

## Are Water Framework Directive stream types biologically relevant? The case of the Mondego river, Portugal

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**Abstract** – Typology schemes are useful in partitioning natural spatial variability and thus helping implementing bioassessment methods. They have become widely accepted in Europe after the publication of the Water Framework Directive (WFD). The major objective of this study was to test if six Portuguese pre-defined physical stream-types (using System-B of the WFD) are concordant with macroinvertebrate assemblages collected in 31 undisturbed sites in a Portuguese river basin. A top-down (stream types defined by physical attributes) and a bottom-up approach (river classes defined by biological communities) were used. No significant differences were found in the mean number of taxa and abundance of different physical stream types and few taxa are characteristic of specific stream types. Discriminant analysis (DA) considering family-level composition revealed that all stream-types were significantly different and the cross-validation process showed that all stream-types had more than 50% of their samples correctly assigned. On the other hand, the bottom-up approach based on a correspondence analysis (CA) showed some overlap of the macroinvertebrate communities of pre-defined stream types, indicating that variations in the macroinvertebrate community structure was primarily related to altitude, mineralisation and a temporal gradient. DA cross-validation and CA results suggest that tested stream types do not account for natural temporal changes known to affect macroinvertebrate communities in this Mediterranean basin and that the WFD typology should account for these natural variations. The exclusion of natural variability could indicate impairment when it does not exist or no impairment when it does exist (type I and II statistical errors).

**Key words:** Freshwater macroinvertebrates / classification / natural variability / Water Framework Directive / Mediterranean basin

### Introduction

Macroinvertebrate stream communities are influenced by climate, geology, stream morphology and hydrology, water chemistry, sediment-size, riparian structure and quality as well as other features (*e.g.*, [Hellawell, 1986](#); [Richards \*et al.\*, 1996](#); [Vinson and Hawkins, 1998](#); [Beisel \*et al.\*, 2000](#); [Sandin, 2003](#); [Munné and Prat, 2004](#)). Patterns of macroinvertebrate communities are generally best explained by a combination of numerous environmental variables, although sometimes single variables might explain a major part of the observed variation ([Wiberg-Larsen \*et al.\*, 2000](#)). The properties of a habitat within an aquatic ecosystem are assumed to determine macroinvertebrate community types ([Bailey \*et al.\*, 2004](#)). Since

stream sites are extremely diverse due to cumulative effects of multiple variables that act at different spatial scales it becomes extremely difficult if not impossible to use their biological communities for biomonitoring purposes without simplifying this variability.

Subdivision of the aquatic landscape into ecoregions, classes and types according to patterns of climate, topography, vegetation, and other factors has been performed extensively in the USA (*e.g.*, [Hawkins \*et al.\*, 2000](#)), Australia (*e.g.*, [Turak and Koop, 2008](#)), and Europe, from early on ([Wasson, 1989](#)) to recent days (*e.g.*, [Moreno \*et al.\*, 2006](#); [Sandin and Verdonshot, 2006](#)). The classification, typology, and/or regionalization of rivers and streams are useful tools for generalising and summarising the natural variability of existing biological conditions. These are useful approaches for partitioning natural spatial variability ([Sandin and Verdonshot, 2006](#)), even if they create

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artificial discontinuities that do not exist in nature (Gerritsen *et al.*, 2000).

The use of a typology approach has become widely accepted in Europe especially after the publication of the European Union (EU) Water Framework Directive (WFD) (European Commission, 2000; Hering *et al.*, 2003, 2004). This Directive requires EU Member States to differentiate the relevant surface water bodies with respect to type using either “System-A” or “System-B”. These water body types define relatively homogeneous river and stream units in terms of geomorphological, physico-chemical, hydrological and/or climatic characteristics (see Wallin *et al.*, 2003 for details).

Both WFD typology systems are top-down approaches, that use previous knowledge, such as abiotic criteria to identify aquatic landscape units (*e.g.*, Munné and Prat, 2004; Ferréol *et al.*, 2005; Sánchez-Montoya *et al.*, 2007). Since catchment geomorphology and climate-related variables are often strong predictors of macroinvertebrate assemblages (Richards *et al.*, 1996; Hawkins *et al.*, 2000; Verdonschot and Nijboer, 2004; Chaves *et al.*, 2005), sites within the same WFD type that were based on these factors are expected to represent relatively distinct ecological units for invertebrate communities (Verdonschot, 2006a; Sánchez-Montoya *et al.*, 2007). Alternatively, in bottom-up approaches, aquatic biological data are used to group similar stream sites (*e.g.*, Wright *et al.*, 1984; Parsons and Norris, 1996; Marchant *et al.*, 1997; Ehlert *et al.*, 2002; Heino *et al.*, 2003; Lorenz *et al.*, 2004; Dodkins *et al.*, 2005; Sánchez-Montoya *et al.*, 2007). To accomplish this purpose biotic data should be collected in reference sites (Hering *et al.*, 2004) since anthropogenic stress reduces natural differences between communities (Verdonschot, 2006a). Additionally, there is a third combined option that consists of testing and refining physically derived classes with a subsequent or simultaneous analysis of biological data from undisturbed sites within those classes (Gerritsen *et al.*, 2000; Dodkins *et al.*, 2005).

Once a typology is established, type-specific biological reference conditions (RC) can finally be defined as required by the WFD, *i.e.* the natural status of each water body type can be characterized. RC will consequently provide the baseline against which anthropogenic impacts of a test site are measured and for which biological community potential and spatial and temporal variability are both described (Reynoldson *et al.*, 1997; Economou, 2002; Wallin *et al.*, 2003; Bailey *et al.*, 2004). Type-specific biological RC should clearly be distinct and natural variation must not be confounded by anthropogenic degradation. Thus, the final aim of an optimal typology in the WFD context should be to assist in accurately defining biological reference conditions and subsequent biological assessment systems to evaluate the ecological quality of stream sites beyond natural variation (European Commission, 2000; Gerritsen *et al.*, 2000; Bailey *et al.*, 2004; Dodkins *et al.*, 2005). Well-defined river and stream types will reduce the likelihood of inferring impairment when it does not exist (type I statistical error) or not detecting impairment when it does exist (type II statistical

error) (Hawkins *et al.*, 2000). In a broader context, an optimal typology will also improve ecological research, conservation planning and biodiversity management (*e.g.*, Hawkins *et al.*, 2000; Verdonschot and Nijboer, 2004; Heino and Mykra, 2006).

Recent studies tested the concordance between several landscape classifications and the variability of stream communities at different spatial scales, from the European (*e.g.*, Moog *et al.*, 2004; Heino and Myrka, 2006; Verdonschot, 2006a), ecoregion (Sandin and Johnson, 2000; Moog *et al.*, 2004) and country levels (*e.g.*, Dodkins *et al.*, 2005), to smaller regions (*e.g.*, Heino and Mykra, 2006; Sánchez-Montoya *et al.*, 2007).

Some studies concluded that major macroinvertebrate distribution patterns are well-distinguished in terms of spatial classes (*e.g.*, Verdonschot and Nijboer, 2004; Sánchez-Montoya *et al.*, 2007) but others found that although stream assemblages often showed statistically significant differences between landscape classes, the actual strength of these classifications was rather weak (Hawkins *et al.*, 2000; Heino and Mykra, 2006). However, very few studies have examined the theoretical validity of the WFD System-B by effectively testing it (Sánchez-Montoya *et al.*, 2007).

