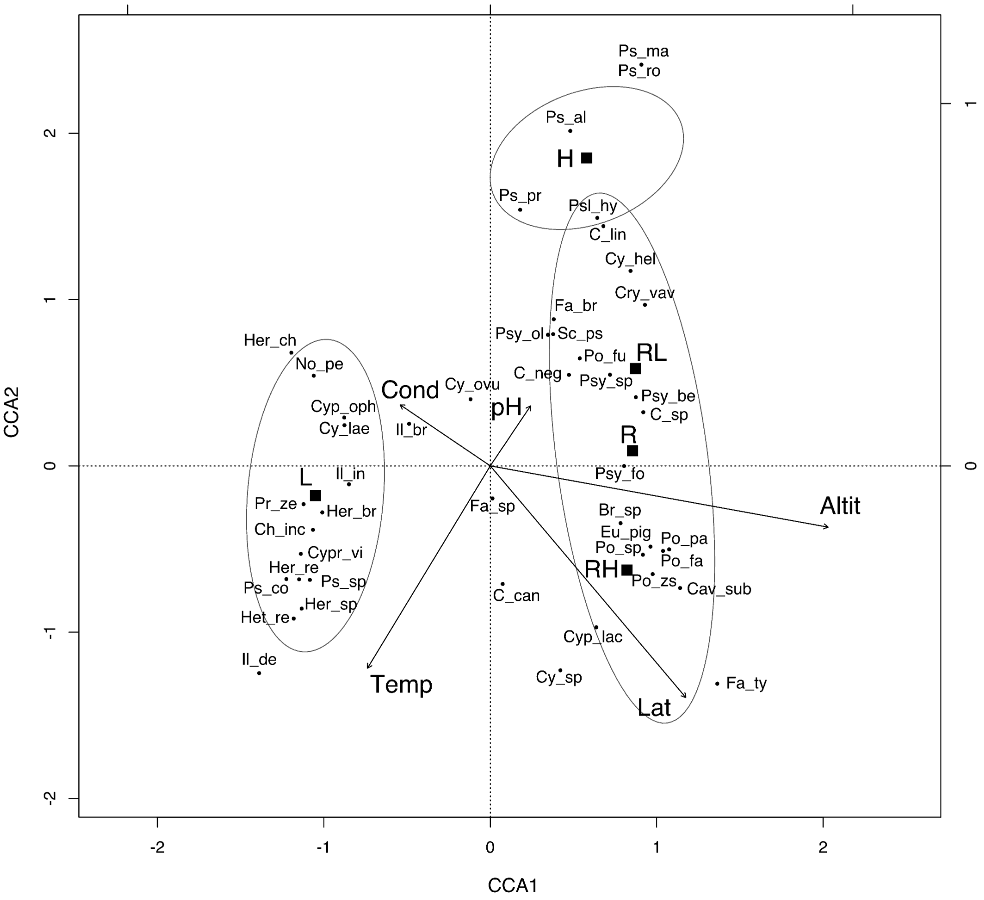

Figure 6.6: Biplot of the species scores and of the environmental variables of the CCA on the focus study data. CCA1: eigenvalue = 0.832; explained variation = 4.9%. CCA2: eigenvalue = 0.327; explained variation = 1.9%. Ellipses indicate groups of species arranged near the centroids of spring types. (See Appendix 2 for the species abbreviations; other abbreviations are as in fig. 6.4).

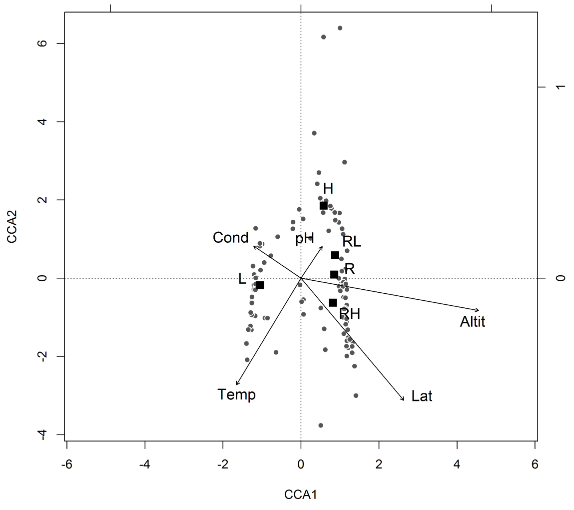

Figure 6.7: : Biplot of the site scores and of the environmental variables of the CCA on the focus study data. CCA1: eigenvalue = 0.832; explained variation = 4.9%. CCA2: eigenvalue = 0.327; explained variation = 1.9%. Ellipses indicate groups of springs arranged near the centroids of spring types. (Abbreviations are as in fig. 6.4).

by a few abundant species and several rare species. This feature assigns to springs a key role in biodiversity conservation (Cantonati et al 2006; Di Sabatino et al. 2003). *Chlamydotheca incisa* also occurred in one spring, and it was the only non-indigenous/naturalised species found in this study. Other freshwater ecosystems seem to host higher numbers of non-native ostracods than springs, although the information on their distribution and invasiveness is based on sparse data (Escrivà et al. 2012). According to Gherardi et al. (2008), ostracods were the most frequent non-indigenous invertebrate taxa in Italian inland waters; for the Iberian Peninsula, García-Berthou et al. (2007) reported eight exotic or naturalised species in rice-fields, three in ponds and pools, and two in lakes and reservoirs. Therefore, springs could be less prone to invasion by exotic taxa because of their environmental harshness and isolation from potential colonists' sources.

Our study revealed that ostracod species richness in limnocrenic springs was significantly greater than in rheocrenes or helocrenes. Different crenobiological investigations report contrasting results about preferences of different taxonomic groups for specific spring types. For example, helocrenic springs hosted a greater diversity of meio- and macrofaunal taxa than other spring types in the study by Gerecke et al. (2011) and Spitale et al. (2012), while Martin and Brunke (2012) found the highest taxonomic richness in rheocrenic springs. Our result may be due to a marked prevalence (62 out of 76) among the limnocrenes included in our database of peculiar springs which are common in Northern Italy floodplains and are locally known as "fontanili". In these areas where water naturally emerges from the ground, relatively deep and large ponds are dug to facilitate groundwater abstraction (Rossetti et al. 2005). Most of these ecosystems are therefore characterised by a variety of lentic (micro)-habitats, a condition that may favour the development of more diverse ostracod communities than in other spring types. Ostracod community compositions in springs clearly show geographical gradients, as highlighted by the CA results. The presence of endemic species characterises specific regions. Slovenian springs host some species, mainly stygophilous or stygobiontic, exclusively distributed in the Balkans, for example *Mixtacandona chappuisi, M. laisi, M. lattingerae,* and *Fabaeformis aemonae* (Meisch 2000). Species as *Candona strumicae* and *Pseudocandona sarsi* were only found in Macedonian springs.

The CCA confirmed the existence of environmental gradients that influence species composition of the ostracod communities in springs both at the continental and at the regional scale. At the regional scale, the observed variation is partly organised along altitude, latitude, and water temperature. Van der Meeren et al. (2010), Stoch et al. (2011), and Külköylüoğlu et al. (2012a) stressed the importance of altitude for ostracod community composition. Several studies highlighted the importance of water temperature in shaping ostracod communities (Mezquita et al. 1999; Van der Meeren et al. 2010; Bottazzi et al. 2011; Külköylüoğlu et al. 2012a, b; Külköylüoğlu & Sarı 2012). At a smaller scale, as shown by other studies (Külköylüoğlu 2005; Pieri et al. 2007; Külköylüoğlu et al. 2012c; Külköylüoğlu & Sarı. 2012), we could also point out a conductivity gradient. Despite several studies emphasising the importance of pH in structuring ostracod communities in springs (Hahn 2000; Bottazzi et al. 2011; Külköylüoğlu et al. 2012c; Külköylüoğlu & Sarı 2012), pH resulted to be non-influential in organising the variation of spring assemblages in the focus area. Spring types affect ostracod-assemblage composition too. Substrata, presence of different microhabitats, water discharge and flow velocity vary between spring types (Martin & Brunke 2012), and they may cause differences in faunal composition. The role of spring type in shaping invertebrate communities was highlighted for macrozoobenthic component of the spring biota (Hahn 2000; Zollhöfer et al. 2000), but little is known about ostracods. Külköylüoğlu & Yılmaz (2006) described differences in ostracod species composition between three springs (rheocrene, limnocrene, and helocrene), but the number of studied sites is too little to reliably detect patterns. On the other hand, Spitale et al. (2012) found that the diversity of meiofaunal organisms differed among spring types but, compared to other taxa, the classification explained little variability for the composition of ostracod communities.

Our analysis was conducted on few environmental factors that were usually available in the bibliographic sources reviewed but, as already reported in the Introduction, it seems likely that other factors are important in the assembly processes too.

