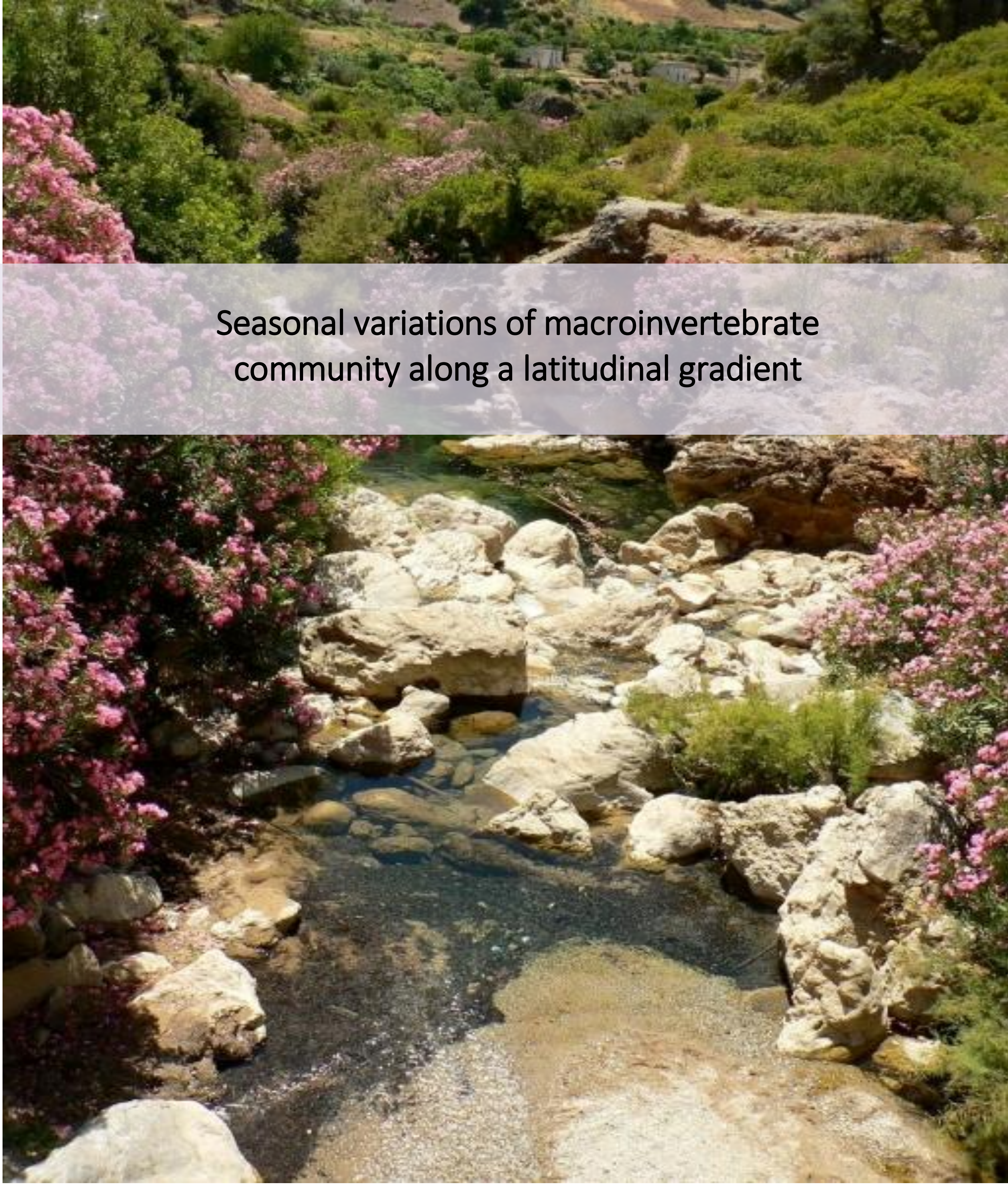
Seasonal variations of macroinvertebrate community along a latitudinal gradient



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Cover photo: a sampling site in Morocco during spring, Kalaa river.

Resum

L’estudi de les comunitat d’organismes a gran escala ha augmentat molt les últimes dècades. La macroecologia és la ciència que unifica processos macroevolutius, ecològics i biogeogràfics per entendre patrons i procesos de les comunitats biològiques a gran escala. Els canvis en latitud són un dels patrons més estudiats en macroecologia. Per altra banda, l’estacionalitat és un procés que condiciona l’ecologia de molts organismes però pocs estudis han analitzat si els patrons latitudinals canvien en funció de l’estació.

Els macroinvertebrats aquàtics són un grup ideal per analitzar patrons macroecològics atès la seva sensibilitat davant de canvis ambientals i la seva enorme diversitat. En aquest treball s’analitzen els canvis estacionals al llarg d’un gradient latitudinal des del nord del Marroc fins a Suècia. S’estudia la comunitat de macroinvertebrats a nivell de gènere de 62 rius perennes corresponents a 6 regions situades al llarg d’aquest gradient. Tot i tenir característiques homogènies, els rius estudiats difereixen en el clima actual i en els esdeveniments geològics del passat propis de la regió.

En general, s’observen canvis en latitud per la majoria dels paràmetres estudiats, incloent la riquesa i la composició de gèneres. Així, a mesura que augmenta la latitud, la riquesa de gèneres decreix i la composició de comunitats canvia. Aquests canvis responen a efectes històrics com ara les glaciacions però també a canvis latitudinals actuals associats a paràmetres climàtics. Aquests canvis latitudinals són més importants a la primavera que a la tardor. De fet, a la tardor la major part de paràmetres no estan relacionats de manera significativa amb la latitud, el que indicaria que les condicions de tardor són més homogènies entre les regions i esborren el senyal latitudinal que trobem a la primavera. En resum, els resultats ens indiquen la necessitat d’incloure l’estacionalitat en els estudis macroecològics sobretot d’organismes que poden presentar grans variacions estacionals (com ara els macroinvertebrats aquàtics) o que inclouen regions on la estacionalitat és rellevant.

Summary

Community ecology of organisms has risen up in the last decades. Macroecology is the science which joins macroevolution, ecology and biogeography processes to understand biological community patterns and processes at large-scale. Latitudinal changes are one of the most studied patterns in macroecology. On the other hand, seasonality is a process that determines the ecology of many organisms. Very few studies, however, have analyzed if latitudinal patterns change seasonally.

Aquatic macroinvertebrates are a suitable group for analyzing macroecologycal patterns due to they respond very well to environmental changes and have a great diversity. In this study seasonal changes are analyzed along a latitudinal gradient from the north of Morocco to Sweden. Macroinvertebrate community is studied at genus level in 62 perennial streams located in 6 regions along this gradient. Although these streams have homogeneous characteristics, they differ in the current climate and in past geological events.

In general, latitudinal changes are observed by the majority of studied parameters, including richness and genera composition. Thus, as latitude increases, genera richness increases and community composition changes. These changes respond to historical effects as glaciations but also to current climatic parameters. These latitudinal changes are more important in spring than in autumn. In fact, in autumn the majority of parameters are not significantly related to latitude, which means that autumn conditions are more homogeneous among regions, erasing the latitudinal pattern found in spring. In summary, results show the need of including seasonality in macroecologycal studies, mainly in those that consider organisms high variable seasonally (such as aquatic macroinvertebrates) or that include regions where seasonality is relevant.

CONTENTS

INTRODUCTION

Organisms vary in space and time. This evidence makes the study of spatial and temporal patterns and processes of organisms a recurrent topic in community ecology (Brown, 1981). Spatial patterns, which have always been a complex challenge for biogeographers and ecologists (Gaston, 2000; Gaston et al., 2009), have been studied at multiple scales: from local habitats to the entire globe. As local communities are integral components of larger biogeographic regions (Witman et al., 2004), it has been proved that regional scale processes influence local ones and *vice versa* (Gaston, 2000; Vinson, & Hawkins, 2012). On the other hand, temporal patterns are also scale dependent. Temporal scales relevant for ecological processes can range from hours to many decades (Mackas et al., 2012). Most community ecology studies have, however, focused at seasonal and interannual scales (Pace et al., 2013). Studies al seasonal scales are more frequent, especially in areas where seasonality is very marked (Nozais et al., 2005; García-Roger et al., 2011; Mackas et al., 2012).

Macroecology is the science that studies the distribution and abundance of species at large spatial and temporal scales (Blackburn & Gaston, 2002; Blackburn, 2004; Gaston & Blackburn, 2007), combining ecological, biogeographical and macroevolutional patterns and processes (Hugueny et al., 2010). One of the most widely study topics in macroecology are latitudinal variations of organisms. There are studies that include organisms, such as mammals (Daan & Aschoff, 1975; Hawkins et al., 2003; Weir & Schluter, 2007), birds (Daan & Aschoff, 1975), amphibians (Huey, 1978; Eikenaar et al., 2012; Alho, 2010; Morrison, & Hero, 2003), reptiles (Eikenaar et al., 2012; Huey, 1978), fish (Houde, 1989; Leggett & Carscadden, 1978; Schultz & Conover, 1997; Hugueny et al., 2010), and invertebrates (Jacobsen et al., 1997; Vinson, & Hawkins, 2012). The great majority of these studies have shown that species richness declines with latitude in both aquatic and terrestrial ecosystems (Gaston, 2000; Witman et al., 2004; Vinson & Hawkins, 2012; Jacobsen et al., 1997).

Seasonal variations of organisms have been also considered in several macroecological studies using mammals (Lovegrove, 2005), birds (Dawson, 2001), amphibians (Morrison & Hero 2003), reptiles (Eikenaar et al., 2012), fish (Grossman, 1998), or invertebrates (Vasconcellos, 2010; Mackas et al., 2012). Obviously, all these studies are performed in regions with significant seasonal variability in temperature and precipitation patterns (e.g., the Holarctic; Grossman et al., 1998) or in the photoperiod (e.g., the tropics; Dawson et al., 2001). However, all these examples consider narrow latitudinal ranges or only include a single sampling site and there are no large-scale latitudinal studies that consider seasonal patterns (Vinson, & Hawkins, 2012).

Freshwater ecosystems, which make up 0.8% of the Earth's surface but host 6% of species biodiversity, are considered more threatened by human impacts than terrestrial or marine ones (Domisch et al., 2013; Dudgeon et al., 2006; Balmford et al., 2005). A better understanding of freshwater biodiversity patterns and processes is needed to better address conservation and management measures in these ecosystems. Most macroecological studies that include freshwater organisms are focus on fish communities (Hugueny et al., 2010; Oberdroff et al., 2011) and very few deal with freshwater macroinvertebrates (Vinson & Hawkins, 2012). In addition, the few existing macroinvertebrate studies are contradictory: some authors agree with the global assumption that there is more aquatic insect diversity in the tropics than in temperate regions while others have found the same diversity in both regions (Jacobsen et al., 1997). The lack of consistent global databases on macroinvertebrates collected with similar methodologies or using the same taxonomic resolution have made this endeavour difficult to address. In addition, the existing macroecological studies on fish are based on species composition recorded per basin (Hugueny et al., 2010), without a proper description of seasonal patterns. Given that macroinvertebrates are more susceptible to change seasonally than fish because their different life cycles and time span (Bonada et al., 2013), the inclusion of seasonality in macroecological studies on macroinvertebrates is needed.

