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APPLIED ISSUES

Concordance between ecotypes and macroinvertebrate assemblages in Mediterranean streams

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SUMMARY

1. According to the guidelines of the European Water Framework Directive, assessment of the ecological quality of streams and rivers should be based on ecotype-specific reference conditions. Here, we assess two approaches for establishing a typology for Mediterranean streams: a top-down approach using environmental variables and bottom-up approach using macroinvertebrate assemblages.
2. Classification of 162 sites using environmental variables resulted in five ecotypes: (i) temporary streams; (ii) evaporite calcareous streams at medium altitude; (iii) siliceous headwater streams at high altitude; (iv) calcareous headwater streams at medium to high altitude and (v) large watercourses.
3. Macroinvertebrate communities of minimally disturbed sites ($n = 105$), grouped using UPGMA (unweighted pair-group method using arithmetic averages) on Bray–Curtis similarities, were used to validate four of the five ecotypes obtained using environmental variables; ecotype 5, large watercourses, was not included as this group had no reference sites.
4. Analysis of similarities (ANOSIM) showed that macroinvertebrate assemblage composition differed among three of the four ecotypes, resulting in differences between the bottom-up and top-down classification approaches. Siliceous streams were clearly different from the other three ecotypes, evaporite and calcareous ecotypes did not show large differences in macroinvertebrate assemblages and temporary streams formed a very heterogeneous group because of large variability in salinity and hydrology.
5. This study showed that stream classification schemes based on environmental variables need to be validated using biological variables. Furthermore, our findings indicate that special attention should be given to the classification of temporary streams.

Keywords: benthic macroinvertebrates, GUADALMED project, Mediterranean streams, typology, Water Framework Directive

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Introduction

Classification of freshwater ecosystems is essential to the development of biological assessment frameworks (Gibson *et al.*, 1996; Gerritsen, Barbour & King, 2000), and during the last few years many classification schemes have been proposed using both geographical and non-geographical criteria (e.g. Naiman *et al.*, 1992; Van Sickle & Hughes, 2000; Johnson, Goedkoop & Sandin, 2004). Ideally, classifications should identify groups of undisturbed (reference) sites where comparable biological communities might be found (Gerritsen *et al.*, 2000), thereby partitioning the natural variation of measurements of ecological quality (e.g. Resh, Norrish & Barbour, 1995; Dodkins *et al.*, 2005). Aquatic macroinvertebrates are widely used in the biological assessment of freshwater ecosystems (Resh *et al.*, 1995), as well as to explain the distribution of aquatic organisms across different spatial scales (Gerritsen *et al.*, 2000; Johnson *et al.*, 2007). Accordingly, as regional climate and catchment geology and morphology are often strong predictors of macroinvertebrate assemblages (Richards, Johnson & Host, 1996; Munné & Prat, 1999), we expect similar assemblages at sites within the same ecotype.

In Europe, there has been renewed interest in regionalization of aquatic ecosystems with the publication of the Water Framework Directive (WFD2000/60/EC; European Commission, 2000). Many classification schemes have been used to test the concordance between landscape patterns and structural and functional aspects of biological communities (Ferréol *et al.*, 2005; Verdonschot, 2006). For instance, two distinct methods have been used to classify sites: a bottom-up approach using biological communities (e.g. Heino *et al.*, 2003; Lorenz, Feld & Hering, 2004; Dodkins *et al.*, 2005) and a top-down approach based on environmental criteria (e.g. Munné & Prat, 2004; Verdonschot & Nijboer, 2004). The latter approach, proposed by the European WFD, recognizes two systems for river classification. Both systems are based on the division of Europe into ecoregions proposed by Illies (Illies & Botosaneanu, 1963), but one approach (system A) differentiates waterbodies according to classes determined using three environmental descriptors: altitude, size and geology, whereas the other approach (system B) is based on five obligatory (latitude, longitude, altitude, size, geology) and several optional variables (Munné & Prat, 2004).

Several regionalization approaches have been developed and tested in Spain, first by Margalef (1983) and later by García de Jalón & González del Tánago (1986), Vidal-Abarca *et al.* (1990) and Bonada *et al.* (2004a). In several Mediterranean catchments, Munné & Prat (2004) tested the two WFD typology approaches (A and B), and concluded that ecotypes obtained by system B gave a better reflection of ecological processes, because key environmental factors, such as hydrological and climatic variables, were considered (e.g. Allan, 1995; Cushing, Cummins & Minshall, 1995). This preliminary work gave rise to the development of an optimal typology for Spanish Mediterranean streams within the context of the GUADALMED Project (http://www.ecostrimed.net). This classification attempts to assess the ecological status of Mediterranean rivers according to the guidelines of the WFD. A preliminary typology of Mediterranean catchments was presented in the first phase of this project (Bonada *et al.*, 2004a). However, to improve on this work, during the second phase of this project a large number of sites and new environmental variables were included.

This study attempts to define stream ecotypes using the WFD system B approach within the Iberian-Macaronesian biogeographic region. Furthermore, to validate this typology, we analysed the concordance between the ecotypes obtained using environmental variables and those obtained using stream benthic macroinvertebrate assemblages by measuring the biotic similarity of reference sites.

Methods

Study area and sampling sites

The study was carried out in 33 catchments located along a latitudinal, thermal and pluviometric gradient along the Spanish eastern coast and the Balearic Islands, and covering a large range of sizes, from large (>14 000 km^2) catchments, such as the Júcar and the Segura rivers, to small (<200 km^2) catchments (Table 1).