6.5 Conclusions

Spring ostracod communities show clear biogeographical patterns, and vary along environmental gradients. Species composition varies along a geographical gradient, and the observed variation is partly organised along altitude, latitude, water temperature, and conductivity. Also spring types are relevant for ostracod assemblage composition.

Many endemic and rare species are found in ambient springs in the Palearctic area. Presence of rare or endemic species should be criteria for setting priority for conservation-oriented site selection, as pointed out by Danielopol et al. (2009). In spite of the ecological relevance of springs, relatively few investigations have dealt with the human impacts on their biota, both at the local (water abstraction, nutrient enrichment, trampling damages by cattle, sediment input, and removal of surrounding vegetation) and at the global (e.g., predicted reduction and increasing irregularity of precipitation due to climate change) scale (Cantonati et al. 2006, 2009; Ilmonen et al. 2012). These neglected environments need conservation guidelines, and more investigations to avoid the loss of biodiversity and of relevant .

6.6 References

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Appendix 6.1: Sources of data

Published articles and reports

References	Number of sites sampled	Number of sites with ostracods	Country	Measured environmental variables												
				Substrate / lithology	Spring type	Hydroperiod	Flow discharge	Water temperature	Dissolved oxygen	Conductivity	Redox potential	pH	Salinity	Alkalinity	Nutrients	Other dissolved ions
Çelekli A. & Külköylüoğlu O., 2007. On the relationship between ecology and phytoplankton composition in a karstic spring (Çepni, Bolu). Ecological Indicators 7: 497-503	1	1	Turkey					X	X	X	X	X	X	X	X	X
Crema S., Ferrarese U., Golo D., Modena P., Sambugar B., & Gerecke R., 1996. Ricerche sulla fauna bentonica ed interstiziale di ambienti sorgentizi in area alpina e prealpina. Report Centro Ecologia Alpina 8: 1-106	29	21	Italy/ Germany	X	X			X		X						
Gerecke, R., Stoch, F., Meisch, C. & Schrankel, I. 2005: Die Fauna der Quellen und des hyporheischen Interstitials in Luxemburg. Ferrantia. ed. T. scientifiques du Musée National d'Histoire naturelle. Luxembourg, Luxemburg	18	16	Luxembourg		X											
Iglikowska A. & Namiotko T., 2010. Freshwater Ostracoda (Crustacea) of Inari Lapland in northern Finland. Annales de Limnologie - International Journal of Limnology 46: 199-206.	2	2	Finland		X											
Iglikowska A. & Namiotko T., 2012. The non-marine Ostracoda of Lapland: changes over the past century. Journal of Limnology 71: 237-244.	4	4	Sweden/Norway/ Finland		X											
Karakaş Sarı P. & Külköylüoğlu O., 2007. Comparative ecology of Ostracoda (Crustacea) in two rheocrene springs (Bolu, Turkey). Ecological Research 23: 821-830	2	2	Turkey		X			X	X	X	X	X	X			
Külköylüoğlu O., 2003. A new report on and the loss of *Scottia pseudobrowniana* kempf, 1971 (ostracoda) from a limnocrene spring in bolu, turkey. Crustaceana 76: 257-268.	1	1	Turkey		X			X	X	X	X	X	X			
Külköylüoğlu O., 2005. Ecological requirements of freshwater Ostracoda (Crustacea) in two limnocrene springs (Bolu, Turkey). Annales de Limnologie - International Journal of Limnology 41: 237-246.	2	2	Turkey		X											
Külköylüoğlu O. & Yılmaz F., 2006. Ecological requirements of Ostracoda (Crustacea) in three types of springs in Turkey. Limnologica - Ecology and Management of Inland Waters 36: 172-180.	3	3	Turkey		X			X	X	X	X	X	X			
Külköylüoğlu O., 2009. Ecological Succession of Freshwater Ostracoda (Crustacea) in A Newly Developed Rheocrene Spring (Bolu , Turkey). Turk. J. Zool. 33: 115-123.	1	1	Turkey		X			X	X	X	X	X	X			
Külköylüoğlu O., Sarı N., & Akdemir D., 2012. Distribution and ecological requirements of ostracods (Crustacea) at high altitudinal ranges in Northeastern Van (Turkey). Annales de Limnologie - International Journal of Limnology 48: 39-51.	17	13	Turkey					X	X	X	X	X	X			
Külköylüoğlu O., Yavuzatmaca M., Akdemir D., & Sarı N., 2012. Distribution and local species diversity of freshwater Ostracoda in relation to habitat in the Kahramanmaraş Province of Turkey. International Review of Hydrobiology 97: 247-261.	33	32	Turkey					X		X	X	X	X			

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References	Number of sites sampled	Number of sites with ostracods	Country	Measured environmental variables												
				Substrate / lithology	Spring type	Hydroperiod	Flow discharge	Water temperature	Dissolved oxygen	Conductivity	Redox potential	pH	Salinity	Alkalinity	Nutrients	Other dissolved ions
Mezquita F., Tapia G., & Roca J.R., 1999. Ostracoda from springs on the eastern Iberian Peninsula: ecology, biogeography and palaeolimnological implications. Palaeogeography, Palaeoclimatology, Palaeoecology 148: 65-85.	114	114	Spain					X	X	X	X	X				X
Mezzanotte E. & Sambugar B., 2004: Contributo alla conoscenza della fauna delle sorgenti. Memorie del Museo Civico di Storia Naturale di Verona - 2ř serie - Monografie naturalistiche 1: 283-292.	7	4	Italy	X	X											
Mori N. & Meisch C., 2012. Contribution to the knowledge on the distribution of Recent free-living freshwater ostracods (Podocopida, Ostracoda, Crustacea) in Slovenia. Natura Sloveniae 14: 5-22.	50	50	Slovenia													
Namiotko T. & Danielopol D.L., 2001. Redescription of two rare hypogean species of the genus *Cryptocandona* Kaufmann (Ostracoda). Crustaceana 74: 557-580.	2	2	Slovakia													
Namiotko T., Meisch C., & Danielopol D.L., 2001. Redescription, taxonomy, distribution and ecology of *Cryptocandona dudichi* (Klie , 1930) (Crustacea , Ostracoda). Bulletin de la Société des Naturalistes Luxembourgeois 102: 109-130.	3	3	Hungary	X	X											
Peterson D., Finger K., Iepure S., Mariani S., Montanari A., & Namiotko T., 2013. Ostracod assemblages in the Frasassi Caves and adjacent sulfidic spring and Sentino River in the Northeastern Apennines of Italy. Journal of Cave and Karst Studies 75: 11-27.	1	1	Italy													
Petkovski T.K. & Meisch C., 1994. Cypria bicolor n. sp., a new crenobiont freshwater ostracod (Crustacea, Ostracoda) from Slovenia. Bulletin de la Société des Naturalistes Luxembourgeois 95: 229-236.	1	1	Slovenia		X											
Petkovski T.K. & Meisch C., 1995. Interesting freshwater Ostracods (Crustacea) from Macedonia. Bulletin de la Société des Naturalistes Luxembourgeois 96: 167-183.	2	2	Macedonia		X	X										
Pieri V., Martens K., Naselli-Flores L., Marrone F., & Rossetti G., 2006. Distribution of Recent ostracods in inland waters of Sicily (Southern Italy). Journal of Limnology 65: 1-8.	1	1	Italy	X												
Roca J.R. & Baltanás A., 1993. Ecology and distribution of Ostracoda in Pyrenean springs. Journal of Crustacean Biology 13: 165-174.	97	97	Spain		X											
Rossetti G., Pieri V., & Martens K., 2005. Recent ostracods (Crustacea, Ostracoda) found in lowland springs of the provinces of Piacenza and Parma (Northern Italy). Hydrobiologia 542: 287-296.	32	32	Italy	X	X		X	X		X		X		X	X	X
Rossetti G., Martens K., Meisch C., Tavernelli S. & Pieri V., 2006. Small is beautiful: diversity of freshwater ostracods (Crustacea, Ostracoda) in marginal habitats of the province of Parma (Northern Italy). Journal of Limnology 65: 121-131.	5	5	Italy		X			X		X		X				