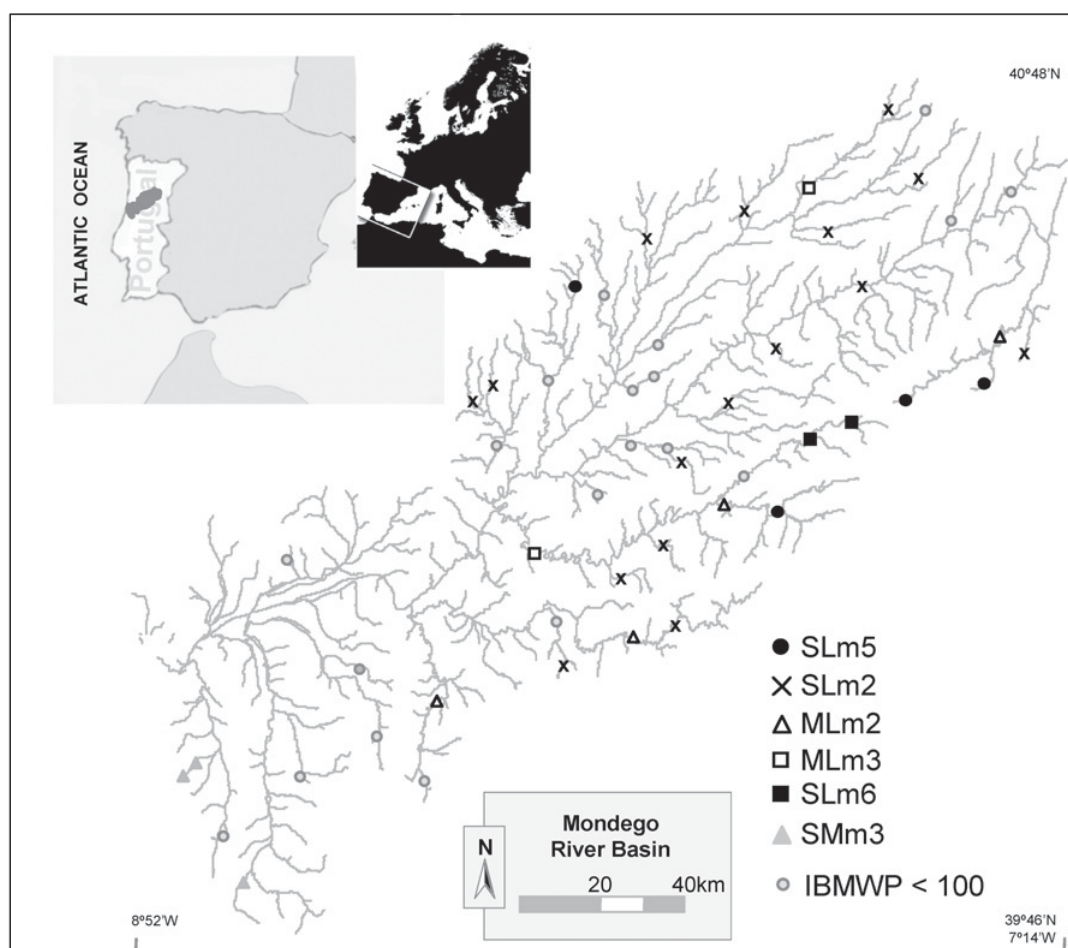
A river and stream typology was defined for Continental Portugal by Alves *et al.* (2004) following the top-down approach indicated by the WFD System-B and twenty-seven river and stream types were identified. The aim of the present paper is to test the robustness and the biological relevance of the geomorpho-climatic classification proposed in the Mondego basin using macroinvertebrate biological data collected in near-natural sites.

## Materials and methods

### Study area and selection of sampling sites

The Mondego River basin (Fig. 1) drains an area of 6645 km<sup>2</sup> into the Atlantic Ocean (Loureiro *et al.*, 1986) and it is located in the central region of Portugal, within ecoregion 1 (as defined by Illies, 1978). Altitude of the drainage basin ranges from sea level to nearly 2000 m. The Mondego itself is the largest entirely Portuguese river with a length of 234 km, beginning at an altitude of 1547 m a.s.l. (Loureiro *et al.*, 1986). Hydrogeological features determine two major areas in the Mondego River basin: an essentially siliceous area in the upper and middle regions of the basin and a mainly calcareous area in its lower section (AMBIO *et al.*, 1999). This basin is located in a region of Mediterranean-type climate, strongly influenced by the Atlantic Ocean. It presents high variability in the annual freshwater discharge and consequently several rivers and streams vary from flooded to dry in the same year (Lima and Lima, 2002).

According to Chaves *et al.* (2006), natural and semi-natural areas cover 43% of the catchment area, but the natural hydrology of the basin has been heavily changed by the construction of several major dams and hundreds



**Fig. 1.** Location of the sampling sites in the Mondego River basin (Portugal) and corresponding stream types according to the WFD-B system (Alves *et al.*, 2004) (see Table 1).

of small weirs. These factors were taken into consideration during site selection, which was initially based on maps, literature review and a preliminary field campaign performed during early spring 2002. The selected sampling sites (Fig. 1) are near-natural systems since they exclude streams in the vicinity of urban, industrial or intensely cultivated areas, or influenced by dams, water withdrawals, high density of roads or other structures or known to have fish stocking and fishing activity. All sites exhibit no or only minor evidence of human disturbance and have the expected riparian vegetation and good apparent in-stream habitat quality (for details see Chaves *et al.*, 2006). For the present study, a site was considered as near-natural only if it had high ecological water quality, *i.e.* a value of the Iberian BioMonitoring Working Party index (IBMWP) higher than 100 during all three sampling events (Jáimez-Cuéllar *et al.*, 2002) (for further information about the use of the IBMWP, see Chaves *et al.*, 2006).

### Macroinvertebrate sampling

Macroinvertebrate communities of the selected sites were sampled during spring and summer 2002 and again in

spring 2004, to account for seasonal variations related to hydrological changes (Chaves *et al.*, 2005) and inter-annual variations related to the irregular precipitation regime (Feio *et al.*, 2006). Some sites were completely dry during the summer campaign and were sampled only when water was present. Macroinvertebrates were always collected by the same operator using a 30 × 30 cm kick-net with a 250 µm mesh size. Care was taken to include all possible habitats over representative sections of the stream (a 100 m stretch), incorporating riffles, runs, and pools if these habitats were present in the stream stretch, in a manner similar to the multi-habitat sampling procedure used in the USA (Barbour *et al.*, 1999). Effort was allocated in proportion to the occurrence of each habitat, resulting in a total sampling area of approximately 3 m<sup>2</sup>. Composite samples were preserved in 100% ethanol, transported to the laboratory where they were rinsed using a 250 µm mesh screen, sorted under magnification and preserved in 70% ethanol.

Sorting of biological samples involved removing large, common and rare organisms during three sequential steps: 1) all specimens larger than 1000 µm were removed; 2) at least 250 specimens <1000 µm were removed using an area based method to allow for the estimation

**Table 1.** River and stream types of the Mondego River basin, following the WFD-B system (Alves *et al.*, 2004). Portuguese morpho-climatic groups were established based on altitude, latitude, longitude, runoff-related, precipitation-related and temperature-related variables. Morpho-climatic groups were numbered according to Alves *et al.* (2004). Shaded types were studied in this work.