The study area is influenced by the Mediterranean climate, with significant spring and autumn rainfalls (Köppen, 1923). Limestone and sedimentary materials are dominant in the catchments, although some siliceous areas are present in the Sierra Nevada mountain range and in the Pyrenees. The sampled

Table 1 Catchment area, annual mean discharge, maximum altitude values and number of sites sampled for the 33 Mediterranean catchments studied along the Spanish eastern coast

Catchment	Area (km^2)	Mean annual discharge ($m^3 s^{-1}$)	Maximum altitude (m.a.s.l.)	No. sampling sites
Adra	743	1.8	2737	5
Aguas	544	0.4	1285	2
Algar	214	1.0	1522	2
Almanzora	2577	1.3	2132	2
Besòs	1038	4.1	1317	2
Carboneras	266	0.1	1077	1
Cenia	204	0.9	1315	1
Chillar	54	0.2	1761	2
Fluvià	1039	9.1	1543	3
Foix	315	0.8	987	1
Francolí	857	1.7	1157	1
Genil	8198	28.0	3304	12
Guadalfeo	1300	6.0	3435	7
Guadalhorce	3147	13.0	1781	5
Guadiana menor	6532	15.0	3108	13
Guadiaro	1416	20.0	1747	9
Jara	58	0.6	772	1
Júcar	18136	52.0	1826	13
Llobregat	4995	25.0	2435	11
Mijares	4026	9.7	1998	9
Muga	795	4.8	1399	2
Palancia	972	2.2	1607	5
Pareis	49	1.1	1203	1
Pollença	70	0.9	1074	4
Segura	14657	23.0	2031	22
Soller	49	0.5	1242	1
Ter	2994	26	2825	9
Tordera	892	5.7	1633	3
Turia	6245	12.0	1987	9
Verde	101	0.5	1586	1
Verde de Marbella	157	2.0	1862	1
Vícar	12	0.004	70	1
Vinalopó	1720	1.4	1213	1

rivers are subjected to high variability in annual discharge, with frequent floods and droughts, which is normal for Mediterranean rivers (Gasith & Resh, 1999). Sclerophyllous and evergreen trees are the dominant vegetation, although in some areas, deciduous and coniferous forests are present. *Ramblas* present a kind of vegetation adapted to particular conditions of high salinity, marked hydrological fluctuations and severe dry periods and floods (Gómez *et al.*, 2004). More information about some of these catchments may be found in Robles *et al.* (2004).

A total of 162 sites were used to define stream ecotypes. These sites represent the network established in the GUADALMED 2 project to characterize the reference condition in Mediterranean streams. The selected sites were considered the least disturbed reaches in the chosen streams, ranging from totally undisturbed to moderately disturbed according to criteria defined by Bonada *et al.* (2004b). The moderately disturbed sites were mainly located in the middle and lower reach of the catchments. Thus, in order to have a robust classification of each site included in the network, not only true reference sites but even moderately disturbed sites were used to define stream ecotypes according to system B.

A quantitative approach to select true reference sites with *a priori* exclusion criteria was used (see Sánchez-Montoya, Suárez & Vidal-Abarca, 2005). Eighteen reference criteria related to riparian vegetation, presence of invasive species, point and diffuse sources of pollution, river morphology, habitat structure and hydrologic conditions were applied at two different spatial scales (site and catchment). Only sites which fulfilled the eighteen criteria were selected as reference sites. Consequently, the macroinvertebrate communities of 105 reference sites (from 162 original sites) were used to validate the ecotypes.

Defining stream ecotypes

Three of the five obligatory variables proposed by the WFD for system B (altitude, geology and size) were considered here. The spatial variables latitude and longitude were not considered because they do not offer discriminatory information because of size of studied area (Munné & Prat, 2004). Moreover, we analysed several optional variables that could provide useful information about Mediterranean streams (Table 2). In order to define ecotypes correctly and following the WFD methodology, the environmental variables used were not influenced by human activities. Environmental data were mainly available from the CEDEX (*Centro de Estudios Hidrográficos*, Spain) database.

Variables were grouped into four classes: hydrological, morphological, geological and climatic (Table 2) according to the methodology proposed by Munné & Prat (2004). The hydrological variables of discharge and dry period percentage were calculated using climatic models (CEDEX, 2005). Hydrological state was calculated using field data from three sampling occasions (spring, summer and autumn) according to the criteria defined by Uys & O'Keeffe

Table 2 Environmental variables used to characterize ecotypes

Groups of variables	Factors	Description
Hydrological	Discharge	Average of annual discharge (m^3 s^{-1})
	Hydrological state	Code for hydrological state (see Table 3)
	Dry period percentage	% of year discharge is equal to 0
Climatic	Temperature	Annual average of air temperature (°C)
	Precipitation	Annual rainfall (mm)
Geological	Surface carbonate geology	% of coverage in the drainage area
	Surface siliceous geology	% of coverage in the drainage area
	Surface evaporate geology	% of coverage in the drainage area
Morphological	Altitude	Metres above sea level
	Drainage slope	Slope/total drained area
	Distance from the origin*	Metres
	Surface drainage area	Total drainage area (km^2)
	Stream order*	Strahler method

Variables marked with an asterisk were later removed because they were highly correlated with other variables within the same group.

Table 3 Code and description of each of the possible hydrological states and their correspondence with the definition given by Uys & O'Keeffe (1997)

Code	Description	Definition	Dry period intensity	Flow predictability
0	Permanent reach	Perennial		↑
1	Surface flow disappears in one season but pools remained	Intermittent		
2	Surface flow disappears in two seasons but pools remained	Intermittent		
3	Surface flow disappears in three seasons but pools remained	Intermittent	increase	increase
4	Totally dry in one season and flow appears in other two seasons	Ephemeral		
5	Totally dry in one season and flow appears in only one season	Ephemeral		
6	Totally dry in one season and only pools remained in the other two seasons	Ephemeral	↓	

(1997) but modified according to dry period intensity (Table 3). Two climatic variables, temperature and rainfall, were obtained using monthly models provided by interpolation of data from meteorological stations (*Instituto Nacional de Meteorología*, Spain). A digital terrain model (Centro Geográfico del Ejérato, Ministerio de Defensa, Spain, 2005) (DTM 30 × 30 m) and Arc/Info software (Version 9.0, ESRI, Redlands, California, U.S.A., 2005) were used to delimit the water drainage area as a new polygon for all sampling sites to calculate drainage area. The other morphological variables (altitude, drainage slope, distance from the origin and stream order) were obtained directly from the digital terrain model. Geological data were calculated by intersecting geological surface with the coverage area, thus obtaining a percentage of the drained area for each geological class: carbonate, siliceous and evaporite.