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References	Number of sites sampled	Number of sites with ostracods	Country	Measured environmental variables												
				Substrate / lithology	Spring type	Hydroperiod	Flow discharge	Water temperature	Dissolved oxygen	Conductivity	Redox potential	pH	Salinity	Alkalinity	Nutrients	Other dissolved ions
Sambugar B., Dessi G., Sapelza A., Stenico A., Thaler B., & Veneri A., 2006. Fauna sorgentizia in Alto Adige. Provincia Autonoma di Bolzano.	62	27	Italy	X	X		X	X		X		X		X	X	X
Stoch F., 1998. Fauna a microcrostacei (copepodi e ostracodi). In: Le sorgenti del Parco Adamello- Brenta. Ricerche idrobiologiche su fonti non captate. (M. Cantonati ed.), Parco Documenti, Parco Naturale Adamello-Brenta, pp 151-159.	23	19	Italy	X						X		X		X	X	X
Stoch F., 2003. Indagini ecologiche e faunistiche sulla meiofauna (Acari e Crostacei) di alcune sorgenti del Parco Naturale delle Prealpi Giulie (Italia nord-orientale). Gortania - Atti del Museo Friulano di Storia Naturale 25: 245-260.	20	3	Italy	X	X			X		X						
Stoch F., Valenti D., Chiesi M., & Tomasin G., 2008. Monitoraggio biologico delle sorgenti salse di Poiano (Reggio Emilia). Proceeding of the XX Italian National Speleological Congress, Iglesias 27-30 April 2007 - Memorie dellíIstituto Italiano di Speleologia, s.II, vol. XXI, 2008, 113-120.	1	1	Italy	X	X		X	X						X	X	X
Zaibi C., Scharf B., Viehberg F.A., Keyser D., & Kamoun F., 2013. Preliminary report on the living non-marine Ostracoda (Crustacea) from Tunisia with the description of a new *Psychrodromus* species. Zootaxa 3626: 499-516.	1	1	Tunisia	X												

Additional unpublished data provided by the authors of the following published articles

References	Number of sites sampled	Number of sites with ostracods	Country	Measured environmental variables												
				Substrate / lithology	Spring type	Hydroperiod	Flow discharge	Water temperature	Dissolved oxygen	Conductivity	Redox potential	pH	Salinity	Alkalinity	Nutrients	Other dissolved ions
Pieri V., Caserini C., Gomarasca S., Martens K., & Rossetti G., 2007. Water quality and diversity of the Recent ostracod fauna in lowland springs from Lombardy (northern Italy). Hydrobiologia 585: 79-87.	28	27	Italy		X			X		X		X		X	X	
Pieri V., Martens K., Stoch F., & Rossetti G., 2009. Distribution and ecology of non-marine ostracods (Crustacea, Ostracoda) from Friuli Venezia Giulia. Journal of Limnology 68: 1-15.	12	12	Italy		X			X	X	X		X				
Stoch F., Gerecke R., Pieri V., Rossetti G., & Sambugar B., 2011. Exploring species distribution of spring meiofauna (Annelida , Acari, Crustacea) in the south-eastern Alps. Journal of Limnology 70: 65-76.	87	80	Italy	X	X	X		X		X		X		X	X	X

Unpublished data

References	Number of sites sampled	Number of sites with ostracods	Country	Measured environmental variables												
				Substrate / lithology	Spring type	Hydroperiod	Flow discharge	Water temperature	Dissolved oxygen	Conductivity	Redox potential	pH	Salinity	Alkalinity	Nutrients	Other dissolved ions
Bottazzi E., 2010. Indagini ecologiche su sorgenti e headwaters dellíalto Appennino parmense. Ph.D. Thesis, University of Parma, Italy.	33	8	Italy	X	X			X		X		X			X	
Cantonati M., Rossetti G., & Segadelli S., 2011. EBERs (Exploring Biodiversity of Emilia Romagna springs) project data.	16	13	Italy		X		X	X	X	X	X	X			X	X
Marchiani C. & Venturelli G., 2006. Studio pilota interdisciplinare per la valutazione e la gestione delle risorse idriche della Riserva Naturale Monte Prinzera. Ricerca realizzata nellíambito del Programma Regionale di Investimenti nelle Aree Protette 2001-2003.	5	5	Italy		X			X	X	X	X	X				
Sambugar B. & Ruffo S., 1997. La fauna acquatica delle sorgenti del Parco delle Dolomiti Bellunesi. Prima parte: Vette Feltrine, Pizzocco, Brendol. Parco nazionale delle Dolomiti Bellunesi, unpublished report.	13	3	Italy	X	X			X								
Sambugar B. & Ruffo S., 1998. La fauna acquatica delle sorgenti del Parco delle Dolomiti Bellunesi. Seconda parte: Monti del Sole, Schiara, Pelf, Pramper, Moschesin. Parco Nazionale delle Dolomiti Bellunesi, unpublished report.	14	3	Italy	X	X		X	X		X						

Appendix 6.2: List of the taxa found in the springs studied. Abbreviations refer to taxa shown in figures.

Taxon	Abbreviation	Occurrence (number of springs)
Bradleystrandesia sp.	Br_sp	1
Bradleystrandesia reticulata		1
Candona sp.	C_sp	20
Candona candida	C_can	18
Candona lindneri	C_lin	7
Candona muelleri		1
Candona neglecta	C_neg	99
Candona peterseni	C_pet	2
Candona strumicae	C_str	1
Candonopsis scourfieldi		3
Cavernocypris sp.		2
Cavernocypris subterranea	Cav_sub	40
Chlamydotheca incisa	Ch_inc	1
Cryptocandona dudichi		3
Cryptocandona phreaticola		2
Cryptocandona reducta		5
Cryptocandona vavrai	Cry_vav	24
Cyclocypris sp.	Cy_sp	3
Cyclocypris globosa		1
Cyclocypris helocrenica	Cy_hel	10
Cyclocypris laevis	Cy_lae	29
Cyclocypris ovum	Cy_ovu	24
Cyclocypris serena		1
Cypria bicolour	Cyp_bic	1
Cypria lacustris	Cyp_lac	25
Cypria ophtalmica	Cyp_oph	82
Cypria reptans	Cyp_rep	16
Cypria sketi	Cyp_ske	3
Cypridopsis sp.		1
Cypridopsis lusatica		2
Cypridopsis vidua	Cypr_vi	37
Darwinula stevensoni		13
Eucypris sp.		7
Eucypris pigra	Eu_pig	38
Eucypris virens		4
Fabaeformiscandona sp.	Fa_sp	7
Fabaeformiscandona aemonae	Fa_ae	5
Fabaeformiscandona breuili	Fa_br	8
Fabaeformiscandona brevicornis		3

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Taxon	Abbreviation	Occurrence (number of springs)
Fabaeformiscandona brisiaca		1
Fabaeformiscandona fabaeformis		1
Fabaeformiscandona lapponica		2
Fabaeformiscandona latens		2
Fabaeformiscandona tyrolensis	Fa_ty	7
Herpetocypris sp.	Her_sp	32
Herpetocypris brevicaudata	Her_br	51
Herpetocypris chevreuxi		2
Herpetocypris helenae		8
Herpetocypris intermedia		13
Herpetocypris reptans	Her_re	16
Heterocypris sp.		3
Heterocypris incongruens	Het_in	41
Heterocypris reptans	Het_re	4
Heterocypris salina		6
Ilyocypris sp.		12
Ilyocypris bradyi	Il_br	59
Ilyocypris decipiens	Il_de	2
Ilyocypris gibba		14
Ilyocypris inermis	Il_in	27
Ilyocypris monstrifica		1
Ilyocypris montana		2
Limnocythere inopinata		2
Metacypris cordata		1
Mixtacandona chappuisi	Mi_ch	8
Mixtacandona laisi	Mi_la	4
Mixtacandona lattingerae	Mi_lat	1
Notodromas monacha		2
Notodromas persica	No_pe	14
Paralimnocythere messanai		12
Paralimnocythere psammophila		1
Penthesilenula malayica		1
Potamocypris sp.	Po_sp	27
Potamocypris arcuata		1
Potamocypris fallax	Po_fa	19
Potamocypris fulva	Po_fu	20
Potamocypris pallida	Po_pa	52
Potamocypris similis		1
Potamocypris unicaudata		1
Potamocypris variegata		1

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Taxon	Abbreviation	Occurrence (number of springs)
Potamocypris villosa	Po_vi	53
Potamocypris zschokkei	Po_zs	59
Prionocypris zenkeri	Pr_ze	28
Pseudocandona sp.	Ps_sp	4
Pseudocandona albicans	Ps_al	16
Pseudocandona cavicola	Ps_ca	16
Pseudocandona compressa	Ps_co	4
Pseudocandona eremita		1
Pseudocandona marchica	Ps_ma	1
Pseudocandona pratensis	Ps_pr	9
Pseudocandona rostrata	Ps_ro	3
Pseudocandona sarsi	Ps_sa	1
Pseudocandona zschokkei		1
Pseudolimnocythere sp.		1
Pseudolimnocythere hypogaea	Psl_hy	1
Psychrodromus sp.	Psy_sp	14
Psychrodromus betharrami	Psy_be	33
Psychrodromus fontinalis	Psy_fo	50
Psychrodromus olivaceus	Psy_ol	30
Psychrodromus peristericus	Psy_pe	2
Psychrodromus tunisicus		1
Sarscypridopsis aculeata		5
Sarscypridopsis lanzarotensis	Sa_la	31
Scottia pseudobrowniana	Sc_ps	13
Tonnacypris lutaria		1
Trajancypris laevis		1

Chapter 7

Are aquatic communities from small water bodies more stochastic in dryer climates? An analysis of ostracod spring metacommunities

Based on this chapter an article is in preparation for submission by
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Roca J.R. & Mesquita-Joanes F.

