1 **Spatial scale effects on taxonomic and biological trait diversity of aquatic**

2 **macroinvertebrates in Mediterranean streams**

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4 Eduardo M. García-Roger[1, 5+], María del Mar Sánchez-Montoya[2], Núria Cid[1], Stefania

5 Erba[3], Ioannis Karaouzas[4], Iraima Verkaik[1], Maria Rieradevall[1], Rosa Gómez[2], M. Luisa

6 Suárez[2], M. Rosario Vidal-Abarca[2], Daniele DeMartini[3], Andrea Buffagni[3], Nikos

7 Skoulikidis[4], Núria Bonada[1], and Narcís Prat[1]

8

9 **Authors' addresses:**

10 [1] *Grup de Recerca "Freshwater Ecology and Management (FEM)", Departament*

11 *d'Ecologia. Universitat de Barcelona, Spain*

12 [2] *Departamento de Ecología e Hidrología. Regional Campus of International Excellence*

13 *"Campus Mare Nostrum". Universidad de Murcia, Spain*

14 [3] *Istituto di Ricerca Sulle Acque (CNR-IRSA), Italy*

15 [4] *Hellenic Centre for Marine Research (HCMR), Greece*

16 [5] *Institut Cavanilles de Biodiversitat i Biologia Evolutiva. Universitat de València, Spain*

17 [+] Corresponding author; E-mail: eduardo.garcia@uv.es

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19 With 5 figures and 6 tables

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1   **Abstract:** We examined the effect of spatial scale on aquatic macroinvertebrate communities in

2   Mediterranean streams from six basins distributed across southern Europe, including Spain, France,

3   Italy, and Greece. We classified the studied streams according to their long-term aquatic regime

4   into the three following types: (i) permanent (P), (ii) intermittent with summer pools (I-P), and (iii)

5   intermittent with summer dry channels (I-D). For each stream type, we analyzed taxonomic and

6   trait diversity, as well as the composition of the macroinvertebrate community, following a spatially

7   nested design at three spatial scales of analysis: microhabitat (substratum patches), mesohabitat

8   (pools vs. riffles), and macrohabitat (streams). In order to assess intrinsic seasonal variability in

9   streams from the Mediterranean region, 20 Surber samples were taken from each stream according

10   to meso- and microhabitat frequency in the wet and the dry season during 2010. Given the need for

11   adaptation to specific hydrological conditions and the fact that microhabitats should encompass the

12   niche requirements of particular taxa, we hypothesized that this spatial scale would have a greater

13   influence on macroinvertebrate taxa composition and biological traits than the other two larger

14   spatial scales in intermittent streams. We observed that patterns in the relative importance of

15   variance components across hierarchical spatial scales changed with time because low flow or

16   droughts altered both mesohabitat prevalence and microhabitat composition. Our results confirm

17   the importance of the microhabitat scale in I-P streams in the wet season but not in the dry one,

18   when a loss of microhabitat diversity occurred. Stream-to-stream variability was more important in

19   P and I-D streams. Our study also explored the relationships between traits and aquatic regimes. We

20   found that aquatic macroinvertebrates inhabiting permanent streams exhibited traits related to the

21   longer duration of life cycles in these rivers (e.g. large size of adult stages) and adaptations to

22   flowing conditions (e.g. modes of aquatic dispersal), whereas aquatic macroinvertebrates inhabiting

23   intermittent streams with summer pools had traits adapted to depositional conditions and ecological

24   preferences for confined habitats (i.e. disconnected pools without flow). Finally, aquatic

25   macroinvertebrates from intermittent streams with summer dry channels had adaptations conferring

26   ability to survive periods with no water (e.g. modes of aerial dispersal, resistant stages). These

27   results indicate that microhabitat conditions should not be neglected as they can play an important

28   role in certain situations.

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32   **Key words**: aquatic macroinvertebrates, biological traits, diversity, Mediterranean region,

33   microhabitats, seasonal changes, spatial scale.

Introduction

Understanding how and why biological community attributes change across multiple spatial scales remains one of the most challenging tasks facing ecology as a general discipline (Levin 1992, Borcard et al. 1992, Dungan et al. 2002, Pavoine & Dolédec 2005, Crist & Veech 2006) and stream ecology in particular (see for review Parsons et al. 2004, Lowe et al. 2006, Johnson & Host 2010, Winemiller et al. 2010). It is widely accepted that any given community descriptor (e.g. diversity, taxonomic or biological trait composition) may generate different patterns across a range of spatial scales, from the macrohabitat to the microhabitat, since different mechanisms are likely to operate at each scale (Morris 1987, Kolasa 1989, Wiens 1989, Levin 1992).

Streams and rivers subdivide easily into clear hierarchical scales of organization (Frissell et al. 1986, Allan 1995), and many studies have attempted to disentangle the relative importance of each scale for various aquatic organism assemblages (see Johnson & Host (2010) for a review of the last decade, but also Robson et al. (2005) for a more specific review of macroinvertebrates in stony perennial streams during the 1993-2005 period). However, the ranges of scales considered have varied from study to study, from eco-regions and catchments (Li et al. 2001, Heino et al. 2004, Townsend et al. 2003, Mykrä et al. 2004, 2008) and streams and reaches (Boyero & Bailey 2001, Boyero 2003, 2005) to mesohabitats and microhabitats (Wang et al. 2003, Finn & Poff 2005, Heino & Korsu 2008, Buffagni et al. 2010).

Despite the variety of responses found in these studies, some general conclusions can be drawn. First, with respect to spatial scale, recognition of the importance of small scales depends on the range of scales considered and the analytical method used (hierarchical vs. non-hierarchical). For instance, when catchments or eco-regions are included in multi-scale comparisons, they usually account for most variation of biological assemblages and community metrics (e.g. taxa richness, diversity). If small patches are included in analyses, their significance emerges only when either disturbance gradients are considered (Wang et al. 2006) or when geographical position is analyzed not as a factor in the spatial hierarchy

1 but as a covariate (Heino 2005, Mykrä et al. 2008). When lower spatial scales are

2 considered (e.g. streams and reaches, mesohabitats and microhabitats), the smallest ones

3 are usually where most of the variation in macroinvertebrate structural and functional

4 measures is observed (Downes et al. 1993, 2000, Robson et al. 2005, Boyero & Bailey

5 2001, Boyero 2003, Heino et al. 2004, Heino & Korsu 2008, Giri et al. 2010).

6

7 Second, analysis of taxonomic composition was the main focus of most of these studies. In

8 the context of the river habitat templet, the assembly of local communities is visualized as a

9 process whereby multiple habitat filters act hierarchically, selecting those organisms

10 endowed with a set of biological traits that allow them to survive, grow, and reproduce

11 under increasingly constraining factors (Poff & Ward 1990, Townsend & Hildrew 1994,

12 Poff 1997, Statzner et al. 2001). However, only a few studies have analyzed trait

13 composition at different hierarchical scales. For example, Lamoroux et al. (2004) analyzed

14 the relationships between habitat and the functional structure of aquatic macroinvertebrate

15 assemblages (12 biological traits) at three spatial scales varying from the microhabitat to

16 the basin scale. These authors found that variability in most biological traits depended

17 largely on microhabitat conditions, but larger-scale effects were not negligible. Similarly,

18 following a nested design and considering only two biological traits (size and feeding

19 habits), Pavoine & Dolédec (2005) found that most of the variation occurred at the lowest

20 spatial scale studied, which was comparable to the microhabitat scale.

21

22 Third and finally, most of the studies have focused on perennial streams in temperate

23 regions, while data on intermittent streams from drier areas such as the Mediterranean are

24 scarce. The Mediterranean region is characterized by high spatial and temporal

25 heterogeneity (di Castri 1981, Gasith & Resh 1999, Blondel et al. 2010, Robson et al.

26 2011) and may offer a very interesting scenario for the question of multi-scale patterns. For

27 instance, the relative importance of spatial scales may change over time due to seasonal

28 episodes of low flow/droughts that may alter pool and riffle prevalence and microhabitat

29 composition within streams. Interestingly, Lind et al. (2006) found that when flow

30 decreases, run mesohabitats more closely resemble pools, and microhabitats become more

31 homogeneous. Moreover, analysis of relationships between habitats and biological traits

1   may be particularly valuable in Mediterranean streams because natural disturbances such as

2   droughts may disrupt aggregate assemblage composition by causing local extinctions,

3   while biological traits adapted to such seasonal disturbances (e.g. formation of resistant

4   stages) are expected to remain strongly associated with local conditions. Droughts are

5   described as ramp disturbances, with progressive effects in stream ecosystem, first isolating

6   pools and then initiating an ecological succession process within each pool until

7   desiccation (Lake 2007, Boulton & Lake 2008). Complete desiccation may or may not take

8   place depending on substrate, groundwater hydrology, and pool morphology, which leads

9   to a particularly high diversity of streams in the Mediterranean region including the

10   following aquatic regimes: (i) permanent, (ii) intermittent with isolated summer pools, (iii)

11   intermittent with dry channels in summer, and (iv) ephemeral (Gallart et al. 2012).