The data analysis primarily follows the work of Munné & Prat (2004) for the definition of stream ecotypes in Mediterranean areas. First, Pearson's correlation was used to identify variables within the same group that were strongly correlated (correlation coefficient >0.8); only variables with correlation coefficient <0.8 were selected for further analyses. Secondly, to summarize environmental complexity, a principal components analysis (PCA) was performed on the groups of environmental variables using the STATISTICA program (Stat Soft, Inc., 1999). From this analysis two variables (axis 1 and 2) were obtained for each group of environmental variables, summarizing most of the variability in the two first dimensions (Legendre & Legendre, 1998). The use of different PCA for each group of variables was performed to maintain a meaningful descriptor unit with the same origin (hydrological, morphological, geological and climatic). Thirdly, a *K*-means analysis (Jain & Dubes, 1988) was performed using the derived PCA-variables to classify the sites into stream ecotypes. This non-hierarchical clustering technique separates objects into pre-established groups, looking for the highest similarities inside each group and differences among groups (Legendre & Legendre, 1998). Finally, a stepwise discriminant analysis using the Wilks's Lambda method (SPSS, Inc., 1999) was used to select the significant derived PCA-variables in defining each stream ecotype.

Concordance between stream ecotypes and macroinvertebrate assemblages

Only macroinvertebrate communities from true reference sites ($n = 105$, see above) were used to validate the previously defined ecotypes. A set of 301 macroinvertebrate samples were used, taken from the 105 reference sites on three sampling occasions (spring, summer and autumn) in 2003. Some sites (ephemeral or intermittent) were not sampled in all studied periods because they were completely dry in some season, most frequently in summer.

A multi-habitat semiquantitative kick-sample, as described by Zamora-Muñoz & Alba-Tercedor (1996), was taken on each sampling occasion using the protocol described by Jáimez-Cuéllar *et al.* (2004). Macroinvertebrate samples were collected from all habitats using a kick-net (mesh size between 250–400 μm) and preserved in 4% formalin. The samples were examined under a stereoscope in the laboratory (see Jáimez-Cuéllar *et al.*, 2004), and at least 200 individuals in each sample were randomly picked and identified to family level, except for Ostracoda, Oligochaeta and Hydracarina. Large uncommon individuals were picked individually, as described in Barbour *et al.* (1999). Family abundances were estimated for the whole sample using four rank abundance categories: 1 = 1–3; 2 = 4–10; 3 = 11–100; 4 = >100 individuals.

The macroinvertebrate assemblage characteristic of the reference condition for each ecotype was determined using IndVal (Dufrêne & Legendre, 1997) on abundance data from all seasons and the PC-ORD program (McCune & Mefford, 1999). A Monte Carlo permutation test with 9999 permutations was used to test the significance for each value. Furthermore, average values of the ratio EPT/OCH (ratio of Ephemeroptera, Plecoptera and Trichoptera taxa to Odonata, Coleoptera and Heteroptera taxa) for each period sampled were calculated to detect differences among ecotypes. This ratio has been shown to very dependent on in-stream hydrological conditions (Rieradevall, Bonada & Prat, 1999; Bonada *et al.*, 2006), with high EPT/OCH values in permanent streams and low EPT/OCH values in intermittent and ephemeral waterbodies.

Stream reference sites were classified using macroinvertebrate family composition; fourth root-transformed abundance data from the combined season's matrix. This matrix represents a combined community, obtained from the average of abundance of each taxon for all sampling occasions. A group-average clustering procedure (UPGMA, unweighted pair-group method using arithmetic averages) based on Bray–Curtis similarities was applied. A total of 31 rare taxa (occurrence at <5% of sites) were removed from the data set because such taxa usually obscure patterns in classification analysis (Gauch, 1982).

The same similarity matrix was used in a non-metric multidimensional scaling (NMDS) (Kruskal & Wish, 1978) to visualize spatial patterns of the community structure among the previously classified clusters. NMDS ordination places the samples in an arbitrary dimensional space such that their relative distances apart match the corresponding pair-wise similarities: nearby samples have similar communities and vice-versa. The correspondence between the similarity matrix and the final ordination plot is explained by a stress value. Stress values equal to zero indicates perfect concordance and values below 0.05 represent very good results, whereas values >0.20 are critical and values >0.30 are not interpretable (Clarke, 1993).

Analysis of similarities (ANOSIM) (Clarke, 1993) on the Bray–Curtis similarities was used to evaluate the degree of separation among the four ecotypes. This analysis was performed using both the three single-season matrix (spring, summer and autumn) and the combined season matrices for the reference data set, to analyse the seasonal effect on the established abiotic typology. In each case, we used presence–absence and rank abundances data to analyse the possible effect of the type of data on the analysis. Each test in ANOSIM produces an R-statistic, which contrasts the similarities of sites within an ecotype with the similarities of sites among ecotypes (when the R value is close to one, similarities between sites within an ecotype are higher than those between sites from different ecotypes, and values close to zero indicate no differences among ecotypes). The number of Monte Carlo permutations was set at 99999. We also did a pair-wise

ANOSIM comparison among stream ecotypes to distinguish among possibly contrasting effects. However, ANOSIM was not used to determine the statistical significance of cluster biological groups, when the underlying argument is circular (Marchant, Wells & Newall, 2000). Cluster, NMDS and ANOSIM analyses were conducted in PRIMER version 6.0. (Clarke & Warwick, 1994).