7.1 Introduction

Explaining the mechanisms of assembly and the main drivers of biotic communities has been traditionally a challenge of ecological research. Metacommunity ecology describes community structure considering local and spatial processes (Leibold et al. 2004). A metacommunity is defined as a set of local communities that are linked by dispersal of multiple potentially interacting species (Wilson 1992). Four metacommunity paradigms were proposed: patch dynamic, species sorting, mass effects, and neutral (Leibold et al. 2004). These metacommunity models do not have clear boundaries, but are probably overlapping conforming a continuous framework (Leibold et al. 2004). In this sense, according to Winegardner et al. (2012) the focus of metacommunity studies should be on the relative role of spatial and environmental control, in other words between deterministic and stochastic processes, rather than on the discrimination between the four paradigms. Determinism and stochasticity imply different assembly rules. Through deterministic processes, the physical habitat selects species by their ecological niches (Poff 1997). Communities are predictable because similar habitats host similar communities. On the other hand, stochastic processes assemble unpredictable communities, not linked to species ecological requests but assembled by random dynamics such as for example dispersal limitation, local extinctions, and ecological drift (Hubbel 2001).

The high connectivity between sites in river systems allows high rate of dispersal for aquatic invertebrates, usually showing not-spatially structured communities (Grönroos et al. 2013), unless a large spatial extent is considered (Escrivà et al. 2015). Conversely, springs are isolated aquatic habitats surrounded by a terrestrial matrix and, consequently, dispersal restrictions among sites is expected for some invertebrate taxa, leading to the presence of spatial patterns in communities (Rádková et al. 2014; Zhai et al. 2015). Studies on other isolated habitats, such as lakes, reported the importance of dispersal limitation in structuring communities for some taxa (Soininen et al. 2011; Heino et al. 2015; Castillo-Escrivà in press). Differently to lakes, the smaller size of springs could cause their less persistence in the landscape. The lower life span of these environments probably implicates less lasting communities because of higher extinction probabilities (Chase 2007).

In addition to isolation and small size, several features make springs interesting sites for ecological studies. They are multiple ecotones that link aquatic (ground, surface, and interstitial waters) and terrestrial ecosystems, and they often show a "mosaic" structure characterised by various microhabitats at a small spatial scale that may sustain high species richness (Cantonati et al. 2012). Springs usually harbour rare and endemic species and high taxa diversity for invertebrates, plants, algae, and bacteria, and some authors have defined them as hot-spots of biodiversity (Cantonati et al. 2012; Ilmonen et al. 2012). For some species, springs are their exclusive (crenobionts) or favourite (crenophiles) habitat (Cantonati et al. 2012). Furthermore, springs are characterised by temperature stability and their aquifer may persist over geological time (Glazier et al. 1991; van der Kamp 1995).

Ostracods are commonly found in springs, where they can be the most abundant invertebrate taxon (Bottazzi et al. 2011). The spatial structure of ostracod communities has been rarely investigated (Escrivà et al. 2015; Zhai et al. 2015; Michelson et al. 2016; Castillo-Escrivà et al. in press), even though ostracods are an important constituent of meiofauna in many freshwater ecosystems (Martens et al. 2008). Spatial structures were found in ostracod metacommunities (Escrivà et al. 2015; Zhai et al. 2015) although ostracods are mostly regarded as good passive dispersers (Martens et al. 2008; Brochet et al. 2010) and many species are eurytopic and widely distributed (Meisch 2000; Martens et al. 2008). On the other hand, many studies (e.g. Curry 1999; Mezquita et al. 2001; Gifré et al. 2002; Pieri et al. 2007) suggested a relevant environmental control on ostracod assemblages. Finally, also historical colonization-extinction events should be taken into account (Poquet & Mesquita-Joanes 2011) in analysing their community structuring processes.

The diversity in metacommunity patterns, i.e. in the relative importance of environment and dispersal, has been partly explained by differences in spatial scale, habitat type, organism size and dispersal modality (Cottenie 2005). Thus the results of a metacommunity analysis on different groups of organisms could be affected by differences in size and dispersal ability. Working on a single organism group in the same habitat but in different geographic areas, facilitates the detection of the role of dispersal, environment and also extinction/colonisation processes in shaping communities. Even taking into account that aquatic ecosystems have been deeply studied in the metacommunity analysis framework (Heino et al. 2015), few studies focused on the same organisms and ecosystems but in different biogeographic areas.

This work is aimed at testing the relative importance of environmental and spatial factors on ostracod assemblages in springs from four areas located in different geographic regions of Europe and under different climatic conditions. We expected differences in assembly dynamics among the four areas. Our hypothesis is that climate differences could affect the community stability and lead to the presence of more stable environmental-driven communities or alternatively to less stable stochastic-driven communities (Heino & Mykrä 2008).

7.2 Methods

7.2.1 Data collection

Four different areas were considered: two in northern Italy (Emilia Romagna region and eastern Alps) and two in the north-eastern Iberian Peninsula (Valencia region and Pyrenees), see fig. 7.1 for geographical location and tab. 7.1 for further details on numbers of sites, range of coordinates and altitude.

Biological and environmental data for Alps were obtained from Stoch et al. (2011) and Sambugar et al. (2006); data for the area of Valencia was gathered from Mezquita et al. (1999). The database on ostracod communities from Pyrenean springs was obtained from Roca and Baltanás (1993) and the corresponding environmental data from Roca (1990). The fourth dataset corresponding to the Italian region of

Table 7.1: Number of springs, coordinates, altitude, and environmental variables for the four areas. Environmental variables: Altit = altitude (m a.s.l.), AMT = annual mean temperature (°C), mT = minimum temperature of coldest month, MT = maximum temperature of hottest month, MDR = mean diurnal range (subtraction between maximum and minimum monthly mean temperature, °C), AP = annual precipitation (mm), PWM = precipitation of the wettest month (mm), PS = precipitation seasonality (standard deviation of the weekly precipitation, expressed as % of the mean), Temp = water temperature, Cond = conductivity (ţS), pH = pH, Oxygen = dissolved oxygen (mg/L), Alcal = Alcalinity (mEq/L), NO3 = NO_3^- concentration (mg/L), NH4 = NH_4^+ concentration (mg/L), Cl = Cl^- concentration (mg/L), SO4 = SO_4^{2-} concentration (mg/L), Na = Na^+ concentration (mg/L), K = K^+ concentration (mg/L), SiO4 =(SiO4)4- concentration (mg/L), Mg = Mg+ concentration, MgCa = Mg+/Ca- ratio, MgCa.NaK = $Mg^{2+}Ca^{2+}/Na^{+}+K^{+}$ ratio, Al.ClSO = Al3+/Cl^-+SO_4^{2-} ratio.

Region	Number of springs	Latitude	Longitude	Altitudinal range (m a.s.l.)	Mean distance from the centroid (Km)	Environmental variables
Emilia Romagna	55	44.4° - 45.1°	9.5° - 11.0°	31 - 1614	30.9	log(Altit), AMT, mT, AP, PWM, PS, Temp, log(Cond), pH, log(NO3), log(NH4)
Alps	100	45.8° - 47.0°	10.5° - 12.3°	170 - 2182	48.3	Altit, AMT, MDR, AP, PWM, PS, Temp, log(Cond), pH, log(Alcal), log(NO3), log(NH4), log(Cl), log(SO4), log(Na), log(K), log(SiO4), MgCa
Valencia	117	38.8° - 40.7°	-1.4° - 0.1°	5 - 1420	72.4	Altit, MDR, MT, AP, PWM, PS, Temp, Oxygen, log(Cond), pH, Alcal, log(NO3), log(NH4), log(Cl), MgCa.NaK, Al.ClSO
Pyrenees	88	41.9° - 42.7°	-0.6° - 1.2°	430 - 2160	49.9	log(Altit), MDR, PS, Temp, Oxygen, log(Cond), Alcal, log(Cl), log(Ca), log(SO4), log(Mg), log(Na), log(K)