Catchment area	Geology	Morpho-climatic group	River or stream type
Small (5–100 km <sup>2</sup> )	Low mineralisation level	Temperature ↑ 3   2   5   6 ↓ Altitude, runoff, precipitation	SLm3
			SLm2
			SLm5
			SLm6
			SMm3
			SHm3
Medium (100–1000 km <sup>2</sup> )	Low mineralisation level	2 3 5	MLm2
			MLm3
			MLm5
Large (1000–10 000 km <sup>2</sup> )	Medium mineralisation level	3	MMm3
	Low mineralisation level	2	LLm2
		3	LLm3
		3	LMm3

of macroinvertebrate total abundance; 3) the entire sample was screened, looking only for non-abundant species, *i.e.* those not removed in the previous steps to guarantee that all *taxa* present were detected. Macroinvertebrates were identified to the family or higher taxonomical levels (*e.g.* Oligochaeta) according to Tachet *et al.* (2000).

### Stream and river types

The typology work of Alves *et al.* (2004) was used to allocate each site into the corresponding river or stream type. In Alves *et al.* (2004), Portuguese morpho-climatic groups were established based on altitude, latitude, longitude (three WFD System-B obligatory descriptors), runoff, precipitation and temperature. Four different morpho-climatic groups were identified in the Mondego River basin and acted as background to establish the river typology according to the WFD System-B (Table 1). When catchment area and geological variables (WFD System-B obligatory variables) were introduced in the model, 13 river and stream physical types were identified within the Mondego River basin (Alves *et al.*, 2004) (Table 1).

### Comparing stream types with macroinvertebrate communities

#### Top-down approach: River types defined by physical attributes

A total of 92 macroinvertebrate samples collected from the undisturbed sites during the three sampling occasions were selected to test the validity of previously defined river and stream types. Prior to all analyses, macroinvertebrate abundance data were  $\log_{10}(x + 1)$  transformed. Univariate

comparisons between stream types of basic community metrics (*taxa* richness, total abundance and abundance of specific *taxa*) were performed among using one-way ANOVA. Discriminant analysis (DA) was used to determine if combinations of abundances of different *taxa* produce a model able to distinguish between stream types and if there were statistically significant differences between the pre-determined groups (stream and river types, with sampling occasions as replicates). DA also identified which *taxa* best discriminated between groups. DA uses a set of response variables to derive discriminant functions (DFs), which are linear combinations of the original variables. One discriminant model was generated starting from a 78 invertebrate *taxa* matrix (mostly families), out of 101 *taxa*, since *taxa* with less than 0.05% of total abundance were *a priori* removed from the analysis for robustness. Employing the selection rule that maximises minimum Mahalanobis distance between groups, stepwise procedures (*F* to enter of 3.84 and *F* to remove of 2.71) were used to choose the combination of *taxa* that best separate those groups. A Wilks'  $\lambda$  test (approximated to the chi-square distribution) was used to examine the significance of the model as a whole (Hair *et al.*, 1998). Statistical significance and the proportion of total variance explained of individual DFs were determined. The *F*-statistic was used to test the  $H_0$  of equality of means (centroids) for each pair of groups. The results of these pairwise group comparisons were corrected through the application of the Dunn-Sidak method using the Holms procedure (Sokal and Rohlf, 1995). Mahalanobis distances were obtained from the *F*-statistics computed for each of these pairwise comparisons by the formula given in Afifi and Clark (1990). DFs were used to classify individual samples into stream types (groups). The expected actual error rates of the classification functions were estimated using cross-validation with the leaving-one-out procedure (Anonymous, 1997). In cross-validation, each

sample is classified by the functions derived from all samples other than that sample. The classification accuracy was tested by the Press's Q procedure (Hair *et al.*, 1998). The potency index and discriminant loadings were used to assess the relative importance of each independent variable in discriminating between groups (Hair *et al.*, 1998). The potency index is a measure of the discriminating power of each independent variable. Discriminant loadings measure the simple linear correlation between each independent variable and each DF. Two-dimensional plots showing group centroids and individual observations were produced for the first three statistically significant DFs. These plots indicate the level of similarity between groups in terms of the discriminant functions. Assignment of *taxa* to a given DF axis was based on a comparison of the magnitude of their canonical loadings (correlation values between DFs and the *taxa*) for each significant DF. Each *taxon* was assigned to the DF with the highest loading. Only *taxa* with loadings  $> 0.30$  (absolute value) were used as axis labels. Direction of the effect of a specific individual *taxon* along a given DF axis was based on the sign (+ or -) of the loadings. Discriminant analysis was conducted using the SPSS statistical software package (Anonymous, 1997).

Finally, the Indicator Value (IndVal, Dufrene and Legendre, 1997) method was used to complement the DA, by identifying significant indicator *taxa* in each stream type (sampling occasions as replicates). The IndVal method determines indicator *taxa* by combining the relative *taxon* abundance with its relative frequency of occurrence in the various groups of sites (*i.e.* stream types). The IndVal for each *taxon* ranges from a minimum of 0% to a maximum of 100%, the latter attained when all specimens of a *taxon* are found in a single group of samples and when the *taxon* occurs in all samples of that group. *Taxa* with an IndVal higher than 25% contribute to the stream type-specificity and only these were considered as characteristic *taxa* (see Dufrene and Legendre, 1997). The significance ( $P < 0.05$ ) of each *taxon* IndVal value was tested using a permutation test (9999 permutations) to determine if *taxa* were characteristic of a stream type (Dufrene and Legendre, 1997). The IndVal procedure was conducted using the labdsv package developed in R software (R Development Core Team, 2006).

### Bottom-up approach: River classes defined using biological variables

Correspondence analysis (CA) was performed on the macroinvertebrate abundance matrix of all *taxa*, for each site and sampling event, to determine if community structure of sites within the same stream type was more similar than that found in different stream types. CA is an indirect ordination technique, with no *a priori* assumptions that is used for bottom-up statistical classification. In CA, the samples are located in a multidimensional space that is based on their taxonomic composition and used for ordination plots (ter Braak and Šmilauer, 2002).

Inter-sampling with Hill's scaling was chosen to optimise the position of the samples in the diagram (ter Braak and Šmilauer, 2002). Since the axes in the resulting ordination plot represent gradients of invertebrate community composition, sites located closer to one another on the plot have more similar community structure. Within the ordination plot, samples were labelled according to stream type. The overlap between them was established by drawing contour lines (not shown) around each type (minimizing overlap) and counting the number of samples within overlapping contour lines. Calculation of the overlap was restricted to the first two ordination axes. Only stream types with an overlap of  $< 25\%$  of the samples were classified as a separate group (Verdonshot, 2006b). CA was performed using CANOCO software for Windows ver. 4.5 (ter Braak and Šmilauer, 2002).