Results

Defining stream ecotypes

Pearson's correlation showed that some of the 13 variables used to define Mediterranean river ecotypes were highly correlated. For instance, discharge, stream order and distance from origin were highly correlated with surface drainage area ($r > 0.8$) and stream order with the distance from origin. Therefore, 11 variables were selected after removing correlated variables within the same group (distance from origin and stream order). Hydrology, geology and stream morphology were thus represented by three variables each plus two more climatic variables. The new PCA-variables were selected with the highest percentages of variance explained (Table 4). After applying several tests with *K*-means analysis (i.e. 4, 5 and 6 groups), we concluded that the five-group classification showed the highest ecological and spatial coherence. Discriminant analysis revealed the importance of hydrological variables in explaining among-group variance, followed by the geological, morphological and climatic variables as is showed in Table 4. Ecotype 1 was characterized by the highest values of the hydrological state; ecotype 2 had the highest percentages of evaporite geology; ecotype 3 had a high percentage of siliceous geology and high altitude; ecotype 4 had the highest percentage of carbonate geology and ecotype 5 had the lowest altitude and the highest discharge and surface drainage area. The average and standard deviation of the environmental variables describing each ecotype are given in Table 5.

It is remarkable that, according to the hydrological state, all the sites of ecotype 1 were composed exclusively of intermittent or ephemeral sites, mainly located in areas (southeast of Spain and Balearic Islands) characterized by a dry climate, with high potential evapotranspiration and low precipitation. Figure 1 shows the spatial distribution of the five ecotypes.

Concordance between Mediterranean stream ecotypes and benthic macroinvertebrate assemblages

IndVal analysis revealed significant differences in macroinvertebrate assemblages among the four ecotypes analysed (Table 6). Ecotype 5 (large watercourses located in the lowlands) was not included in this analysis because all sites were considered as impaired.

Ecotype 1 (temporary streams) had only one family (Dytiscidae) as an indicator taxon, whereas the other three ecotypes had from 10 to 31 significant indicator families. In ecotype 2 (evaporite headwater streams at medium altitude), Mollusca, Heteroptera and Trichoptera were the dominant orders, each characterized by three families, Odonata and Crustacea were each represented by two families and Ephemeroptera and Planaridae were each represented by one family. Ecotype 3 (siliceous headwater streams at high altitude) had the highest number of indicator taxa. All indicator Plecoptera families (four) were in this ecotype. Trichoptera appeared as the most represen-

Table 4 Principal component analysis (PCA) axes selected to define stream ecotypes, amount of variance explained (% variation), axis interpretation, names and order of Discriminant Function Analysis (DFA) selection

Group of variables	PCA axis selected	% variation	Interpretation of PCA axis	PCA-variables	DFA
Hydrological	PCA axis 1	50.3	Dry period	Hydro-axis 1	2
	PCA axis 2	28.2	Hydrological state versus discharge	Hydro-axis 2	1
Climatic	PCA axis 1	58.5	Precipitation versus temperature	Clim-axis 1	7
Geological	PCA axis 1	68.2	Carbonate versus siliceous geology	Geol-axis 1	3
	PCA axis 2	31.8	Evaporitic geology	Geol-axis 2	4
Morphological	PCA axis 1	51.8	Altitude	Morp-axis 1	5
	PCA axis 2	25.4	Total area drained and specific slope	Morp-axis 2	6

Table 5 Average and SD (in parentheses) values of the environmental variables for five Mediterranean stream ecotypes

Mediterranean stream ecotypes	No. sampling sites	Siliceous geology (% drainage area)	Carbonate geology (% drainage area)	Evaporite geology (% drainage area)	Hydrologic state	Discharge ($m^3 s^{-1}$)
1	24	27 (±38)	69 (±37)	4 (±6)	Intermittent/ Ephemeral	0.1 (±0.4)
2	32	13 (±22)	67 (± 21)	19 (±11)	Perennial	0.9 (±2.0)
3	25	97 (±7)	3 (±7)	0.1 (±0.4)	Perennial	0.3 (±0.7)
4	71	9 (±17)	88 (±17)	2 (±4)	Perennial	1.0 (±1.9)
5	10	20 (±31)	70 (±28)	9 (±4)	Perennial	13.8 (±5.3)

Mediterranean stream ecotypes	Stream order	Surface drainage area (km^2)	Altitude (m)	Temperature (°C)	Precipitation (mm)	Definition of ecotypes
1	1.1 (±0.3)	22 (±35)	645 (±523)	14 (±2)	700 (±311)	Temporary
2	1.9 (±0.8)	200 (±290)	446 (±266)	15 (±2)	541 (±188)	Evaporite calcareous at medium altitude
3	1.3 (±0.6)	47 (±118)	1177 (±589)	13 (±3)	720 (±225)	Siliceous headwaters at high altitude
4	1.7 (±0.9)	149 (±38)	840 (±343)	14 (±2)	689 (±192)	Calcareous headwaters at medium and high altitude
5	4.3 (±0.7)	3490 (±1675)	239 (±187)	16 (±1)	553 (±212)	Large watercourses