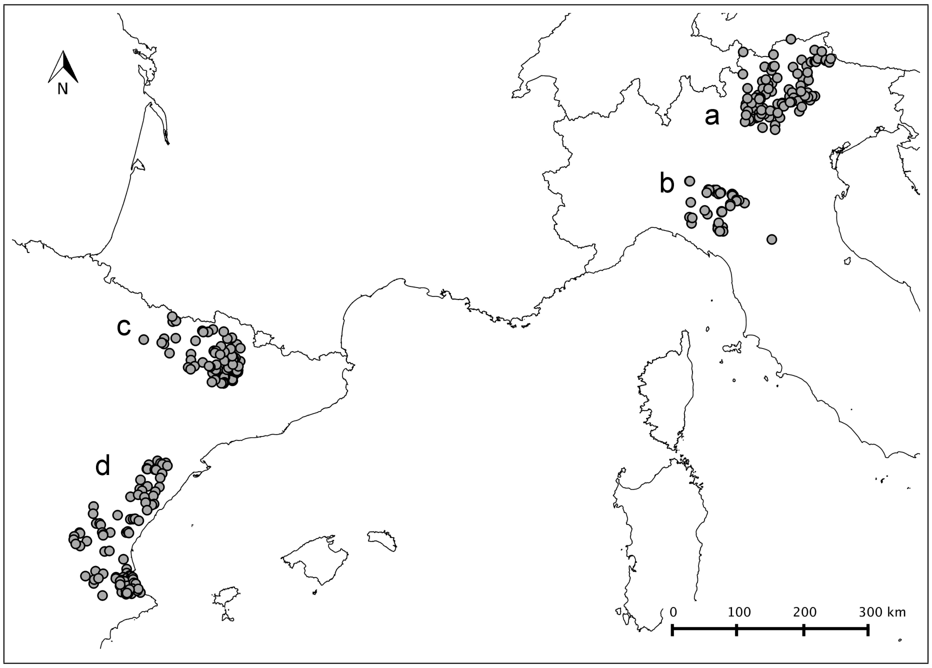

Figure 7.1: Study areas. a = Alps; b = Emilia Romagna; c = Pyrenees; d = Valencia

Emilia Romagna included published and new data from four studies: data for sites E1-E7 were presented by Bottazzi et al. (2008); those from springs E21-E51 by Rossetti et al. (2005); sites E52-E56 by Pieri in Marchiani & Venturelli (2006). Finally we included new data from sites E8-E20; here, ostracods were collected in 2013 and 2104 with a hand net (frame dimensions: 10x10 cm; mesh size: 250 µm) performing 10 replicas of 15 seconds in each site and sampling the substrate for an area equal to the net frame. A multi-habitat proportional approach was used: every microhabitat was sampled for a number of repetitions proportional to its percentage cover in the spring. The following environmental variables were measured in field: pH, conductivity, water temperature, altitude, and geographical coordinates. Concentrations of NO_3^-, NO_2^-, NH_4^+, and SRP (Soluble Reactive Phosphorus) were obtained in the laboratory using standard methods (APHA 2005). Ostracod data were recoded as species presence/absence for all the datasets. Only springs inhabited by ostracods were taken into account for analysis. Present climatic variables for each site of the four datasets were extracted from the Worldclim database (Hijmans et al. 2005). Monthly precipitation data from 1909 to 2009 were collected from the Climatic Researc Unit (CRU) time-series database (Jones & Harris 2008) with 0.5 degrees spatial resolution. Geographic information was handled with the QGIS software. (QGIS Development Team 2013).

The full environmental database presented 14 missing values from 10 sites. In order to not eliminate these sites from the analysis, missing values were replaced with estimated values using a multiple regression analysis for each area (using the known environmental variables as predictors).

7.2.2 Statistical analysis

In order to allow comparisons in different-sized regions, environmental heterogeneity between the four areas was tested with Anderson's PERMDISP procedure for the analysis of multivariate homogeneity of group dispersions (Anderson 2006). Environmental variables were tested for normality (Shapiro's test) and log-transformed if necessary. Correlation between variables was checked for each area and only not-correlated data ($|r|<0.8$) were kept. Correlated ionic concentrations were replaced with ionic ratios (calculated using concentration in mEq/L) in order to avoid the elimination of many variables and o allow evalution of the influence of water ionic composition rather than concentration. Tab. 7.1 shows the considered ecological variables for each study area.

Differences in species composition between areas were tested with PERMANOVA (Anderson & Walsh 2013). Biological data was related to environmental factors using canonical redundancy analysis (RDA). As suggested by Legendre and Gallagher (2001), presence/absence data were Hellinger-transformed prior to the RDAs. Spatial structure in the data was investigated with principal coordinates of neighbour matrices (PCNM) based on geographic coordinates (Borcard et al. 2004). Positive eigenvectors produced by this analysis were used as explanatory variables in RDA. The significance of environmental and spatial variables to the RDA models was evaluated by permutation test in a forward selection at the 5% significance level (Legendre & Legendre 2012). Variation explained by the model was assessed using the R2 adjusted for multivariate analysis (Legendre & Legendre 2012). In the same way significance of axes of spatial RDA was assessed. Significant variables were retained and used for the variation partitioning analysis in the RDA models. Variation partitioning separated the percentage of the variation in community composition explained by different fractions: pure environmental (environmental without the spatial fraction), pure spatial (spatial without the environmental fraction), residual (unexplained), and the variation shared by spatial and environmental variables (Borcard et al. 1992). The significance of the different fractions (pure environmental, pure spatial, shared, and total) was evaluated by 100 permutations of the original data (Borcard et al. 1992). Based on the CRU time-series database, the mean of historical monthly precipitations was calculated for every site. Site means of different areas were compared with ANOVA pairwise comparisons (with Bonferroni's correction) in order to understand past differences in historical climate. Prior to this, data were checked for normality (Shapiro's test) and homogeneity of variances (Bartlett's test). All the analyses were performed with R software (R Core Team 2013), using the packages vegan (Oksanen et al. 2013), PCNM (Legendre et al. 2012), and packfor (Dray et al. 2011).

7.3 Results

Overall 67 ostracod species were found: 29 in Emilia Romagna and in Valencia area, 21 in the Pyrenees, and 30 in the Alps. Species richness varied from 1 to 7 species per site. The most frequent species in the whole study area was *Cypria ophtalmica*, which occurred in 66 sites. The most frequent in the Alps was *Potamocypris pallida*; in Emilia Romagna it was *Cypria ophtalmica*, in Valencia *Herpetocypris brevicaudata*, and in the Pyrenees *Candona neglecta*. *Cyclocypris ovum, Cypria ophtalmica*, and *Potamocypris zschokkei* were found in all the four areas. PERMANOVA assessed differences in community compositions between regions ($F = 19.045$; $p = 0.001$). Tab. 7.2 shows the species occurrences in each region.

Homogeneity of environmental factors among the four areas was confirmed by PERMDISP ($F = 0.722$; $p = 0.539$). Tab. 7.3 reports the p values of the ANOVA pairwise comparison tests of historical monthly precipitation between the 4 different areas, and fig. 7.2 shows the boxplot of each region. Precipitation amounts were significantly different in every pairwise comparison, and decrease in the following region order: Alps, Emilia Romagna, Pyrenees, and Valencia.

Table 7.2: Species occurrence (number of springs) for each region.

Species	Emilia Romagna	Alps	Valencia	Pyrenees
Darwinula stevensoni			9	
Candona sp.		1		
Candona candida		3		
Candona gr. *neglecta*	2	5		
Candona neglecta	3	6		46
Candona lindneri	1	1		
Fabaeformiscandona sp.		2		
Fabaeformiscandona brevicornis		1		
Fabaeformiscandona gr. *breuili*	1			
Fabaeformiscandona breuili	1	1		
Fabaeformiscandona tyrolensis		7		
Pseudocandona marchica	1			
Pseudocandona rostrata	1			
Pseudocandona lobipes	1			
Pseudocandona compressa	2			
Pseudocandona pratensis			8	
Pseudocandona albicans	1	1		1
Cryptocandona vavrai	1	3		1
Candonopsis kingsleii	1			
Cypria ophtalmica	34	7	1	24
Cypria lacustris		1		
Cyclocypris sp.		1		
Cyclocypris laevis	10			
Cyclocypris ovum	3	2	4	3
Ilyocypris gibba			10	4
Ilyocypris decipiens	1			1
Ilyocypris bradyi			15	12
Ilyocypris inermis	1		7	1
Ilyocypris montana				2
Notodromas persica	5		4	1
Cypris bispinosa			1	
Eucypris virens			1	
Eucypris pigra		13		2
Prionocypris zenkeri	6			
Bradleystrandesia sp.		1		
Herpetocypris reptans	2			
Herpetocypris brevicaudata	1		41	1
Herpetocypris chevreuxi				1
Herpetocypris helenae			6	
Herpetocypris intermedia			11	16
Psychrodromus sp.	1	6		

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Species	Emilia Romagna	Alps	Valencia	Pyrenees
Psychrodromus olivaceus	3	2		4
Psychrodromus fontinalis	3	30		
Psychrodromus betharrami	6	16		1
Heterocypris incongruens			18	2
Heterocypris salina			3	2
Scottia pseudobrowniana		4		
Cypridopsis sp.			1	
Cypridopsis vidua	3		21	
Cypridopsis lusatica			2	
Sarscypridopsis aculeata			5	
Sarscypridopsis lanzarotensis			31	
Cavernocypris subterranea		16		
Potamocypris sp.		5		
Potamocypris zschokkei	1	16	4	24
Potamocypris fulva	5	4		
Potamocypris pallida	3	33		
Potamocypris fallax		10		
Potamocypris variegata			1	
Potamocypris villosa		1	31	16
Potamocypris arcuata			1	
Paralimnocythere messanai			12	
Paralimnocythere psammophila			1	
Pseudolimnocythere sp.	1			
Cyprideis torosa			4	
Loxoconcha elliptica			2	
Xestolebis nitida			2	

Tab. 7.4 reports the significant exploratory variables and the total explained variation of the parsimonious environmental and spatial RDAs of each region. The environmental accounted variation ranged from 23.22% (Emilia Romagna) to 2.33% (Pyrenees). Among regions, different variables were retained by forward selection, with the exception of altitude that was selected both in Emilia Romagna and in Valencia.