## Results

### Site selection

Fifty-two sites were chosen during the preliminary visual inspection but only 33 sites met the high ecological water quality criterion (Table 2, Fig. 1). Eight of the 13 river or stream types identified in the Mondego River basin were represented by the 33 selected sites. Most of the selected sites had small catchment areas (5–100 km<sup>2</sup>) and none was larger than 640 km<sup>2</sup>. The majority of the selected sites (64%) were located at mid-height altitudes (200–800 m), and 27% and 9% were positioned at lower and higher altitudes, respectively. Most of the sites were located in siliceous river-beds (91%), while the remaining sites were predominantly calcareous (Table 2). Only two sites were completely dry during summer but around 20% had stagnated water or pools (Table 2). Stream types SLm3 (site 31) and MLm5 (site 17) included only one site each and therefore could not be used for subsequent comparisons (Tables 1 and 2, Fig. 1) leaving a total of six stream types for further analysis.

### Top-down approach: River types defined by physical attributes

No significant differences were found in either *taxa* richness ( $F = 0.89$ ;  $df = 5, 86$ ;  $P = 0.49$ ; Fig. 2) or total abundance ( $F = 1.54$ ;  $df = 5, 86$ ;  $P = 0.19$ ; Fig. 2) between stream types. Twenty-two insect families, three mollusk families and four other arthropod and oligochaete groups showed significant differences in abundance between stream types (Table 3). More than 30 *taxa* were common to all stream types and about 20 occurred only at one or two sites with abundances less than 0.05% of the total for those sites.

The stepwise DA analysis resulted in a significant model ( $\chi^2 = 278.6$ ;  $df = 60$ ;  $P < 0.001$ ) that discriminated the six groups of streams based on only 12 of the 78 independent variables (*taxa*) used. Additionally, very few of the *taxa* recorded in any of the stream types, were

**Table 2.** Overview of the characteristics of sites investigated in the Mondego River basin. Descriptors include altitude (A) – low (L): < 200 m, mid (M): 200–800 m, high (H) > 800 m; Geology (G) – siliceous streambed (Sil), calcareous streambed (Cal); Catchment area (Ca) – very small (vs): < 10 km<sup>2</sup>, small (s): 10–100 km<sup>2</sup>, medium (m): 100–1000 km<sup>2</sup>; Mean discharge (Md) – low (L): < 0.05 m<sup>3</sup>.s<sup>-1</sup>, mid (M): 0.05–1.00 m<sup>3</sup>.s<sup>-1</sup>, high (H): 1.00–5.00 m<sup>3</sup>.s<sup>-1</sup>; predominant substrate size (S) – silt and clay (Sc): < 0.06 mm, sand (Sd): 0.06–2 mm, pebbles and gravel (Pg): 2–64 mm, cobble, boulders and stones (Cb): > 64 mm; and Strahler's stream order (O). Stream type according to *Alves et al. (2004)* is also given – for a brief description see *Table 1*. IBMWP values for each sampling occasion – > 100 (x), < 100 (0). Sites with an IBMWP > 100 in all sampling occasions were selected for the present study (x, in IBMWP Total), except stream types MLm5 and SLm3 due to lack of sites.

	Rivers (stretches)	Descriptor						Type	IBMWP			Total
		A	G	Ca	Md	S	O		Sp 02/	Sm 02/	Sp 04	
1.	Mondego (C. S <sup>a</sup> Maria)	H	Sil	vs	L	Sd / Cb	1	SLm5	x/ x/ x			x
2.	Quêcere	H	Sil	s	L	Cb	1	SLm5	x/ x/ x			x
3.	Caldeirão	M	Sil	vs	L	Cb	1	SLm2	x/ x/ x			x
4.	Mondego (Trinta)	M	Sil	m	M	Pg / Cb	2	MLm2	x/ x/ x			x
5.	Tamanhos	M	Sil	s	M	Sd / Pg	2	SLm2	0/ Dry/ 0			0
6.	Coja	M	Sil	s	M	Sd / Cb	1	SLm2	x/ x/ x			x
7.	Carapito	M	Sil	s	M	Sd / Cb	1	SLm2	x/ x/ x			x
8.	Dão	M	Sil	vs	M	Pg	1	SLm2	x/ 0/ 0			0
9.	Muxagata	M	Sil	s	M	Sc / Cb	2	SLm2	x/ 0/ 0			0
10.	Gouveia	M	Sil	s	M	Sd / Cb	2	SLm2	x/ x/ x			x
11.	Ludares	M	Sil	s	M	Sd / Pg	1	SLm2	x/ Dry/ x			x
12.	Santos Evos	M	Sil	s	M	Cb	1	SLm2	x/ x/ x			x
13.	Coja	M	Sil	m	M	Sd	2	MLm3	x/ x/ x			x
14.	Tourais	M	Sil	s	L	Sc / Pg	1	SLm2	x/ x/ x			x
15.	Alva (Sabugueiro)	H	Sil	s	M	Sd / Cb	1	SLm6	x/ x/ x			x
16.	Caníça	M	Sil	s	M	Pg / Cb	1	SLm6	x/ x/ x			x
17.	Alva (S. Gião)	M	Sil	m	H	Pg / Cb	2	MLm5	x/ x/ x			x
18.	Alvôco	M	Sil	m	H	Pg / Cb	2	MLm2	x/ x/ x			x
19.	Louriga	M	Sil	s	M	Cb	1	SLm5	x/ x/ x			x
20.	Mortágua (Mortágua)	L	Sil	m	M	Sc / Cb	2	MLm3	0/ 0/ 0			0
21.	Falheiros	L	Sil	s	M	Pg / Cb	1	SLm2	x/ x/ x			x
22.	Mortágua (Vila Boa)	L	Sil	s	M	Pg / Cb	1	SLm2	x/ x/ x			x
23.	Criz (P <sup>va</sup> do Lobo)	L	Sil	m	M	Cb	3	MLm5	x/ x/ 0			0
24.	Criz (C <sup>po</sup> Besteiros)	M	Sil	s	M	Sd	1	SLm5	x/ x/ x			x
25.	Asnes	M	Sil	s	M	Sd	2	SLm2	x/ x/ x			x
26.	Dinha	M	Sil	s	M	Cb	2	SLm5	x/ x/ 0			0
27.	Beijós	M	Sil	s	M	Sc / Pg	1	SLm3	x/ x/ 0			0
28.	Cabanas (Cabanas)	M	Sil	s	M	Sd	1	SLm3	0/ 0/ 0			0
29.	Cabanas (Carregal Sal)	M	Sil	s	M	Sd / Pg	1	SLm3	x/ Dry/ 0			0
30.	Cavalos (Tábua)	L	Sil	s	M	Sd / Cb	2	SLm3	x/ 0/ 0			0
31.	Covelo	L	Sil	vs	L	Pg / Cb	1	SLm3	x/ Dry/ x			x
32.	Cavalos (S. Geraldo)	M	Sil	s	M	Pg	2	SLm2	x/ 0/ 0			0
33.	Ribelas	M	Sil	s	M	Sd	1	SLm2	x/ x/ x			x
34.	Seia	M	Sil	s	M	Sd / Cb	2	SLm2	x/ x/ x			x
35.	Mata	M	Sil	s	M	Cb	1	SLm2	x/ x/ x			x
36.	Alva (Arganil)	L	Sil	m	M	Cb	3	MLm2	x/ 0/ 0			0
37.	Folques	L	Sil	s	M	Cb	1	SLm2	x/ x/ x			x
38.	Ceira (Casal Novo)	M	Sil	s	M	Cb	2	SLm2	x/ x/ x			x
39.	Ceira (Colmeal)	M	Sil	m	M	Cb	2	MLm2	x/ x/ x			x
40.	Sótão (Casal Ribeira)	M	Sil	s	M	Cb	2	SLm2	x/ 0/ 0			0
41.	Sótão (Penedo)	M	Sil	s	M	Sd / Cb	1	SLm2	x/ x/ x			x
42.	Alva (Moura Morta)	L	Sil	m	H	Cb	3	MLm3	x/ x/ x			x
43.	Moinhos	L	Cal	vs	M	Sd	1	SHm3	x/ x/ 0			0
44.	Alcabideque	L	Cal	s	M	Sd / Pg	1	SHm3	0/ 0/ 0			0
45.	Mouros	L	Cal	s	L	Sc / Cb	1	SHm3	0/ 0/ 0			0
46.	Dueça	M	Sil	s	-	Pg	1	SHm3	Dry/ Dry/ Dry			0
47.	Corvo	L	Sil	m	M	Pg / Sd	2	MLm2	x/ x/ x			x
48.	Anços	L	Cal	vs	M	Pg / Sd	1	SMm3	0/ x/ 0			0
49.	Venda Nova	L	Cal	s	L	Pg	1	SMm3	x/ x/ x			x
50.	Sto Amaro (Headwaters)	L	Cal	vs	L	Sd	1	SMm3	x/ x/ x			x
51.	Sto Amaro (Louriçal)	L	Cal	vs	M	Sd / Pg	1	SMm3	x/ x/ x			x
52.	Crespos	L	Cal	vs	M	Sd	1	SMm3	0/ x/ 0			0