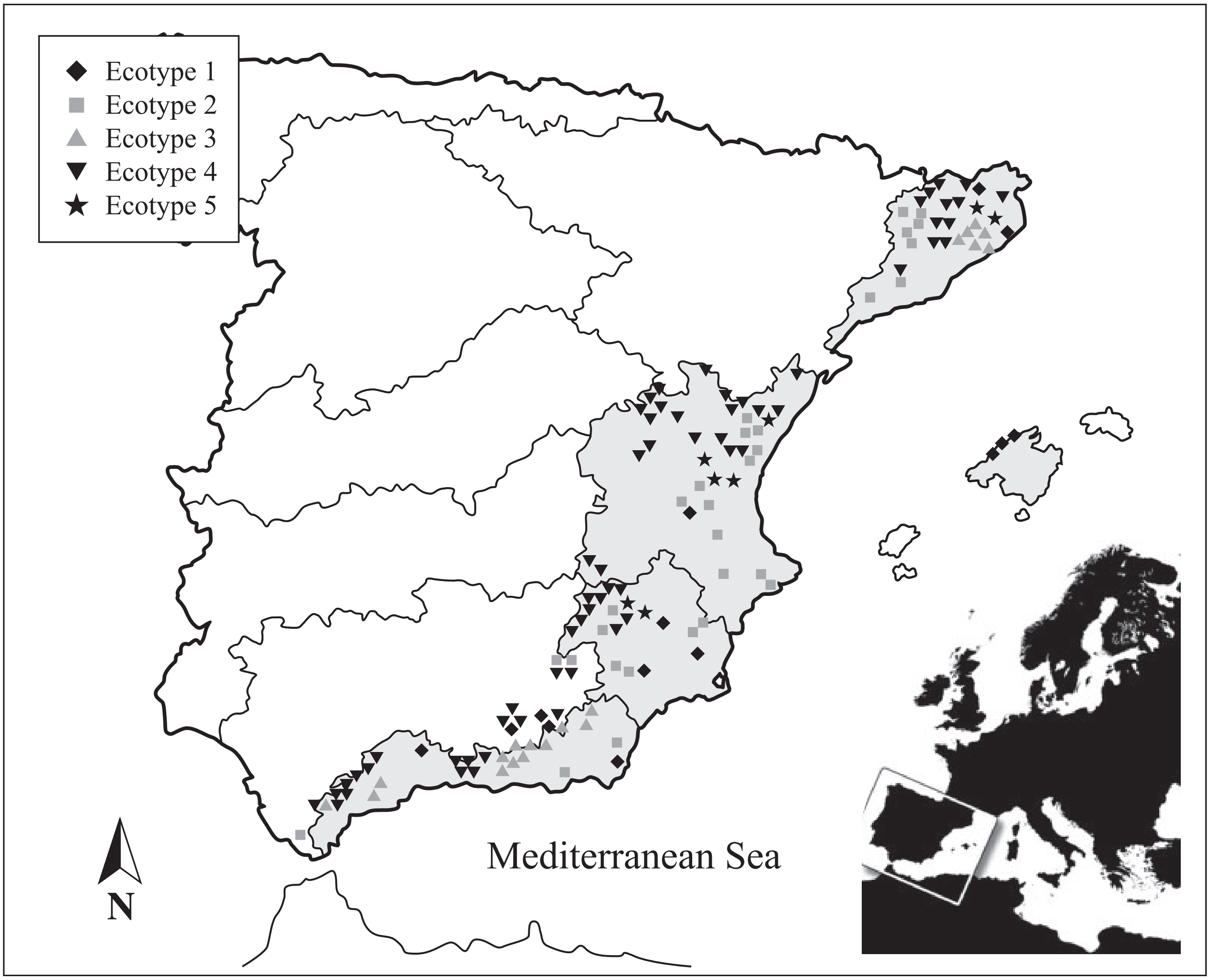

Fig. 1 Spatial distribution of the five ecotypes (n = 162) according to system B.

Table 6 IndVal results for the four Mediterranean stream ecotypes based on 105 reference sites

Ecotype 1			Ecotype 2			Ecotype 3			Ecotype 4		
Order	Family	I.V.	Order	Family	I.V.	Order	Family	I.V.	Order	Family	I.V.
C	Dytiscidae	32.3	H	Gerridae	38.6	P	Perlidae	51.8	O	Aeshnidae	38.0
			M	Physidae	35.7	D	Blephariceridae	50.0	C	Gyrinidae	37.0
			O	Gomphidae	35.2	E	Heptageniidae	46.8	D	Stratiomyidae	36.3
			T	Psychomyiidae	33.0	X	Planariidae	44.9	E	Leptophlebiidae	32.9
			E	Caenidae	31.9	E	Ephemerellidae	43.8	O	Cordulegasteridae	30.6
			T	Hydroptilidae	29.8	D	Limoniidae	36.9	D	Athericidae	29.1
			X	Gammaridae	29.2	D	Psychodidae	35.6	C	Scirtidae	28.3
			O	Calopterygidae	27.2	D	Dixidae	35.5	M	Hydrobiidae	28.2
			M	Planorbidae	27.1	P	Nemouridae	35.4	C	Haliplidae	24.5
			X	Dugesiidae	26.4	D	Empididae	34.1	X	Sialidae	22.0
			H	Corixidae	25.7	C	Hydraenidae	33.6			
			M	Thiaridae	20.3	T	Limnephilidae	33.5			
			X	Atyidae	19.5	X	Hydracarina	32.6			
			H	Mesoveliidae	19.4	T	Sericostomatidae	32.5			
			T	Ecnomidae	19.3	P	Leuctridae	32.1			
						X	Ostracoda	31.7			
						X	Oligochaeta	31.6			
						T	Rhyacophilidae	31.3			
						T	Hydropsychidae	31.2			
						T	Brachycentridae	30.9			
						T	Leptoceridae	30.9			
						T	Philopotamidae	30.9			
						C	Elmidae	30.7			
						D	Ceratopogonidae	30.0			
						T	Lepidostomatidae	29.2			
						E	Baetidae	29.1			
						T	Glossosomatidae	28.6			
						D	Chironomidae	27.8			
						P	Capniidae	26.7			
						T	Odontoceridae	20.8			
						D	Thaumaleidae	18.2			

Families with most significant presence in each ecotype (P-value < 0.05) and their indicator value (I.V.) are presented together with the corresponding Order for each Family; C, Coleoptera; M, Mollusca; H, Heteroptera; O, Odonata; D, Diptera; E, Ephemeroptera; T, Trichoptera; P, Plecoptera; X, Others.

ted order (10 families), followed by Diptera, Ephemeroptera and Coleoptera (eight, three and two families, respectively). Finally, in ecotype 4 (calcareous headwater streams at medium and high altitude) the most represented orders were Coleoptera (three families), Diptera and Odonata (two families each).