12   Furthermore, inter-annual variability in the region (dry vs. wet years) may periodically alter

13   the long-term hydrological regime of each stream type.

14

15   In this study, we tested the relative contribution of different spatial scales to variation in

16   aquatic macroinvertebrate assemblages and their taxonomic and biological trait diversities

17   in the aforementioned stream types. Following Frissell et al. (1986), we used the following

18   operational nested scales: (i) macrohabitat, which corresponds to streams, (ii) mesohabitat,

19   which corresponds to the pool/riffle system within a reach, and (iii) microhabitat, which

20   corresponds to different substratum types within a mesohabitat. In order to capture the

21   temporal heterogeneity of Mediterranean streams, we included two sampling seasons.

22   Inter-annual differences have also been shown to change macroinvertebrate structure and

23   trait composition in Mediterranean streams (Bêche et al. 2006, Bêche & Resh 2007), but in

24   this study we concentrated on a shorter time period and a wide spatial extent. Given the

25   need for organisms to adapt to specific hydrological conditions and the fact that

26   microhabitats should encompass the niche requirements of particular taxa, we hypothesized

27   that the microhabitat scale would have a greater influence on macroinvertebrate taxa

28   composition and biological and ecological traits than the other two larger spatial scales in

29   intermittent streams. Finally, we tested for the existence of significant associations between

30   biological and ecological traits and each aquatic regime. In general, we expected that (i)

31   aquatic macroinvertebrates from permanent streams would have traits adapted to dominant

1 flow conditions (e.g. organisms temporarily attached, filter feeders), (ii) aquatic

2 macroinvertebrates from intermittent streams with isolated summer pools would have traits

3 related to life under confined conditions, typically characterized by reduced levels of

4 oxygen and depositional regimes (e.g. deposit feeders), and (iii) aquatic macroinvertebrates

5 from intermittent streams with dry channels in summer would have traits conferring the

6 ability to escape the periods of habitat disappearance (e.g. short aquatic stages, resistant

7 structures, modes of aerial dispersal).

8

9 **Methods**

10

11 **Study sites**

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13 This study was conducted in six stream basins throughout the European Mediterranean

14 region (Fig. 1). The streams belong to five circum-Mediterranean eco-regions according to

15 the classification by Illies (1978): Ibero-Macaronesian region (Taibilla), Pyrenees

16 (Vallcebre), Western plains (La Vêne), Italy-Corsica-Malta region (Biferno and

17 Candelaro), and Hellenic Western Balkan region (Evrotas), thus providing sufficient

18 variation of the aquatic macroinvertebrate communities at the macrohabitat scale. All

19 streams were the subject of MIRAGE (Mediterranean Intermittent River ManAGEment)

20 studies, a EU-funded research project, and are located in areas with minimal human

21 influence on stream hydrology and water quality.

22

23 **Study design and sampling**

24

25 We used a nested sampling design to estimate the components of variation in the structure

26 and composition of aquatic macroinvertebrate communities associated with the three

27 successive spatial scales introduced earlier: (i) macrohabitat (hereafter referred to as

28 stream), (ii) mesohabitat, and (iii) microhabitat.

29

30 From each of the six basins, we surveyed streams differing in their hydrological regime.

31 Each stream was classified according to its long-term aquatic regime as (i) permanent (P),

1   (ii) intermittent with summer pools (I-P), or (iii) intermittent with summer dry channels (I-

2   D). When long-term hydrological data were available, we used the flow permanence and

3   seasonal predictability metrics proposed by Gallart et al. (2012) to classify the streams into

4   the abovementioned types. In the absence of such data, we relied on field surveys,

5   bibliographic information, and expert advice. See Table 1 for a list of the studied streams

6   and their classification.

7

8   A reach measuring 25-30 m long in each stream was sampled in spring and summer 2010.

9   These two sampling periods represented the wet and dry seasons in the Mediterranean

10 region (García-Roger et al. 2011). Within reaches, we sampled pool (velocity < 0.03 m s^{-1})

11 and riffle mesohabitats based on their frequency of occurrence. Within mesohabitats, we

12 sampled microhabitats, also based on frequency of occurrence. Thus, 20-22 Surber samples

13 were taken proportionally to the relative occurrence of pool-riffle mesohabitats and

14 microhabitats (see Table 2) within each reach in each sampling period (427 samples in

15 total). Water depth (cm) and velocity (m s^{-1}) were measured for each sample, and samples

16 were fixed with formaldehyde (4% final concentration). The number of batches of 20-22

17 samples per stream was high enough to reach an asymptote in taxon accumulation curves

18 (data not shown, but previously demonstrated for a subset of the studied streams by García-

19 Roger et al. (2011)). Surber dimensions were adapted to stream size (ranging from 0.025 to

20 0.09 m^2; 250 µm mesh), so the final percentage of habitat sampled in each stream was

21 standardized to ~1% of the reach studied to allow comparisons across streams (on average,

22 the sum of the 20 Surber samples in each stream represented 1.14 % ± 0.08 SE). We found

23 no significant differences in taxa richness per sample for either of the two different Surber

24 sizes used in our dataset (Kruskal-Wallis rank sum test, *p*-value = 0.722).

25

26 For each stream on each sampling occasion we recorded the following physicochemical

27 parameters: temperature (ºC), conductivity (µS cm^{-1}), pH, and dissolved oxygen

28 concentration (mg L^{-1}) and its saturation percentage (Table 1). Additionally, water samples

29 of 250 mL were collected, filtered upon arrival at the laboratory through 0.45 µm

30 membrane filters, kept in cool conditions (4 ºC), and analyzed to determine concentrations

31 of nitrates (mg NO$_3^-$ L^{-1}) and nitrites (mg NO$_2^-$ L^{-1}) using HPLC, ammonia (mg NH$_4^+$ L^{-1})

1   through chemical titration, and phosphates (mg PO_4^{3-} L^{-1}) following Murphy & Riley

2   (1962). Discharge (L s^{-1}) was obtained from automatic gauging stations or by measuring

3   channel width and water velocity at a minimum of five equal subsections of the stream

4   cross-section at each reach.

5

6   **Sample identification and preliminary data handling**

7

8   Macroinvertebrate samples were examined under a stereoscope at 10x, with all individuals

9   counted and identified to family level following Tachet et al. (2010), except for

10   Oligochaeta (82 taxa in total). We set taxonomic resolution at family level as a trade-off

11   between comparing streams from sufficient eco-regions and obtaining enough information

12   on the structure and composition of their macroinvertebrate communities. The usefulness

13   of the family level for detecting changes in the structure and functional composition of

14   stream macroinvertebrate communities has been demonstrated in previous studies (Dolédec

15   et al. 2000, Gayraud et al. 2003, Bonada et al. 2006, 2007b, Vieira et al. 2006).

16

17   Macroinvertebrate trait data were obtained from Tachet et al. (2010). We used 63

18   categories of 11 biological traits (Table 3a) and 50 categories of 10 ecological traits (Table

19   3b). Biological traits and their respective trait categories describe macroinvertebrate

20   variation in morphology (1. maximum potential size), life-cycle features (2. duration, 3.

21   number of cycles per year, 4. aquatic stages), reproduction, resilience or resistance ability

22   (5. reproductive modes, 6. dispersal, 7. resistant forms, 8. locomotion), physiology (9.

23   respiration), and feeding behaviour (10. Food and 11. feeding habits). Ecological traits and

24   trait categories reflect environmental preferences and behaviours associated with such

25   preferences. Following Usseglio-Polatera et al. (2000), the ecological traits considered in

26   this study focused on variables that reflected the spatial distribution of aquatic

27   macroinvertebrates (1. longitudinal, 2. transversal, and 3. altitudinal), their microhabitat

28   preferences (4. substrate, 5. water velocity, 6. temperature, 7. salinity, 8. pH, 9. saprobity),

29   and the productivity of the inhabited water bodies (10. trophic status).