Fig. 7.3 displays the triplots of environmental RDAs for each region. First axis was related to annual precipitation and altitude in Emilia Romagna, to conductivity and amount of precipitation of the wettest month in the Alps, and to altitude in Valencia. Second axis was related to minimum air temperature in Emilia Romagna, to Na^+ concentration in the Alps, and to NO_3^- concentration in Valencia. In the Pyrenees, springs were organised by Mg^{2+} concentration, with the upper left part of the plot characterised by higher concentration. The spatial accounted variation ranged from 16.00% (Emilia Romagna) to 3.93% (Pyrenees). Appendix 7.1 provides maps for each region displaying the scores of the communities on the significant RDA axes. Emilia Romagna, Alps and Pyrenees spatial data produced one significant RDA axis, whereas in Valencia area three axes were significant. Results of variation partitioning were shown in fig. 7.4. All the fractions (pure spatial, pure environmental and shared variation) were significant. The whole model (pure spatial+pure environmental) accounted for 22.7% in Emilia Romagna, 13.8% on Alps, 8.1% in Valencia, and 6.2% on Pyrenees. Variance explained by environmental variables decreased in the following area order: Alps, Emilia Romagna, Pyrenees, and Valencia. Environmental factors resulted more relevant than spatial ones in Alps and Emilia Romagna, viceversa in Pyrenees and Valencia.

7.4 Discussion

Ostracods show a biogeographic structure at a large (continental) scale in freshwater ecosystems (Baltanás & Danielopol 2013): almost all the families have about 90% of species considered as endemic, i.e. they occur in only one zoogeographical region (Martens et al. 2008). Such geographic patterns appear less clear at a local scale (Baltanás & Danielopol 2013) but they were identified in springs of the western Palearctic region, with the occurrence of local endemisms and rare species (Rosati et al. 2014). Biogeographic patterns partly affected the composition of regional ostracod fauna in the studied springs even though it was not enough to describe the regional diversity of communities. Differences in regional assemblages were also found to be due to the species affinities to local habitat characteristics, such as the tolerance to organic pollution that determined the high frequency of *Cypria ophtalmica* in springs of the exploited agricultural area of Emilia Romagna (Meisch 2000) or the presence of cold stenothermic species on the Alps and Pyrenees.

Ostracod assemblages resulted affected by both spatial and local environmental factors, but with different percentages of variance explained. The fundamental importance of environmental features on ostracod assemblages was previously known. Water chemistry and conductivity were known to be important drivers of ostracod communities in springs (Mezquita et al. 1999; Pieri et al. 2007), so as water temperature (Mezquita et al. 1999). Altitude was less investigated and was found to affect crenic invertebrate (Stoch et al. 2011) and ostracod communities in different ecosystems (Van der Meeren et al. 2010). Few studies analysed the influence of present climatic factors such as precipitation or air temperature on crenic ostracods, but we demonstrated that these factors could be important also at the regional scale. Indeed precipitation and temperature could affect flow regime, which were well-known drivers of ostracod communities in springs and in other freshwater ecosystem (Roca & Baltanás 1993; Mezquita et al. 2005). The significance of spatial factors indicated that communities showed a spatial structure, thus dispersal of ostracods were limited in the studied area. Low connectivity between springs and passive dispersal of ostracods could explain such spatial patterns, as also found by Castillo-Escrivà et al. (in press) in isolated endorheic lakes. Emilia Romagna showed the highest portion of variance explained by

Table 7.3: p values of ANOVA pairwise comparison tests of precipitation data.

Region	Alps	Emilia Romagna	Pyrenees
Emilia Romagna	$< 2*10-16$		
Pyrenees	$< 2*10-16$	7*10-10	
Alps	$< 2*10-16$	$< 2*10-16$	$< 2*10-16$

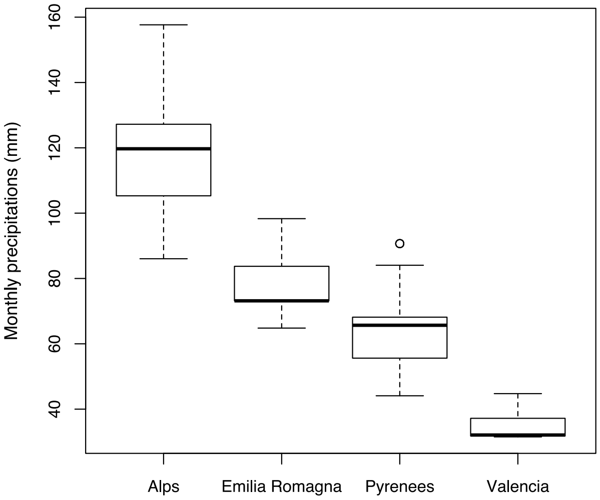

Figure 7.2: Boxplot of means of monthly precipitations of each site in the different areas

Table 7.4: Results of RDAs of environmental and spatial variables. For abbreviations see table 1.

Region	Environmental accounted variation (%)	Selected environmental variables	Spatial accounted variation (%)	Selected PCNM variables
Emilia Romagna	23.22%	Altit mT AP Temp	16.00%	V2 V4 V3
Alps	12.52%	Cond PWM Na	4.56%	V2 V5
Valencia	5.14%	Altit NO3	7.14%	V1 V8 V21 V24 V6
Pyrenees	2.33%	Mg	3.93%	V9 V19 V20