The EPT/OCH ratio, used to study the characteristics of macroinvertebrate assemblages in each ecotype, was highest in ecotype 3 (Table 7). The average of the other ecotypes was lower and more similar among the remaining three ecotypes. Moreover, EPT/OCH varied seasonally, with the highest values noted in wet periods (ecotype 3 in spring = 4.7) and the lowest values noted in the summer or the driest periods (ecotype 1 in summer = 0.7).

Table 7 Mean values of EPT/OCH ratio for each period sampled and the overall mean ± SD

	Spring	Summer	Autumn	mean (±SD)
Ecotype 1	2.3	0.7	1.7	1.56 ± 0.80
Ecotype 2	1.8	1.3	1.8	1.61 ± 0.28
Ecotype 3	4.7	3.2	3.7	3.88 ± 0.78
Ecotype 4	2.0	1.3	2.0	1.75 ± 0.41

The UPGMA classification using Bray–Curtis distances discriminated 10 biological groups, with a similarity of approximately 62% (Fig. 2). The majority of biological groups (1–4 and 8–10) were small and belonged to ecotype 1. The fifth group was composed of three sites, two of which belonged to ecotype 2 and

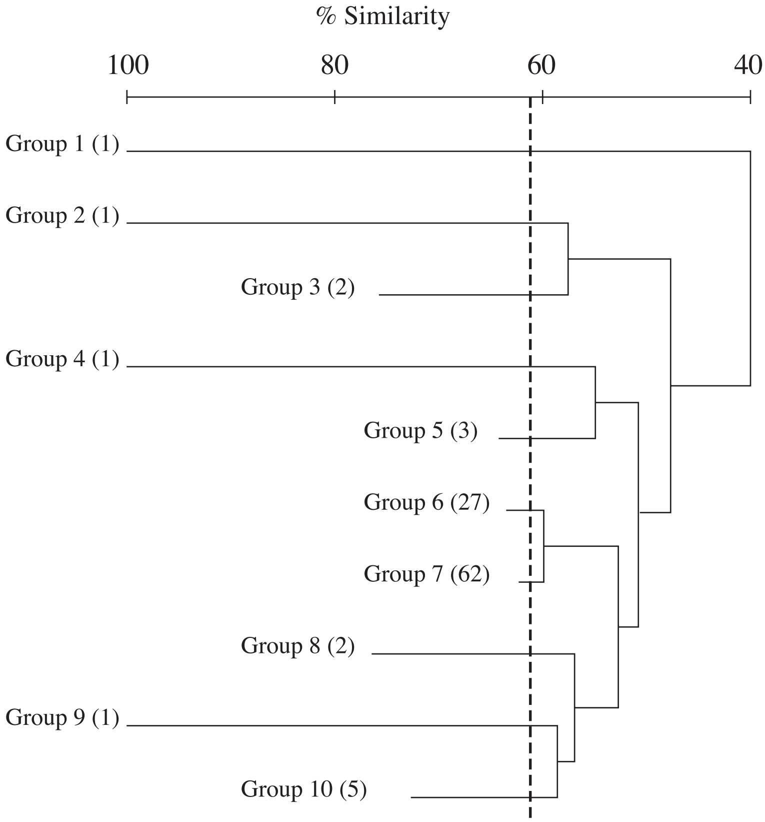

Fig. 2 Group average clustering (UPGMA) dendrogram of the reference sites (n = 105) based on their Bray–Curtis similarities. The number of sites in each group is shown in parentheses.

one to ecotype 4. The sixth group consisted of 27 sites of which 18 belonged to ecotype 3 (representing 82% of the sites classified in that ecotype). Furthermore, five sites of this group were classified into ecotype 4, three sites into ecotype 1 and one site into ecotype 2. Finally, the seventh group ($n = 62$ sites) was the largest biological group, composed of 46 sites belonging to ecotype 4 (representing 88% of the sites classified in that ecotype), 10 sites classified into ecotype 2 (representing 77% of sites in that ecotype), four sites classified into ecotypes 3 and 2 sites classified into ecotype 1. In summary, most of the sites classified as ecotype 3 (siliceous high altitude streams, 81.8%) or ecotype 4 (calcareous medium and high altitude streams, 88%) clustered together, and therefore, were described by a particular macroinvertebrate assemblage. On the other hand, ecotype 1 (temporary streams) was composed of nine different biological groups from cluster analysis.

The NMDS ordination of sites had a stress value of 0.21, and showed a slight overlap among ecotypes (Fig. 3). The best two-dimensional solution showed that most of the sites corresponding to ecotype 1 were located in the upper part of the graph and well separated from the rest of the ecotypes, lying along the second NMDS axis. Ecotype 2 was located next to ecotype 4, in the right-lower part of the graph. The two ecotypes composed mainly by sites from the seventh biological group were well separated from the sixth biological group, which was composed mainly by ecotype 3 (siliceous high altitude sites).