30

1 The trait database assigned affinities of genera to each trait category using a fuzzy coding

2 approach (Chevenet et al. 1994, Bonada et al. 2007a), but because we worked at the family

3 level we averaged and re-scaled (0-5 scale) the affinities of all genera recorded within a

4 family, as in previous studies (Dolédec et al. 2000, Gayraud et al. 2003). In order to assess

5 effects of the level of taxonomic resolution on the structure of biological and ecological

6 trait data, we followed Gayraud et al. (2003) and calculated the Difference in Trait

7 Structure index (DTS) at the family and genus levels for a subset of our studied streams

8 (Cal Rodó, Can Vila, and La Rogativa) for which we had samples (n = 242) identified to

9 the genus level (except for Chironomidae, which were identified to sub-family). These

10 streams covered a gradient of summer drought conditions. DTS was computed for each

11 sample as follows:

$$DTS = \frac{1}{2} \sum_{i=1}^{n} |p_i - q_i|,$$

12

13

14 where p_i is the relative frequency of the trait category i at the first taxonomic level (i.e.

15 genus), q_i is the relative frequency of the same trait category at the second taxonomic level

16 (i.e. family), and n is the number of trait categories in the trait. This measure varies from

17 zero (if relative frequencies of trait categories at both identification levels are identical) to

18 one (if relative frequencies of trait categories at the two identification levels are totally

19 different). It should be noted that relative frequencies of trait categories were obtained from

20 *samples x trait* matrices computed respectively for genus and family data as described in

21 the general procedure of data analysis (see below). To limit the number of variables

22 describing differences in data structure of the 11 biological plus 10 ecological traits at the

23 genus and family levels, we derived an overall index named Difference in Functional

24 Structure (DFS) by averaging the 21 DTSs for each sample.

25

26 **Data analyses**

27

28 As a first step, we studied the environmental heterogeneity associated with each spatial

29 scale. We used Principal Components Analysis (PCA) to assess differences between

30 reaches in the environmental variables measured in each season. Since the environmental

1   variables are measured with different scales and units, we computed PCA on standardized

2   values. Uncorrelated environmental variables with a high power of differentiation

3   (Pearson's $r < 0.8$) were retained for analysis. At lower spatial scales, we explored inter-

4   seasonal changes in the relative frequencies of different mesohabitats and microhabitats by

5   means of Fisher's exact tests on count data for each stream studied. We used Simpson's

6   diversity index to describe changes between seasons in the relative frequency of each

7   mesohabitat and microhabitat type, and repeated-measures ANOVA to test for significant

8   differences in diversity between seasons for each aquatic regime (P, I-P, or I-D).

9

10   Our macroinvertebrate analyses were based on two sets of data in the form of matrices. The

11   first was a standard *samples x taxa* matrix (427 x 82) containing log-abundances at family

12   level. The second was a *samples x trait categories* matrix (427 x 113) obtained by

13   multiplying *samples x taxa* log-abundances (427 x 82) and *taxa x trait category* affinity (82

14   x 113) matrices. For each Surber sample, we computed Rao's taxonomic and trait diversity

15   (H_D) as follows:

$$H_D = \sum_{i=1}^{n} \sum_{j=1}^{n} p_i \ p_j \ d_{ij},$$

16

17

18   where p_i is the relative frequency of taxon (or trait category) i, p_j is the relative frequency

19   of taxon (or trait category) j, and d_{ij} is the distance between the ith and j^{th} taxa (or trait

20   categories) according to Rao (1982) and Champely & Chessel (2002). We obtained

21   taxonomic distances at family and order levels following Clarke & Warwick's (1998)

22   procedure. For traits, we computed Rao's diversity for each trait and sample, although we

23   finally derived an average value. This was a weighted average, where weights were the

24   numbers of trait categories within each biological trait. No distance measurement was used

25   between traits, d_{ij} being set to 1 for traits, and so Rao's diversity was equal to the Gini-

26   Simpson diversity index.

27

28   The variation due to each spatial scale was separately assessed for each metric with nested

29   Generalized Linear Models (GLMs; Nelder & Wedderburn 1972), with the above-

30   mentioned three nested factors. We analyzed the data from the wet and dry seasons

1   separately to look for changes in multi-scale variation partitioning associated with

2   hydrological change. The GLMs had the following general equation:

3

4 $$Y_{ijkl} = \mu + \alpha_i + B_{ij} + C_{ijk} + \varepsilon_{ijkl}$$

5

6   where Y_{ijkl} is the expected value of any given metric for a single observation, μ equals the

7   parametric mean of each metric distribution, α_i is the fixed effect of the ith stream, B_{ij} is the

8   contribution for the jth mesohabitat of the ith stream, C_{ijk} is the contribution for the kth

9   microhabitat of the jth mesohabitat of the ith stream, and ε_{ijkl} is the error term of the lth item

10   in the kth microhabitat of the jth mesohabitat of the ith stream (which corresponds to within-

11   microhabitat Surber-sample variation). It is important to note that levels within nested

12   factors, especially mesohabitat and microhabitat, did not represent a random sample of all

13   the possible levels but were deliberately arranged, so factors were treated as fixed. Hence,

14   each factor was compared against the error term (Quinn & Keough 2002). In the GLMs, we

15   used deviances to estimate the percentage of variation accounted for by each spatial scale.

16   The community composition of samples was analyzed through Permutational Multivariate

17   Analysis of Variance using distance matrices of macroinvertebrate abundance data

18   (Anderson 2001) with the same factors and the same analytical constraints as in the above-

19   cited GLMs. In total, 999 permutations were performed on the Euclidean distance matrix

20   obtained from log-transformed macroinvertebrate abundances.

21

22   Finally, we used the fourth-corner methodology to look for associations between stream

23   types and aquatic macroinvertebrate traits (Legendre et al. 1997). This procedure measured

24   the linkages between three tables: a table L (427 x 82) containing the log-abundances of 82

25   macroinvertebrate taxa in 427 samples, a second table R (427 x 3) with the three aquatic

26   regimes (P, I-P, and I-D) coded as dummy variables for each sample, and a third table Q

27   (82 x 113) describing 113 trait categories for the 82 taxa. Through matrix algebra, the

28   fourth-corner method estimates parameters describing the relationships between habitat

29   characteristics and traits. The link between the aquatic regime and traits was measured

30   through a correlation coefficient whose significance was tested by a permutation procedure.

31   According to the update by Dray & Legendre (2008) of the fourth-corner method, we first

1   permuted samples to test the null hypothesis that taxa log-abundances and aquatic regimes

2   were unrelated (permutation model 2, repeated 999 times). Next we permuted taxa to test a

3   second null hypothesis that taxa log-abundances and traits were unrelated (permutation

4   model 4, repeated 999 times). Only when both permutation tests were significant did we

5   consider that aquatic regimes and particular traits were effectively linked. In a conservative

6   manner, we considered p-values for each association equal to the maximum of p-values of

7   the two permutation tests. The Holm correction for multiple tests was applied on the

8   statistics obtained from the fourth-corner analysis.

9

10   All statistical analyses were performed with packages from R 2.13.1 statistical software (R

11   Development Core Team 2011). We used the *base* package for GLMs, repeated-measures

12   ANOVA and Fisher's exact tests, the *vegan* package for PCA and Permutational

13   Multivariate Analysis of Variance, and the *ade4* package for the computation of taxonomic

14   distances and fourth-corner analyses.

15

16   **Results**

17

18   **Heterogeneity associated with each spatial scale**

19

20   PCA summarized environmental differences between the streams (Fig. 2a-b). The first axis

21   explained 37.2% of total variance and was positively related to temperature (loading =

22   0.425), and negatively to conductivity (loading = -0.447) and oxygen concentration

23   (loading = -0.442). The second axis, which explained 21.4% of variance, was negatively

24   related to nitrate concentration (loading = -0.651), and was interpreted as a negative

25   gradient of moderate eutrophy. When comparing the wet (Fig. 2a) and the dry season (Fig.

26   2b), we observed that ellipsoids of data dispersion at 95% confidence increased for P and I-

27   P streams, reflecting a higher variability of conditions in the dry season. Of course, there

28   were no data for I-D streams in the dry season.

29

30   Seasonal differences were also noticeable at the mesohabitat and microhabitat scales,

31   especially in I-P and I-D streams (Fig. 3). The prevalence of riffles decreased in the dry

season, although the change was only marginally significant in P streams (Fisher's Exact test; p-value = 0.0797), in which the variety of mesohabitats as estimated by Simpson's diversity index did not differ significantly between seasons (repeated-measures ANOVA, p-value = 0.420). In I-P streams, pools became the dominant mesohabitat by a highly significant margin in the dry season (Fisher's Exact test; p-value < 0.001), during which heterogeneity at this spatial scale decreased significantly (repeated-measures ANOVA, p-value = 0.003).