Environmental variables change seasonally and contribute to seasonal community patterns in marine and freshwater streams (Mackas et al., 2012). Freshwater macroinvertebrates are affected by environmental variables as temperature, pH, oxygen, and nutrient concentration among others (Bini et al., 2014; Heino et al., 2015). Some of these variables are also known to vary in latitude (e.g., temperature; Jacobsen et al., 1997). In this context, macroinvertebrates are ideal organisms to test for seasonal changes along latitudinal gradients. In this thesis, seasonal macroinvertebrate community changes will be analysed along a large latitudinal gradient in the Palearctic region, including regions with Mediterranean, temperate and subarctic climates. Mediterranean climate regions are characterized by hot and dry summers and cold and wet winters having a strong seasonality (with floods occurring in autumn and droughts in summer) (Aschmann,1973; Gasith & Resh, 1999; Bonada et al., 2007b), whereas temperate regions are less seasonal and have mild summers and colder and wetter winters (Gasith & Resh, 1999; Bonada et al., 2007b). Subarctic regions suffer from winter droughts due to the ice covering the river streambed (Irons et al., 1993). Spring snowmelt causes flood peaks and a secondary flood peak can occur in autumn (Friberg et al., 2013). These regions also differ in past geological events, such as glaciations, that should be considered in any macroecological study (Gaston, 2000; Vinson & Hawkins, 2012). The Mediterranean region in Europe was used as refuge during Pleistocene glaciations, experiencing an exceptional level of speciation for many taxonomical groups including macroinvertebrates (De Figueroa et al., 2013). These glaciations also caused taxonomic losses in northern European regions, which have been used to explain the observed latitudinal changes in macroinvertebrate species richness (Bonada et al., 2007a; Domisch et al., 2013; Bonada, & Resh, 2013).

Our main hypothesis was that latitudinal changes in macroinvertebrate richness and communities should be found regardless the season but that spring communities would show stronger latitudinal changes than autumn communities. Macroinvertebrate diversity should decrease and community should change with latitude due to current environmental latitudinal changes and past geological events that resulted in southern speciation and northern extinctions (Bonada et al., 2007a; Domisch et al. 2013; Bonada & Resh 2013). However, autumn patterns would be less distinct than spring patterns because the presence of floods in autumn is universal along the gradient. Flood events mobilize the substrate, increase suspended organic matter and inorganic sediments, and wash out flora and fauna, homogenizing communities (Poff and Ward, 1989; Robinson et al., 2003). Instead, spring environmental conditions should display larger latitudinal changes and reflect different climatic characteristics. For example, even perennial Mediterranean rivers have a significant decrease in their base flow in spring and summer, with significant effects on macroinvertebrate communities (Bonada & Resh, 2013). In order to address this hypothesis macroinvertebrate richness, abundance, EPT (Ephemeroptera, Plecoptera, Trichoptera), OCH (Odonata, Coleoptera, Heteroptera) and global compositions were analysed. EPT and OCH metrics are considered indicators of seasonal changes between Mediterranean and temperate invertebrate communities (Bonada et al., 2012).

MATERIALS AND METHODS

Sampling sites

Data from the RICHABUN project (CGL2007-60163/BOS), funded by the Spanish Ministry of Education and Science/FEDER (Fondo Europeo de Desarrollo Regional), were used. This project aims to analyze latitudinal patterns of macroinvertebrates at community and population level from Sweden to Morocco. It includes samples from 6 regions that have different climatic characteristics, from Mediterranean (Rif in Morocco and the Betic region in Spain and the), to temperate (Picos de Europa in Spain, Jura in France, and Carpathians in Slovakia), and to subartic (Jämtland in Sweden) (*Fig. 1*). These regions belong to different biogeographical regions: Mediterranean, Atlantic, continental, alpine, and boreal, respectively (Illies, 1978) *(Annex 1*). This study will only consider samples at community level. The different regions will be referred in the text as Rif, Betic, Picos, Jura, Carpathians, and Sweden.

A total of 66 sites were sampled in spring and autumn 2008, with 11 sites per region. Ten sites for Picos and 8 for Rif were finally used (i.e., 62 sites in total) because difficulties to find appropriate rivers that matched the criteria below. Despite rivers in the Mediterranean climate regions have a higher frequency of flow intermittence (Bonada & Resh, 2013), only sites with perennial flow were considered to avoid extreme confounding factors. For the same reason, sampling sites were as similar as possible within and among regions. Thus, selected sites were all headwaters (i.e., stream order 1 or 2), non-impacted (i.e., Reference Criteria higher of equal to 7; Bonada et al., 2002), had a high habitat diversity (i.e., Fluvial Habitat Index higher of equal to 60; Pardo et al., 2002), a good riparian quality (i.e., Riparian Quality Index higher of equal to 75; Munné et al., 2003), a calcareous geology (i.e., conductivity higher than 400 µS/cm), and were located between 400 and 1000 m.a.s.l. (i.e., all these factors are considered to influence of macroinvertebrate richness and abundance; Bonada et al., 2005). Area covered by all sites within a region was around 40.000km^2 to avoid any type of species/area effects. Site selection was done using maps and visual observations in the field. All sites belonged to different rivers (see *Annex 2* for a general description of each particular site and *Annex 3* for some example pictures of the sampling sites).

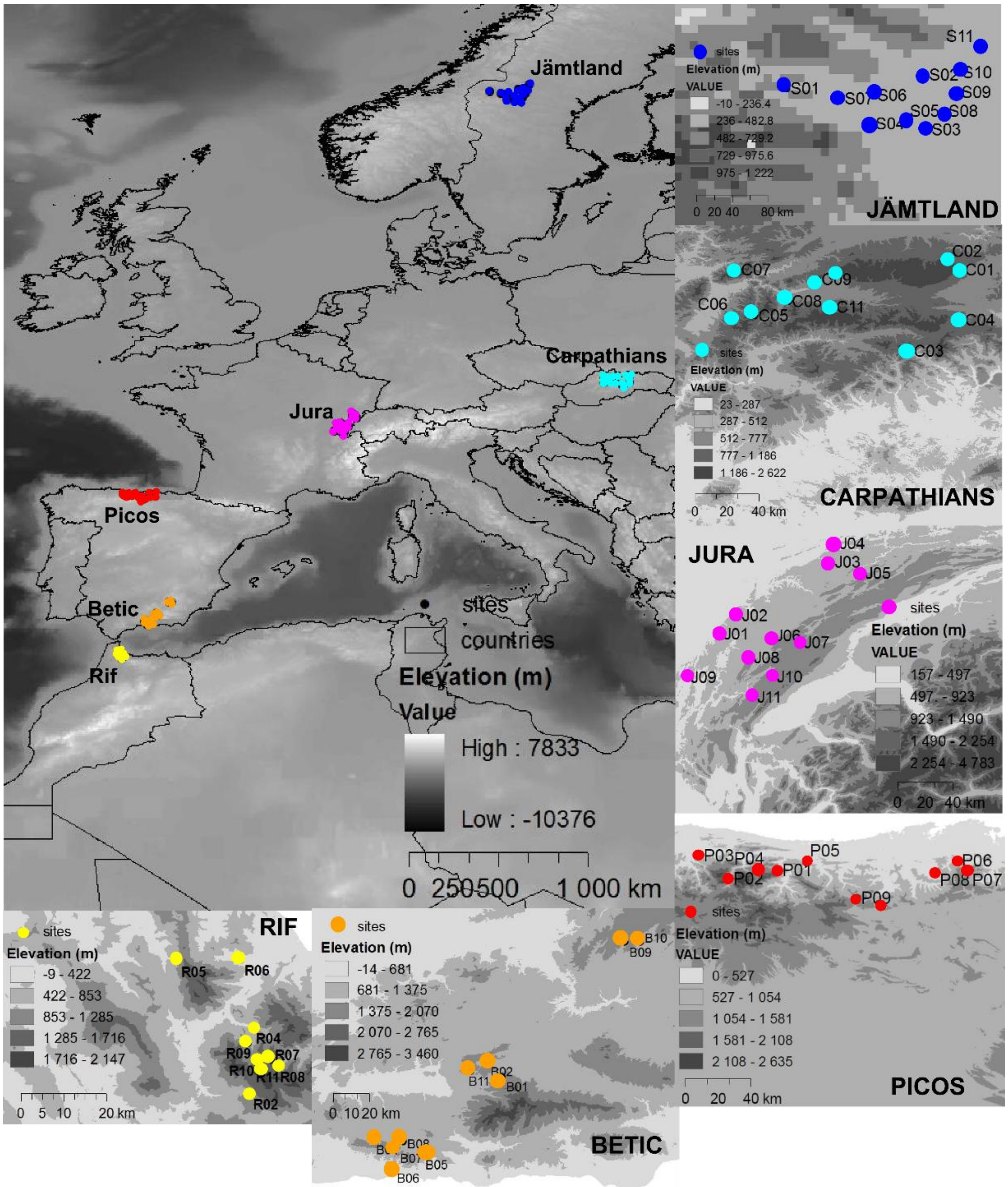

Fig. 1. *Location of sampling sites of each region in an elevation map (courtesy by Cesc Múrria).*

Environmental data

Several environmental variables were taken in each site and season (*Table 1*). In addition, an index to assess the influence of floods between spring and autumn was

Fig. 2*. Five painted rocks in the river section of a Carpathians site in spring.*

designed. This index (called *disturbance index*) was estimated by painting five large, medium, and small rocks, placed them in spring across the river section, and localized and counted in autumn (*Fig. 2*). As larger stones need higher floods to be moved than small stones, the disturbance index considered the number of moved rocks weighted by its size (i.e., large*30 + medium*20 + small*10).

Macroinvertebrate data

For each site and season, 3 quantitative random samples were collected using a Hess sampler with a mesh size of 250µm and a surface of 0.07m^2. Once the sampler was placed, the substrate was vigorously moved during 3 minutes and all material was collected and preserved in formalin 4%. In addition, a semiquantitative kick-sample was collected by sampling all available habitats until no new macroinvertebrate families were collected after 2 consecutive samples (i.e., about 30 minutes). This sample was preserved in alcohol 96%.

All samples were sorted in the laboratory and macroinvertebrates were identified at genus level using several keys (Sansoni, 1988; Waringer, 1997; Vieira-Lanero, 2000; Tachet et al., 2010). Diptera was not considered in this study because of their difficulty in taxonomy identification. A total of 182 genera were found for all regions.

Macroinvertebrates were expressed as densities (individuals/m^2) for quantitative samples and as rank of abundances (1 for 1-3 individuals, 2 for 4-10 individuals, 3 for 11-100 individuals, and 4 for >100 individuals) for semiquantitative samples. To make sure all semiquantitative samples included all taxa; those genera collected in the quantitative sample but not included in the semiquantitative sample were included with their corresponding abundance range. All semiquantiative samples but only quantitative samples from 4 randomly selected sites in Rif, Carpathians, and Sweden were analyzed in this study. These quantitative samples were the ones sorted and identified by the author of this thesis. Quantitative samples were used to analyze abundance and diversity patterns, whereas semiquantitative samples were used to analyze richness and community patterns. Genera richness, abundance, EPT and OCH were compared among regions and between seasons.

