

# The influence of natural flow regimes on macroinvertebrate assemblages in a semiarid Mediterranean basin

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## ABSTRACT

The investigation of flow–ecology relationships constitutes the basis for the development of environmental flow criteria. The need to understand hydrology–ecology linkages in natural systems has increased owing to the prospect of climate change and flow regime management, especially in water-scarce areas such as Mediterranean basins. Our research quantified the macroinvertebrate community response at family, genus and species level to natural flow regime dynamics in freshwater streams of a Mediterranean semiarid basin (Segura River, SE Spain) and identified the flow components that influence the composition and richness of biotic assemblages. Flow stability and minimum flows were the principal hydrological drivers of macroinvertebrate assemblages, whereas the magnitude of average and maximum flows had a limited effect. Perennial stable streams were characterized by flow sensitive lotic taxa (Ephemeroptera, Plecoptera and Trichoptera) and intermittent streams by predominately lentic taxa (Odonata, Coleoptera, Heteroptera and Diptera). Relatively minor biological changes were recorded for intermediate flow regime classes along a gradient of flow stability. Seasonal variation and minimum flows are key hydrological components that need to be considered for river management and environmental flows in the Segura River basin and other Mediterranean basins. The anthropogenic modification of these parameters, due to both human activities and climate change, would probably lead to significant changes in the structure and composition of communities in perennial stable streams. This would be characterized by a reduction of flow sensitive Ephemeroptera, Plecoptera and Trichoptera taxa and an increase in more resilient Odonata, Coleoptera, Heteroptera and Diptera taxa. Copyright © 2012 John Wiley & Sons, Ltd.

KEY WORDS natural flow regime; flow stability; minimum flows; macroinvertebrate composition; richness; Segura River basin; semiarid Mediterranean streams

Received 24 November 2011; Revised 10 April 2012; Accepted 11 April 2012

## INTRODUCTION

The search for links between instream ecology and hydrology has become one of the fundamental issues in contemporary river science (Vaughan *et al.*, 2009). Empirical investigation of regional flow–ecology relationships constitutes the basis for the development of environmental flow criteria (Arthington *et al.*, 2006; Poff *et al.*, 2010). In addition, the need to understand ecology–hydrology linkages in natural systems has been highlighted by the need to define reference conditions against which modified dynamics can be compared (Tockner *et al.*, 2003). These needs are particularly pressing in the light of predicted climate change (European Environment Agency 2008) and anthropogenic modification of natural flow regimes, especially in water-scarce areas such as Mediterranean basins.

Instream hydrological variability, encapsulating elements of the entire flow regime such as the daily, seasonal and annual patterns of discharge, the frequency, timing, predictability and duration of extreme flows (high and low), rates of change in discharge and the magnitude of flows, are widely recognized as key ecological organizers in fluvial ecosystems

(Richter *et al.*, 1996; Poff *et al.*, 1997; Hart and Finelli 1999; Bunn and Arthington 2002). Spatial variation of these characteristics is determined by variations in climate and mediated by basin geology, topography and vegetation (Winter 2001). These hydrological and environmental factors influence the physical habitat for aquatic and riparian biota determining the conditions for reproduction and recruitment and affecting the availability of trophic resources, refuges during adverse situations and opportunities for dispersal (Naiman *et al.*, 2008). Consequently, flow variability has strong ecological implications that shape the structure and function of riverine ecosystems from the local to regional scales and from days (ecological effects) to millennia (evolutionary effects) (Lytle and Poff 2004). It has been hypothesized that sites with similar hydrological characteristics should share similar faunal community composition, traits and ecosystem functioning (Poff and Ward 1989). Therefore, as Arthington *et al.* (2006) and Poff *et al.* (2010) suggested, ecological responses of flow regimes to a given anthropogenic change should be broadly similar in rivers with similar natural flow regimes.

This hypothesis provides a powerful foundation to predict ecological responses to future flow regime changes, constituting the key element of a new holistic framework for developing scientifically credible regional environmental flows: the *Ecological Limits of Hydrologic Alteration*

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(Arthington *et al.*, 2006; Kennard *et al.*, 2010; Poff *et al.*, 2010). Therefore, identifying and quantifying specific relationships between flow regimes and biological communities in undisturbed river ecosystems are essential steps to ensure sustainable river management (Arthington *et al.*, 2006; Jowett and Biggs 2009). Such relationships have been studied in general at the regional scale, with the use of macroinvertebrates (e.g. Monk *et al.*, 2006; Konrad *et al.*, 2008; Kennen *et al.*, 2010; Armanini *et al.*, 2011), fisheries (e.g. Poff and Allan 1995; Pegg and Pierce 2002; Kennard *et al.*, 2007; Snelder *et al.*, 2009) or multiple taxonomic groups (e.g. Jowett and Duncan 1990; Clausen and Biggs 1997). However, the strength and nature of relationships between the flow regime and the biological assemblage vary depending on the geographical region, the floral or faunal group considered and the taxonomic resolution analysed.

In some areas, such as Mediterranean-climate regions, organisms have to withstand high intrannual and interannual hydrological variability, together with frequent natural flow extremes (floods and droughts) (Gasith and Resh 1999). Species may respond over evolutionary time scales by developing morphological, physiological and/or life-history traits to bear such stresses (Poff *et al.*, 1997; Bonada *et al.*, 2007a; Bonada *et al.*, 2007b). Previous studies of Mediterranean streams (e.g. Bonada *et al.*, 2002; Jáimez-Cuellar *et al.*, 2002; Vivas *et al.*, 2002; Bonada *et al.*, 2004; Mellado 2005; Sanchez-Montoya *et al.*, 2007; Argyroudi *et al.*, 2009) as well as other semiarid areas (e.g. Boulton and Lake 2008) have highlighted the importance of flow permanence on the composition and structure of macroinvertebrate communities. A progressive replacement of Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa by Odonata, Coleoptera and Heteroptera taxa has been reported as flow permanence decreases (Sanchez-Montoya *et al.*, 2007; Argyroudi *et al.*, 2009) or hydrological connectivity is reduced (Bonada *et al.*, 2006); although Diptera has also been associated with river sections with low or no flows and dominate lentic habitats in Southeast Spain (Vivas *et al.*, 2002). Consequently, flow stability and hydrological extremes (especially low flows) are expected to be the most important components of Mediterranean flow regimes shaping instream assemblages; although its relative importance is still unclear.

The aim of this study was to quantify the effect of different flow regimes on macroinvertebrate communities. We utilized a dataset containing stream macroinvertebrate records at family, genus and species level across a semiarid Mediterranean region that encompasses a wide gradient of hydrological regimes (Belmar *et al.*, 2011) to test the following predictions: (1) flow stability and minimum flows should be the principal hydrological drivers of macroinvertebrate assemblage composition and richness; (2) an increase in the explanatory power of hydrology should occur as taxonomic resolution increases; and (3) a replacement of taxa should take place along a hydrological gradient from permanent streams with stable discharges to streams with high flow intermittence and flow variability. In general, a decrease in the percentage of flow-sensitive EPT families should occur as an increase in the percentage of more resilient Odonata, Coleoptera, Heteroptera and Diptera (OCHD) families takes place.

## METHODS

### Study area

Located in the Southeast of Spain, the Segura River basin drainage network, including coastal watercourses draining to the Mediterranean Sea, was selected as the study area. The management area of the Segura River basin, one of the most arid zones of the Mediterranean region, includes watercourses with highly heterogeneous flow regimes. These water bodies range from perennial rivers, with low seasonal and interannual flow variability, to highly seasonal ephemeral streams (Belmar *et al.*, 2011). This variability is due to a strong climatic and altitudinal gradient from NW to SE, despite its relatively small size (18 870 km<sup>2</sup>). Climate ranges from wet (>1000 mm mean annual precipitation) and cold in the high elevation mountains of the NW (>1000 m above sea level) to semiarid and hot in the SE lowlands (<350 mm mean annual precipitation). Mean annual temperatures range between 10 and 18 °C (CHS 2007). The lithology of the plains is characterized by limestone (karst) and Miocene and Triassic marls, with some small influences of volcanic strata. In contrast, calcites and dolomites dominate the mountainous headwaters. The vegetation is varied and ranges from Mediterranean conifer forests in the NW mountains to arid and semiarid shrublands in the SE lowlands. This gradient in altitude and climate is coupled with an anthropogenic population density gradient. The river network has low population densities in the forested headwaters, intermediate densities in the agricultural midlands (with major flow regulation) and highly populated cities in the lowlands (Mellado 2005). Agricultural (52.1%), forest and seminatural (45.2%) and artificial (2.1%) are the dominant land uses in the Segura basin (estimated from Corine Land Cover 2000), making the Segura River basin one of the most regulated in Europe (Ministerio de Medio Ambiente (MMA) 2004). Water resource demands exceed 224% of that available, and only 4% of runoff reaches the mouth of the river (Zimmer 2010). This has resulted in overexploitation of the surface waters, an interbasin transfer from the Tagus River (a mean of 325 hm<sup>3</sup> yr<sup>-1</sup>), a mean groundwater extraction of around 478 hm<sup>3</sup> y<sup>-1</sup> (over 80% of natural recharge) and a high regulatory capacity of 770 hm<sup>3</sup> (over 90% of the natural input) due to 24 dams over 10 m in height (Grindlay *et al.*, 2009; Grindlay *et al.*, 2011).