A great variety of substrata were observed at the microhabitat scale (Fig. 3). Differences in microhabitat composition were especially marked between streams. In Table 4, we show the results of Fisher's Exact tests for changes in microhabitat composition between seasons for P and I-P streams. While in general P streams did not vary in microhabitat composition between seasons (only KOL at Evrotas showed a significant change in microhabitat composition), I-P streams, with only one exception (2010 was a wet year in Can Vila), shifted in microhabitat composition between seasons to a dominance of organic substrates (Table 4). However, total microhabitat diversity did not differ in either P streams (repeated-measures ANOVA, p-value = 0.799) or I-P streams (p-value = 0.147), although a slight tendency to homogenization was observed in the latter.

Effect of taxonomic resolution on the functional description of the aquatic macroinvertebrate communities

Prior to further analyses, we confirmed that taxonomic resolution (family cf. genus) had little bearing on the functional description of the communities based on biological traits. Despite differences between the subset of three streams considered (Fig. 4), overall median similarity was high, at nearly 80% (DFS-values ~ 0.2), indicating that reducing taxonomic resolution to family level had only minor effects on the functional description of the aquatic macroinvertebrate communities.

1 **Relative importance of spatial scales**

2

3 The hierarchical structure of stream habitats affected the significance and relative

4 importance of the different spatial scales, as shown by our nested analyses (Table 5).

5 In general, results for each aquatic regime were consistent for the different metrics used.

6

7 The relative importance of each spatial scale differed between aquatic regimes, and patterns

8 also varied between seasons (Fig. 5). In all cases, a substantial proportion of variation in

9 community composition and metrics was explained. For P streams in the wet season,

10 results were consistent across metrics, and streams accounted for the largest proportion of

11 explained variation in all cases (30-40%), followed by microhabitats (20-30%) and

12 mesohabitats (4-20%). All scales were significant for all metrics (Table 5a). In the dry

13 season, partitioning of explained variance was similar, but the proportion of unexplained

14 variance increased. Among explained variance, the relative importance of the microhabitat

15 scale decreased compared with the wet season. No significant effects were associated with

16 this spatial scale in any metric (Table 5b).

17

18 For I-P streams in the wet season, the scale accounting for the largest proportion of

19 variance for two of the three metrics was the microhabitat (25-40%), followed by streams

20 (10-30%) and mesohabitats (8-12%). Interestingly, differences in trait diversity between

21 mesohabitats were not significant in the wet season (Table 5a). The largest spatial scale

22 accounted for most of the variance in community composition, which was expected given

23 the wide biogeographical range explored in this study. In the dry season, the relative

24 importance of all spatial scales decreased, offset by an increase in unexplained variation

25 due to within-sample variability (40-65%). Nevertheless, the partitioning of explained

26 variance did not differ from that observed in the wet season, with microhabitats accounting

27 for most variation (16-30%). For the diversity metrics, significant effects were observed at

28 the microhabitat scale in the dry season, while no differences were seen for either

29 mesohabitats or streams (Table 5b). In the case of community composition, most variation

30 was explained (approx. 60%). Interestingly, significant effects were observed at all spatial

31 scales for this metric in the dry season.

1 For I-D streams, with data available only in the wet season, the largest spatial scale

2 accounted for most of the variation in all metrics (45-90%). And for the two diversity

3 metrics, it was the only significant factor. Again, significant effects were observed for

4 community composition at all scales, with differences between streams, between riffles and

5 pools within streams, and between microhabitats within mesohabitats (Table 5a).

6

7 **Relationships between aquatic regimes and aquatic macroinvertebrate traits**

8

9 The fourth-corner analysis produced 41 significant relationships between trait categories

10 and aquatic regimes (Table 6). Only 7 of the 21 biological (life-cycle duration, number of

11 cycles per year, food) and ecological traits (altitude, temperature, salinity, pH) had no

12 significant relationships.

13

14 P streams harbored larger macroinvertebrates (> 2-4 cm maximal size) than I-P streams

15 (0.5-1 cm maximal size), typically for the entire life cycle (i.e. up to adults), with

16 adaptations for aquatic dispersal (either active or passive). In contrast, I-D streams were

17 associated with aerial passive mechanisms of dispersion. Adaptations to flow conditions in

18 each aquatic regime were also significant for respiration modes (i.e. gills in P streams, and

19 spiracles in I-P streams) and feeding habits (e.g. aquatic macroinvertebrates from I-P

20 streams were typically deposit feeders, likely because of depositional conditions in summer

21 pools).

22

23 There were a handful of significant relationships between ecological traits and the habitat

24 characteristics of each aquatic regime. Streams with P aquatic regimes usually had flowing

25 channels with hard, mineral substrates, whereas I-P streams typically had lentic habitats (no

26 flow) as ponds, pools, or marshes, with soft substrates (e.g. muds), and I-D streams

27 typically had clean, oligotrophic, temporary headwaters (i.e. crenon, epirithron).

28

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31

1 **Discussion**

2

3 **Aquatic regimes and the relative importance of spatial scales on community metrics**

4

5 It is generally assumed that the spatial scale at which biological communities exhibit the

6 greatest variation is the scale over which important physicochemical gradients or biotic

7 interactions control assemblage composition (Li et al. 2001). Interestingly, we observed

8 that regardless of the metrics used to describe aquatic macroinvertebrate communities,

9 streams differing in aquatic regimes also differed in the spatial scale at which the highest

10 proportion of variation was explained (the macrohabitat scale in P and I-D streams, but the

11 microhabitat in I-P streams). In general, for the wet season, all nested models displayed a

12 considerable amount of explained variation (range: 40-60%). The hydrological conditions

13 during the dry season differed between aquatic regimes. While P streams kept pool-riffle

14 sequences, I-P streams typically lost their riffles and were reduced to a chain of

15 disconnected pools, and I-D streams completely dried. The present study also demonstrates

16 that environmental changes in the dry season typically increased the proportion of

17 unexplained variation, but patterns of explained variation remained the same for each

18 aquatic regime. Furthermore, this study supports the use of nested designs for examining

19 spatial scales of variation in Mediterranean streams with different aquatic regimes.

20

21 At the macrohabitat scale, P streams exhibited a wide range of physicochemical conditions

22 in both the wet and dry seasons. This was consistent with the importance of this spatial

23 scale in explained variation in both seasons for all metrics considered. Under P aquatic

24 regimes, the mesohabitat scale accounted for a much lower proportion of variation for all

25 metrics, despite the existence of riffles and pools and their persistence in the dry season.

26 Previous studies have pointed out that taxon diversity does not differ in general between

27 mesohabitats in P streams when inter-annual differences in discharge are low (Scullion et

28 al. 1982, McElravy et al. 1989, Carter and Fend 2001), and at this spatial scale, nor did trait

29 diversity. To clarify, this does not mean that similar traits were associated with pools and

30 riffles, but that the diversity of traits and trait categories was similar in both mesohabitats.

31 This suggests that although trait structure may change in response to the main

1 environmental factors in each mesohabitat, as evidenced by the fourth-corner analysis, the

2 variety of life-history strategies needed to cope with the particular constraints in each

3 mesohabitat remains more or less constant. Note, too, that pool and riffle communities have

4 been shown to differ in taxonomic composition (García-Roger et al. 2011). The importance

5 of the microhabitat scale, nested within mesohabitats, was also low for this aquatic regime.

6 Moreover, we observed that P streams were in general more stable with respect to

7 microhabitat composition between seasons than streams subjected to the I-P aquatic regime

8 (Table 4).

9

10 Under the I-P aquatic regime, we found that the microhabitat scale accounted for most of

11 the explained variation in the two diversity metrics considered in this study. It might be

12 argued that the diminished importance of the stream spatial scale could be attributed to the

13 lower number of I-P streams studied compared with P streams. Nevertheless, our result is

14 similar to previous findings by Downes et al. (1993) and Boyero (2003), who observed that

15 lower spatial scales explained most of the variation in metrics related to richness and

16 diversity in Australian and Mediterranean streams with even fewer stream replicates. The

17 importance of the microhabitat scale was also highlighted by Robson et al. (2005), who

18 employed a nested approach to partition the variation due to several spatial scales from

19 microhabitats (individual stones) to stream types (perennial and intermittent) in Australia.

20 However, in that case, the largest source of variation in taxa richness was found to be

21 stream type, which could obscure differences at lower spatial scales.