Statistical analyses

Environmental differences among regions were tested using a Wilcoxon paired test (Hollander & Wolfe, 1973) for non parametric samples. A Principal Component Analysis (PCA) with scaled variables was also applied to assess general environmental patters between regions and for each season using the *ade4* R package (Dray & Dufour, 2007). Site scores along the first and second PCA axes were fitted in a linear mixed model with latitude to look for latitudinal gradients on general environmental data using the *lme4* R package (*lmer* function) (Bates et al., 2015). Latitude was used as fixed factor and region as random factor. Linear mixed models were used instead of linear models because sites were grouped by region. The same model structure was used in all linear mixed models computed below.

For macroinvertebrate quantitative data, a Kruskal-Wallis test (Hollander & Wolfe, 1973) was applied to test for abundance and diversity differences between seasons for all regions, whereas a Wilcoxon paired test was computed to test differences among regions. Abundance was expressed as density (ind/m^2) by dividing genus abundances by the total sample surface (0.21m^2). Simpon's diversity (D') was used as a diversity index.

For macroinvertebrate semiquantitative data, local genus richness (α-richness) was calculated and related to latitude by fitting a linear mixed model for each season. γ-richness was also calculated per region.

In order to analyse latitudinal changes in community composition, EPT and OCH richness was firstly computed and related to latitude using a linear mixed model for each season. Secondly, the genera significantly found in each region and season were investigated using the *stats* and *indicspecies* R packages (Cáceres & Legendre, 2009), and those with an occurrence >70% were also identified. Finally, a Correspondence Analysis (CA), an ordination technique that extracts continuous axes of variation from species abundance data (Ter Braak, 1986), was built with the *ade4* R package (Dray & Dufour, 2007) to look for general patterns in community composition. A between-analysis among regions was assessed to know if region overall differed in their composition. This analysis is based on a random test that compares an observed value (i.e., among regions variability) to the distribution of 999 simulated values. Site scores along the first and second CA axes were fitted in a linear mixed model with latitude. In order to know how well main biological variability was explained by latitude, environment, and both factors together, linear mixed models were built using CA scores of the first and second axes and related to first PCA scores (for environment) and latitude.

Finally, environmental and biological data were analyzed together using a Canonical Correspondence Analysis (CCA) (see Ter Braak, 1986) to assess which proportion of the biological data variability is explained by environmental variables and which one by latitude.

All tests were computed using the version 3.2.0 of the R software (R Core Team, 2016) and the limiting *p-value* for considering statistical significant results was 0.05.

RESULTS

Environmental characterization

Mean and standard deviation values of main physicochemical variables for each region in spring and autumn are shown in *Table 1*. Higher discharge values were found in autumn respect spring ones for Betic and Carpathians. Seasonal differences on oxygen concentration and saturation were found for Betic, Picos, Jura, and Sweden. Temperature was significantly higher in spring in all regions except in the Jura. pH differences were only significant in Sweden and conductivity was significantly higher in autumn for Betic, Picos, and Jura. Furthermore, the disturbance index showed that the Rif was the region with more flood events whereas Sweden the region with less flood events (*Fig. 3*). Mid-latitude regions had more variability.

PCA built with the variables in *Annex 4* displayed differences among regions and between seasons (*Fig. 4 a* and *b*). The first axis of spring PCA (*Fig. 4 a*) explained 22.75% of the total variance and was correlated positively by discharge, oxygen concentration, minimum and maximum of stream width, and chlorine (loading = 0.68, 0.71, 0.79, 0.72 and 0.76, respectively), and slightly negatively related with magnesium (loading = -0.61). The second axis explained the 14.94% of variance and was mainly positively related with conductivity and oxygen concentration (loading = 0.70 and 0.68) and barely negatively related with temperature (loading = -0.44).
The autumn PCA (*Fig. 4 b*) was explained in the first axis by a 24.10% of variance and positively related with oxygen concentration and percentage of oxygen saturation (loading = 0.68 both), with minimum and maximum river width (loading = 0.77 and 0.70, respectively) and negatively related with magnesium (loading = -0.71). The second axis explained a 15.75% of variance and was positively related with pH (loading = 0.57) and negatively with conductivity and calcium (loading = -0.69 and -0.65, respectively). Spring samples showed larger regional differences than autumn samples (*Fig. 4 a* and *b*). Jura was the region with most dissimilar environmental conditions, especially in spring. Linear mixed models neither showed significant latitudinal changes in physicochemistry parameters for the spring first and second PCA axis (first axis: Chi-square= 0.9, df = 1, *p*=0.321; second axis: Chi-square= 3.8, df = 1, *p*=0.050) and nor for the first and second autumn PCA axis (first axis: Chi-square= 3.7, df=1, *p*=0.053; second axis: Chi-square=0.8, df=1, *p*=0.362).

Table. 1. *Means and standard deviations of the physicochemical variables of each region and season. Each variable was measured in spring (S) and autumn (A). Statistically significant results between seasons are shown with an asterisk after applying a Kruskal-Wallis test (p value <0.05).*

	Discharge (l/s)			**pH**			**Temp (ºC)**			**Conductivity (µS/cm)**		
Season	S	A		S	A		S	A		S	A	
Rif	167.25 ± 106.15	238.550 ± 285.61		8.68 ± 0.51	8.31 ± 0.23		12.35 ± 2.09	10.69 ± 2.16	*	434.88 ± 90.22	443.25 ± 87.87	
Betic	67.65 ± 57.02	96.78 ± 78.85	*	8.04 ± 0.54	7.71 ± 1.16		11.49 ± 2.98	8.64 ± 1.84	*	397.00 ± 97.38	507.80 ± 99.17	*
Picos	309.05 ± 271.81	238.66 ± 199.74		8.17 ± 0.31	8.34 ± 0.14		19.29 ± 0.31	9.55 ± 0.25	*	207.40 ± 39.55	258.30 ± 45.23	*
Jura	356.91 ± 339.25	855.09 ± 1235.75		7.95 ± 0.66	7.63 ± 0.47		9.76 ± 2.76	8.74 ± 1.65		405.64 ± 87.82	447.82 ± 68.94	*
Carpathians	133.45 ± 78.69	312.73 ± 379.81	*	NA	8.05 ± 0.27		9.33 ± 1.50	7.58 ± 1.12	*	319.82 ± 87.79	325.91 ± 86.94	
Sweden	114.73 ± 131	106.64 ± 96.57		7.43 ± 0.15	8.07 ± 0.16	*	10.85 ± 1.88	7.02 ± 1.56	*	235.27 ± 97.10	276.18 ± 141.12	

	Oxygen ppm (mg/l)			**Oxygen % (saturation)**			**Max. width (m)**			**Min. width (m)**	
Season	S	A		S	A		S	A		S	A
Rif	10.39 ± 0.67	10.48 ± 0.58		102.13 ± 5.01	97.48 ± 5.09		5.76 ± 1.92	5.91 ± 2.37		1.53 ± 0.93	1.75 ± 1.54
Betic	9.55 ± 1.23	7.00 ± 0.79	*	99.78 ± 12.54	68.60 ± 5.70	*	3.85 ± 1.11	3.53 ± 1.17		1.35 ± 1.10	1.28 ± 0.75
Picos	11.43 ± 0.31	10.67 ± 0.25	*	91.19 ± 35.31	99.46 ± 1.70	*	6.03 ± 1.89	5.80 ± 1.19		2.57 ± 1.34	2.58 ± 1.33
Jura	13.38 ± 1.54	11.00 ± 1.04	*	123.70 ± 12.08	96.30 ± 6.38	*	7.66 ± 3.19	7.69 ± 3.12		4.80 ± 2.98	4.49 ± 2.69
Carpathians	11.47 ± 0.69	11.89 ± 0.48	*	103.25 ± 5.38	100.63 ± 2.22		4.84 ± 1.37	5.66 ± 2.55		2.16 ± 1.01	2.35 ± 1.31
Sweden	10.12 ± 0.96	10.86 ± 0.83	*	94.33 ± 7.20	93.36 ± 5.93	*	5.49 ± 2.51	4.36 ± 2.19	*	2.69 ± 2.30	2.18 ± 1.74

Table. 2. *The five most abundant macroinvertebrate genera (number of individuals) collected in the regions where quantitative samples were obtained (Rif, Carpathians, and Sweden) separated by season: spring (S) and autumn (A).*

Rif				**Carpathians**				**Sweden**			
S		A		S		A		S		A	
Baetis	4921	*Hydropsyche*	310	*Rhithrogena*	718	*Gammarus*	1227	*Baetis*	529	*Baetis*	604
Serratella	188	*Micrasema*	150	*Leuctra*	662	*Leuctra*	697	*Elmis*	349	*Elmis*	112
Esolus	174	*Galba*	126	*Protonemura*	546	*Baetis*	481	*Leuctra*	186	*Leuctra*	108
Habrophlebia	129	*Baetis*	112	*Gammarus*	472	*Protonemura*	413	*Wormaldia*	121	*Nemoura*	68
Caenis	124	*Leuctra*	64	*Limnius*	373	*Rhithrogena*	326	*Hydraena*	117	*Hydraena*	44

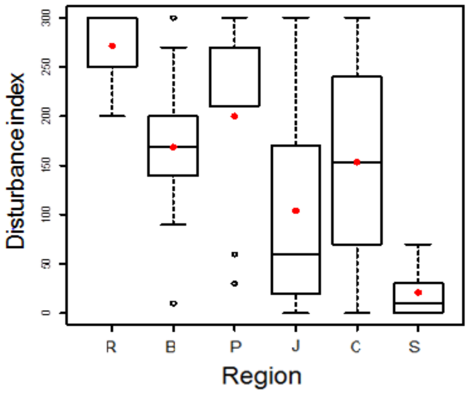

***Fig. 3**. Boxplot of the disturbance index per region and arranged by latitude: Rif (R), Betic (B), Picos (P), Jura (J), Carpathians (C), and Sweden (S). In each box, median is shown by a wide horizontal line whereas mean is shown with a red bullet. Outliers are plotted as white bullets.*