### Hydrological data

A drainage network was derived from a 25-m digital elevation model (DEM) developed by the *Instituto Geológico Nacional* and layers available from the website of the Spanish Ministry of Environment, using the ArcGIS software (v 9.2) and the ArcHydro extension (v 1.2) (ESRI, Redlands, CA, USA). The network comprises sections that link each network junction or node, and each node was associated with its corresponding watershed (derived from the DEM). The minimum watershed area to define a river section was 10 km<sup>2</sup>, resulting a hydrological network with 390 river sections.

The hydrological classification developed for the Segura River basin in Belmar *et al.* (2011) was used to define distinct natural hydrological regimes. This classification was developed using 73 indices based on the ‘indicators of hydrologic alteration’ (Mathews and Richter 2007). These flow indices represent a wide range of ecologically relevant flow statistics (Richter *et al.*, 1996; Olden and Poff 2003; Monk *et al.*, 2006; Mathews and Richter 2007; Monk *et al.*, 2007) and comprise monthly and annual flow statistics including measures of duration of droughts as well as the central tendency and dispersion of flow magnitude (average, low and high flow conditions). Indices related to the frequency, duration and rate of change of high flow events were not used by Belmar *et al.* (2011) because of the absence of daily flow data. Natural flows were derived from a monthly rainfall–runoff model developed by the Centre for Hydrographic Studies (Ministry of Environment and Public Works, Spain), for the period 1980/1981–2005/2006. The classification of the flow regimes recorded comprised eight flow-regime classes (names are provided throughout to aid interpretation) principally characterized by the magnitude of mean annual flow, the duration of droughts and the interannual variation of flow (Table I). The resulting flow regimes can be placed into four broad hydrological groups: (1) main stem rivers, with perennial flow throughout the year, low interannual variation and an average annual discharge greater than  $10 \text{ m}^3 \text{ s}^{-1}$  (class 1, *large rivers*) or between 2 and  $10 \text{ m}^3 \text{ s}^{-1}$  (class 2, *medium rivers*); (2) perennial stable streams, which only difference with respect to main stem rivers is their reduced average discharge, between  $0.3$  and  $2 \text{ m}^3 \text{ s}^{-1}$  (class 3, *creeks*) or lower than  $0.3 \text{ m}^3 \text{ s}^{-1}$  (class 4, *headwater streams*); (3) perennial seasonal streams, which eventually cease flowing (although perennial surface water persists) and with peak discharges in winter (class 5, *winter peak flow seasonal streams*) or spring (class 6, *spring peak flow seasonal streams*); and (4) temporary streams, including *intermittent streams* (class 7), which do not flow for between 20 and 50% of the time, and *ephemeral streams*, that do not experience flow for more than 50% of the time (class 8). Indices and classes were assigned to their corresponding river section.

#### Macroinvertebrate data

Macroinvertebrate abundance data at family, genus and species level were compiled from the *Biodiversidad* database (*Ecología Acuática* research group, Department

of Ecology and Hydrology, University of Murcia, Spain). Species data were available for beetles (Coleoptera), which have been recorded in all kinds of water bodies in the region and have been shown to be good indicators of aquatic biodiversity (Bilton *et al.*, 2006; Sanchez-Fernandez *et al.*, 2006). Samples had been taken along 100-m stream transects with the use of a kick net (500–1000  $\mu\text{m}$ ) and following the multihabitat protocol (Jáimez-Cuéllar *et al.*, 2002). Baseline macroinvertebrate samples were collected between 1980 and 2006.

A minimum of five samples per hydrological class were selected, ensuring that they had been collected in freshwater streams (conductivity  $< 5000 \mu\text{s cm}^{-1}$ ), above water regulation infrastructures (e.g. dams or weirs) and abstraction areas and in absence of significant evidences of anthropogenic alteration. However, with the use of the aforementioned criteria, two classes did not have any biological data: *large rivers* (class 1), owing to the absence of reference conditions, and *ephemeral streams* (class 8), where no sampling had been undertaken owing to their frequent dry status.

Every sample was collected during the spring or early summer from a different sampling site (Figure 1). This period is considered the most representative of the annual macroinvertebrate community composition in Mediterranean streams (Bonada *et al.*, 2009). Each site was paired with the closest downstream node in the drainage network. To avoid pseudoreplication, when there was more than one site (and sample) available for the same node, only the closest to the hydrological node was selected. The final dataset consisted of 35 samples associated with 84 macroinvertebrate families, and 133 genera, and 43 samples associated with 110 Coleoptera species (Appendix A).

#### Environmental data

Climatic, topographic and geologic variables that were assumed to control hydrological processes (Snelder *et al.*, 2005) were derived from different Geographic Information System (GIS) layers available for the watershed. Average annual precipitation and air temperature were derived from 1-km grid maps created by the Spanish Ministry of Environment by means of interpolation using data from the Spanish weather stations network (Estrela *et al.*, 1999). Drainage area, mean altitude and slope were calculated using the *Instituto Geológico Nacional's* DEM. Geology

Table I. Mean and standard deviation of the mean annual flow (MADIS), time with zero flow ( $D_L$ ) and coefficient of variation in annual flows ( $CV_{\text{INTER}}$ ) for the natural flow regime classes defined in the Segura River basin (Belmar *et al.*, 2011).

Hydrological class	Number of stream sections	MADIS ( $\text{m}^3 \text{ s}^{-1}$ )	$D_L$ (%)	$CV_{\text{INTER}}$
Class 1: Perennial large size rivers	17	11.30 ( $\pm 0.74$ )	0.00 ( $\pm 0.00$ )	0.52 ( $\pm 0.01$ )
Class 2: Perennial medium size rivers	31	3.76 ( $\pm 2.26$ )	0.00 ( $\pm 0.00$ )	0.50 ( $\pm 0.13$ )
Class 3: Perennial stable creeks	21	1.00 ( $\pm 0.45$ )	0.00 ( $\pm 0.00$ )	0.32 ( $\pm 0.09$ )
Class 4: Perennial stable headwater streams	43	0.18 ( $\pm 0.17$ )	0.00 ( $\pm 0.00$ )	0.26 ( $\pm 0.13$ )
Class 5: Perennial winter peak flow seasonal streams	26	0.37 ( $\pm 0.09$ )	2.31 ( $\pm 2.06$ )	1.39 ( $\pm 0.29$ )
Class 6: Perennial spring peak flow seasonal streams	110	0.06 ( $\pm 0.06$ )	4.46 ( $\pm 6.32$ )	0.81 ( $\pm 0.30$ )
Class 7: Temporary intermittent streams	101	0.04 ( $\pm 0.04$ )	24.88 ( $\pm 13.15$ )	1.71 ( $\pm 0.38$ )
Class 8: Temporary ephemeral streams	41	0.01 ( $\pm 0.01$ )	61.90 ( $\pm 20.21$ )	3.43 ( $\pm 0.84$ )