The global R from the ANOSIM test showed significant differences in assemblage composition among the four stream ecotypes using both abundance (R = 0.399, $P < 0.001$) and presence-absence ($R = 0.409$, $P < 0.001$) data (Table 8). However, examination of the six possible pair-wise tests showed no significant differences between ecotype 2 and 4, with either data sets. When single season matrices were used, the global R showed significant differences but they were always lower than the combined matrix.

Discussion

The reference condition approach (e.g. Reynoldson *et al.*, 1997) is often used in constructing ecological classification schemes (Gerritsen *et al.*, 2000; Bailey,

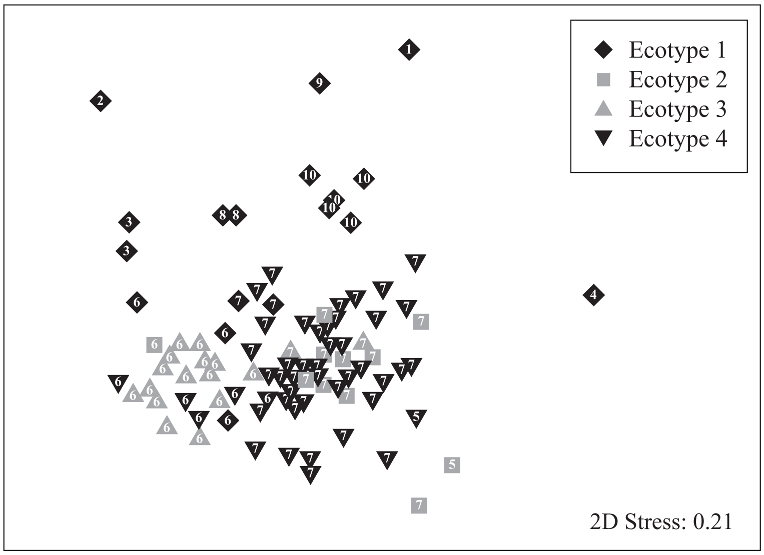

Fig. 3 Non-metric multidimensional scaling (NMDS) ordination of sites of the reference sites based on the Bray–Curtis similarities. Labels identify the stream groups defined by UPGMA, and symbols identify ecotypes obtained by system B.

Table 8 ANOSIM results for global and pair-wise comparisons among the Mediterranean stream ecotypes, based on the reference data sets of combined (three seasons) and each individual season and using macroinvertebrate presence–absence and rank abundance data

	Pair-wise comparisons between stream ecotypes						
	Stream ecotypes	Ecotypes 3 & 4	Ecotypes 2 & 3	Ecotypes 3 & 1	Ecotypes 4 & 2	Ecotypes 1 & 4	Ecotypes 1 & 2
Data Type	Global *R*	*R*	*R*	*R*	*R*	*R*	*R*
All seasons ($n = 105$)							
Abundance	0.399***	0.273**	0.506***	0.444***	0.170ns	0.654***	0.262*
Pres/abs	0.409***	0.303**	0.486**	0.410***	0.180ns	0.660***	0.265*
Spring ($n = 105$)							
Abundance	0.302***	0.161 NS	0.552***	0.393***	0.113 NS	0.512***	0.225 NS
Pres/abs	0.307***	0.183*	0.533***	0.358***	0.126ns	0.507***	0.225 NS
Summer ($n = 93$)							
Abundance	0.230**	0.104 NS	0.479**	0.813***	0.132 NS	0.481**	0.302 NS
Pres/abs	0.239**	0.122 NS	0.482***	0.793***	0.136 NS	0.475**	0.303 NS
Autumn ($n = 103$)							
Abundance	0.189**	0.060 NS	0.499***	0.423***	0.019 NS	0.313**	0.278**
Pres/Abs	0.194**	0.071 NS	0.485**	0.400***	0.020 NS	0.327**	0.281**

Significant *R* values; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, non-significant.

Norris & Reynoldson, 2004), as human disturbance reduces the natural differences between biological communities (Verdonschot, 2006). In the present study, five stream ecotypes were identified using a combination of hydrological, geological, climatic and morphological criteria (top-down approach). The hydrological variables (discharge, dry period percentage and hydrological state) had the highest power of discrimination, distinguishing perennial from intermittent and ephemeral streams. About 65% of studied sites were selected as reference sites. All sampling sites included in ecotype 5 (large watercourses) showed evidence of significant human disturbance, resulting in no reference macroinvertebrate communities being found. As a result, top-down versus bottom-up comparison could not be made for these watercourses. The lower reaches of most streams in Europe (e.g. Ehler *et al.*, 2002; Lorenz *et al.*, 2004; Nijboer *et al.*, 2004; Dodkins *et al.*, 2005) and in other Mediterranean areas (e.g. Prat & Munné, 2000; Bonada *et al.*, 2004a) are in the same condition.

We hypothesized that the stream ecotypes established here would be environmentally homogeneous, with similar hydrological and morphological conditions, and therefore would contain similar biological communities. Accordingly, both top-down and bottom-up approaches would give similar results about the families of macroinvertebrates comprising each group (e.g. Hughes & Larsen, 1988; Hawkins & Norris, 2000). Several recent studies have analysed the concordance between environmental classifications and biological communities (Hawkins & Vinson, 2000; Moog *et al.*, 2004; Snelder *et al.*, 2004; Heino & Mykra, 2006), but to our knowledge this is the first study from the Mediterranean area where temporary rivers predominate. Although a recent study established three major stream types in Europe: mountain, lowland and mediterranean (Verdonschot & Nijboer, 2004), we consider this classification to be insufficient for capturing the variability present in streams influenced by Mediterranean climate (Gasith & Resh, 1999).