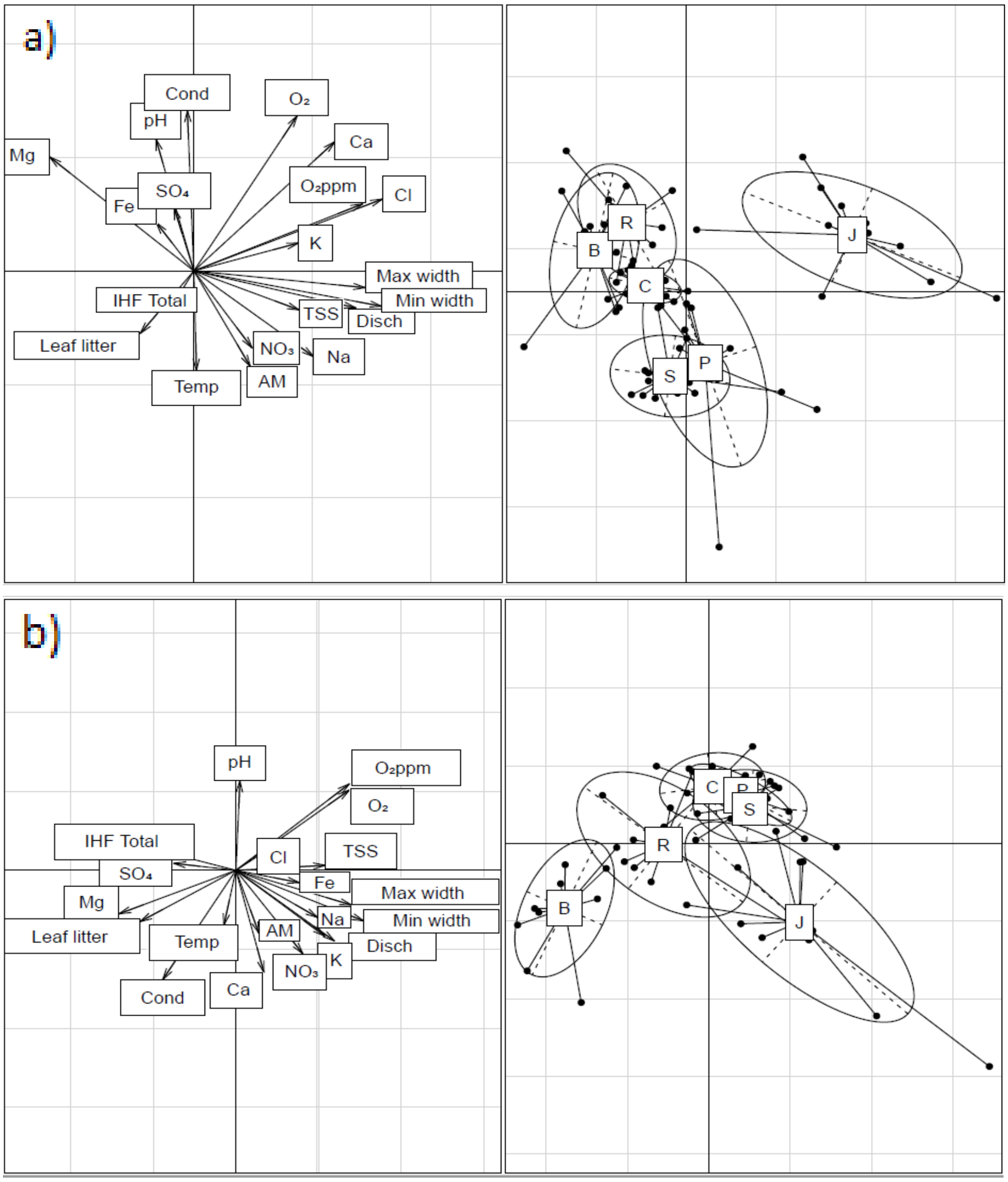

***Fig. 4.** a) Spring and b) autumn PCA of environmental parameters showing the effects of each variable (left panels) and the spatial position of the regions according to these variables (right panels). The ellipses envelop 70% of the data. See **Annex 4** for a description of each variable and the code used here. AM corresponds to algae and moss.*

Abundance and diversity

Rif was the region with more macroinvertebrate abundance followed by Carpathians and Sweden (*Table 2*). *Baetis* and *Leuctra* were the only genera present in the three regions where quantitative samples were obtained. Boxplots of macroinvertebrate density in Rif, Carpathians, and Sweden showed that there is a decreasing density as we move from Rif to Sweden in spring (*Fig. 5*). However, this trend was not find in autumn, where the Carpathians was the region with higher densities (*Fig. 5*). Kruskal-Wallis tests were significant for spring (Chi-squared = 6, df = 2, *p*=0.049) and non-significant for autumn (Chi-squared = 5.8, df = 2, *p*=0.054). Any of the posterior post-hoc Wilcoxon tests comparing regions were significant.

Simpson diversity (D) was visually higher for Carpathians and Sweden in spring, whereas in autumn diversity increased in the Rif and decreased in Sweden (*Fig. 6*). However, no significant differences were found with *Kruskal-Wallis* tests (spring: Chi-squared = 2.5, df = 2, *p*=0.290; autumn: Chi-squared = 2, df = 2, *p*=0.360).

Richness and composition

The number of genera collected in each region (γ-richness) is summarized in *Annex 5.* Gamma diversity decreases from Betic to Rif/Picos (with the same γ-richness), Jura, Carpathians, and Sweden in spring; and from Betic, Rif, Carpathians, Jura, Picos, and Sweden in autumn. Betic is thus the region with higher gamma diversity and Sweden with the lowest. Local richness values (α-diversity) showed a significant decrease with latitude after applying a mixed linear model for spring (Chi-squared=6, df=1, *p*=0.014) but not for autumn (Chi-squared=3.65, df=1, p=0.055) (*Fig. 6*).

Exclusive genera of each region are shown in *Annex 5.* Overall, spring was the season with more exclusive genera. In both seasons Betic (12 genera in spring and 8 in autumn), Rif (11 genera in both seasons), and Picos (10 genera in spring and 4 in autum) were the regions with more exclusive genera. Jura was the region with less exclusive genera in both occasions.

The number of EPT and OCH genera of each region and season were plotted (*Fig. 7*). Results from linear mixed models were not statistically significant for EPT (spring: Chi-squared=0.085, df=1, *p*=0.7; autumn: Chi-squared=0, df=1, *p*=0.9). In spring, the highest EPT was found in mid-latitudes (Picos, Jura, and Carpathians), whereas in

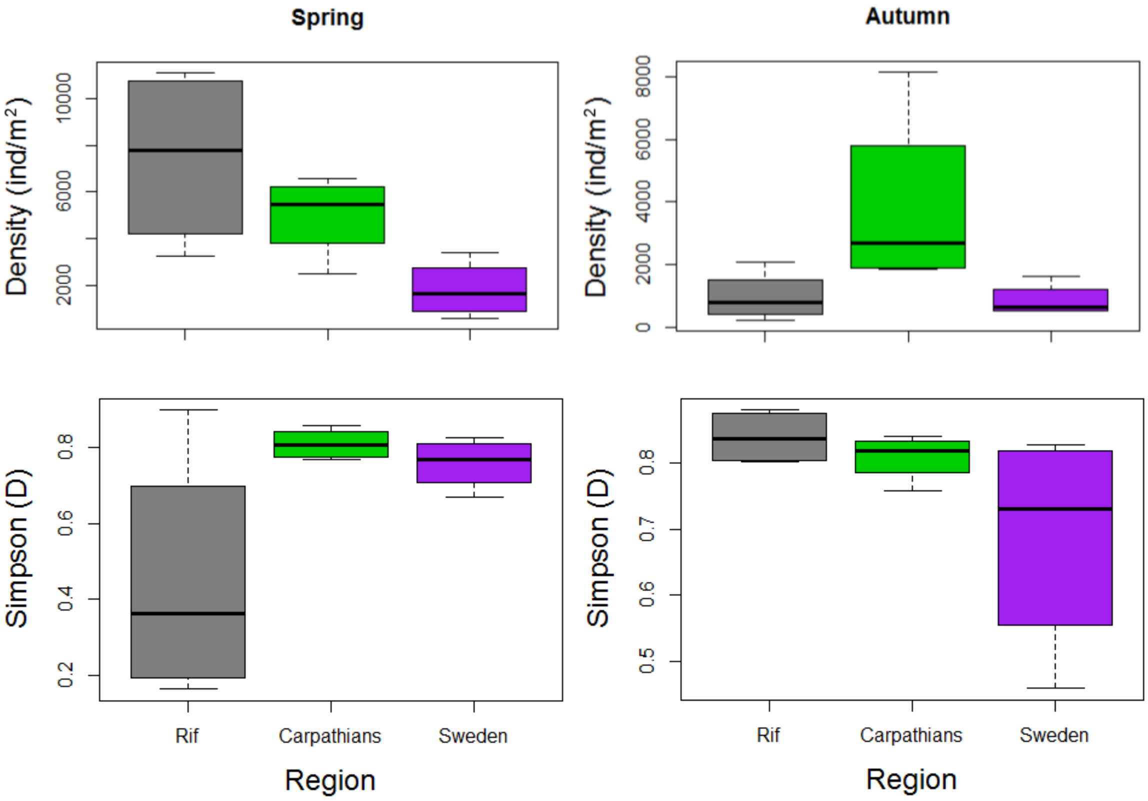

***Fig. 5**. Boxplots of abundance (in density) and diversity (Simpson index) in the three regions from where quantitative samples were obtained (Rif, Carpathians, and Sweden) separate by season. In each box, median is shown by a wide horizontal line quartiles.*

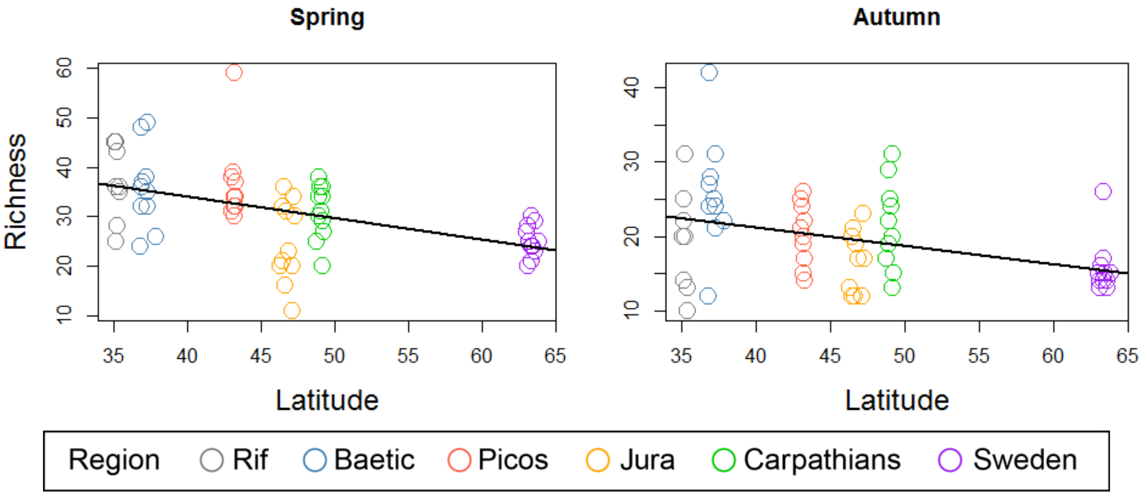

***Fig. 6**. Relationship between genera richness per site and latitude in spring (left) and autumn (right). Each region is shown in a different color. The black line represents only the tendency after a linear mixed model.*