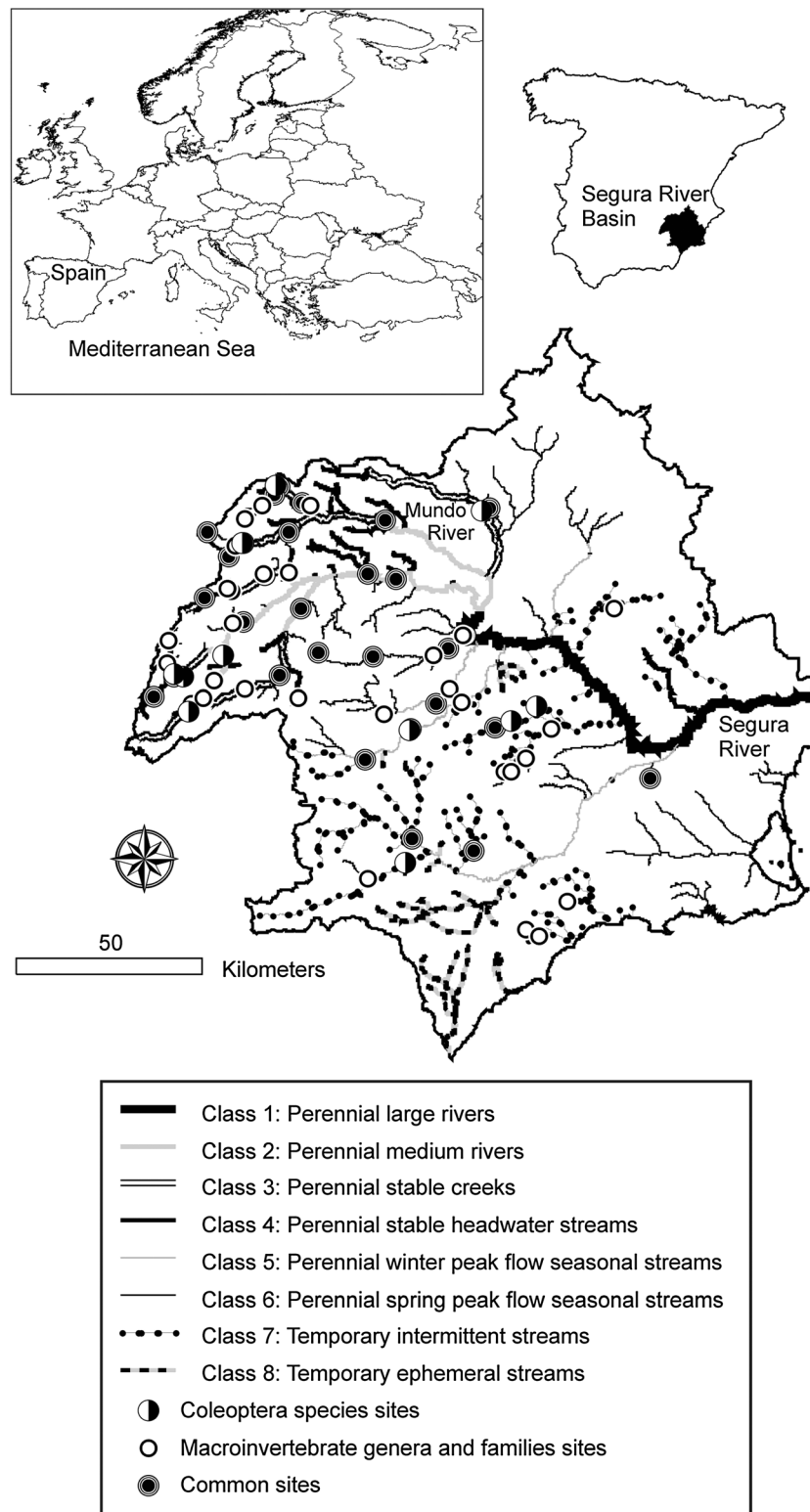


Figure 1. Location of the study area, hydrological classes in the river network and sampling sites.

was characterized by the percentage of karst area in each watershed and derived from the 'Spain's Map of Karst' 1:1 000 000 developed by the *Instituto Geológico y Minero de España* and, indirectly, through water conductivity (recorded for every biological sample). We hypothesized that the karstic surface would control groundwater storage and baseflow (Snelder and Biggs 2002) and that higher conductivities would reflect the

predominance of sedimentary marls that result in flashy hydrographs that reflect precipitation patterns (Bracken *et al.*, 2008).

#### Data analysis

A principal component factor analysis (PCFA) (i.e. a principal components analysis (PCA) combined with a

varimax rotation) was used to examine dominant patterns of intercorrelation among the hydrological indices (Belmar *et al.*, 2011) and to identify subsets of indices that describe the major sources of variation while minimizing redundancy (i.e. multicollinearity). The varimax rotation allows obtaining a clearer pattern of loadings (indices clearly marked by high loadings for some axes and low loadings for others) and, therefore, a better interpretation of the meaning of each axis. The hydrological characteristics of each stream in the network were defined through the corresponding PCFA scores (hydrological components) and hydrological class.

Rare taxa (those collected at fewer than 5% of sampling sites) were removed for multivariate analyses. Abundance data were transformed by means of the Beals smoothing function (Beals 1984; McCune 1994) to reduce noise by enhancing the pattern of joint occurrences. This function is appropriate in the current investigation, because the data consist of a large number of small sample units (Peck *et al.*, 1995) and fulfil the requirements established by De Cáceres and Legendre (2008).

For each taxonomic level analysed, we performed a nonmetric multidimensional scaling (NMDS) ordination on the basis of Bray–Curtis distances among the sampling sites. The strength of the correlation between the NMDS axes and the environmental variables, as well as the hydrological components, was plotted as vectors. In addition, the individual variables and components were analysed using Pearson coefficients. Covarying (redundant) environmental variables were removed for subsequent model development because the primary objective of the research was to determine the most important flow components influencing macroinvertebrate assemblages and not to distinguish the independent effect of hydrological and environmental drivers.

Distance-based linear models (DistLM) were developed to assess the importance of hydrological components driving taxonomical differences among sites. DistLM calculates a multivariate multiple regression analysis between any symmetric distance matrices, including a permutation test, as described by McArdle and Anderson (2001). The final models were selected following a forward stepwise procedure. For each taxonomic level, marginal tests determined the variance explained by each flow component, and the sequential procedure discarded the variance shared by more than one thereby avoiding the overestimation of their effect on the community.

Similarly, generalized linear models (GLM) were employed to determine how hydrological components (independent variables) affected faunal richness patterns. Models were constructed using log-transformed data following a forward stepwise procedure, assuming a Gaussian error distribution for the dependent variables. These variables were the richness of Coleoptera species, the number of macroinvertebrate genera, the number of macroinvertebrate families and the ratio EPT/EPTOCHD (defined by the richness of Ephemeroptera, Plecoptera, Trichoptera, Odonata, Coleoptera, Heteroptera and Diptera families). The latter is based on the EPT/EPTOCH ratio, which is used to characterize temporary and lotic–lentic conditions in Mediterranean-climate regions (Bonada *et al.*, 2006).

A nonmetric single-factor analysis of similarity (ANOSIM) was used to test whether assemblage composition differed among hydrological classes and, therefore, if natural regimes can be used to differentiate distinct groups of invertebrate communities. Global  $R$  indicates if assemblages are randomly grouped (i.e.  $R=0$ ) or not (usually  $0 < R \leq 1$ , although negative values are possible *sensu* Clarke (1993)).  $R$  pairwise values were also obtained for each pair of classes, indicating whether intraclass similarities were greater than interclass ones ( $R$  value close to 1).

Indicator taxa were defined for each hydrological class with the use of the indicator species analysis (IndVal) of Dufrene and Legendre (1997). This analysis generates an indicator value index (IV) for each taxon and class, calculated on the basis of the specificity (maximum when a taxon only occurs in one class) and fidelity (maximum when all sites in a class have the taxon) of each taxon to each class.

All permutation tests (DistLM, ANOSIM and IndVal) were undertaken using 999 permutations. PCFA was undertaken in STATISTICA v 6 (StatSoft, Inc 2001). NMDS and IndVal were conducted using PC-ORD software v 4.42 (McCune and Grace 2002). ANOSIM and DistLM were undertaken in PRIMER v 6 (Clarke and Gorley 2006). GLM were performed using the R statistical software v 2.12.2 (R Development Core Team 2008).

## RESULTS

### *Hydrological components*

The three first PCFA axes were selected to represent the set of hydrological indices because all of them explained greater than 10% of the variance (46, 28 and 12%, respectively), and the fourth axis only explained an additional 4%. The first axis was positively correlated with mean and maximum monthly flows (Table IIa), representing the flow magnitude component of the indicators of hydrologic alteration. The second axis was negatively correlated with the interannual coefficients of variation in monthly flows, the intrannual coefficient of variation in maximum monthly flows and the percentage of time without flows. These variables characterize the interannual and intrannual variability of the flow regime and, as a result, this axis was defined as the flow stability component (Table IIb). The third axis, magnitude of minimum flows, was correlated with all the minimum monthly flows and their average value (Table IIc).

These three hydrological components (PCFA axes) displayed significant positive correlations with mean altitude and precipitation in the watershed and negative correlations with mean temperature (Table III). In addition, karst surface and slope were positively correlated with flow stability and minimum flows, whereas drainage area was associated with the magnitude of flow. As anticipated, conductivity displayed a negative association with flow magnitude and stability.

### *Hydrological components determining assemblage composition*

The macroinvertebrate NMDS ordinations for different taxonomic resolutions identified similar patterns (Figure 2).

Table II. Pearson correlation coefficients between the three first axes from the principal component factor analysis (PCFA) and the 73 hydrological indices.