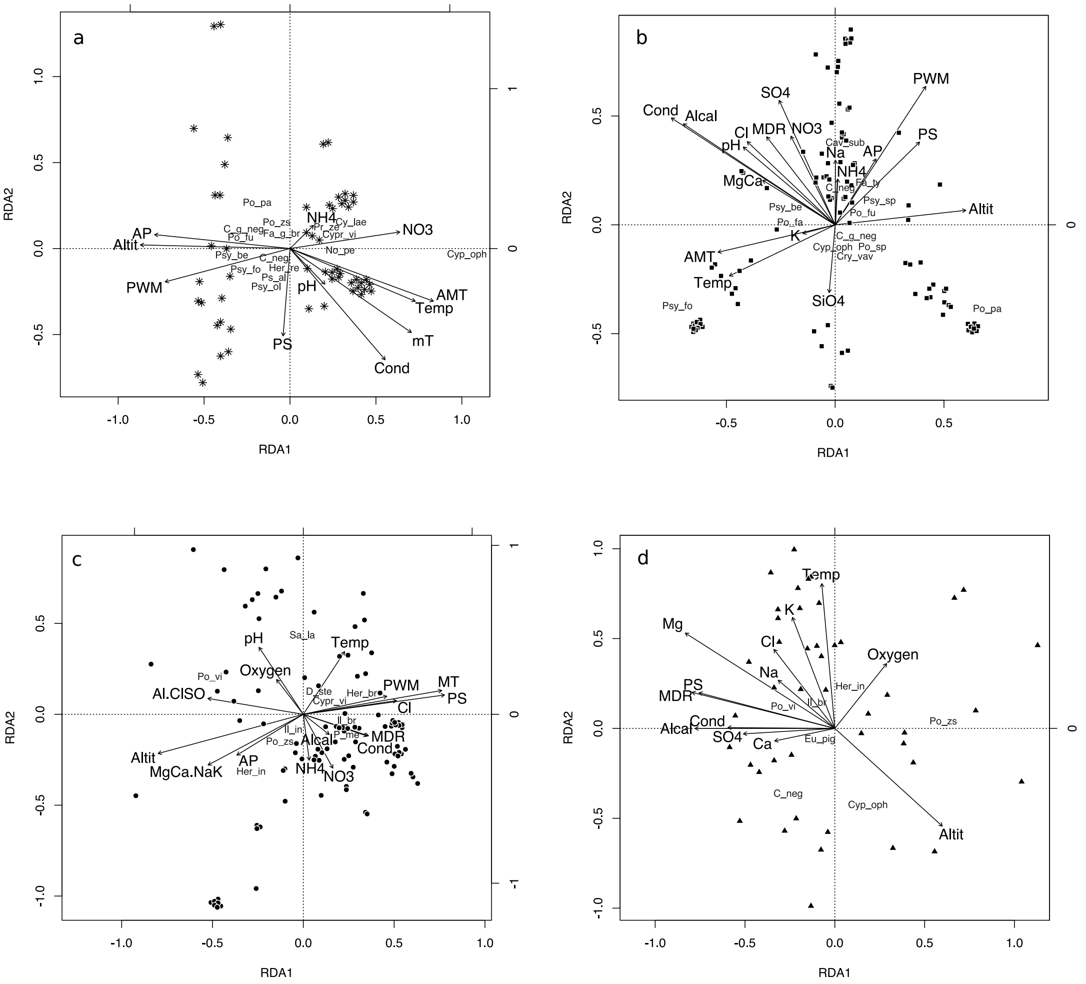

Figure 7.3: RDA triplots for each area: a) Emilia Romagna, b) Alps, c) Valencia, d) Pyrenees. C_neg = *Candona neglecta*; C_gr_neg = *Candona* gruppo *neglecta*; Cy_lae = *Cyclocypris laevis*; Cy_ovu = *Cyclocypris ovum*; Cyp_oph = *Cypria ophtalmica*; Cypr_vi = *Cypridopsis vidua*; Cav_sub = *Cavernocypris subterranea*; Cry_vav = *Cryptocandona vavrai*; D_ste = *Darwinula stevensoni*; Eu_pig = *Eucypris pigra*; Fa_ty = *Fabaeformiscandona tyrolensis*; Her_br = *Herpetocypris brevicaudata*; Her_re = *Herpetocypris reptans*; Her_in = *Herpetocypris intermedia*; Het_in = *Heterocypris incongruens*; Il_in = *Ilyocypris inermis*; Il_br = *Ilyocypris bradyi*; No_pe = *Notodromas persica*; P_me = *Paralimnocythere messanai*; Po_fa = *Potamocypris fallax*; Po_fu = *Potamocypris fulva*; Po_pa = *Potamocypris pallida*; P_vi = *Potamocypris villosa*; Po_zs = *Potamocypris zschokkei*; Pr_ze = *Prionocypris zenkeri*; Psy_be= *Psychrodromus betharammi*; Psy_fo = *Psychrodomus fontinalis*; Psy_ol = *Psychrodromus olivaceus*; Ps_al = *Pseudocandona albicans*; Ps_sp = *Psychrodromus* sp. Sa_la = *Sarscypridopsis lanzarotensis*. Only the most interesting species are shown

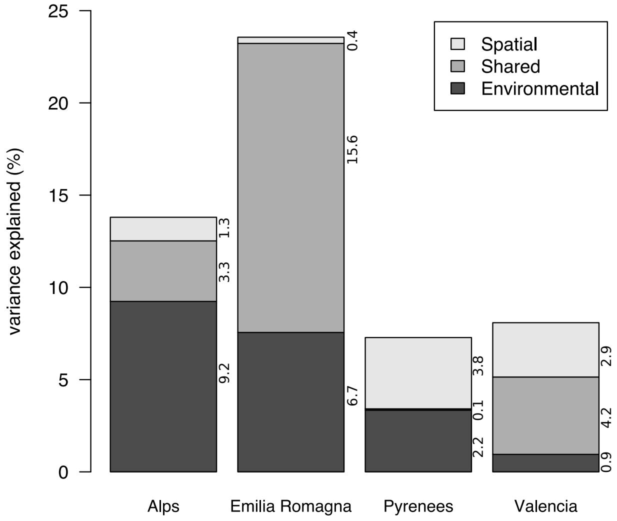

Figure 7.4: Results of variation partitioning

the shared effect of environmental and spatial factors. Two types of springs were sampled in this area: mountain and lowland springs, characterised by different ecological features and different biological communities. Since mountain springs were located in the southern part of the region and lowland in the northern, the environment variables showed a strong spatial structure that resulted in such high percentage of variance explained by both factors.

The amount of variation explained by environmental factors in our study decreased with the increasing of climate aridity, from the Alps to the Valencia Mediterranean area, as supported by the analysis of precipitations in the last century. The reduced size of springs makes them prone to drying events, especially in southern Mediterranean areas, where low-precipitation dramatically affect the aquifer recharges. Frequent disturbances may lead to extinction-colonization dynamics that influence the metacommunity processes. Such disturbances may provoke local extinctions followed by colonisations from nearby sites. Patch-dynamic processes may thus take place, where disturbed sites act as sinks for species and undisturbed nearby sites as sources. Early recolonizations after disturbance lead to random assemblages driven by distances and community composition of near sources of colonists (Lepori & Malmqvist 2009). These stochastic processes may reduce the match between organisms and their environmental requirements, leading to a low amount of variation that can be explained by environmental variables (Heino & Mykra 2008; Grönroos et al. 2013; Heino et al. 2015). Heino and Mykrä (2008) failed to detect a source-sink dynamic in the macroinvertebrate community of disturbed rivers, and they explained it by the high connectivity between disturbed and undisturbed sites in those environments that facilitates the colonisation. These authors hypothesized a more important role of such dynamics at the between-drainages scale. High connectivity explains also the higher variance explained by environmental variables in ostracod communities of Iberian rivers, compared to Valencian and Pyrenean springs (Escrivà et al, 2015). On the contrary, the isolation of springs determines the dominance of stochastic processes in more disturbed (e.g. in drier Mediterranean climates) sites, as shown by the gradient of variance explained by environmental factors in the studied communities. Zhai et al. (2015) results on ostracod communities of Carpathian springs were consistent with this hypothesis, since the amount of variance explained by environmental variables was comparable to the continental areas of our study. Furthermore, post-disturbance colonisation from nearest sites should be testified by spatial structures of communities (Heino & Mykrä 2008). Indeed, spatial factors in Iberian springs explain more variance compared to Alps and Emilia Romagna models, although differences in size of areas prevent a sound comparison of spatial control between regions. In other words, stochastic processes such as dispersal, local extinction, and random colonisations seem to prevail in community assemblages of drier Mediterranean climate, due to the instability of sites in terms of persistence in the landscape. In more continental, wetter climates, higher persistence and stability of sites probably allowed the species to occupy their niche since long time, even from the end of the last glaciation (Horsák et al. 2012). The settled communities are expected to best fit the ecological characteristics of sites, and deterministic processes, such as species sorting, dominated the assemblages.

7.5 Conclusions

In conclusion, ostracod assemblages in springs are driven by environmental and spatial factors but with different strengths. The spatial structures indicate dispersal limitation in the studied areas, probably because of spring isolation. Due to the frequent occurrence of site desiccation, stochastic processes such as dispersal, local extinction and random colonisation might be major structuring factors for spring metacommunities in drier climates. Assemblages from more continental and wetter climates, where site are less disturbed by drought, are probably mainly driven by deterministic processes. In addition, water abstraction for drinking or irrigation was recognised as a relevant threat for spring communities (Cantonati et al. 2009). The impact of anthropic flow reduction could be more severe in arid climates, affecting the persistence of springs and the assembly dynamics.

The study of present assemblages and their relationships with different climates may help to foresee the responses of communities in a global change scenario. Given their role as proxies for climate and ecosystem changes (Holmes & Chivas 2002), ostracods could serve as ideal model organisms for these studies.

7.6 References

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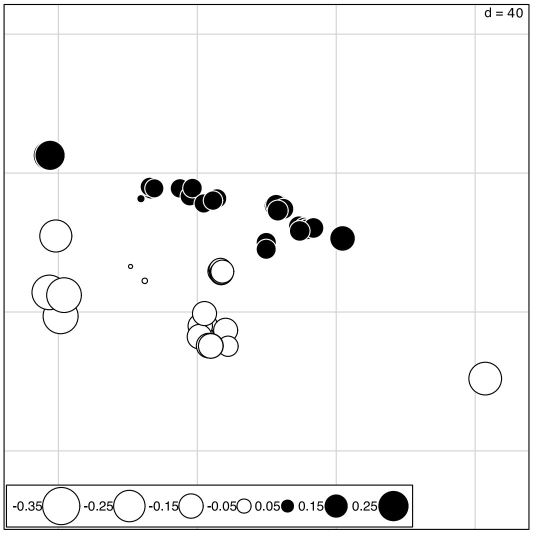
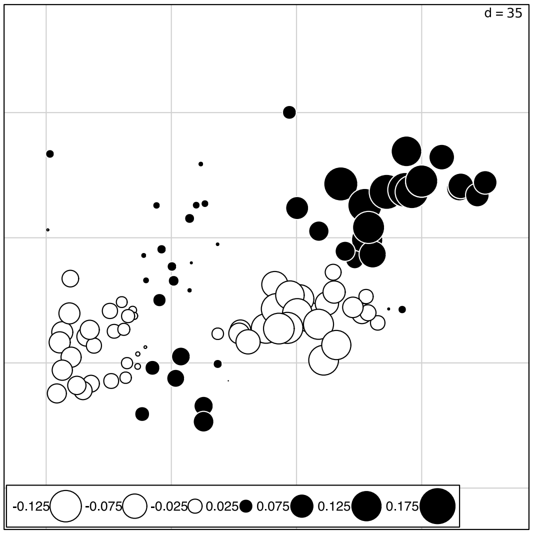
Appendix 7.1