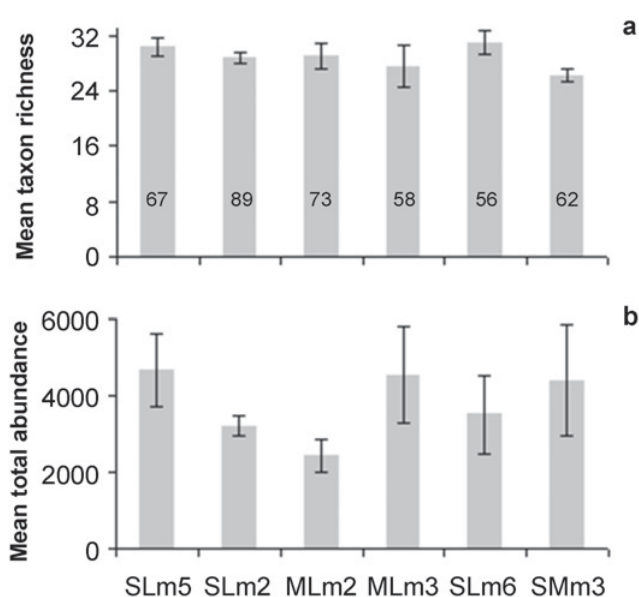


Fig. 2. Mean *taxon* richness (a) and mean total abundance (b) in each physical stream type with respective standard errors.

classified as characteristic of a stream type according to the IndVal analysis (Table 3). Only one family was found characteristic for three river types and a maximum number of five *taxa* were found characteristic for one type. Ten of the 16 *taxa* were selected both for IndVal and DF function analysis (Table 3), and the six *taxa* used in axis labels in Figure 3 were selected by both analyses. However, despite the relative low number of *taxa* selected for the discriminant model, pairwise comparisons of stream type centroids indicated significant differences between all types (Table 4). Mahalanobis distances between centroid pairs were always greater between other stream types and stream type SMM3 and secondly between other stream types and SLM6 with the exception of MLM3 and SMM3 since they were more distant from SLM6 (Table 4, Fig. 3). The DA cross-validation error rates for all stream types were less than 50%. Most of the misclassified samples were originally assigned to stream type SLM2. Samples for stream type SMM3 were 100% correctly classified (nine samples). Nine sites were correctly classified during all sampling events, while all other sites were misclassified for at least in one sampling event. Fifteen of the 34 misclassified samples were collected during spring 2002, 13 during spring 2004, and only six during summer 2002. Cross-validation results were significantly better than chance (Press's  $Q = 142.46$ ;  $df = 1$ ;  $P < 0.001$ ).

*Taxa* included in the significant DA model were: Limoniidae, Aphelocheiridae, Leptophlebiidae, Hydrobiidae, Tricladida, Brachycentridae, Gammaridae, Coenagrionidae, Oligoneuriidae, Thremmatidae, Rhyacophilidae and Baetidae (ordered by entrance in the model, Table 3). Although the first four DFs were statistically significant, most of the variance in family level community composition between stream types was explained by the first two DFs (Table 5). DF-1 accounted for about 43% of the variability and primarily represents the difference

between stream type SMM3 and all other types (Figs. 3a and 3b). Stream types with higher values along DF-1 exhibit increasing similarity to stream type SMM3 and had higher abundances of Limoniidae while those with lower values along this axis are more similar to stream type SLM6 and showed higher abundances of Leptophlebiidae (Fig. 3d, Table 3). DF-2, which accounted for approximately 23% of the variability, predominantly represents differences between stream type SLM6 and MLM3 and all other stream types (Figs. 3a and 3c). Stream types with higher values along DF-2 were characterized by higher abundances of Leptophlebiidae, Brachycentridae and Thremmatidae (Fig. 3d, Table 3). The remaining DFs each explained less than 20% of the variance. DF-3 discriminated stream type SLM5 from the others (Figs. 3b and 3c) and correlates positively with Coenagrionidae and negatively with Hydrobiidae (Fig. 3d, Table 3). Although not shown in Figure 3, DF-4 discriminated stream type MLM2 from all the remaining types, with high positive correlations with Gammaridae, Aphelocheiridae and Oligoneuriidae and a high negative correlation with Haliplidae (Table 3).

#### Bottom-up approach: River classes defined using biological variables

The CA ordination of 78 macroinvertebrate *taxa* and 92 samples is shown in Figure 4. The first two axes explained 13.8% of the total variance in biological data with a total inertia of 2.21. Samples seem to follow a complex gradient of temporal and spatial factors (Fig. 4a). Samples belonging to temporary and permanent streams are separated along the first axis but they do not form distinct groups (Fig. 4a) showing that there is not a clear segregation of the WFD Portugal river types using the assemblages of macroinvertebrates. A temporal gradient segregating samples collected during summer from those collected in spring was found along the second axis indicating the importance of seasonal changes. A spatial gradient of altitude and mineralisation was also identified (Fig. 4a). A CA-derived typology is, therefore, hard to obtain. When the correspondent pre-defined physical stream type is associated to each sample (Fig. 4b), the variation within and the overlap among stream types is substantial, and only the stream type SLM6 is clearly separated from all the remaining, while SLM2 strongly overlap with most stream types (Table 6).

## Discussion

### Resemblance of river types and macroinvertebrate assemblages

The identification of near-natural sites allows testing if classifications based on river types and biological communities coincide, although there was a lack of studies on this topic until recent years (Hawkins *et al.*, 2000; Heino and

**Table 3.** *Taxa* selected as significant in three different analysis: 1) ANOVA showing significant differences in abundance among stream types (df = 5, 86;  $P < 0.05$ ). 2) Discriminant loadings and potency index of *taxa* included in the DA significant model and other important *taxa* (loading > 0.30 in absolute value, in bold). 3) Significant indicator *taxa* among stream types ( $P < 0.05$ ) in the IndVal analysis. *Taxa* in bold were used as axis labels in Figure 3d.