Variable	Description	PCFA axis			
		First	Second	Third	
(a)	MA 1	Mean monthly flow (October)	<b>0.98</b>	0.13	0.02
	MA 2	Mean monthly flow (November)	<b>0.98</b>	0.13	0.02
	MA 3	Mean monthly flow (December)	<b>0.99</b>	0.12	0.05
	MA 4	Mean monthly flow (January)	<b>0.98</b>	0.14	0.04
	MA 5	Mean monthly flow (February)	<b>0.98</b>	0.14	0.04
	MA 6	Mean monthly flow (March)	<b>0.99</b>	0.12	0.03
	MA 7	Mean monthly flow (April)	<b>0.98</b>	0.14	0.02
	MA 8	Mean monthly flow (May)	<b>0.98</b>	0.15	0.04
	MA 9	Mean monthly flow (June)	<b>0.98</b>	0.15	0.03
	MA 10	Mean monthly flow (July)	<b>0.97</b>	0.16	0.04
	MA 11	Mean monthly flow (August)	<b>0.97</b>	0.16	0.05
	MA 12	Mean monthly flow (September)	<b>0.98</b>	0.13	0.02
	MA 16	Mean annual flow divided by catchment area	0.18	0.50	0.35
	MEDDIS/A	Median annual discharge divided by catchment area	0.22	0.52	0.35
	MH1	Mean of the maximum monthly flows (October)	<b>0.96</b>	0.08	0.01
	MH2	Mean of the maximum monthly flows (November)	<b>0.96</b>	0.06	0.07
	MH3	Mean of the maximum monthly flows (December)	<b>0.91</b>	0.00	0.05
	MH4	Mean of the maximum monthly flows (January)	<b>0.97</b>	0.14	0.08
	MH5	Mean of the maximum monthly flows (February)	<b>0.97</b>	0.15	0.11
	MH6	Mean of the maximum monthly flows (March)	<b>0.94</b>	0.03	0.02
	MH7	Mean of the maximum monthly flows (April)	<b>0.98</b>	0.10	0.04
	MH8	Mean of the maximum monthly flows (May)	<b>0.98</b>	0.15	0.08
	MH9	Mean of the maximum monthly flows (June)	<b>0.98</b>	0.13	0.00
	MH10	Mean of the maximum monthly flows (July)	<b>0.98</b>	0.13	-0.03
	MH11	Mean of the maximum monthly flows (August)	<b>0.98</b>	0.13	-0.03
	MH12	Mean of the maximum monthly flows (September)	<b>0.95</b>	0.05	-0.04
	MH13	Mean of the mean maximum flows for all months	<b>0.98</b>	0.08	0.04
	MADIS	Mean annual flow for all years	<b>0.98</b>	0.14	0.03
	RANGE	Maximum annual discharge minus minimum annual discharge	<b>0.98</b>	0.06	-0.05
	Q1	Percentile flow with the annual discharge exceeded 1% of time	<b>0.99</b>	0.09	0.01
	Q50	Median annual flow for all years	<b>0.97</b>	0.14	0.03
(b)	CVA 1	Coefficient of variation (October)	-0.08	<b>-0.83</b>	-0.30
	CVA 2	Coefficient of variation (November)	-0.12	<b>-0.86</b>	-0.15
	CVA 3	Coefficient of variation (December)	-0.09	<b>-0.84</b>	-0.19
	CVA 4	Coefficient of variation (January)	-0.19	<b>-0.88</b>	-0.21
	CVA 5	Coefficient of variation (February)	-0.21	<b>-0.89</b>	-0.17
	CVA 6	Coefficient of variation (March)	-0.19	<b>-0.81</b>	-0.25
	CVA 7	Coefficient of variation (April)	-0.26	<b>-0.90</b>	-0.20
	CVA 8	Coefficient of variation (May)	-0.02	<b>-0.91</b>	-0.19
	CVA 9	Coefficient of variation (June)	0.02	<b>-0.83</b>	-0.35
	CVA 10	Coefficient of variation (July)	0.09	<b>-0.82</b>	-0.37
	CVA 11	Coefficient of variation (August)	0.09	<b>-0.84</b>	-0.36
	CVA 12	Coefficient of variation (September)	-0.03	<b>-0.81</b>	-0.34
	MA 13	Range divided by median monthly flow	-0.06	<b>-0.90</b>	-0.03
	MA 14	Interquartile divided by median monthly flow	0.09	<b>-0.80</b>	0.05
	CVINTRA	Coefficient of variation in mean monthly flows	0.02	<b>-0.90</b>	-0.03
	MA 15	Mean minus median monthly flow divided by median monthly flow	-0.15	<b>-0.73</b>	0.06
	MA 17	Range divided by median annual flow	-0.22	<b>-0.93</b>	-0.10
	MA 18	Interquartile divided by median annual flow	-0.17	<b>-0.83</b>	-0.05
	MA 19	Mean minus median annual flow divided by median annual flow	-0.17	<b>-0.84</b>	0.03
	CVH	Coefficient of variation in mean maximum monthly flows	-0.27	<b>-0.79</b>	-0.08
	DL	Percentage of months with zero flow	-0.38	<b>-0.75</b>	-0.24
	CVINTER	Coefficient of variation in annual flows for all years	-0.21	<b>-0.92</b>	-0.25
	Q5/Q50	Q5 divided median monthly flow	-0.23	<b>-0.88</b>	-0.08
	Q10/Q50	Q10 divided median monthly flow	-0.21	<b>-0.87</b>	-0.06
	STDEV	Standard deviation of annual discharge	<b>0.99</b>	0.07	-0.09
	AMAX/Q50	Maximum annual discharge divided by Q50	-0.23	<b>-0.92</b>	-0.08
	AMIN/Q50	Minimum annual discharge divided by Q50	-0.25	0.63	0.42
	IH	Q5 divided mean monthly flow	0.08	-0.04	-0.27
	IL	Q95 divided mean monthly flow	-0.26	0.60	0.48

Continues

Table II. (Continued)

Variable	Description	PCFA axis		
		First	Second	Third
(c) ML 1	Mean minimum monthly flow (October)	0.02	0.19	<b>0.92</b>
ML 2	Mean minimum monthly flow (November)	0.04	0.19	<b>0.92</b>
ML 3	Mean minimum monthly flow (December)	0.03	0.19	<b>0.92</b>
ML4	Mean minimum monthly flow (January)	0.11	0.20	<b>0.77</b>
ML 5	Mean minimum monthly flow (February)	0.08	0.18	<b>0.88</b>
ML 6	Mean minimum monthly flow (March)	0.04	0.18	<b>0.93</b>
ML 7	Mean minimum monthly flow (April)	0.10	0.23	<b>0.78</b>
ML 8	Mean minimum monthly flow (May)	0.03	0.17	<b>0.93</b>
ML 9	Mean minimum monthly flow (June)	0.00	0.17	<b>0.90</b>
ML 10	Mean minimum monthly flow (July)	0.01	0.17	<b>0.90</b>
ML 11	Mean minimum monthly flow (August)	0.04	0.16	<b>0.89</b>
ML 12	Mean minimum monthly flow (September)	0.05	0.16	<b>0.88</b>
ML 13	Mean of the mean minimum flows for all months	0.06	0.20	<b>0.96</b>

Coefficients higher than |0.70| are in bold. Horizontal lines separate indices associated to the three flow components represented by the axes: (a) magnitude (average and maximum flows), first axis (46% of the variance); (b) flow stability, second axis (28% of the variance); and (c) minimum flows, third axis (12% of the variance).

Sites were structured along a flow stability gradient from perennial headwater streams (left side, class 4) to intermittent streams (right side, class 7); although some classes were widely dispersed (particularly class 6 – spring peak flow seasonal streams). This gradient was associated with several environmental variables and hydrological components (PCFA axes). Perennial stable streams (classes 3 and 4) were predominately located on karstic rocks and sites in higher altitude areas with steeper slopes, higher flow stability and relatively high minimum flows. In contrast, intermittent streams were associated to low slopes, reduced flow stability and low minimum flows, but higher conductivity and air temperature.

The DistLMs indicated that hydrological components accounted for a significant proportion of the variance in the macroinvertebrate community that increased with taxonomic resolution (Table IV): 28% for families, 30% for genus and 38% for Coleoptera species. In all cases, flow stability and minimum flows were the dominant hydrological drivers of taxonomical differences among sites.

*Response of taxonomic richness to hydrological components*

The GLMs showed a moderate effect of hydrological variables on the richness of macroinvertebrate families, genera and species (Table V). However, the model

Table III. Pearson correlation coefficients between environmental variables and hydrological components (the three first axes from the principal component factor analysis, respectively).