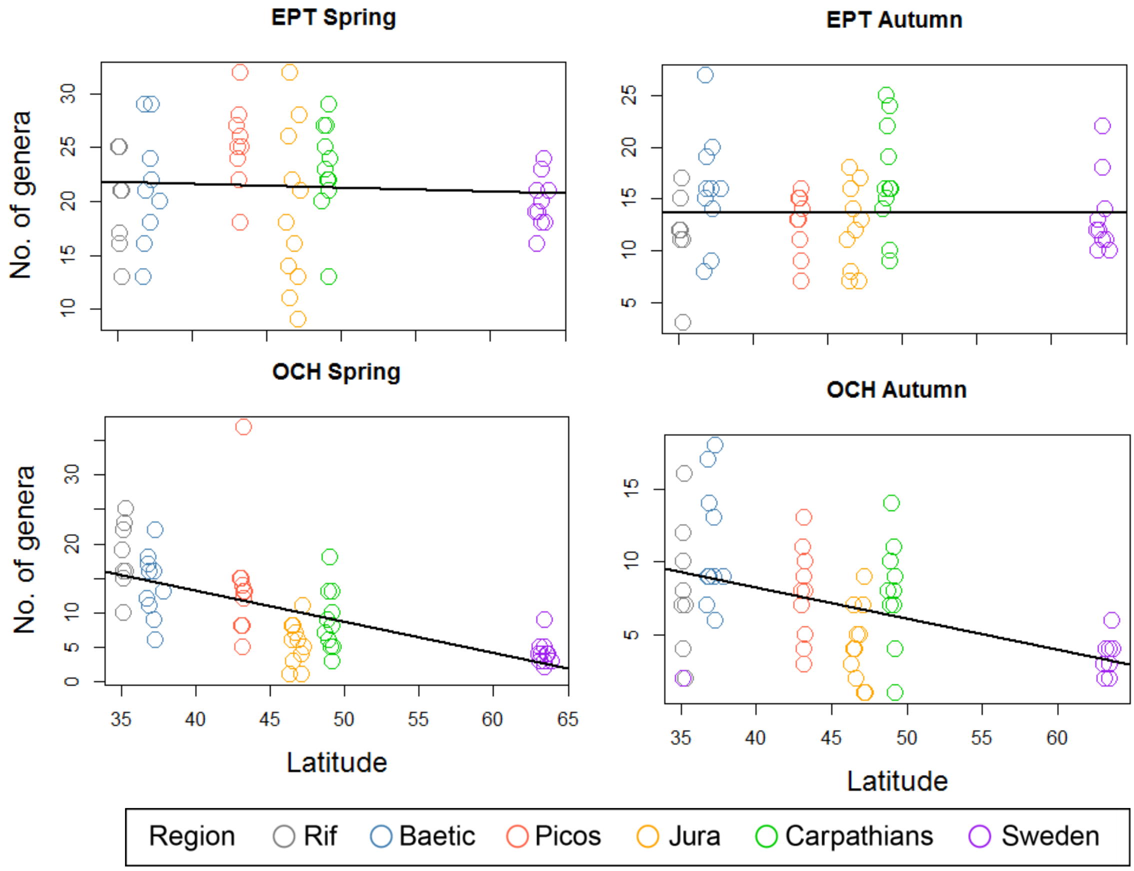

Fig. 7. *Relationship between the number of EPT (*Ephemeroptera, Plecoptera and Tricoptera*) and OCH (*Odonata, Coleoptera and Hemiptera*) genera per site and latitude in spring (left) and autumn (right). Each region is shown in a different color. The black line represents only the tendency after a linear mixed model.*

Table. 3. *Spring significant genera of each region after applying a statistical significance of species site-group associations (p<0.05). Regions: Rif (R), Baetic (B), Picos (P), Jura (J), Carpathians (C) and Sweden (S).*

R	B	P	J	C	S
Agabus	*Agapetus*	*Ancylus*	*Perlodes*	*Drusus*	*Alainites*
Allogamus	*Boyeria*	*Baetis*		*Ecclisopteryx*	*Amphinemura*
Anacaena	*Calopteryx*	*Dupophilus*		*Gammarus*	*Bathyomphalus*
Aquarius	*Capnioneura*	*Ecdyonurus*		*Glossosoma*	*Chaetopteryx*
Calamoceras	*Centroptilum*	*Echinogammarus*		*Leuctra*	*Elodes*
Deronectes	*Hydrocyphon*	*Serratella*		*Limnius*	*Heptagenia*
Euleuctra	*Hydropsyche*	*Habroleptoides*		*Lithax*	*Hydraena*
Haliplus	*Hydroptila*	*Isoperla*		*Melampophylax*	*Philopotamus*
Helophorus	*Microvelia*	*Oreodytes*		*Planaria*	*Polycentropus*
Hydrometra	*Normandia*	*Polycelis*		*Potamophylax*	*Radix*
Hydroporus	*Onychogomphus*	*Silo*		*Protonemura*	*Sericostoma*
Laccobius	*Pomatinus*	*Siphonoperla*		*Rhithrogena*	*Sialis*
Mesophylax	*Scirtes*			*Rhyacophila*	*Siphlonurus*
Ochthebius	*Tinodes*			*Schmidtea*	
Schizopelex					
Velia					

Table. 4. *Autumn significant genera of each region after applying a statistical significance of species site-group associations (p<0.05). Regions: Rif (R), Betic (B), Picos (P), Jura (J), Carpathians (C), and Sweden (S).*

R	B	P	J	C	S
Calamoceras	*Agapetus*	*Ancylus*	*Drusus*	*Baetis*	*Chaetopteryx*
Deronectes	*Calopteryx*	*Ecdyonurus*	*Potamophylax*	*Brachyptera*	*Diura*
Hydroporus	*Capnioneura*	*Echinogammarus*		*Elmis*	*Nemoura*
Schizopelex	*Centroptilum*			*Gammarus*	*Polycentropus*
	Hydrocyphon			*Habroleptoides*	
	Hydropsyche			*Limnius*	
	Onychogomphus			*Perlodes*	
	Paraleptophlebia			*Philopotamus*	
	Pomatinus			*Rhithrogena*	
	Sericostoma			*Rhyacophila*	
	Wormaldia				

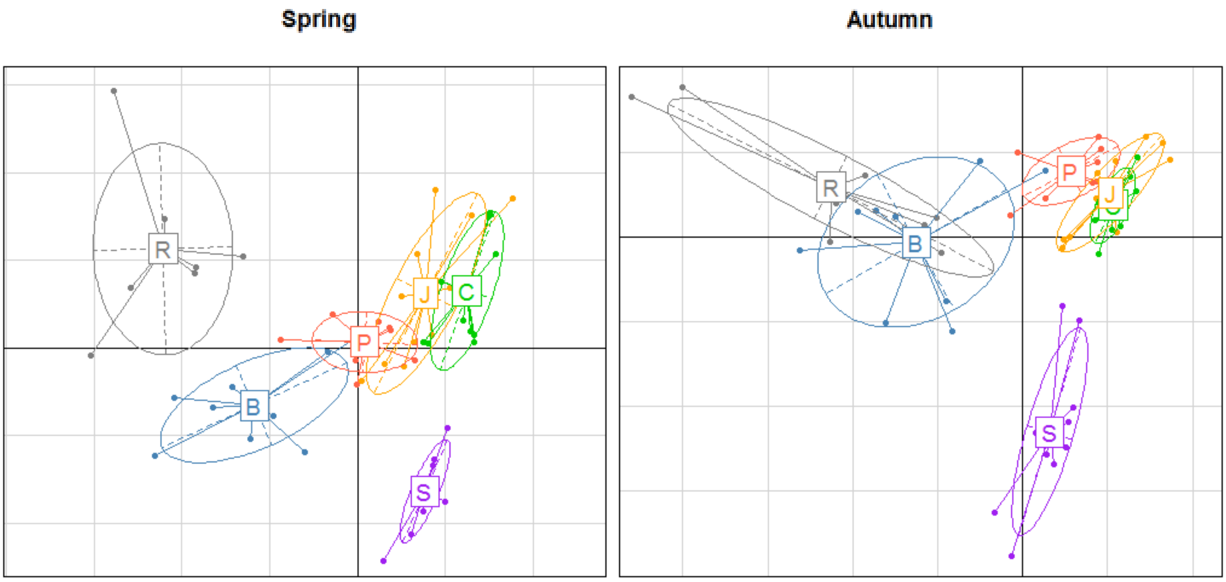

Fig. 8*. Correspondence Analysis (CA) of the macroinvertebrate genera abundance in spring (left panel) and autumn (right panel). Regions: Rif (R), Baetic (B), Picos (P), Jura (J), Carpathians (C) and Sweden (S), named from lower to high latitude). Labels indicate the gravity centre of the ellipses that envelop 70% of the data.*

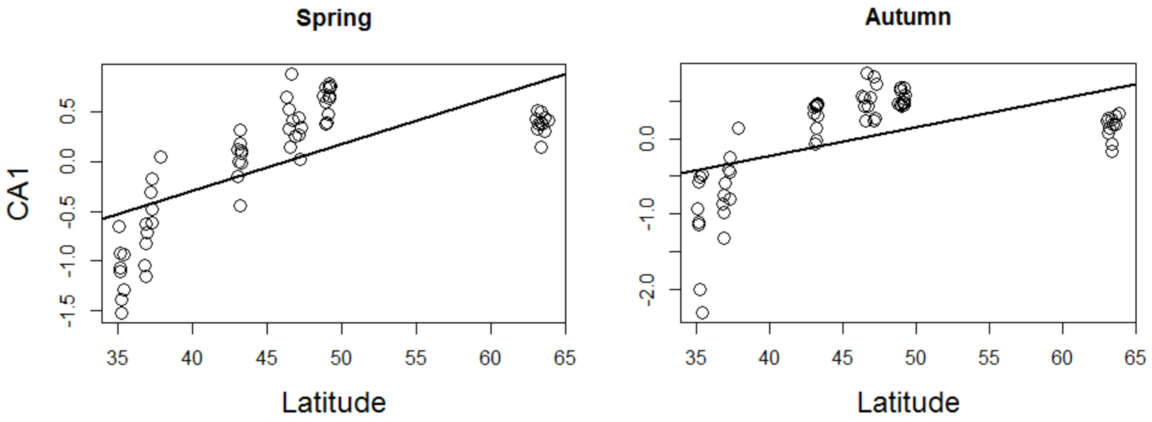

Fig. 9*. Relationship between the CA first axis and latitude in spring (left) and autumn (right). The black line represents only the tendency after a linear mixed model.*

autumn regional changes were less clear. A significant latitudinal effect was however found for OCH in both seasons (spring: Chi-squared=8.3, df=1, *p*=0.003; autumn: Chi-squared=5.2, df=1, *p*=0.022). OCH genera had a decreasing tendency in latitude from the Rif to Sweden with the maximum OCH genera collected in the Betic region (*Fig. 7*).

The more significant species of each region in spring and autumn are presented in *Table 3* and *4*, respectively. Except for the Jura, there were more singular species in all regions in spring than in autumn. This pattern was especially true for Rif, Picos and Sweden.