Macroinvertebrate family composition differed between Mediterranean stream ecotypes. Siliceous headwater high altitude streams (ecotype 3) had the highest number of indicator taxa (31). Indicator taxa consisted mostly of EPT taxa commonly found in soft-water high altitude streams and including a significant proportion of shredding invertebrates, such as Lepidostomatidae and Nemouridae (e.g. Alba-Tercedor, González & Puig, 1992; Williams & Feltmate, 1992; Wiggins, 1996; Tachet *et al.*, 2000). These communities are similar to those found in the temperate rivers of Europe. Moreover, similarities have also been found concerning species traits of temperate and Mediterranean faunas (see Bonada, Dolédec & Statzner, in press).

Streams of ecotype 2 had taxa characteristic of the middle courses of streams (see Table 6) with lentic-type habitats (e.g. Williams and Feltmate, 1992; Merrit & Cummins, 1996; Bonada *et al.*, 2006). For instance Mollusca, such as Thiaridae, Physidae and Planorbidae, and Crustaceans such as Atyidae also showed a preference for streams at middle altitudes and having moderate to high mineralization.

In general, we found good agreement between the selection of indicator taxa in this study and those from other studies in the Mediterranean region in Spain (Bonada *et al.*, 2004a; Jáimez-Cuellar, 2004; Vivas *et al.*, 2004; Mellado, 2005); a finding that moreover supports the conjecture that these ecotypes have some ecological significance. The importance of Coleoptera in temporary streams has been emphasized by several authors (e.g. Moreno *et al.*, 1997; Beauchard, Gagneur & Brosse, 2003), hence our finding that only one indicator taxon (Dytiscidae) was characteristic of ecotype 1 or temporary streams was unexpected. One explanation for the lack of other indicator taxa may be as a result of the wide variety of temporary streams, such as *ramblas* (Vidal-Abarca, Suárez & Ramírez, 1992; Moreno *et al.*, 1997) and karstic ephemeral springs (Pardo & Álvarez, 2006) in this ecotype. Another explanation may be related to the temporal and spatial changes that affect temporary streams during the annual cycle. For example, a recent study showed the influence of drought on habitat loss in permanent and intermittent streams in the Mediterranean-climate area (Bonada *et al.*, 2006). These authors showed that different macrohabitats (riffles, connected pools and disconnected pools) had different macroinvertebrate assemblages. Riffle habitats had a predominance of EPT taxa, whereas pool habitats had a predominance of OCH taxa. Furthermore, our study supports the use of the EPT/OCH ratio in discriminating community assemblages. EPT taxa predominated in headwater systems (ecotype 3) with low temperatures and the presence of riffles, whereas OCH taxa were more abundant in calcareous, evaporitic streams at lower altitudes and having a predominance of pool habitats (ecotypes 1 and 2). Thus, in temporary streams we found the lowest EPT/OCH values in summer because of drought-induced changes in habitat (Lake, 2003).

As discussed, the largest difference between the communities defined from top-down versus bottom-up approaches was found for ecotype 1 or temporary streams. High variability in annual discharge and sequential floods and droughts that occur on an annual cycle are two characteristics of Mediterranean climate streams (e.g. Vidal-Abarca, Suárez & Ramírez, 1992; Gómez *et al.*, 2004; Robles *et al.*, 2004). In the present study, all sites of ecotype 1 were intermittent or ephemeral and hence suffered intense drought, usually in summer. Both expansion and contraction processes were prevalent, affecting the physical and chemical properties of the water and subsequently the composition of macroinvertebrate assemblages (e.g. Power *et al.*, 1988; Williams & Feltmate, 1992; Williams, 1996; Stanley, Fisher & Grimm, 1997; Resh *et al.*, 1998; Lake, 2000; Acuña *et al.*, 2005). Moreover, intermittent streams showed different stages of drying and the presence of different macrohabitats (from a riffle-pool sequence to isolated pools) that clearly influenced the composition of macroinvertebrate assemblages (Logan & Brooker, 1983; McCulloch, 1986; Bonada *et al.*, 2006). Although droughts in Mediterranean regions are predictable and periodic (Gasith & Resh, 1999; Lake, 2003), their intensity can vary because of interannual variations in weather (Bonada, Rieradevall & Prat, in press). Drought events can also result in the stream channel drying up partially or completely (Sabater *et al.*, 2001; Beauchard *et al.*, 2003; Butturini *et al.*, 2003), and some studies have shown that watercourse desiccation can influence community development even the year after the drought (e.g. Boulton & Lake, 1992; Acuña *et al.*, 2005). In addition to stress related to seasonal and interannual variations in water quantity, sites of ecotype 1 also differed in natural salinity (180–12 400 $\mu S\ cm^{-1}$), which is also known to affect macroinvertebrate community composition (e.g. Williams & Feltmate, 1992; Moreno *et al.*, 1997). Hence, our findings support previous studies regarding the importance of temporal and spatial heterogeneity as predictors stream communities (e.g. Townsend & Hildrew, 1994); and this is especially evident for communities of intermittent streams.

In conclusion, temporary streams and siliceous headwater streams at high altitude had very different communities. This is a result that needs to be considered when establishing class boundaries for classification of ecological status (e.g. Buffagni *et al.*, 2006). By contrast, mineralized stream ecotypes (calcareous versus evaporitic) were not found to have different biological communities, suggesting that reference conditions and class boundaries (according to macroinvertebrates) should be similar. As noted, ecotype 1 'temporary streams' was the most diverse group, and this large variability may justify further division of this ecotype into subgroups to assurance proper application of the WFD methodology (Thornes & Rowntree, 2006). Finally, it must be considered that hydroclimatic models predict that global climate change will increase the frequency and severity of floods and droughts across Europe, thus increasing the proportion of streams with Mediterranean characteristics in areas that are now temperate (Arnell, 1999; Bonada *et al.*, in press). This makes more urgent the characterization of macroinvertebrate communities and its relationship with different ecological status, if macroinvertebrates are to be used in monitoring programmes that are compliant with the requirements of WFD.

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