Environmental variable	Flow magnitude	Flow stability	Minimum flows
Mean precipitation (mm)	<b>0.26</b>	<b>0.64</b>	<b>0.39</b>
Conductivity ( $\mu\text{S cm}^{-1}$ )	<b>-0.28</b>	<b>-0.54</b>	-0.21
Mean altitude (m)	<b>0.34</b>	<b>0.64</b>	<b>0.34</b>
Mean slope (°)	0.24	<b>0.37</b>	<b>0.27</b>
Karst surface (%)	0.21	<b>0.36</b>	<b>0.37</b>
Mean temperature (°C)	<b>-0.37</b>	<b>-0.57</b>	<b>-0.27</b>
Drainage area ( $\text{km}^2$ )	<b>0.83</b>	-0.16	-0.14

Significant correlations ( $p < 0.05$ ) are in bold.

obtained for the EPT/EPTOCHD ratio explained 36% of the variance with the use of flow magnitude and flow stability as independent variables. Gradual changes to the relative richness of EPT families were observed from perennial to intermittent hydrological classes, decreasing along the flow magnitude gradient, whereas the OCHD families displayed the opposite pattern (Figure 3).

*Differences in assemblage composition among hydrological classes*

The hydrological classes identified supported significantly different invertebrate assemblages at the family (ANOSIM,  $R = 0.39$ ;  $p\text{-value} < 0.05$ ), genus (ANOSIM,  $R = 0.34$ ;  $p\text{-value} < 0.05$ ) and species taxonomic level (ANOSIM,  $R = 0.40$ ;  $p\text{-value} < 0.05$ ) (Table VI). Pairwise comparisons revealed significant assemblage differences at all taxonomic resolutions between the extremes of the hydrological gradient, perennial stable streams (creeks and headwaters, classes 3 and 4 respectively) and intermittent streams (class 7). Differences between creek and medium river communities (class 2) as well as between creeks and perennial seasonal streams with peak flows during the winter (class 5) increased with the taxonomic resolution, except for the genus level. However, intermittent streams and perennial seasonal streams, both with winter (class 5) and spring peak flows (class 6), differed at the genus or at the genus and species levels, respectively. No significant differences were found both between creeks and headwater streams or within seasonal streams (winter and spring peak flows) (Table VI).

The IndVal analyses determined indicator families for medium rivers (class 2), headwater streams (class 4), spring peak flow seasonal streams (class 6) and intermittent streams (class 7) (Table VII). Medium rivers were characterized by Polycentropodidae (Trichoptera) and Potamanthidae (Ephemeroptera). Headwater streams were defined by six families of Trichoptera (particularly Philopotamidae, with the highest IV) and one Crustacea (Astacidae). Spring peak flow seasonal streams were characterized by

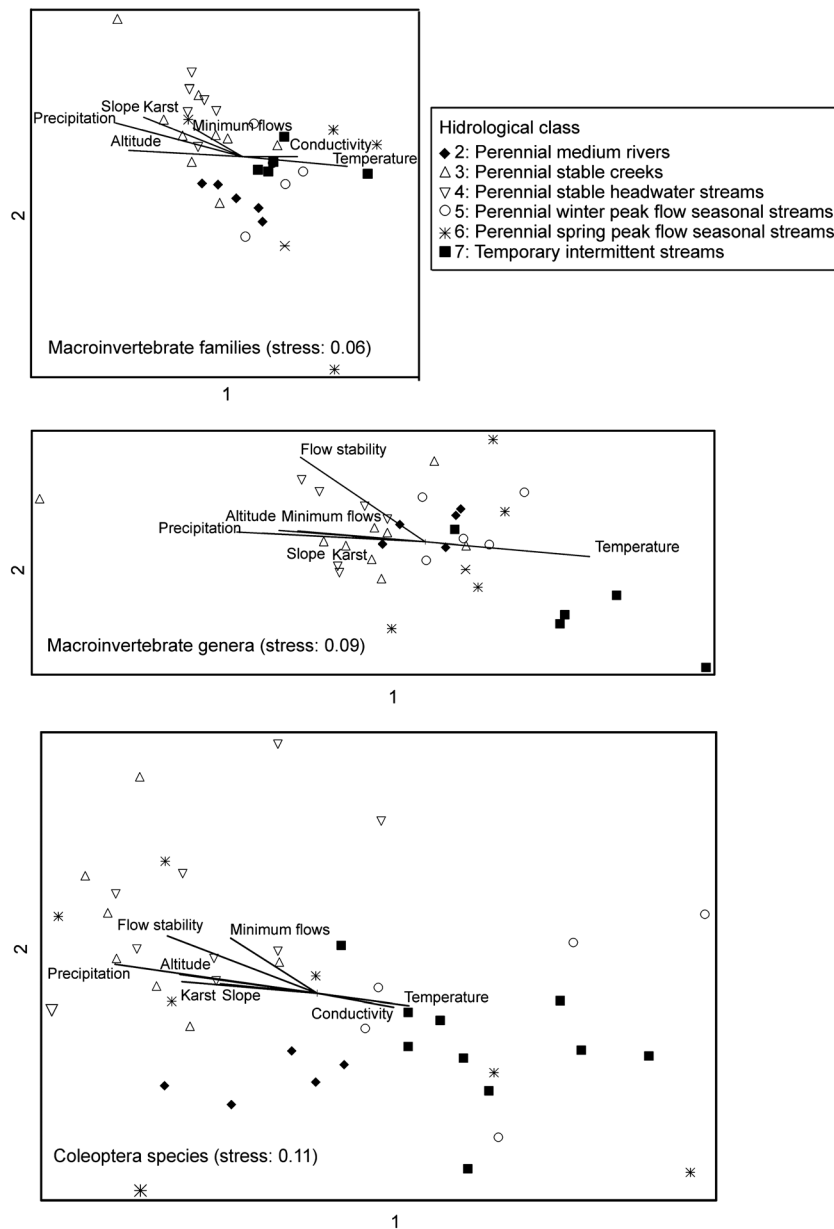


Figure 2. Nonmetric multidimensional scaling (NMDS) plots of sites for each taxonomic level. The magnitudes of the correlations between the NDMS axes and the hydrological components as well as the environmental variables are shown as vectors.

Table IV. Results of the distance-based linear models for each taxonomic level.

Hydrological component	Macroinvertebrate families		Macroinvertebrate genera		Coleoptera species	
	Marginal (%)	Sequential (%)	Marginal (%)	Sequential (%)	Marginal (%)	Sequential (%)
Flow magnitude	7	6*	6	5	4	3*
Flow stability	12***	9**	24***	24***	27***	27***
Minimum flows	13***	13***	11***	6*	16***	8***
Total (%)		28		30		38

\* $p \leq 0.05$ ;  
 \*\* $p \leq 0.01$ ;  
 \*\*\* $p \leq 0.001$  significance levels.

Syrphidae (Diptera), which presented the highest IV in the Segura basin. Intermittent streams were defined by the presence of Coenagrionidae and Libellulidae (Odonata), Pleidae (Heteroptera) and Noteridae and Hydrophilidae (Coleoptera).

Indicator genera were found for all classes except creeks (class 3) and winter peak flow seasonal streams (class 5). Medium rivers (class 2) and headwater streams (class 4) were characterized by Ephemeroptera: *Habrophlebia* and *Potamanthus* for the former and *Epeorus* and *Rhithrogena*



Table V. Generalized linear models for the different dependent variables, on the basis of richness.

Dependent variable	Variance explained (%)	Explanatory hydrological components
EPT/EPTOCHD	36	Flow magnitude,** flow stability*
Macroinvertebrate families	21	Minimum flows**
Macroinvertebrate genera	24	Minimum flows**
Coleoptera species	17	Minimum flows**

\* $p \leq 0.05$ ;  
 \*\* $p \leq 0.01$ ;  
 \*\*\* $p \leq 0.001$  significance levels.

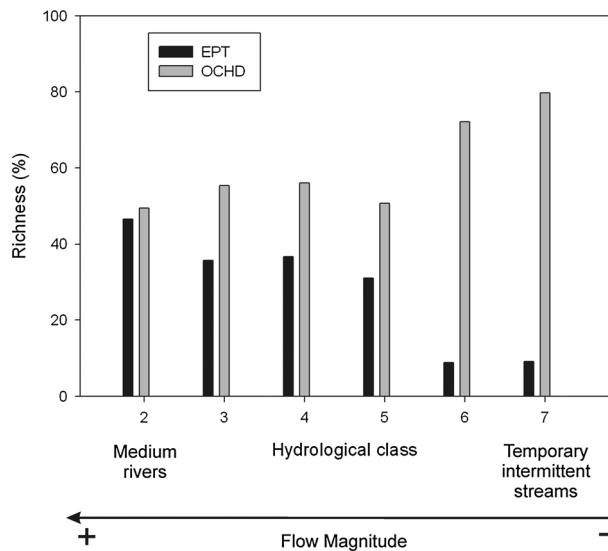


Figure 3. Variation of the percentage of families of the EPT (Ephemeroptera, Plecoptera and Trichoptera) and OCHD (Odonata, Coleoptera, Heteroptera and Diptera) groups in the different hydrological classes along the flow magnitude gradient.

for the latter. Headwaters were also characterized by seven Coleoptera genera (*Oreodytes*, *Graptodytes*, *Esolus*, *Limnebius*, *Normandia*, *Hydrocyphon* and *Oulimnius*), two Trichoptera (*Rhyacophila* and *Sericostoma*), one Crustacea (*Austropotamobius*) and two Plecoptera (*Perla* and *Isoperla*). Spring peak flow seasonal streams (class 6) were characterized by one genus of Coleoptera (*Dytiscus*), Hirudinea (*Helobdella*), Mollusca (*Pseudamnicola*) and Odonata (*Platycnemis*), with identical IVs. Intermittent streams (class 7) highlighted the highest number of indicator genera, with the highest IVs for two Diptera (*Dasyhelea* and *Anopheles*), two Heteroptera (*Heliocoris* and *Anisops*), two Odonata (*Anax* and *Sympetrum*) and two Coleoptera (*Enochrus* and *Berosus*).