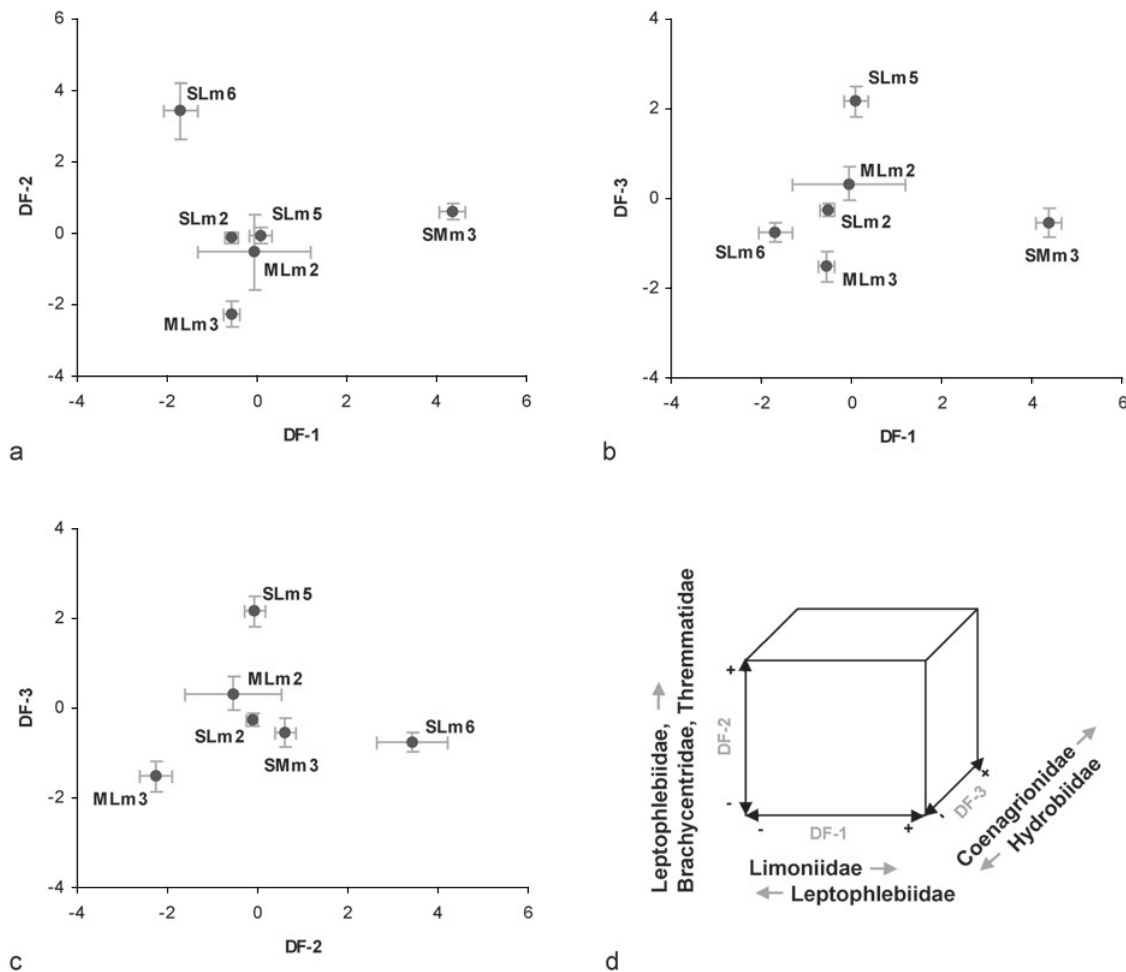
<i>Taxa</i>	ANOVA		DA					Potency index	IndVal		Stream type
	<i>F</i>	Sig.	DF-1	DF-2	DF-3	DF-4	DF-5		IndVal	<i>P</i>	
Tricladida	2.48	0.04	0.08	0.24	0.21	−0.06	0.29	0.03			
Oligochaeta	2.86	0.02									
Ostracoda	4.72	< 0.01							37.65	0.031	<b>SMm3</b>
Gammaridae	6.92	< 0.01	<b>0.32</b>	0.01	−0.04	<b>0.46</b>	−0.10	0.08			
Hydracarina	3.63	0.01									
<b>Hydrobiidae</b>	7.01	< 0.01	0.24	−0.23	<b>−0.44</b>	−0.09	<b>0.32</b>	0.08	42.87	0.025	<b>SMm3</b>
Lymnaeidae	2.22	0.05									
Sphaeriidae	2.23	0.05									
Chloroperlidae	2.40	0.04							34.93	0.043	<b>SLm6</b>
Perlidae	2.30	0.05									
Nemouridae	4.16	< 0.01									
Baetidae*			0.13	0.11	−0.04	−0.10	<b>0.35</b>	0.02	38.01	0.040	<b>SMm3</b>
Caenidae*			0.06	−0.11	<b>0.31</b>	0.22	0.04	*			
Oligoneuriidae	3.75	< 0.01	−0.07	−0.26	−0.14	<b>0.36</b>	0.19	0.04	37.91	0.013	<b>MLm3</b>
<b>Leptophebiidae</b>	7.33	< 0.01	<b>−0.30</b>	<b>0.34</b>	0.13	−0.24	−0.23	0.08	52.80	0.003	<b>SLm6</b>
Calopterygidae	2.84	0.02									
<b>Coenagrionidae</b>	3.52	0.01	0.02	−0.01	<b>0.42</b>	−0.17	<b>0.35</b>	0.04	24.27	0.034	<b>SLm5</b>
Cordulegasteridae	3.71	< 0.01							34.20	0.033	<b>SLm2</b>
Lestidae									33.85	0.011	<b>MLm3</b>
Aphelocheiridae	6.42	< 0.01	−0.05	−0.21	<b>0.42</b>	<b>0.43</b>	0.24	0.07	44.83	0.008	<b>MLm2</b>
<b>Brachycentridae</b>	8.89	< 0.01	−0.20	<b>0.54</b>	−0.08	0.14	<b>0.53</b>	0.10	65.97	0.002	<b>SLm6</b>
Calamoceratidae*			−0.08	<b>−0.31</b>	0.08	0.07	−0.17	*			
Glossosomatidae*			0.04	−0.07	−0.23	−0.14	<b>0.31</b>	*			
Hydropsychidae*			0.04	0.06	0.14	0.11	<b>0.43</b>	*			
Philopotamidae									45.56	0.047	<b>MLm3</b>
Polycentropodidae	4.15	< 0.01									
Rhyacophilidae	3.29	0.01	−0.16	−0.15	−0.11	−0.17	<b>0.68</b>	0.04	58.82	0.002	<b>MLm3</b>
Sericostomatidae	2.90	0.02									
<b>Thremmatidae</b>	5.21	< 0.01	−0.14	<b>0.41</b>	−0.16	0.02	<b>0.34</b>	0.06	41.54	0.004	<b>SLm6</b>
Haliplidae	4.00	< 0.01	−0.09	−0.07	−0.05	<b>−0.31</b>	0.03	*			
Hydrophilidae	2.76	0.02									
Hydraenidae	2.35	0.05	0.04	0.09	0.11	0.03	<b>0.32</b>	*			
Elmidae	5.31	< 0.01	0.03	0.15	0.11	−0.02	<b>0.33</b>	*			
Scirtidae	2.83	0.02							38.69	0.019	<b>SLm6</b>
Chironomidae	2.79	0.02									
<b>Limoniidae</b>	7.00	< 0.01	<b>0.37</b>	0.23	−0.01	−0.11	0.06	0.08	60.83	0.002	<b>SMm3</b>
Ephydriidae	4.39	< 0.01									
Psychodidae*			−0.08	0.21	−0.11	0.12	<b>0.37</b>	*			
Simuliidae*			0.01	−0.01	0.05	0.00	<b>0.35</b>	*			

\*Variable not included in the DA model.