22

23 For taxonomic composition, we observed that the stream scale was responsible for the

24 highest proportion of explained variation, which makes sense considering the wide

25 geographical range covered in this study. The proportion of variation explained by the three

26 spatial scales considered decreased in the dry season, but the relative importance of the

27 spatial scales in explained variation remained nearly constant. Mesohabitat and

28 microhabitat heterogeneity decreased in the dry season (Table 4), which is consistent with

29 the decrease in the importance of these two spatial scales observed in our nested analysis.

30 Microhabitats dominating pools during this season were essentially organic (e.g. deposits

31 of organic matter, submerged and emergent macrophytes) as previously reported by

1 García-Roger et al. (2011). Unexplained variation in taxa and trait diversity metrics, as well

2 as in taxonomic composition, increased in the dry season due to inter-sample variation, a

3 pattern also observed by Heino & Korsu (2008) and Giri et al. (2010), which ultimately

4 may be a consequence of divergent community trajectories in the remaining pool habitats

5 (e.g. differences in the degree and duration of disconnection, Bonada et al. 2006).

6 Finally, we observed that I-D streams presented a wider range of conditions (according to

7 the PCA biplot in Fig. 2a) than I-P and P streams in the wet season. The huge variability

8 observed in I-D streams is consistent with the relative importance of the macrohabitat scale

9 (i.e. stream), as found also by Bêche et al. (2006). The strong effect of stream-to-stream

10 variability on all metrics studied in these streams likely prevented the detection of

11 differences at lower spatial scales. Moreover, it has been argued by Gallart et al. (2012)

12 that the characteristics of the aquatic macroinvertebrates inhabiting I-D streams (i.e. the

13 aquatic assemblage and the set of traits) are likely to differ from stream to stream in this

14 aquatic regime because of the large variability in habitat predictability associated with

15 them.

16

17 **Relationships between aquatic regimes and aquatic macroinvertebrate traits**

18

19 It has long been recognized that biological traits of macroinvertebrate communities such as

20 respiration, reproduction, locomotion, life cycle duration, and dispersal capacity differ

21 according to stream flow duration and predictability, and thus habitat persistence (Williams

22 1996). The analysis of biological traits in stream macroinvertebrate communities has

23 received a great impetus in the last decade (Usseglio-Polatera et al. 2000, Vieira et al. 2006,

24 Statzner & Bêche 2010), and Bonada et al. (2007b) have shown that different biological

25 traits are associated with permanent, intermittent, and ephemeral streams, which differ in

26 flow regimes and substrata. Statzner & Bêche (2010) have reviewed trait category

27 responses reported in the literature in relation to changes in discharge, emphasizing that

28 biological trait responses to flow variation are extraordinarily diverse, and thus it is

29 difficult to establish unequivocal relationships between aquatic regimes and traits. We

30 found results consistent with previous studies of Mediterranean-climate streams (Bêche et

31 al., 2006, Bonada et al., 2007b) and extended these analyses by including ecological traits,

1   which reflect the environmental preferences of aquatic macroinvertebrates and the

2   behaviours associated with those preferences (Vieira et al. 2006).

3

4   In contrast to the results obtained by Bonada et al. (2007b), who found only one significant

5   trait associated with permanent streams (aquatic eggs), we found a large set of significant

6   biological and ecological traits for P streams. For biological traits, the long-term stability of

7   P streams appears to permit the development of large-sized macroinvertebrates (>2-4 cm in

8   maximal size) and wholly aquatic life cycles (i.e. up to adult stages). Moreover, stable

9   conditions are compatible with asexual modes of reproduction since clonal proliferation

10 allows a more rapid colonization of the habitat than sexual reproduction (Bilton et al.

11 2001). Consistent with the long-term persistence of flow, the mechanisms of dispersal that

12 were significantly associated with P streams were either active or passive but always

13 aquatic-based. The production of drought resistant forms is unnecessary in a permanent

14 habitat, which explains why we found a negative relationship between this aquatic regime

15 and the ability to produce a particular kind of protective case (cocoons). For ecological

16 traits, we found that aquatic macroinvertebrates from P streams preferentially inhabit river

17 channels with substrates composed of flags, boulders, cobbles, and pebbles. According to

18 the NC Division of Water Quality (2010), the bed of P streams is often comprised of

19 coarser particles than other temporary stream types (e.g. I-P and I-D) due to consistent

20 stream-forming flows, which transport finer particles downstream.

21

22 I-P streams had positive associations with smaller maximal body sizes (>0.5-1 cm). We

23 believe that enclosure and overcrowding in the remaining isolated pools may typically lead

24 to an increase in stress conditions. For instance, increasing competition and predation may

25 lead to smaller sizes. Consistently, we found negative associations in I-P streams with

26 larger macroinvertebrate sizes (>2-4 cm) and with aquatic modes of dispersal, both of

27 which were positively associated with P streams. These results are similar to those of

28 Bonada et al. (2007b) and Finn et al. (2008, reviewed by Statzner & Bêche 2010).

29 Considering the prevalence of lentic conditions in the dry season and the progressive

30 depletion of dissolved oxygen in the remaining pools, our finding of a positive association

31 between I-P streams and spiracles for respiration seems reasonable. Furthermore,

1   depositional conditions in pools would select for organisms feeding on fine detritus (i.e.

2   deposit feeders). Ecological traits confirmed this supposition, since we found positive

3   associations between I-P streams and more lentic habitats (ponds, pools, disconnected side-

4   arms, marshes) with deposition of fine and soft substrates (e.g. muds). Consistent with our

5   results, Bonada et al. (2007b) found that intermittent sites were dominated by pool-favoring

6   taxa. We observed that the frequency of Ephemeroptera, Plecoptera, and Trichoptera

7   (riffle-favoring taxa, Bonada et al. 2007b) decreased in the dry season, likely because of

8   metamorphosis to their adult state (see data in García-Roger et al. 2011). Along these lines,

9   it has been argued that during the wet season, riffle-favoring taxa may dominate the benthic

10   communities, but some of them may be replaced by pool-favoring taxa (e.g. Odonata,

11   Coleoptera, and Heteroptera) in the dry season (Bonada et al. 2007b).

12

13   Wiggins et al. (1980) suggested that one major life history strategy that allows organisms to

14   successfully exploit temporary waters is that of emigrating from the habitat before it dries

15   and returning when flow resumes. Accordingly, we found aquatic macroinvertebrates in I-

16   D streams that possess the ability to aerially disperse to cope with hydrological constraints.

17   Despite the huge variation in environmental conditions among I-D streams, all were

18   typically associated with clean, oligotrophic headwaters (i.e. crenon, epirithron).

19

20   In summary, our study has demonstrated that Mediterranean streams with differing aquatic

21   regimes also exhibit different multi-scale patterns of spatial variation in the structure and

22   composition of aquatic macroinvertebrate communities and in metrics related to taxa and

23   trait diversity. While the macrohabitat scale (i.e. stream-to-stream variation) accounted

24   primarily for variation in P and I-D streams, the microhabitat scale was most relevant for I-

25   P streams. In all aquatic regimes, we observed that patterns in the relative importance of

26   variance components across hierarchical spatial scales changed over time due to predictable

27   seasonal episodes of low flow or droughts that alter both mesohabitat prevalence and

28   microhabitat composition. Interestingly, we observed that the different aquatic regimes

29   exerted different constraints on aquatic macroinvertebrates, resulting in different biological

30   and ecological traits being associated with each stream type.

31

9

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5

Figure captions

Fig. 1. Map of the study area showing the locations of the stream basins studied.

Fig. 2. PCA biplot of stream environmental variables for a) the wet season and b) the dry season. Stream type is represented by symbols: (■) P, (○) I-P, and (Δ) I-D. 95% confidence ellipsoids for each stream type are shown. Codes for streams are in Table 1.

Fig. 3. Relative frequency of meso- (P: pools, R: riffles) and micro-habitats (see legend) in each stream type (P, I-P, I-D; see text for description) in a) the wet season and b) the dry season. Codes for streams are in Table 1. Dashed vertical lines separate streams.

Fig. 4. Box-plots of Difference in Functional Structure (DFS) comparing identifications at the genus and family levels in CR, CV, and ROG. Boxes and lines indicate respectively the range of the central 50 and 90% of DFS values.

Fig. 5. Variation partitioning for the metrics analysed with GLMs (Rao's taxonomic diversity and Rao's trait diversity) and permutational ANOVA (assemblage taxonomic composition) for each stream type in a) the wet season and b) the dry season.