Coleoptera indicator species were detected for all classes except spring peak flow seasonal streams (class 6) (Table VII). Medium rivers (class 2) were primarily characterized by *Hydraena manfredjaechi* and *Normandia nitens*; creeks (class 3) by *Hydraena exasperata*; headwater streams (class 4) by *Helophorus alternans*; winter peak flow

Table VI. Result of the analysis of similarity.

Classes	Macroinvertebrate families	Macroinvertebrate genera	Coleoptera species
2, 5	0.22	0.15	0.50**
2, 7	0.59*	0.62*	0.49**
3, 2	0.26*	0.06	0.56**
3, 5	0.25*	0.20	0.76***
3, 6	0.49**	0.30*	0.05
3, 7	0.50**	0.53**	0.86***
4, 2	0.85**	0.67**	0.37**
4, 3	0.00	-0.02	-0.09
4, 5	0.81**	0.80**	0.66***
4, 6	0.53**	0.77**	0.09
4, 7	0.88**	0.86**	0.77***
5, 7	0.29	0.42*	0.17
6, 2	0.27**	0.33**	-0.01
6, 5	0.12	0.02	0.16
6, 7	0.09	0.44*	0.38**
Global R	0.39***	0.34***	0.40***

Class 2, perennial medium rivers; class 3, perennial stable creeks; class 4, perennial stable headwater streams; class 5, perennial winter peak flow seasonal streams; class 6, perennial spring peak flow seasonal streams; and class 7, temporary intermittent streams.

\* $p \leq 0.05$ ;  
 \*\* $p \leq 0.01$ ;  
 \*\*\* $p \leq 0.001$  significance levels.

seasonal streams (class 5) by *Eretes griseus* and *Ranthus suturalis*; and intermittent streams (class 7) by *Ochthebius delgadoi*.

## DISCUSSION

### *The importance of hydrological components on macroinvertebrate assemblages*

The research presented herein supports the general hypothesis that streams with similar flow regimes express greater than random similarity in macroinvertebrate assemblages composition (Resh *et al.*, 1988; Poff 1996). Our results demonstrate relatively strong relationships between community composition and the flow regimes at different taxonomic levels. The strength of these relationships increased with taxonomic resolution suggesting that the species level data yields the strongest relationships and that, where it is available, it should be used in ecohydrological investigations (Monk *et al.*, 2012). Flow stability and minimum flows were shown to be the principal hydrological drivers/descriptors of the macroinvertebrate community assemblages in the Segura River basin. Similar results were reported by Chinnayakanahalli *et al.* (2011) in western USA, where baseflows and seasonality were the main predictors of invertebrate composition. However, these results contrast with studies performed in temperate-maritime regions where the magnitudes of mean flows or high flows were reported to be the best predictors of macroinvertebrate assemblages (Clausen and Biggs 1997; Monk *et al.*, 2006; Monk *et al.*, 2008).

Flow stability and minimum flows are major determinants of habitat availability and connectivity that affect

Table VII. Indicator taxa ( $IV \geq 25$  and  $p \leq 0.05$ ) for each hydrological class and taxonomic level.

Hydrological class	Macroinvertebrate families	IV (%)	Macroinvertebrate genera	IV	Coleoptera species	IV (%)
2. Perennial medium rivers	Polycentropodidae	31	<i>Habrophlebia</i>	28	<i>Hydraenamanfredjaechi</i>	47
	Potamanthidae	27	<i>Potamanthus</i>	27	<i>Normandianitens</i>	47
3. Perennial stable creeks					<i>Limnius intermedius</i>	44
					<i>Ochthebius difficilis</i>	34
					<i>Limnius opacus</i>	28
					<i>Pomatinius substriatus</i>	25
					<i>Hydraena exasperata</i>	55
					<i>Ilybius meridionalis</i>	50
					<i>Ochthebius bellieri</i>	46
					<i>Limnius volckmari</i>	34
					<i>Agabus brunneus</i>	32
					<i>Hydroporus marginatus</i>	30
					<i>Ochthebius bonnairei</i>	30
					<i>Anacaena bipustulata</i>	29
					<i>Deronectes moestus</i>	29
					<i>Hydraena carbonaria</i>	29
					<i>Hydraena capta</i>	27
					<i>Hydraena rufipennis</i>	26
	4. Perennial stable headwater streams					<i>Stictonectes epipleuricus</i>
					<i>Agabus didymus</i>	25
5. Perennial winter peak flow seasonal streams						

6. Perennial spring peak flow seasonal streams	Syrphidae	85	<i>Dytiscus</i>	35	
			<i>Helobdella</i>	35	
			<i>Pseudamnicola</i>	35	
			<i>Platynemis</i>	35	
7. Temporary intermittent streams	Noteridae	35	<i>Dasyhelea</i>	63	<i>Ochthebius delgadoi</i>
	Pleidae	35	<i>Anopheles</i>	63	<i>Enochrus politus</i>
	Coenagrionidae	30	<i>Helicorisa</i>	63	<i>Helophorus fulgidicollis</i>
	Libellulidae	28	<i>Anisops</i>	63	<i>Laccophilus minutus</i>
	Hydrophilidae	26	<i>Anax</i>	52	<i>Ochthebius europallens</i>
			<i>Enochrus</i>	48	<i>Ochthebius grandipennis</i>
			<i>Sympetrum</i>	48	<i>Ochthebius viridis fallaciosus</i>
			<i>Berosus</i>	45	<i>Ochthebius jaimet</i>
			<i>Sigara</i>	45	<i>Helochaeres lividus</i>
			<i>Plea</i>	45	
			<i>Ischnura</i>	45	
			<i>Noterus</i>	42	
			<i>Potamopyrgus</i>	42	
			<i>Cercion</i>	42	
			<i>Libellula</i>	42	
			<i>Helochaeres</i>	41	
			<i>Bidessus</i>	40	
			<i>Procambarus</i>	40	
			<i>Limnophora</i>	40	
			<i>Tipula</i>	40	
			<i>Microvelia</i>	40	
			<i>Agabus</i>	36	
			<i>Dryops</i>	32	
			<i>Laccobius</i>	32	
			<i>Orthetrum</i>	32	
			<i>Gerris</i>	30	
			<i>Nebrioporus</i>	27	
			<i>Cloeon</i>	27	
			<i>Micronecta</i>	25	

aquatic macroinvertebrate assemblages. Flow stability reflects seasonal and interannual patterns of variation, associated with the predictability of flows (Poff 1996) and the stability of habitat conditions in terms of depth, flow velocity and hydraulic forces (Suen and Herricks 2009). The variation of stream flow velocity configures stream morphology, water temperature, bed stability and consequently the availability of aquatic habitats for instream organisms (Jowett and Duncan 1990). Minimum flows represent an extreme of the flow, particularly in the dry season, and reflect the magnitude of seasonal droughts (Smakhtin 2001). Habitat heterogeneity is reduced under low flow conditions because wetted width, water depth and flow velocity also diminishes (Walters and Post 2011). In addition, extreme low flows can reduce longitudinal connectivity and increase physical stresses transforming streams into series of isolated pools with higher water temperature and elevated conductivity (Stanley *et al.*, 1997). Consequently, droughts have been recognized as an important part of the natural flow regime in intermittent streams (Boulton 2003; Lake 2003; Sheldon and Thoms 2006; Chase 2007). Species inhabiting intermittent streams must have physiological, behavioural or life-history adaptations to cope with higher conductivities, predation pressures and habitat isolation, such as short life histories, generalist feeding, aerial respiration or active aerial dispersal (e.g. Bonada *et al.*, 2007b). Under these conditions, dispersal abilities and distances between or along water bodies have been found to be primary determinants of community composition (McAbendroth *et al.*, 2005), because active movement when the riverbed is dry is limited to a small number of taxa such as dytiscid and hydrophilid beetles (Boulton *et al.*, 2006; Larned *et al.*, 2010).