Several genera were found in >70% sampled sites per region, especially in spring (*Annex 6*). Picos in spring and Rif in autumn were the regions with the highest number of genera with >70% of occurrence. Rif and Betic shared many species with similar occurrence values such *Hydropsyche* or *Allogamus*. In spring, *Baetis, Leuctra,* and *Elmis* genera were found in all regions. In autumn only *Leuctra* was found in all regions (*Annex 6*).

The resulting CA (*Fig. 8*) showed a more isolated spatial distribution among the regions in spring than in autumn. In autumn, regions are more similar in macroinvertebrate composition and three main groups were identified: Rif-Betic, Picos- Jura-Carpathians, and Sweden was isolated from the others. Axes 1 and 2 explained 9.6% and 6.7% of the total variability in spring and 8.5% and 6.4% in autumn. The between components analysis was statistically significant in both seasons (*p*<0.01) and the between-region difference was higher in spring than in autumn (between-class variance = 0.24 and 0.21 spring and autumn respectively).

Latitudinal effects were significant when considering the first CA axis (*Fig. 9*) for spring but non-significant for autumn (spring, first axis: Chi-squared=6.1, df=1, *p*=0.013; autumn, first axis: Chi-squared=3.8, df=1, *p*=0.051). However, the second CA axis showed significant relationship with latitude for the autumn but not for spring (spring, second axis: Chi-squared=2.8, df=1, *p*=0.093; autumn, second axis: Chi-squared=5.4, df=1, *p*=0.020).

Main environmental variability among sites (i.e., first PCA axis) was not related to main biological variability among sites. Thus, the spring linear mixed model between first CA axis and the first PCA axis was not significant (Chi-squared=2.1, df=1, *p*=0.142), whereas a significant latitudinal effect was found when combining the first PCA axis and the latitude (Chi-squared=8.1, df=1, *p*=0.017). When comparing latitudinal models run with and without the first PCA axis, no significant differences were found (Chi-squared=3.8, df=1, *p*=0.155).

The spring CCA found that environmental variables explained 42.27% of the total variance and latitude only explained 7.71% of this variance (total inertia = 3.96). The autumn CCA found that the environmental variables explained 44.21% of the total variance and latitude only explained 6.29% of the total variance (total inertia = 4.94).

DISCUSSION

General latitudinal patterns

Latitudinal patterns were observed from north of Morocco to Sweden in the sense predicted: richness decreased and community changed as latitude increased. This general latitudinal pattern could be mainly related to multiple past factors and current environmental factors (as a slight effect) acting together. On the one hand, past geological events as glaciations caused some taxonomic losses in high latitudes favouring the migration of several species and an increase of speciation events in the south of Europe (Hewitt, 2004; Bonada & Resh, 2013). In warm climates the speciation rate is usually faster than in cold climates (Jacobsen et al., 1997), supporting the latitudinal richness tendency observed in this study. Gamma diversity, for example, was higher in southern than northern latitudes in our study dealing with macroinvertebrates. On the other hand, environmental conditions as climate characteristics have determined current ecological niches (i.e. suitable habitat for determinates organisms) (Bonada et al., 2007a). In fact, some authors as Jacobsen et al. (1997) postulate that temperature and conductivity were the most related variables with macroinvertebrate diversity. In our case, especially temperature affected Betic and Jura regions in autumn.

Richness overall decreased with increasing latitude, which agrees with many existing macroecological studies (e.g., Jacobsen et al., 1997; Bonada et al., 2007a). For macroinvertebrates, a higher alpha and gamma diversity have been recorded nearest to Ecuador (Jacobsen et al., 1997) which has been related to environmental heterogeneity (Miserendino, 2001). Regarding abundance and diversity, no clear latitudinal patterns were observed considering spring and autumn together, indicating that local characteristics are probably related to changes in species abundance with consequent effects on diversity indexes. In agreement with our finding, the few existing studies that analyse latitudinal changes in marine ciliate abundance did not found a clear pattern when moving for the Ecuador northwards (Huaxue et al., 2013). Not many studies have analysed latitudinal patterns in abundance because of the lack of comparable data. Most macroecological studies use data coming from different sources that make abundance comparison a challenge (Blackburn, 2004).

Concerning changes in macroinvertebrate community, OCH genera followed a latitudinal pattern whereas EPT did not. Macroinvertebrate communities in Mediterranean rivers are adapted to droughts and floods and present resistance and resilience strategies against disturbances (i.e. less permeable cuticles, high dispersal abilities; Hershkovitz & Gastih, 2013). During low flows, Mediterranean rivers are also composed by communities dominated by lentic-like taxa, such as OCH (Bonada et al., 2006). Coleoptera and Heteroptera diversity is actually highest in the Mediterranean than in temperate Europe (Domisch et al., 2013), especially in the extreme south of Europe (Bonada et al., 2012). This would explain the latitudinal observed pattern of OCH. In contrast, EPT did not show any latitudinal change, despite these genera are more commonly found in riffle-like habitats and thus in more temperate climates (Bonada et al., 2007a; Bonada & Resh, 2013; Tilbian, 2013). Other historical factors could also explain these EPT and OCH patterns. OCH are especially diverse in Mediterranean regions whereas among the EPT, Plecoptera is primarily a temperate group but Ephemeroptera and Trichoptera seem to be spread to temperate and tropical regions (Jacobsen et al., 1997, Vinson & Hawkins, 2012), eroding latitudinal patterns. The tendency of Trichoptera richness to be less variable than the two others can be explained by their trophic diversity compared to other aquatic insects (Vinson & Hawkins, 2012).

Macroinvertebrate composition was related to local environmental variables but they did not explain the overall latitudinal trend. Latitudinal changes in composition could be again explained by past historical and current environmental variables acting at regional scales. Glaciations simplified northern communities and favoured speciation in southern ones (Hewitt, 2004). The results from our CA actually showed this pattern, as Mediterranean communities were more different among them than temperate ones. In addition, regional characteristics of each region are important. Sites of our study had similar local environmental characteristics, but they differ in climate (a regional characteristic). Because of temperature and precipitation patterns, Mediterranean climates have marked seasonal changes that affect macroinvertebrates. This is more relevant for intermittent rivers but is also significant in perennial ones (Rieradevall et al., 1999). Macroinvertebrate communities are significantly different between Mediterranean and temperate climate rivers (Bonada et al., 2007a), explaining the large change observed between these climatic regions in our CA.

Seasonal latitudinal patterns

Mediterranean regions are characterised by summer drought and autumn flood disturbances (Bonada & Resh, 2013) whereas subarctic regions are mainly influenced by spring floods as a result of snowmelt (Korhonen & Kuusisto, 2010; Veijalainen et al., 2010). Our disturbance index seemed to agree with this pattern, as higher flood disturbance events occurred in lower latitudes between spring and autumn. This can explain why we could not register nearly any disturbance in Sweden in the disturbance index because the disturbances had already happened.

Latitudinal patterns were more evident for spring than for autumn, which agrees with our hypothesis. Rif had extreme changes in abundance and diversity between seasons. Assuming that in autumn in the Mediterranean climate there are some flow events that homogenize the stream conditions (Bonada & Resh, 2013), this pattern could be explained by the *intermediate-disturbance hypothesis*. According to this hypothesis, the maximum richness is reached when intermediate levels of disturbance are present (Jacobsen et al. 1997). In comparison to Carpathians and Sweden, Rif had the highest mean value of the disturbance index showing that this was the region with more environmental changes between seasons. The presence of flood events in the Rif would reduce the abundance of some particular genera from spring to autumn, increasing the autumn diversity. Carpathians and Sweden had minimal variability between seasons which partially also agrees with the higher stability found in the disturbance index. This was especially true for Sweden where rivers are dominated by snowmelt dynamics (Friberg et al., 2013) that most likely occurred before the sampling of this study took place.

Latitudinal changes in richness and community composition varied between spring and autumn, whereas EPT and OCH changes along latitude were uniform in both seasons. Despite we were unable to quantify climatic differences along the studies latitudinal gradient, spring climatic conditions (i.e., temperature, precipitation and thus hydrological characteristics) are supposed to be more different than autumn climatic conditions. In spring, rivers in Mediterranean regions are dominated by a high diversity of riffle-like and lentic-like organisms that will shift to a community completely dominated by lentic-like taxa if rivers become intermittent (a typical feature of Mediterranena rivers; Bonada & Resh, 2013). Instead, rivers in subarctic regions are supposed to be recovering from snowmelt floods, with more simplified communities (Mustonen et al., 2016). In autumn, flood events occur along the gradient and, despite they are more frequent in southern latitudes (i.e., Rif as shown by the disturbance index), environmental conditions are more uniform and communities are simpler after flood events (Robinson et al., 2004). All this could explain why the latitudinal signal in autumn was less obvious than in spring.

One limitation of this study is the large latitudinal gap between Carpathians and Sweden. Samples from an intermediate region would probably increase the power of the latitudinal gradient. In addition, the inclusion of winter and summer samples would also help us to provide a more robust explanation of seasonal latitudinal changes in macroinvertebrate communities.

The majority of macroecology studies do not consider seasonality and how it could affect the well-known patterns (Vinson & Hawkins, 2012). Most likely this is because most of them are based on already existing data, very often from different sources, and seasonality is an issue within the data that is difficult to rule it out. In addition, most macroecological studies are performed using relatively long-lived organisms (e.g., fish; Oberdorff et al., 2011) that are less susceptible to display seasonal variability within a year. Seasonal variability should be considered especially in those macroecological studies dealing with relatively short-live organisms or that consider regions with marked seasonal patterns.

CONCLUSION

Latitudinal changes of macroinvertebrate communities from north Morocco to central Sweden varied depending on the season and the metric considered. Linear changes in abundance and diversity were more evident in spring than in autumn. Richness and community composition showed siginificant linear latitudinal changes in spring but not in autumn. EPT and OCH showed significant linear pattern in both seasons. These patterns are more explained by large-scale factors (past historical events and current climate) than by local environmental characteristic. Results from this study indicate the need to consider multiple seasons when studying latitudinal patterns in macroecology because these cannot be universal. This is especially relevant for taxonomic groups that have seasonal changes in community composition (e.g. macroinvertebrates) and for regions with high seasonality (e.g., Mediterranean and subarctic climates). Accounting for these temporal variability in macroecological patterns will help to better understand the nowadays biodiversity.