Fig. 1

Fig. 2

Conductivity (-0.447), O$_2$ (-0.442) — Temperature (0.425)

a) Wet season

P   I-D

BLA   FM
IS   ROG
         RM
SEN
      CC
KOL
                        I-P
            CR   CV
RAS

PC2 21.4%

Nitrate (-0.651)

b) Dry season

P

SEN
              CR
              CV
KOL   IS
                        I-P
           ROG
BLA
   CC

Nitrate (-0.651)

PC1

37.2%

Fig. 3

Fig. 4

Fig. 5

a) Wet season

P

I-P

I-D

b) Dry season

Deviance explained or cumulative R^2

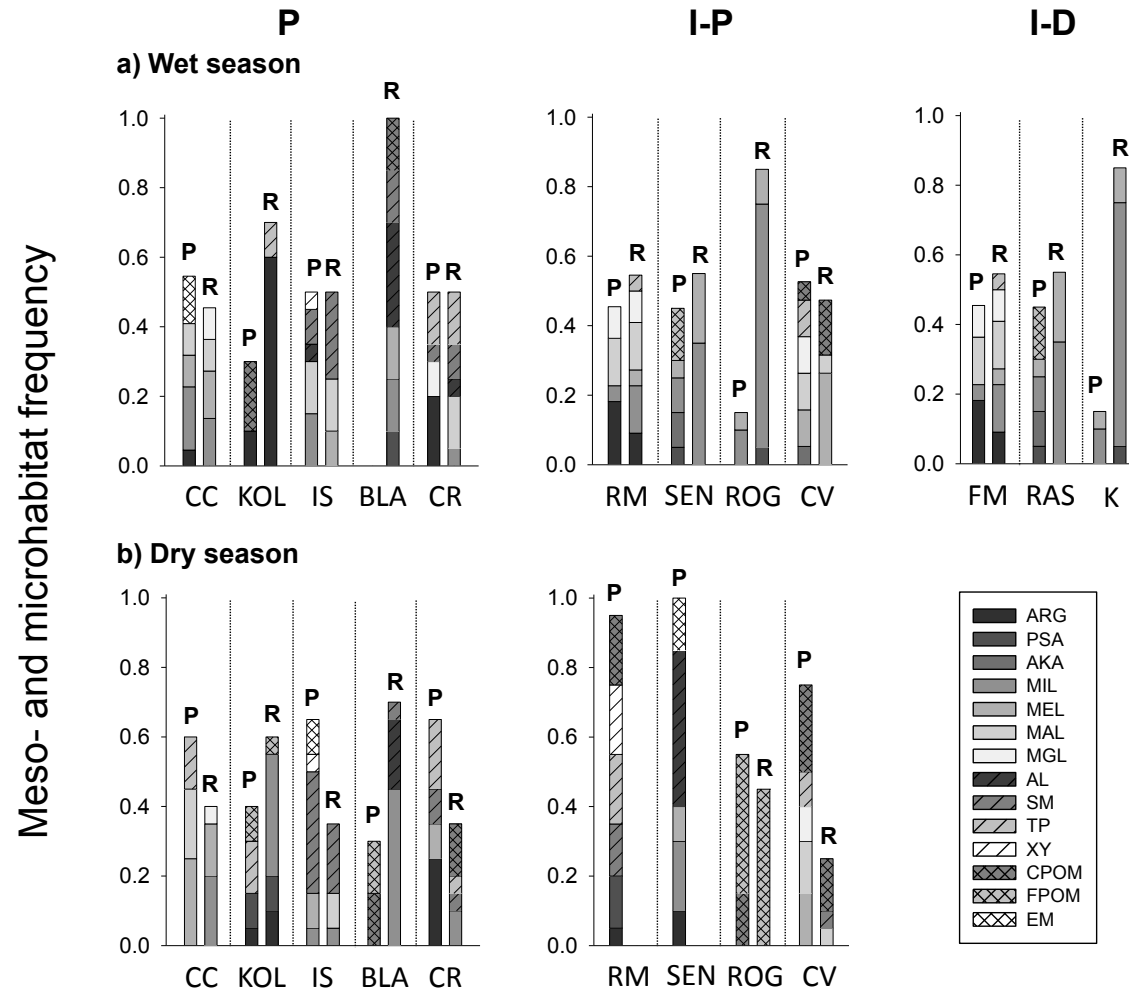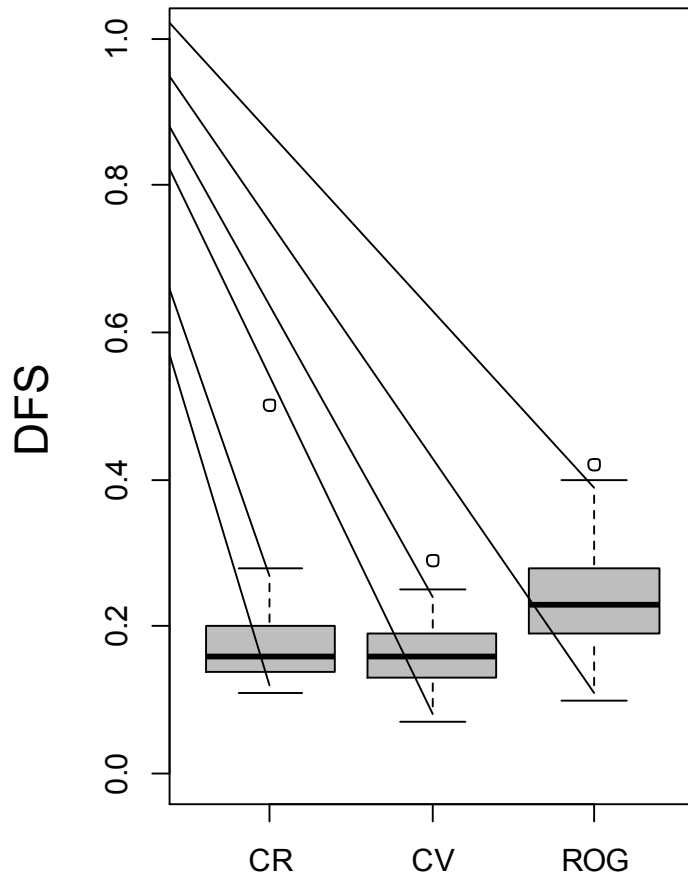Rao Taxa | Rao Trait | Taxa assembl.