Our results indicate a moderately strong relationship between flow regime and faunal richness at the different taxonomic resolutions, weaker than that between flow regime and community composition (especially at species level). Other studies have also reported a moderate effect of minimum flows (Walters and Post 2011), flow seasonality or the number of days with zero flow (Chinnayakanahalli *et al.*, 2011).

In Mediterranean regions, ephemeral and intermittent streams are recognized to be significantly less diverse than perennial streams (Bonada *et al.*, 2007b) and differ in community composition (e.g. Bonada *et al.*, 2006; Argyroudi *et al.*, 2009). Our results found a strong relationship between flow magnitude and stability and the ratio of EPT/EPTOCHD. This supports the findings of Bonada *et al.* (2006) and Sanchez-Montoya *et al.* (2007), who reported a decrease in EPT richness as hydrological isolation and the length of the dry period (temporality) increased. EPT taxa in particular tend to occur in riffles, whereas pools support the majority of OCHD taxa (Vivas *et al.*, 2002; Oscoz *et al.*, 2011). Therefore, riffle permanence has a strong effect on the structure of benthic assemblages in streams (Feminella 1996).

#### *Biological significance of hydrological classes*

The six hydrological classes examined in this study indicate distinct macroinvertebrate assemblages at all of

the taxonomic resolutions considered. Taxonomic differences were greatest among the classes at both extremes of the flow stability gradient and are similar to the results reported by other studies in the Iberian Peninsula (Sanchez-Montoya *et al.*, 2007) and in the Segura basin (Millan *et al.*, 2006; Diaz *et al.*, 2008; Carbonell *et al.*, 2011). However, when the other classes were considered, only minor and gradual biological changes along the gradient were detected. Consequently, a simpler classification with four broad hydrological types (Belmar *et al.*, 2011) is more appropriate for management purposes in the Segura River basin and other semiarid Mediterranean basins: (1) main stem rivers (classes 1 and 2); (2) perennial stable streams (classes 3 and 4); (3) perennial seasonal streams (classes 5 and 6); and (4) temporary streams (classes 7 and 8).

We found a clear agreement between the selection of indicator taxa in this study and those from other studies in the Mediterranean region in Spain (e.g. Bonada *et al.*, 2004; Mellado 2005; Sanchez-Montoya *et al.*, 2007). Headwater streams were characterized by taxa that inhabit the upper reaches of rivers with colder and oxygen-rich waters, in areas of cobbles and small boulders. These sites supported the greatest presence of Trichoptera families, such as Philopotamidae, and were also characterized by the presence of typically reophilic Ephemeroptera (*Epeorus* and *Rhitrogena*) and Plecoptera (*Perla* and *Isoperla*) genera. In general, these taxa are considered to have high oxygen requirements, and their presence is associated with good water quality (Jacobsen *et al.*, 2003). Medium rivers were characterized by Ephemeroptera genera, such as *Potamanthus* and *Habrophlebia*, typical of reaches of large rivers where low to moderate flow velocities, associated with gravel and sand substrates, predominate (Puig *et al.*, 1984). Intermittent streams were associated with taxa from shallow standing waters or those with reduced velocities, such as numerous Coleoptera (e.g. *Enochrus*, *Berosus* and *Noterus*), Odonata (e.g. *Anax*, *Sympetrum* and *Ischnura*) and Heteroptera (e.g. *Heliocorisa*, *Anisops* and *Sigara*), with highly mobile adults (Bilton *et al.*, 2001) and short life-history development times (Velasco *et al.*, 1990; Barahona *et al.*, 2005). The importance of Coleoptera in temporary streams highlighted in this study has also been demonstrated in several previous studies (Picazo *et al.*, 2012).

#### *Implications to river restoration and conservation*

With the results presented, the magnitude of monthly minimum flows and the interannual and intrannual natural variation of flows are two key flow components for the definition of environmental flows in Mediterranean basins. Currently, many historically perennial streams have already become intermittent as a result of excessive abstraction and impoundment, whereas others exhibit an inverse seasonal pattern as a result of water release from reservoirs during the summer months (Belmar *et al.*, 2010). Such hydrological modifications could become more intense in the future as a result of climate change (European Environment Agency 2008), which is expected to intensify suprasedasonal droughts and lead to

more anthropogenic water withdrawals. This may lead to the depletion of groundwater in local aquifers and, therefore, flow intermittency in previously perennial streams. Such intermittency could result in significant changes to the faunal community, increasing the risk of local extinctions of drought-sensitive taxa. This effect has already been documented in desert streams (Bogan and Lytle 2011), where simplified pools composed of the most tolerant and resilient species have been described (*sensu* Cote and Darling 2010). Therefore, the conservation and, where appropriate, restoration of natural hydrological variability is crucial for the maintenance of riverine ecosystem integrity (i.e. ecosystem structure and function) (Thoms 2006; Vaughan *et al.*, 2009).

Future research should focus on how the degree of hydrological alteration affects aquatic communities and ecosystem functioning. Aquatic macroinvertebrates are ideal candidates for the development of hydroecological models to quantify the effects of flow reduction (Castella *et al.*, 1995; Niu and Dudgeon 2011a; Niu and Dudgeon 2011b). Using the four broad hydrological types stated, we will be

able to provide a reference framework in the near future to achieve a more sustainable management of ecohydrological resources in the Segura River basin and other Mediterranean basins, fulfilling the objectives of the *Ecological Limits of Hydrologic Alteration* and the European Union Water Framework Directive.

#### ACKNOWLEDGEMENTS

We wish to thank the University of Murcia and the *Fundación Séneca* for their financial support to Óscar Belmar and Cayetano Gutiérrez-Cánovas, respectively, by means of predoctoral grants; the Euromediterranean Institute of Water for its support to the project 'Hydrological classification of the rivers and streams in the Segura basin and associated macroinvertebrate communities'; the *Confederación Hidrográfica del Segura* for providing the climatic data and the SIMPA model; and the *Instituto Geográfico Nacional* as well as the *Instituto Geológico y Minero de España* for the GIS data.

#### Appendix A:

Taxa collected in the Segura basin grouped by taxonomic level.

<b>Hirudinea</b>	<i>Dryops gracilis</i> (Karsch, 1881)
<u>Erpobdellidae</u>	<i>Dryops sulcipennis</i> (Costa, 1883)
Dina	Pomatinus
<u>Glossiphoniidae</u>	<i>Pomatinus substriatus</i> (Müller, 1806)
Helobdella	<u>Dytiscidae</u>
<b>Mollusca</b>	<i>Eretes griseus</i> Motschulsky 1849
<u>Ancylidae</u>	<i>Hygrotus confluens</i> (Fabricius, 1787)
Ancylus	<i>Hyphydrus aubei</i> Ganglbauer, 1892
Ferrissia	<i>Ilybius meridionalis</i> Aubé, 1836
<u>Hydrobiidae</u>	<i>Meladema coriacea</i> Castelnau, 1834
Mercuria	<i>Rhantus suturalis</i> (McLeay, 1825)
Potamopyrgus	<i>Stictotarsus duodecimpustulatus</i> (Fabricius, 1792)
Pseudamnicola	Agabus
<u>Lymnaeidae</u>	<i>Agabus biguttatus</i> (Olivier, 1795)
Lymnaea	<i>Agabus bipustulatus</i> (Linnaeus, 1767)
<u>Melanopsidae</u>	<i>Agabus brunneus</i> (Fabricius, 1798)
Melanopsis	<i>Agabus didymus</i> (Olivier, 1795)
<u>Physidae</u>	<i>Agabus nebulosus</i> (Forster, 1771)
Physella	<i>Agabus nitidus</i> (Fabricius, 1801)
<u>Planorbidae</u>	<i>Agabus paludosus</i> (Fabricius, 1801)
Gyraulus	<i>Agabus ramblae</i> Millán & Ribera, 2001
Planorbarius	Bidessus
<u>Sphaeriidae</u>	<i>Bidessus minutissimus</i> (Germar, 1824)
Pisidium	Deronectes
<b>Crustacea</b>	<i>Deronectes depressicollis</i> (Rosenhauer, 1856)
<u>Astacidae</u>	<i>Deronectes fairmairei</i> (Leprieur, 1876)
Austropotamobius	<i>Deronectes hispanicus</i> (Rosenhauer, 1856)
<u>Atyiidae</u>	<i>Deronectes moestus</i> Leprieur, 1876
Atyaephyra	Dytiscus
<u>Cambaridae</u>	Graptodytes
Procambarus	<i>Graptodytes fractus</i> (Sharp, 1880-82)
<u>Gammaridae</u>	<i>Graptodytes ignotus</i> (Mulsant, 1861)
Echinogammarus	<i>Graptodytes varius</i> (Aubé, 1836)
<b>Insecta</b>	Hydroglyphus
<b>Coleoptera</b>	<i>Hydroglyphus geminus</i> (Fabricius, 1792)
<u>Dryopidae</u>	<i>Hydroglyphus signatellus</i> (Klug, 1834)
Dryops	Hydroporus