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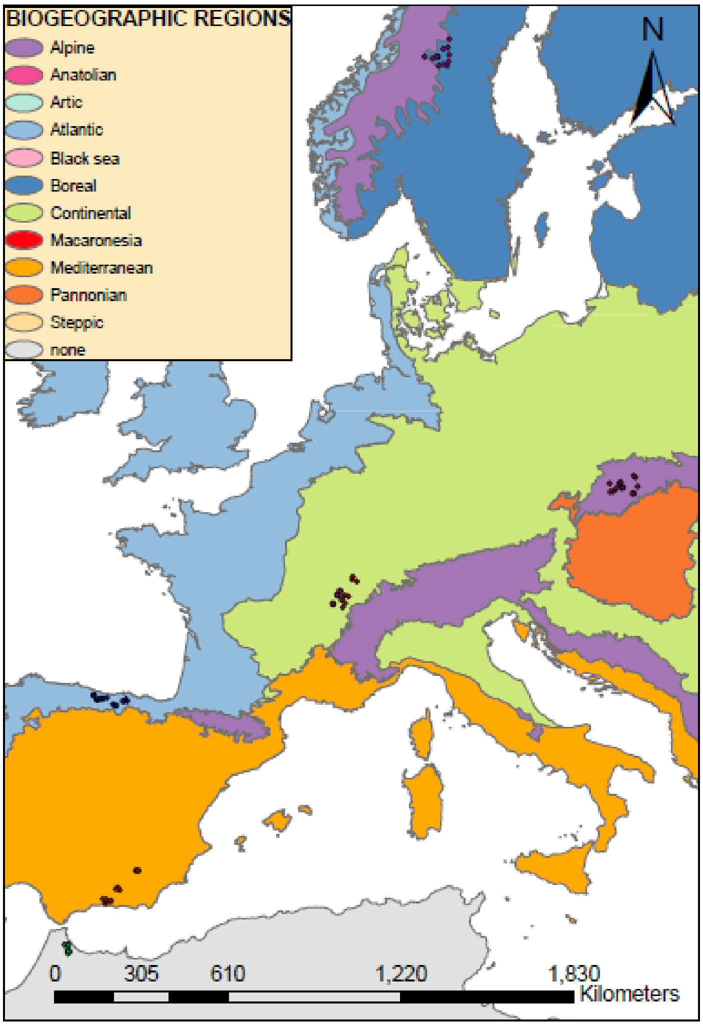
Annex 1

Location of sites (in black bullets) in each biogeographical region according to Illies, 1978.



Annex 2

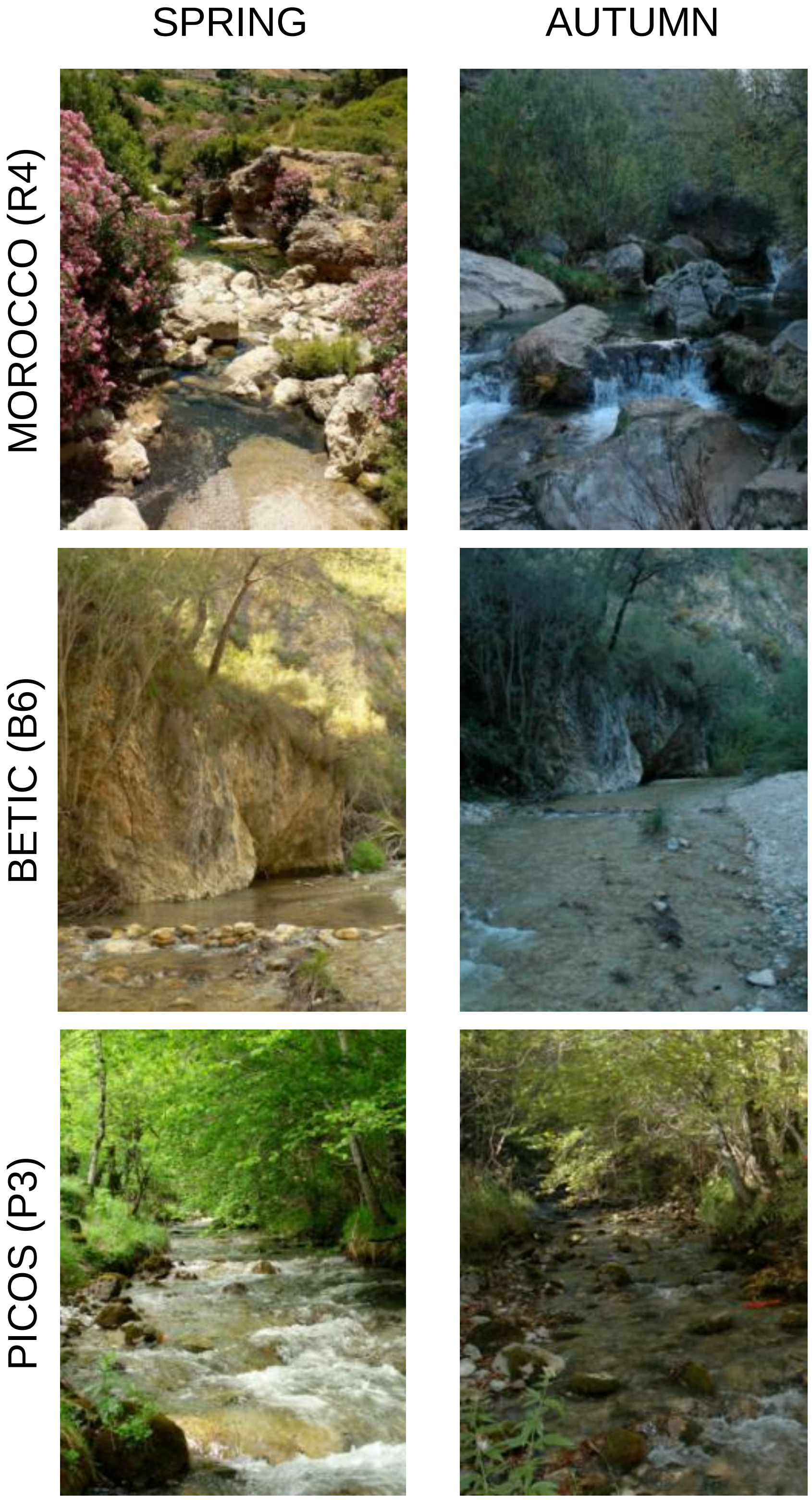
General information of each sampling point including the code (a letter identifying the region and a number), the name of the river, the toponym and the coordinates.

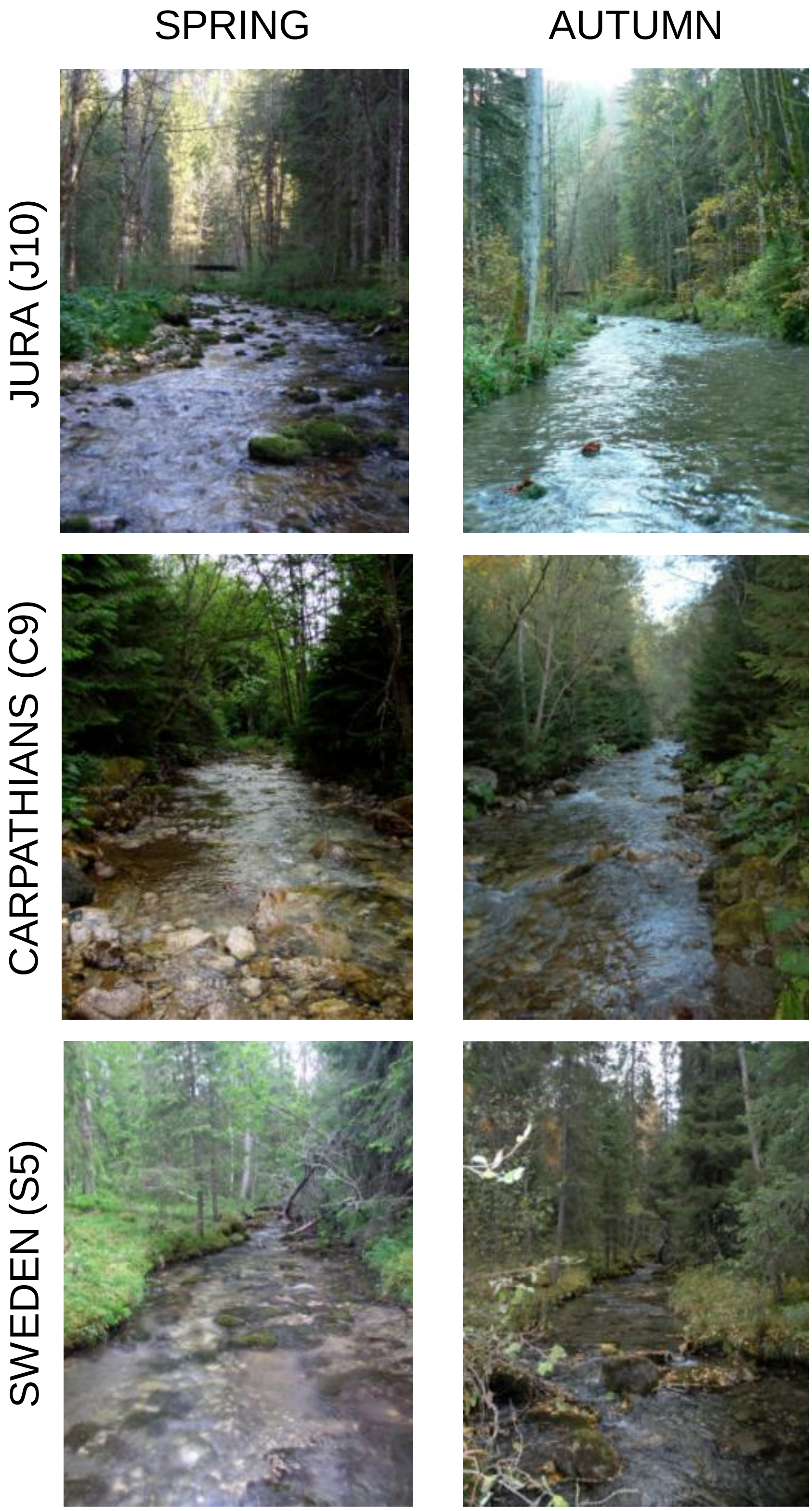
Region	Country	Code	Basin	River	Toponym	Time zone	X UTM	Y UTM
Rif	Morocco	R02	Laou	Maggo	Nord Village Maggo	30	300922	3886531
		R04	Laou	Kalaa	Akchour	30	301741	3901957
		R05	Martil	Arozane	Beni Moussa	30	287668	3917976
		R06	Laou	Tassakisté	Afechtal	30	299953	3918008
		R07	Adelmane	Madissouka	Madissouka	30	305220	3894410
		R08	Adelmane	Béni M'Hammed	Beni M'Hammed	30	306250	3892767
		R09	Laou	Farda	Afaska	30	300840	3898468
		R10	Laou	Farda	Imizzare	30	302047	3894314
Betic	Spain	B01	Genil	Aguas Blancas	Tocón	30	468724	4120836
		B02	Guadiana Menor	Arroyo de Prado Negro	Venta del Molinillo	30	462069	4128997
		B04	Genil	Arroyo del Cerezal	Cortijo del Cerezal	30	412084	4087044
		B05	Verde	Verde	Bco. de los Madroñales	30	433371	4078887
		B06	Chíllar	Chíllar	Cerro Fuerte Perro	30	421142	4071685
		B07	Genil	Venta Vicario	Loma de Ubares	30	422525	4082424
		B08	Genil	Cebollón	Cruz Los Llanos	30	425609	4085782
		B09	Guadiana Menor	Castril	Bco. de las Palomas	30	521920	4193742
		B10	Guadiana Menor	Guardal	Cortijo de la Natividad	30	528538	4193848
		B11	Genil	Darro	Fuente la Teja	30	455172	4123899
Picos	Spain	P01	Deva	Bejes	Bejes	30	366633	4788602
		P02	Cares	Peguera		30	344732	4783904
		P03	Sella	Covadonga	Cangas de Onís	30	331987	4797950
		P04	Cares	Duje	Invernales del Texu	30	357394	4787575
		P05	Nansa	Venta Fresnedo	Venta Fresnedo	30	378943	4793029
		P06	Asón	Bustablado	El avellanal-Bustablado	30	446621	4793034
		P07	Asón	Asón	Asón	30	451113	4786155
		P08	Pisueña	Pisueña	Valvanuz	30	436587	4785140
		P09	Saja	Quericado	Balcón de la Cardosa (Puerto Palombera)	30	401378	4771378