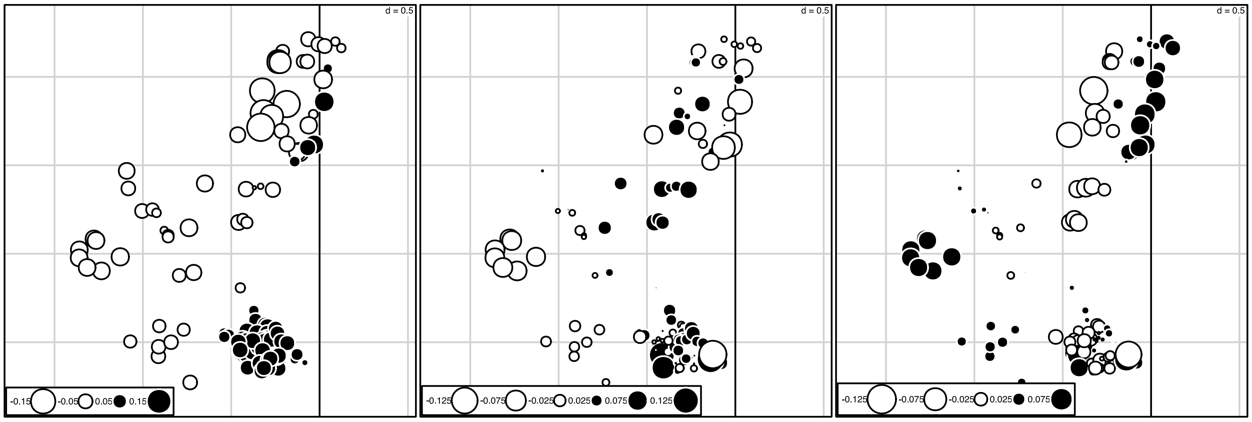
Maps of springs of each region. Colours and size of points represent the scores of the significant axis of RDA on spatial variables. Geographic scale of the map is given by a grid, which size in Km is given with the letter d in the upper part of the plot



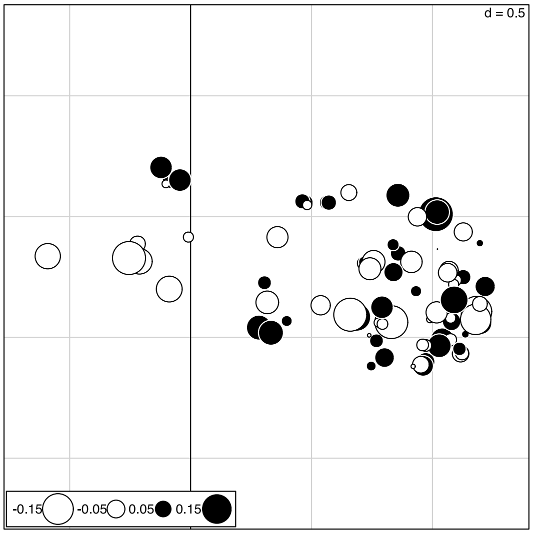
Map of Emilia Romagna springs.



Map of Alps springs.



Maps of Valencia springs.



Map of Pyrenees springs.

Chapter 8

General conclusions

8.1 Main results

With respect to the research hypotheses and questions, the main outcomes of the thesis can be summarised as follows.

1. Biological sampling in springs is affected by several methodological problems and moreover can cause disturbance. In this work different methods were compared: the net with proportional multi-habitat approach and the combined use of traps and the washing of vegetation sample. The use of the net provides more accurate and complete information, but also significant impacts on the biotic and abiotic components of springs. For these reasons, this method is only recommended for biodiversity inventories. On the other hand, traps and vegetation washing are still reliable methods with less negative effects on spring ecosystems, thus they are more suitable for ecological studies focused on the analysis of the community structure.

2. Local environmental factors significantly influence the invertebrates in springs. Invertebrate communities of Mount Prinzera are affected by water chemistry, composition or heterogeneity of in-springs habitat, hydroperiod or discharge fluctuations. When compared to springs of the same region, pH is one of the candidates for explaining the absence (or the low presence) of adult ostracods in Prinzera springs. The permanent springs show seasonal changes in the concentration of main ions, whereas conductivity, pH and water temperature are more stable. The thermal stability probably determines the lack of seasonal changes in structure of macroinvertebrate communities of permanent springs.

3. The niche filtering of springs on ostracod assemblages were studied by the analysis of functional diversity of communities from Emilia Romagna. Springs result to sustain almost the 50% of the regional species pool, and several species are found exclusively in crenic habitats. This is the first study analysing functional diversity of ostracods; a list of functional traits was compiled and six functional groups were identified. Analysing the regional species pool, functional diversity in springs result not significantly different than that expected by chance. Springs do not to limit functional similarity between coexisting species. Probably, the range of ostracod considered functional traits is too narrow to make them subjected to spring environmental filtering. This findings lead to conclude that, given the satisfaction of niche requirements of ostracod species, assembly processes in springs are probably affected by stochastic drivers as dispersal, speciation and local extinctions.

4. In addition to environmental variables, also spatial factors affect spring invertebrates. Indeed, significant spatial patterns are identified in invertebrate communities (macroinvertebrates, ostracods, and water mites) of the Emilia Romagna region. The characteristic isolation of springs

limits dispersal of taxa in the sites of the studied area, influencing more passive than active dispersers. Anyway, spatial factors explain only a low percentage of the total variation, and all invertebrate communities result mainly affected by environmental variables. Regional climatic variables influence communities less than local factors, and for ostracods and water mites their effect is negligible.

5. At continental scale, spring ostracod communities show clear biogeographic patterns and are organised along environmental gradients. Species composition varies along a geographic gradient, and the observed variation is mainly explained by altitude, latitude, water temperature, and conductivity. Also spring typologies (helocrene, rheocrene, and limnocrene) are relevant for ostracod assemblage composition. The presence of endemic and rare species characterises specific regions.

6. Analysing springs from four areas in Italy and Iberian Peninsula, I observed that climate affect the assembly processes of spring ostracods. The amount of variation explained by environmental factors decreases with the increasing of climate aridity. Frequent disturbances, as site desiccations, may provoke local extinctions followed by colonisations from near sites, leading to a source-sink dynamics. Early recolonisations after disturbance produce random assemblages, reducing the match between organisms and their environmental requirements, and therefore a low amount of variation is explained by environmental variables. Thus, stochastic processes mainly drive these assemblages, whereas communities from more wet climates result mainly driven by deterministic processes.

8.2 Conclusive remarks and future prospects

This work confirmed that niche-based processes are important in shaping invertebrate communities in springs, and environmental drivers explain a relevant percentage of community variation. Surprisingly niche-filtering processes have limited effect on ostracod communities, even though this result could be taxon-dependent. At the same time, spatial patterns are detected in all the studied communities, revealing a limitation to dispersal among springs for some taxa. Anyway, environmental control is prevalent if compared to spatial factors, underlining the importance of deterministic processes. Stochastic processes become more influential in arid climates, where the disturbance linked to frequent desiccations lead to a species source-sink dynamics. Finally, the existence of biogeographic gradients in ostracod distributions and the presence of local endemisms are confirmed at large scale. In conclusion, stochastic and deterministic processes are not mutually exclusive, but they contribute together to shape invertebrate communities in springs.

Studies about assembly processes with a comprehensive metacommunity approach give important indications about spring management. Springs host a high fraction of the regional pool species and several endemisms were found living in these environments from all the Western Palearctic. These results underline the relevance of springs for the maintenance of freshwater biodiversity and suggest the enhancing of conservation efforts. In the source-sink dynamics observed in impacted sites, the presence of sites suitable for colonisation after a local extinction could prevent the loss of biodiversity in the area. Thus I suggest the protection of diverse springs in the same area instead of single springs scattered in different areas. This may prevent also the loss of specie with limited dispersal abilities. Finally, this work may help to foresee the responses of communities in a global change scenario, by studying the present assemblages and their potential evolution under different climates.

To conclude, some future prospects can be suggested. Several aspects of spring community assembly remain to be further addressed. Little is known about the historical dynamics, for example the role of glaciation events, which create the biogeographical patterns and the occurrence of several endemisms that I highlighted in this study. Analysing the distribution of the phylogenetic diversity could reveal specific patterns and could improve the understanding of historical processes. The analysis should be done for different taxa and contemplate the disentangling between functional and phylogenetic diversity.

Besides spatial and environmental factors, also biotic interactions could affect the community compositions. The role of intra and inter-specific interactions could enhance the knowledge of assembly processes in spring invertebrate communities.

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