*Continues*

## Appendix A (Continued)

- Hydroporus discretus* Fairmaire, 1859  
*Hydroporus lucasi* Reiche, 1866  
*Hydroporus marginatus* (Duftschmid, 1805)  
*Hydroporus nigrata* (Fabricius, 1792)  
*Hydroporus pubescens* (Gyllenhal, 1808)  
*Hydroporus tessellatus* Drapiez, 1819  
Laccophilus  
*Laccophilus hyalinus* (De Geer, 1774)  
*Laccophilus minutus* (Linnaeus, 1758)  
Nebrioporus  
*Nebrioporus bucheti cazorlensis* (Lagar, Fresneda & Hernando, 1987)  
*Nebrioporus clarki* (Wollaston, 1862)  
Oreodytes  
Stictonectes  
*Stictonectes epipleuricus* (Seidlitz, 1887)  
*Stictonectes optatus* (Seidlitz, 1887)  
Yola  
*Yola bicarinata* (Latreille, 1804)  
Elmidae  
Elmis  
*Elmis aenea* (Müller, 1806)  
*Elmis maugetii maugetii* Latreille, 1798  
*Elmis rioloides* (Kuwert, 1890)  
Esolus  
*Esolus parallelepipedus* (Müller, 1806)  
Limnius  
*Limnius intermedius* Fairmaire, 1881  
*Limnius opacus* Müller, 1806  
*Limnius volckmari* (Panzer, 1793)  
Normandia  
*Normandia nitens* (Müller, 1817)  
*Normandia sodalis* (Erichson, 1847)  
Oulimnius  
*Oulimnius troglodytes* (Gyllenhal, 1827)  
*Oulimnius tuberculatus perezii* Sharp, 1872  
Potamophilus  
Riolus  
*Riolus cupreus* (Müller, 1806)  
*Riolus illiesi* Steffan, 1958  
Gyrinidae  
Aulonogyrus  
*Aulonogyrus striatus* (Fabricius, 1792)  
Gyrinus  
*Gyrinus dejeani* Brullé, 1832  
Orectochilus  
*Orectochilus villosus* (Müller, 1776)  
Halipidae  
*Peltodytes rotundatus* (Aubé, 1836)  
Halipus  
*Halipus lineatocollis* (Marsham, 1802)  
*Halipus mucronatus* Stephens, 1832  
Helophoridae  
Helophorus  
*Helophorus alternans* Gené, 1836  
*Helophorus brevipalpis* Bedel, 1881  
*Helophorus fulgidicollis* Motschulsky, 1860  
*Helophorus occidentalis* Angus, 1983  
*Helophorus nubilus* Fabricius, 1776  
*Helophorus seidlitzii* Kuwert, 1885  
Hydraenidae  
Hydraena  
*Hydraena capta* Orchymont, 1936  
*Hydraena carbonaria* Kiesenwetter, 1849  
*Hydraena exasperata* Orchymont, 1935  
*Hydraena hernandoi* Fresneda & Lagar, 1990  
*Hydraena manfredjaechi* Delgado & Soler, 1991  
*Hydraena pygmaea* Waterhouse, 1833  
*Hydraena quilisi* Lagar, Fresneda & Hernando, 1987  
*Hydraena rufipennis* Boscá Berga, 1932  
*Hydraena servilia* Orchymont, 1936  
Limnebius  
*Limnebius cordobanus* Orchymont, 1938  
*Limnebius maurus* Balfour-Browne, 1978  
*Limnebius oblongus* Rey, 1883  
Ochthebius  
*Ochthebius auropallens* Fairmaire, 1879  
*Ochthebius bellieri* Kuwert, 1887  
*Ochthebius bonnairei* Guillebau, 1896  
*Ochthebius delgadoi* Jäch, 1994  
*Ochthebius difficilis* Mulsant, 1844  
*Ochthebius dilatatus* Stephens, 1829  
*Ochthebius (Enicocerus) exsculptus* Germar, 1824  
*Ochthebius grandipennis* Fairmaire, 1879  
*Ochthebius jaimeii* Delgado & Jäch, 2007  
*Ochthebius quadrioveolatus* Wollaston, 1854  
*Ochthebius tudmirensis* Jäch, 1997  
*Ochthebius viridis fallaciosus* Ganglbauer, 1901  
Hydrochidae  
Hydrochus  
*Hydrochus grandicollis* Kiesenwetter, 1870  
*Hydrochus nooreinus* Henegouven & Sáinz-Cantero, 1992  
Hydrophilidae  
Anacaena  
*Anacaena bipustulata* (Marsham, 1802)  
*Anacaena globulus* (Paykull, 1798)  
*Anacaena lutescens* (Stephens, 1829)  
*Coelostoma hispanicum* (Küster, 1848)  
*Hydrophilus pistaceus* (Castelnau, 1840)  
Berosus  
*Berosus hispanicus* Küster, 1847  
Enochrus  
*Enochrus ater* (Kuwert, 1888)  
*Enochrus politus* Küster, 1849  
Helochares  
*Helochares lividus* (Forster, 1771)  
Laccobius  
*Laccobius bipunctatus* (Fabricius, 1775)  
*Laccobius hispanicus* Gentili, 1974  
*Laccobius gracillis gracillis* Motschulsky, 1849  
*Laccobius moraguesi* Régimbart, 1898  
*Laccobius neapolitanus* Rottenberg, 1874  
*Laccobius obscuratus* Rottenberg, 1874  
*Laccobius sinuatus* Motschulsky, 1849  
*Laccobius ytenensis* Sharp, 1910  
Noteridae  
Noterus  
*Noterus laevis* Sturm, 1834  
Scirtidae  
Cyphon  
Elodes  
Hydrocyphon  
**Diptera**  
Anthomyiidae  
Limnophora  
Athericidae  
Atrichops  
Ibisia  
Ceratopogonidae  
Dasyhelea  
Chironomidae  
Chironomini

Continues

## Appendix A (Continued)

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Corynoneura	Pleidae
Tanytarsini	Plea
Culicidae	Veliidae
Anopheles	Microvelia
Diamesinae	Velia
Dixidae	<b>Odonata</b>
Empididae	Aeshnidae
Ephydriidae	Anax
Hemerodromiinae	Boyeria
Limoniidae	Calopterigydae
Eloeophyla	Calopteryx
Pseudolimnophila	Coenagrionidae
Orthoclaadiinae	Cercion
Simuliidae	Ischnura
Stratiomyidae	Pyrhosoma
Oxycera	Cordulegastridae
Syrphidae	Cordulegaster
Tabanidae	Gomphidae
Tabanus	Gomphus
Tanypodinae	Onychogomphus
Tipulidae	Libellulidae
Tipula	Libellula
<b>Ephemeroptera</b>	Orthetrum
Baetidae	Sympetrum
Baetis	Platycnemididae
Centroptilum	Platycnemis
Cloeon	<b>Plecoptera</b>
Procloeon	Leuctridae
Caenidae	Leuctra
Caenis	Nemouridae
Ephemerellidae	Nemoura
Ephemerella	Protonemura
Serratella	Perlidae
Torleya	Dinocras
Ephemeridae	Eoperla
Ephemera	Perla
Heptageniidae	Perlodidae
Ecdyonurus	Isoperla
Epeorus	<b>Trichoptera</b>
Rhithrogena	Beraeidae
Leptophlebiidae	Brachycentridae
Habroleptoides	Micrasema
Habrophlebia	Drusinae
Paraleptophlebia	Hydropsychidae
Polymirtacidae	Cheumatopsyche
Ephoron	Hydropsyche
Potamantidae	Hydroptilidae
Potamanthus	Agraylea
<b>Heteroptera</b>	Hydroptila
Aphelocheiridae	Lepidostomatidae
Aphelocheirus	Lasiocephala
Corixidae	Leptoceridae
Heliocorisa	Athripsodes
Micronecta	Limnephilidae
Sigara	Allogamus
Gerridae	Halesus
Aquarius	Stenophylax
Gerris	Limnephilinae
Hydrometridae	Philopotamidae
Hydrometra	Polycentropodidae
Naucoridae	Psychomyiidae
Naucoris	Metatype
Nepidae	Tinodes
Nepa	Rhyacophilidae
Notonectidae	Rhyacophila
Anisops	Sericostomatidae
Notonecta	Sericostoma

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