		P10	Besaya	Sietefuentes	Santiurde de Reinosa	30	410937	4767916
Jura	France	J01		Seille	Ladoye sur Seille	31	705064	5182147
		J02		Cuisance	Arbois-Mesnay	31	713872	5195242
		J03		Creuse		32	303653	5233731
		J04		Cuisancin	Val de Cuisancin	32	306298	5244225
		J05		Consolation		32	318320	5225192
		J06		Ain	Source de l'Ain	32	272608	5181699
		J07		Doubs	Mouthe	32	286520	5176321
		J08		Hérisson	Ilay	31	720827	5165883
		J09		Surans	Gigny	31	689928	5150437
		J10		Les Rousses	Les Rousses	32	272141	5152462
		J11		Flumen	Les Moulins	31	723570	5137480
Carpathians	Slovakia	C01		Čierna voda		34	449776	5450795
		C02		Biela		34	444575	5457173
		C03		Hrdzavý		34	427947	5399800
		C04		Veľká Biela		34	449423	5419073
		C05		Ľubochnianka		34	363712	5423384
		C06		Gaderský Potok		34	353927	5422856
		C07		Varínka		34	357299	5452508
		C08		Biely Potok		34	377962	5432064
		C09		Prosiečanka		34	390500	5445687
		C10		Suchý Potok		34	396458	5447595
		C11		Demänovka		34	396241	5427999
Jämtland	Sweden	S01				33	417031	7041763
		S02				33	487313	7056033
		S03				33	488227	6993936
		S04				33	459457	7000839
		S05				33	477112	7002018
		S06				33	460940	7033043
		S07				33	444223	7029700
		S08				33	496967	7007949
		S09				33	503707	7031954
		S10				33	503977	7058788
		S11				33	513583	7085473

Annex 3

Pictures of six sampling sites one of each region in spring and autumn. Information of each site can be found in Annex 2*.*




SPRING
AUTUMN
JURA (J10)
CARPATHIANS (C9)
SWEDEN (S5)

Annex 4

List of environmental variables to build the PCA.

Variables	Description (units)
Discharge	River discharge (in l/s)
pH	pH value
Temperature	Water Temperature (ºC)
Conductivity	Conductivity value (in microS/cm)
Oxygen (ppm)	mg/l of Oxygen concentration
Oxygen (%)	% of Oxygen saturation
Max width	Max width of the sampled reach (in m)
Min width	Min width of the sampled reach (in m)
% Algae+Moss	% of algae and moss in the sampled reach (algae+leaf litter=100%)
% Leaf litter	% of leaf litter in the sampled reach (algae+leaf litter=100%)
K	Potassium concentration (mg/l)
Na	Sodium concentration (mg/l)
Mg	Magnesium concentration (mg/l)
Ca	Calcium concentration (mg/l)
Fe	Iron concentration (mg/l)
TSS	Total suspended solids concentration (mg/l)
Cl	Chloride concentration (mg/l)
NO3	N-Nitrate concentration (mg/l)
SO4	Sulphates concentration (mg/l)
IHF Total	Totat value of the Fluvial Habitat Index (sum from different subparts: inclusion, riffle frequency, substrate composition, depth/velocity regime, shade percentage, heterogeny elements and aquatic vegetation cover) according to Pardo et al., 2002.

Annex 5

Number of different genera per region (γ-richness) in each season: spring (S) and autumn (A).

Region	S	A
Rif	92	70
Betic	100	76
Picos	92	54
Jura	73	55
Carpathians	72	56
Sweden	56	42

Spring and autumn exclusive genera per region. The total number of exclusive genera per region is presented below. Regions: Rif (R), Betic (B), Picos (P), Jura (J), Carpathians (C) and Sweden (S), named from lower to high latitude.

Spring

R	B	P	J	C	S
Aulonogyrus	*Bidessus*	*Anomalopterygella*	*Coelambus*	*Anisus*	*Glyphotaelius*
Calamoceras	*Coenagrion*	*Beraea*	*Dictyogenus*	*Brachycentrus*	*Heptagenia*
Chaetarthria	*Crocothemis*	*Diplectrona*	*Niphargus*	*Bythiospeum*	*Hydatophylax*
Copelatus	*Glossiphonia*	*Dupophilus*	*Piscicola*	*Ecclisopteryx*	*Molannodes*
Euleuctra	*Guadalgenus*	*Ithytrichia*		*Eubria*	*Procloeon*
Mesophylax	*Helochares*	*Micronecta*		*Schmidtea*	*Siphlonurus*
Micropterna	*Hydrochus*	*Noterus*			
Notonecta	*Hygrotus*	*Pachyleuctra*			
Peltodytes	*Laccophilus*	*Stictotarsus*			
Platycnemis	*Lasiocephala*	*Theodoxus*			
Schizopelex	*Oxyethira*				
	Stenophylax				
11	12	10	4	6	6

Ututumn

R	B	P	J	C	S
Aulonogyrus	*Asellus*	*Helobdella*	*Gyraulus*	*Apatania*	*Capnopsis*
Calamoceras	*Athripsodes*	*Helophorus*	*Hydatophylax*	*Brachycentrus*	*Gerris*
Cloeon	*Bidessus*	*Lasiocephala*		*Electrogena*	*Heptagenia*
Cloeon	*Bythinella*	*Synagapetus*		*Electrogena*	*Oligostomis*
Gyrinus	*Bythiospeum*			*Electrogena*	
Haliplus	*Metalype*			*Oreodytes*	
Laccophilus	*Nepa*			*Schmidtea*	
Lymnaea	*Pyrrhosoma*				
Paracymus					
Physa					
Schizopelex					
11	8	4	2	7	4

Annex 6

Genera with more than 70% of occurrence in each region for spring and autumn separately.

Spring

Rif		**Betic**		**Picos**		**Jura**		**Carpathians**		**Sweden**	
Baetis	100	*Baetis*	100	*Baetis*	100	*Baetis*	100.0	*Baetis*	100.0	*Alainites*	100.0
Habrophlebia	100	*Hydropsyche*	100	*Ecdyonurus*	100	*Protonemura*	100.0	*Leuctra*	100.0	*Baetis*	100.0
Leuctra	100	*Leuctra*	100	*Elmis*	100	*Elmis*	90.9	*Limnius*	100.0	*Chaetopteryx*	100.0
Agabus	87.5	*Hydraena*	90	*Serratella*	100	*Leuctra*	90.9	*Protonemura*	100.0	*Hydraena*	100.0
Hydraena	87.5	*Ecdyonurus*	80	*Esolus*	100	*Rhithrogena*	90.9	*Rhithrogena*	100.0	*Leuctra*	100.0
Hydropsyche	87.5	*Polycentropus*	80	*Hydraena*	100	*Rhyacophila*	90.9	*Rhyacophila*	100.0	*Rhyacophila*	100.0
Isoperla	87.5	*Protonemura*	80	*Polycelis*	100	*Gammarus*	81.8	*Elmis*	90.9	*Amphinemura*	90.9
Protonemura	87.5	*Sericostoma*	80	*Protonemura*	100	*Ecdyonurus*	72.7	*Gammarus*	90.9	*Elmis*	90.9
Velia	87.5	*Agapetus*	70	*Rhithrogena*	100	*Esolus*	72.7	*Hydraena*	90.9	*Heptagenia*	90.9
Allogamus	75	*Allogamus*	70	*Rhyacophila*	100	*Hydraena*	72.7	*Isoperla*	90.9	*Philopotamus*	90.9
Caenis	75	*Elmis*	70	*Sericostoma*	100	*Limnius*	72.7	*Glossosoma*	81.8	*Sericostoma*	90.9
Calamoceras	75	*Esolus*	70	*Ancylus*	90			*Planaria*	81.8	*Elodes*	81.8
Elmis	75	*Hydroptila*	70	*Epeorus*	90			*Sericostoma*	81.8	*Isoperla*	81.8
Ephemerella	75	*Onychogomphus*	70	*Isoperla*	90			*Allogamus*	72.7	*Potamophylax*	81.8
Esolus	75	*Rhyacophila*	70	*Silo*	90			*Drusus*	72.7	*Ephemera*	72.7
Helophorus	75	*Tinodes*	70	*Echinogammarus*	80			*Esolus*	72.7	*Polycentropus*	72.7
Hydroporus	75			*Hydropsyche*	80			*Potamophylax*	72.7	*Sialis*	72.7
Rhyacophila	75			*Leuctra*	80						
				Limnius	80						
				Siphonoperla	80						
				Tinodes	80						

Autumn

Rif		**Betic**		**Picos**		**Jura**		**Carpathians**		**Sweden**	
Baetis	100	*Baetis*	100	*Baetis*	100	*Rhyacophila*	100.0	*Baetis*	100.0	*Baetis*	100.0
Hydropsyche	87.5	*Hydropsyche*	100	*Ecdyonurus*	100	*Baetis*	90.9	*Protonemura*	100.0	*Leuctra*	100.0
Leuctra	87.5	*Sericostoma*	90	*Elmis*	100	*Elmis*	81.8	*Rhyacophila*	100.0	*Rhyacophila*	100.0
Hydraena	75	*Leuctra*	80	*Rhithrogena*	100	*Ecdyonurus*	72.7	*Gammarus*	90.9	*Diura*	90.9
Protonemura	75	*Protonemura*	80	*Limnius*	90	*Gammarus*	72.7	*Hydraena*	90.9	*Hydropsyche*	81.8
		Rhyacophila	80	*Hydraena*	80	*Rhyacophila*	100.0	*Leuctra*	90.9	*Nemoura*	81.8
		Elmis	70	*Polycelis*	80	*Baetis*	90.9	*Rhithrogena*	90.9	*Hydraena*	72.7
		Hydraena	70	*Rhyacophila*	80	*Elmis*	81.8	*Elmis*	81.8	*Philopotamus*	72.7
		Limnius	70	*Ancylus*	70	*Ecdyonurus*	72.7	*Limnius*	81.8	*Baetis*	100.0
				Echinogammarus	70	*Gammarus*	72.7	*Sericostoma*	81.8	*Leuctra*	100.0
				Leuctra	70			*Ecdyonurus*	72.7	*Rhyacophila*	100.0
				Protonemura	70					*Diura*	90.9
				Sericostoma	70					*Hydropsyche*	81.8
										Nemoura	81.8
										Hydraena	72.7
										Philopotamus	72.7