Mykra, 2006). The ecological relevance of all typology schemes must be demonstrated since the underlying variables and approaches used to determine the classes in each scheme differ greatly (Sandin and Verdonschot, 2006). Macroinvertebrate communities of the pre-defined physical stream types (Alves *et al.*, 2004) used in the present study were shown to be ecologically and statistically different by the both the DA model and pairwise comparisons of group centroids (Fig. 3 and Table 4). Although more than 30 *taxa* (family-level resolution), occurred across all stream types (Table 3), 12 *taxa* were sufficient

to significantly discriminate between the six stream types. Furthermore, the studied physical types seem to have some ecological meaning. The Mahalanobis distances obtained in the DA model indicated that, SMm3 and SLm6 types had the greatest differences in macroinvertebrate community structure, which seems to reflect the physical characteristics of those stream types. Although both types included sites with small catchment areas, SMm3 had a higher level of mineralisation than SLm6 and both stream types were at opposite ends of the morpho-climatic gradient (Table 1). According to Hawkins *et al.*





**Fig. 3.** Discriminant functions (DF-1, -2 and -3) scores for centroids of each stream type with corresponding standard error (a, b and c). *Taxa* assigned to each DF based on the highest loading (> 0.30 in absolute value) (d).

(2000) and references therein, classifications of streams partitioned biotic variation best when they differed in topography or climate or both, such as in the present study. Those authors also stated that classification systems work even better when local factors (*e.g.*, stream size, water depth, substrate composition) are used to classify sites. Chaves *et al.* (2005) showed that some of the large-scale variables included in the typology of Alves *et al.* (2004) for the Mondego river might act as proxies, to some extent, for local-scale variables, as suggested by the hierarchical theory of river formation (Parsons *et al.*, 2004). Water physicochemical factors such as conductivity and TDS are very different in the two hydrogeological regions found in this basin while altitude seems to reflect a sediment grain-size gradient (see Fig. 3 in Chaves *et al.*, 2005). Between-site natural variability in Trichopteran assemblage structure (Feio *et al.*, 2005) and overall macroinvertebrate community structure of the Mondego River basin (Chaves *et al.*, 2005) have been shown to be related primarily to altitude and conductivity (alkalinity). Thus large-scale variables used for stream typology employed in this study seem to include some of the local spatial variation in the Mondego River basin, which may

be the reason for the significant differences between DA centroids of different stream types. WFD System-B stream types inevitably include ecoregion information and incorporate more detailed spatial environmental information leading to more specific/restricted physical boundaries. As a result, the physical stream types defined include local environmental factors that act more directly upon the invertebrate communities than the large scale factors used to define the typology (*e.g.*, Boyero, 2003) and thus inadvertently reflect macroinvertebrate distribution patterns.

### Ecological meaning of the Mondego stream types

Although the IndVal approach is very different and has distinct assumptions, it confirmed the DA results to some extent since characteristic *taxa* selected by the IndVal method were nearly the same as those selected by the DA model. Consequently, the studied typology seemed to have some ecological meaning but simultaneously raised some concerns because there was only a small number of indicator *taxa* for each stream type and many *taxa* occurred in all stream types or occurred only sporadically at

**Table 4.** Paired comparisons between group centroids of stream types using log-transformed family level abundance data (df = 12, 75). Provided are *F* statistics with *P* values (corrected by the Dunn-Sidak method using the Holms procedure) and Mahalanobis distances (MD) for each comparison.

Stream type		MLm3	SLm2	SLm5	SLm6	SMm3
MLm2	<i>F</i> test	3.802	4.916	4.641	6.619	9.903
	<i>P</i>	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	MD	6.055	3.276	4.927	10.541	12.267
MLm3	<i>F</i> test		3.031	5.614	7.580	8.981
	<i>P</i>		0.002	< 0.001	< 0.001	< 0.001
	MD		3.629	8.941	16.095	15.892
SLm2	<i>F</i> test			4.590	6.039	13.629
	<i>P</i>			< 0.001	< 0.001	< 0.001
	MD			3.059	7.230	11.494
SLm5	<i>F</i> test				7.211	9.790
	<i>P</i>				< 0.001	< 0.001
	MD				11.484	12.127
SLm6	<i>F</i> test					11.869
	<i>P</i>					< 0.001
	MD					21.003

a given site. In addition, there were no significant differences in *taxa* richness and total abundance among stream types. Finally, CA ordination plots showed no easily identifiable groups and a great overlap between pre-defined types might be indicative of a weak classification when all the assemblage is used (see Heino *et al.*, 2003; Heino and Mykra, 2006).

Verdonschot and Nijboer (2004) indicate that differences between stream types are only partly explained by qualitative differences in species lists but are also influenced by differences in abundances of individual species. Here, the abundances of some *taxa* varied among types indicating that differences in types of invertebrate communities should be related to variations in the proportion of some specific *taxa*. However all of the problems described might be related to the use of a higher taxonomic resolution such as family-level, which increases the number of common *taxa* (see Chaves *et al.*, 2008). The family resolution is a broad taxonomic level that might include several different species, which, for the present study, could be characteristic of different stream types. Nevertheless, distinct conclusions on the optimal taxonomic resolution have been obtained in different studies. Sánchez-Montoya *et al.* (2007) tested a stream ecotype classification with family-level macroinvertebrate composition, obtaining from 1 to nearly 30 significant indicator *taxa* in different ecotypes. Hawkins *et al.* (2000) indicated that genus/species-level data produce stronger classifications than family-level data, but the contrary is also true. In contrast, Moog *et al.* (2004) concluded that finer spatial resolution requires finer taxonomic resolution and Verdonschot (2006b) showed that family-level data provides a less distinct separation of reference sites. In the