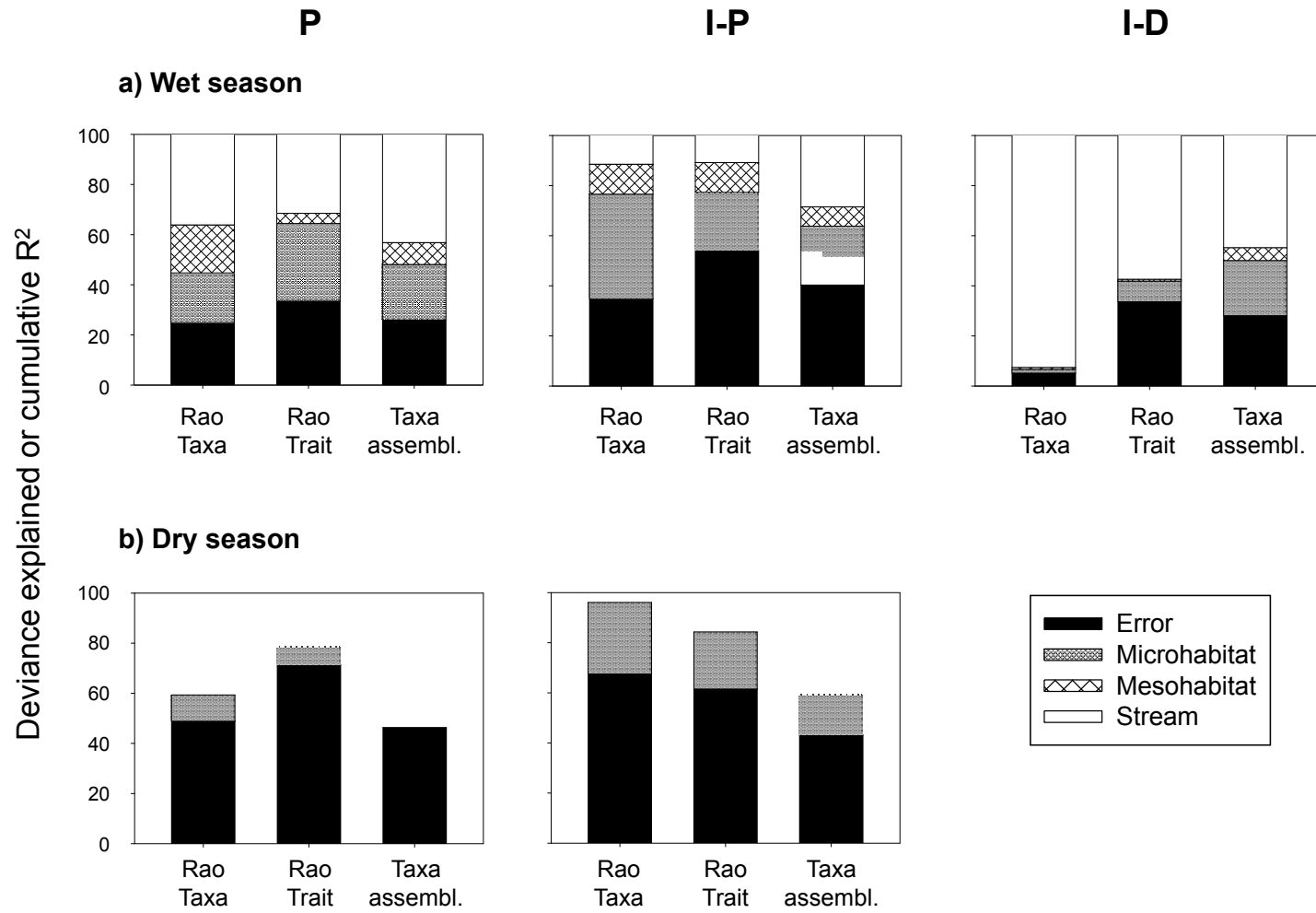Error
Microhabitat
Mesohabitat
Stream
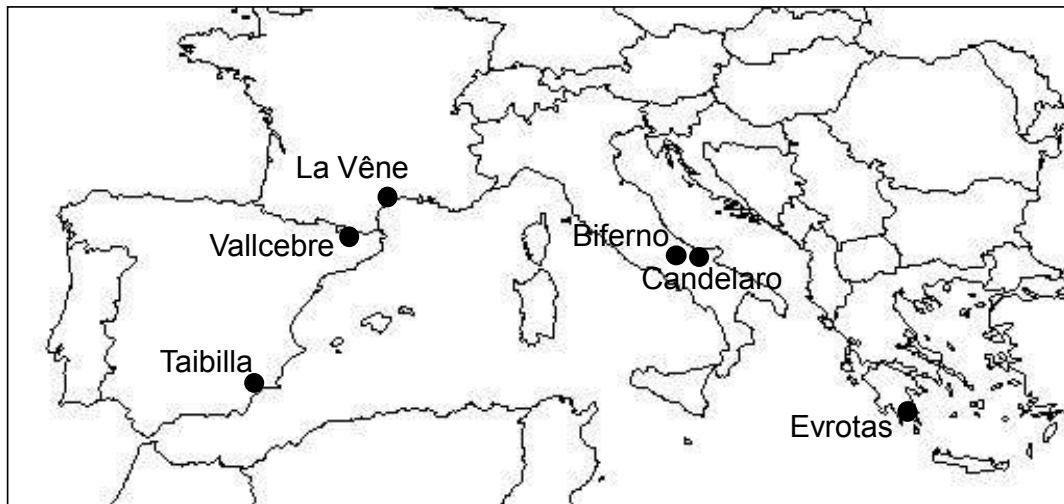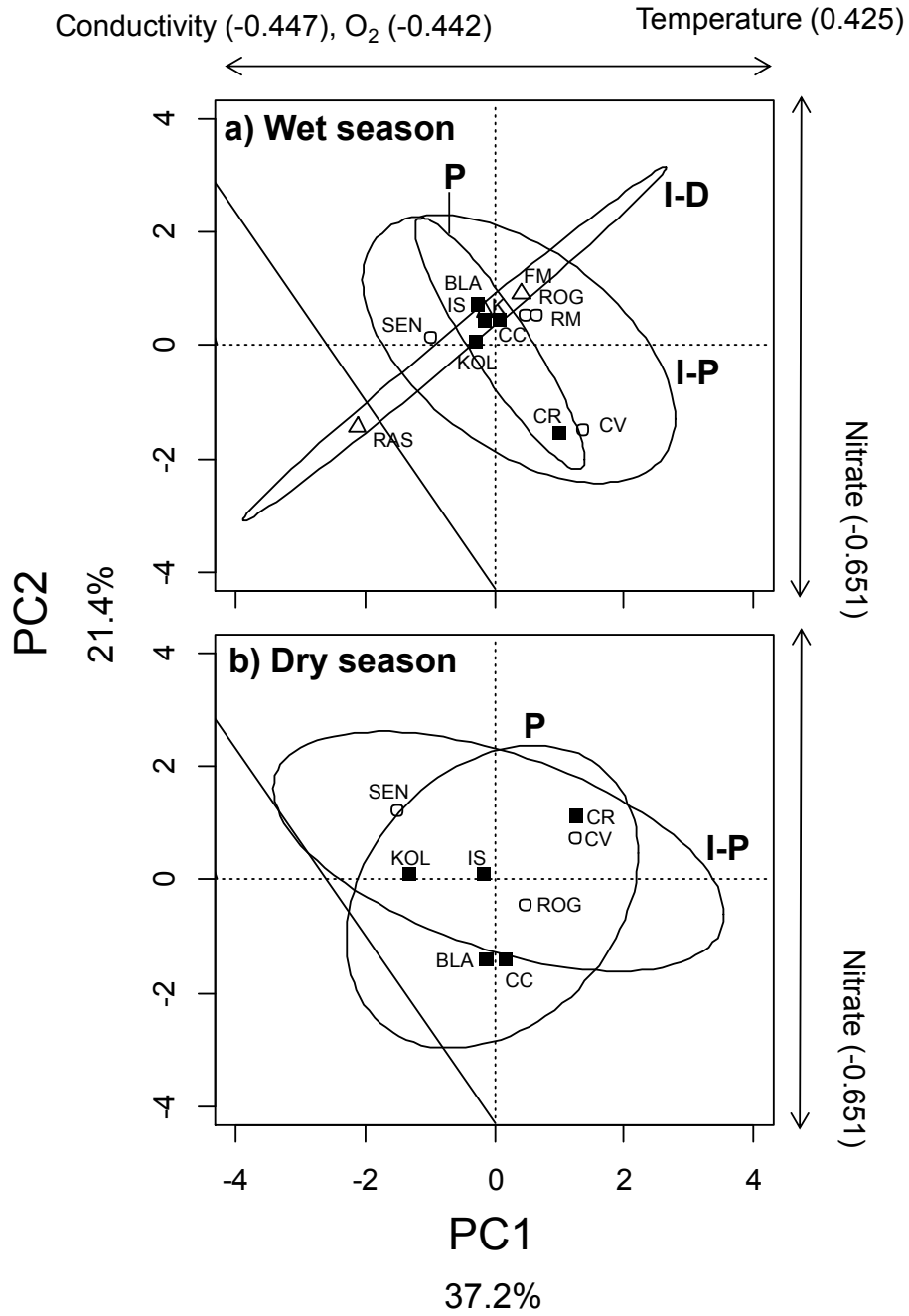
Table 1. Environmental characteristics of the streams studied. Each stream was classified according to its long-term aquatic regime. Only uncorrelated variables (Pearson's $r < 0.8$) are shown. Values are provided for the wet (W) and dry (D) season respectively. Dashes indicate that no data were available due to the complete drying of the stream channel in the dry season.

Basin	Stream	Aquatic regime	Source	Temp (º C)		Cond (µS cm^{-1})		O$_2$ (mg l^{-1})		Nitrate (mg l^{-1})		Phosphate (mg l^{-1})		Depth (m)		Velocity (m s^{-1})	
				W	D	W	D	W	D	W	D	W	D	W	D	W	D
Biferno	RM	I-P	Field surveys	13.0	n.a.	684	n.a.	10.71	n.a.	0.18	n.a.	0.001	n.a.	0.18	n.a.	0.16	n.a.
Candelaro	CC	P	Field surveys	16.4	21.0	535	429	9.93	10.80	0.67	0.77	0.014	0.051	0.20	0.04	0.22	0.31
	FM	I-D	Field surveys	15.4	-	445	-	9.31	-	0.51	-	0.009	-	0.07	-	0.13	-
Evrotas	KOL	P	Gallart et al. 2012	11.1	23.2	385	429	7.42	7.03	3.08	3.03	0.023	0.080	0.18	0.07	0.25	0.21
	SEN	I-P	Field surveys	21.3	23.0	496	475	8.28	6.86	3.85	2.57	0.170	0.080	0.46	0.22	0.50	0.11
	RAS	I-D	Field surveys	12.6	-	264	-	8.35	-	0.11	-	0.160	-	0.26	-	0.23	-
Taibilla	BLA	P	García-Roger et al. 2010	21.2	23.7	756	560	8.06	7.49	1.24	1.73	0.031	0.046	0.24	0.04	0.34	0.26
	ROG	I-P	García-Roger et al. 2010	13.5	15.2	714	618	9.35	8.88	5.05	4.12	0.025	0.052	0.16	0.01	0.09	0.01
Vallcebre	CR	P	García-Roger et al. 2010	14.7	10.9	1780	1342	10.04	9.57	6.47	5.45	0.020	0.035	0.46	0.13	0.50	0.10
	CV	I-P	García-Roger et al. 2010	10.6	10.7	1951	1395	10.41	9.50	5.78	3.52	0.030	0.040	0.22	0.08	0.12	0.07
La Vêne	IS	P	Tournoud et al. 2005	15.3	16.6	520	565	9.90	8.70	0.13	0.63	0.011	0.058	0.19	0.16	0.08	0.09
	K	I-D	Gallart et al. 2012	14.2	-	559	-	8.65	-	0.15	-	0.016	-	0.18	-	0.06	-

n.a. data not available for the study period.

Table 2. List and description of microhabitats (see García-Roger et al. 2011for more details)

Microhabitat		
Organic		Code
Algae - Filamentous algae, algal tufts		AL
Submerged macrophytes (including bryophytes)		SM
Living part of terrestrial plants - Fine roots, floating riparian vegetation		TP
Xylal - Tree trunks, dead wood, branches, roots		XY
CPOM - Deposits of coarse particulate organic matter		CP
FPOM - Deposits of fine particulate organic matter		FP
Emergent macrophytes		EM
Mineral	Wentworth class	
Argyllal < 6 μm	Clay	ARG
Psammal > 6 μm to 2 mm	Fine Silt - Coarse Sand	PSA
Akal > 2 mm to 2 cm	Gravel	AKA
Microlithal > 2 cm to 6 cm	Coarse Gravel - Pebble	MIL
Mesolithal > 6 cm to 20 cm	Cobble	MEL
Macrolithal > 20 cm to 40 cm	Boulder	MAL
Megalithal > 40 cm	Boulder	MGL

Table 3. List of a) biological and b) ecological traits of aquatic macroinvertebrates, and their respective trait categories, considered in this study.

a) Biological trait	Trait category	b) Ecological trait	Trait category
1. Maximal size	≤ 0.25 cm > 0.25-0.5 cm > 0.5-1 cm > 1-2 cm > 2-4 cm > 4-8 cm > 8 cm	1. Transversal distribution	River channel Banks, connected side-arms Ponds, pools, disconnected side-arms Marshes, peat bogs Temporary waters Lakes Groundwaters
2. Life cycle duration	≤ 1 year > 1 year	2. Longitudinal distribution	Crenon Epirithron
3. Number cycles per year	< 1 = 1 > 1		Metarithron Hyporithron Epipotamon
4. Aquatic stages	Egg Larva Nymph Adult		Metapotamon Estuary Outside river system
		3. Altitude	Lowlands (< 1000 m.a.s.l.) Mountain level (1000-2000 m.a.s.l.) Alpine level (> 2000 m.a.s.l.)
5. Reproduction	Ovoviviparity Isolated eggs, free Isolated eggs, cemented Clutches, fixed Clutches in vegetation Clutches, terrestrial Asexual reproduction	4. Susbtrate (preferendum)	Flags, boulders, cobbles and pebbles Gravel Sand Silt Macrophytes
6. Dispersal	Aquatic passive Aquatic active Aerial passive Aerial active		Microphytes Twigs, roots Organic detritus, litter Mud
7. Resistant forms	Eggs, gemmules, statoblasts Cocoons Housings Diapause or dormancy None	5. Flow velocity (preferendum)	Nil Slow (< 25 cm s^{-1}) Moderate (25-50 cm s^{-1}) Fast (> 50 cm s^{-1})
8. Locomotion	Flier Surface swimmer Full water swimmer Crawler	6. Temperature (preferendum)	Cold (< 15ºC) Warm (> 15 ºC) Eurythermic
	Burrower Interstitial Temporarily attached Permanently attached	7. Salinity (preferendum)	Freshwater Brackish water
9. Respiration	Tegument Gill	8. pH (preferendum)	≤ 4 > 4-4.5 > 4.5-5
	Plastron Spiracle Hydrostatic vesicle		> 5-5.5 > 5.5-6 > 6
10. Food	Microorganisms Detritus < 1 mm	9. Saprobity	Xenosaprobic Oligosaprobic β-Mesosaprobic
	Dead plant ≥ 1 mm Living microphytes Living macrophytes		α-Mesosaprobic Polysaprobic
	Dead animals ≥ 1 mm Living microinvertebrates Living macroinvertebrates Vertebrates	10. Trophic status	Oligotrophic Mesotrophic Eutrophic
11. Feeding habits	Absorber Deposit feeder Shredder Scraper Filter feeder Piercer Predator Parasite		

Table 4. *P*-values from Fisher's exact tests of the change in microhabitat composition between the wet and dry season in each stream. Only P and I-P streams are shown.

Stream basin	P		I-P	
	Stream	***p*-value**	**Stream**	***p*-value**
Biferno	-	-	RM	< 0.001
Candelaro	CC	0.192	-	-
Evrotas	KOL	< 0.001	SEN	0.001
La Vêne	IS	0.435	-	-
Taibilla	BLA	0.147	ROG	< 0.001
Vallcebre	CR	0.531	CV	0.194

Table 5. *P*-values from significance tests associated with generalized linear models (GLMs) applied to taxonomic and biological trait community metrics, and permutational ANOVA of community composition data for a) the wet and b) the dry season. For Rao's diversities of both taxa and biological traits we assumed a Gaussian distribution and used an identity link function

a) Wet season

Effect	df	P Rao (taxa)	P Rao (traits)	P Taxa assembl.	I-P Rao (taxa)	I-P Rao (traits)	I-P Taxa assembl.	I-D Rao (taxa)	I-D Rao (traits)	I-D Taxa assembl.
Stream	4	< 0.001	< 0.001	0.001	< 0.001	0.026	0.001	< 0.001	< 0.001	0.001
Mesohabitat within Stream	4	< 0.001	0.023	0.001	0.002	0.766	0.001	0.169	0.840	0.033
Microhabitat within Mesohabitat within Stream	28	0.003	< 0.001	0.003	< 0.001	0.038	0.001	0.862	0.908	0.018

b) Dry season

Effect	df	P Rao (taxa)	P Rao (traits)	P Taxa assembl.	I-P Rao (taxa)	I-P Rao (traits)	I-P Taxa assembl.
Stream	4	< 0.001	0.058	0.001	0.985	0.085	0.001
Mesohabitat within Stream	4	0.003	0.544	0.021	0.904	0.362	0.001
Microhabitat within Mesohabitat within Stream	28	0.672	1.000	0.067	0.024	0.021	0.002

1 **Table 6.** Results of fourth-corner analysis. Significant positive (+) and negative (-) associations (*P*
2 < 0.05) and non-significant associations (0) between trait categories and aquatic regimes are shown.
3 After the application of the Holm correction, significant associations are shown in bold.
4
5

Trait	Trait Category	P	I-P	I-D
Maximal size	> 0.5-1 cm	-	+	0
	> 2-4 cm	+	-	0
Aquatic stages	Adult	+	0	0
Reproduction	Ovoviviparity	+	0	0
	Asexual reproduction	+	0	0
Dispersal	Aquatic passive	+	-	0
	Aquatic active	+	0	0
	Aerial passive	0	0	+
Resistant forms	Cocoons	-	0	0
Respiration	Gill	+	-	0
	Plastron	+	0	0
	Spiracle	-	+	0
Locomotion	Full water swimmer	+	0	0
Feeding habits	Deposit feeder	0	+	0
Transversal distribution	River channel	+	-	0
	Ponds, pools, disconnected side-arms	0	+	-
	Marshes, peat-bogs	-	+	0
	Temporary waters	-	+	0
Longitudinal distribution	Crenon	0	0	+
	Epirithron	0	0	+
	Epipotamon	0	-	0
	Metapotamon	0	0	0
Substrate	Flags, boulders, cobbles and pebbles	+	-	0
	Mud	0	+	0
Flow velocity	Nil	0	+	0
	Slow	+	0	0
	Medium	0	-	0
Saprobity	Xenosaprobic	0	0	+
	Oligosaprobic	0	0	+
Trophic status	Oligotrophic	0	-	+
	Mesotrophic	0	0	-