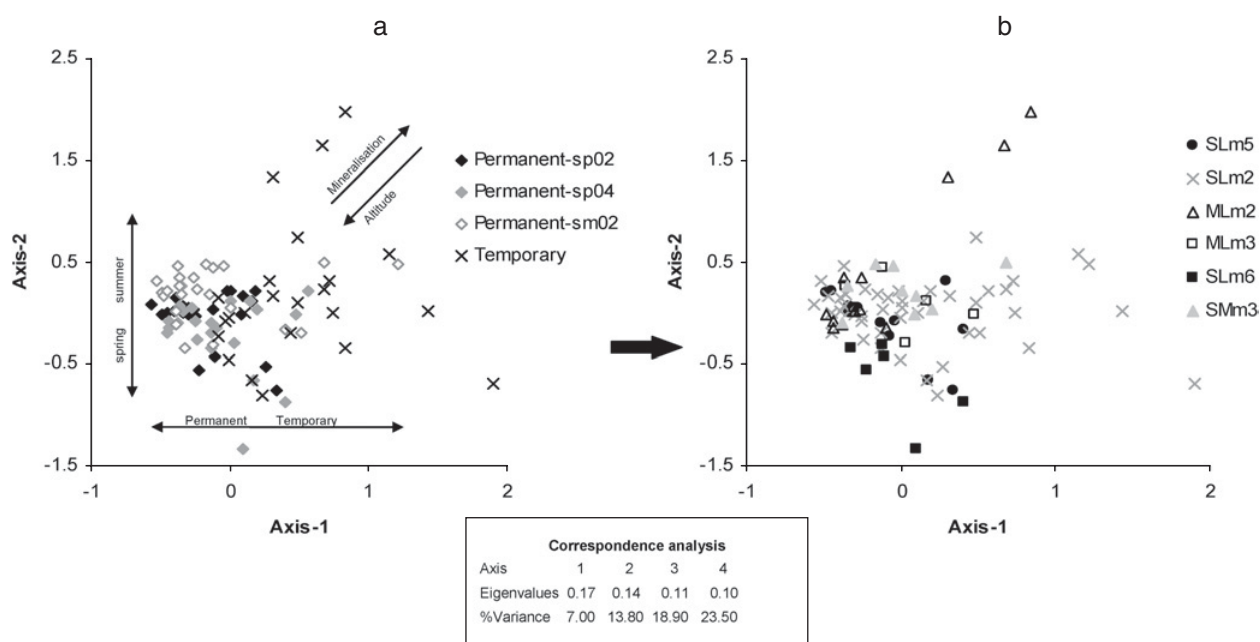
present study, the family-level was sufficient to discriminate stream-types and provided some type-specific indicator *taxa*.

Excluding rare *taxa* (less than 0.05% of the total abundance) did not result in the low number of indicator *taxa* for these stream types since they were found at very low frequency, appearing only in one or two of the sampled sites. Thus, these *taxa* could not characterize any stream type. Moreover, it would not be a powerful approach to base an assessment system on rare *taxa* that could be easily missed during field campaigns because insuring their collection would involve a great deal of effort (Hawkins *et al.*, 2000).

### Spatial and temporal dilemmas of the typology system

The significant cross-validation procedure of the DA model showed a high percentage of correctly classified samples within stream types. Most sites were sampled during three different occasions to include the seasonal or/and inter-annual natural variation of the macroinvertebrate community previously documented in the Mondego River basin (Chaves *et al.*, 2005; Feio *et al.*, 2006) and confirmed again in this study. During the cross-validation procedure, some samples belonging to the same site but collected in different occasions were placed in different stream types, indicating that the tested typology was not able to cope with temporal natural variability. Temporal variation is not reflected by the typology system, thus, when it occurs it might be interpreted as human-induced variability, incurring in a type I statistical error. Samples collected during the dry season (summer) failed less than spring samples during the DA cross-validation procedure. This might be due to the lower diversity of habitats and consequent higher homogeneity of the biological community during summer within each stream type.

The present study also indicates that there might be some problems with spatial allocation using the physical typology tested. For instance, in the CA ordination diagram, a site included in the MLm2 separated completely from all the remaining samples of that stream type. This site should probably have been included within a different stream type with higher mineralisation values as indicated by the presence of shrimps and gammarids in the samples. Furthermore, in the cross-validation procedure of the significant DA model, one sample of this site was placed in type SMm3, the only stream type with medium mineralisation values considered in the present study. As a result of this misplacement, gammarids were incorrectly selected as being characteristic of the MLm2 type in the DA model. Some studies have suggested the modification or refinement of landscape class boundaries using biological data (Dodkins *et al.*, 2005). However, morphoclimatic group 2 stream types, with medium catchment areas but higher mineralisation levels (see Table 1) do not exist in the Mondego River basin or in Portugal according to Alves *et al.* (2004). Thus, an adjustment



**Fig. 4.** Ordination (CA) of family invertebrate abundance. Samples are represented by different symbols according to the legend within each plot: a) samples are marked according to sampling occasions and temporary vs. permanent streams; most probable temporal and spatial patterns are indicated by arrows with the arrowhead pointing in the direction of increasing influence; b) samples are marked according to the pre-defined physical stream types by *Alves et al. (2004)*. Eigenvalues and cumulative % of variance explained by the first four axes are given.

**Table 5.** Tests of significance for the discriminant functions (DF), eigenvalues and variance explained due to stream types.

Test of function(s)	Tests of significance				DF	Eigenvalue	Variance explained	
	Wilk's $\lambda$	Chi-square	df	Sig.			Proportion	Cumulative
<b>1 through 5</b>	0.033	278.626	60	< 0.001	<b>1</b>	2.376	43.4	43.4
<b>2 through 5</b>	0.113	178.846	44	< 0.001	<b>2</b>	1.261	23.0	66.4
<b>3 through 5</b>	0.255	111.946	30	< 0.001	<b>3</b>	0.930	17.0	83.3
<b>4 through 5</b>	0.493	58.021	18	< 0.001	<b>4</b>	0.762	13.9	97.2
<b>5</b>	0.869	11.554	8	0.172	<b>5</b>	0.151	2.8	100.0

**Table 6.** Percentage of overlap between stream types, according to the two first ordination axes of the CA.

Source stream type	Overlap with	Overlap %	Source stream type	Overlap with	Overlap %
SLm5	SLm2	<b>17.0</b>	MLm3	SLm5	25.0
	MLm2	<b>16.6</b>		SLm2	<b>21.3</b>
	MLm3	33.3		MLm2	25.0
	SLm6	<b>0.0</b>		SLm6	<b>0.0</b>
	SMm3	<b>11.1</b>		SMm3	55.6
SLm2	SLm5	83.3	SLm6	SLm5	<b>0.0</b>
	MLm2	50.0		SLm2	<b>2.1</b>
	MLm3	83.3		MLm2	<b>0.0</b>
	SLm6	<b>16.6</b>		MLm3	<b>0.0</b>
	SMm3	55.5		SMm3	<b>0.0</b>
MLm2	SLm5	33.3	SMm3	SLm5	25.0
	SLm2	<b>19.2</b>		SLm2	36.2
	MLm3	33.3		MLm2	25.0
	SLm6	<b>0.0</b>		MLm3	50.0
	SMm3	<b>22.2</b>		SLm6	<b>0.0</b>

Only stream types with an overlap of samples < 25% were identified as an individual group (bold).

to include some MLm2 type sites into a hypothetical MHm2 stream type seems impossible.

## Conclusions

An accurate top-down stream classification leads to environmentally discrete groups of sites that should be strongly related to the composition and distribution of the biota. Although the lack of reference or near-natural sites in the Mondego River basin for all river and stream types defined by *Alves et al. (2004)* was a limitation to this study, the physical typology tested was apparently successful at identifying the available stream types using the biota. Nonetheless, issues concerning seasonal variations need to be better addressed, since these temporal variations can constrain the correspondence between stream types and biological assemblages. Natural variability should ideally be incorporated into a given typology prior to the examination of anthropogenic effects on rivers and streams since pre-defined physical stream types do not necessarily translate into an expected/desired clear discrimination of biota.

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