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Fish-based groups for ecological assessment in rivers: the importance of environmental drivers on taxonomic and functional traits of fish assemblages

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ABSTRACT

Key-words: *freshwater fish, water framework directive, river-types, ecological quality assessment, Portugal*

The use of river-types is of practical value, serving as groups for which assessment procedures can be developed and applied. An abiotic typology was set by the Portuguese Water Agency, mainly based on 6 major morphoclimatic regions. However, to be biologically meaningful, this typology should fit the distribution patterns of the biological quality elements communities proposed in Water Framework Directive under the lowest possible human pressure. This study aimed to identify and characterize fish-based geographical groups for continental Portugal and their environmental and geographical discriptors, using taxonomic and functional traits. Sampling took place between 2004 and 2006 during Spring. Fish fauna from 155 reference sites was analysed using a multivariate approach. Cluster Analysis on fish composition identified 10 fish-groups, expressing a clear correspondence to the river basin level, due to the restrict basin distribution of many species. Groups showed a wider aggregation in 4 regions with a larger geographical correspondence, statistically supported by Similarity Analysis, both on fish composition and mostly on fish metrics/guilds. Principal Components Analysis revealed major environmental drivers associated to fish-groups and fish-regions. Fish-groups were hierarchically grouped over major and local regions, expressing a large-scale response to a North-South environmental gradient defined by temperature, precipitation, mineralization and altitude, and a regional scale response mainly to drainage area and flow discharge. From North to South, fish-regions were related to the morphoclimatic regions. Results contributed to reduce redundance in abiotic river-types and set the final typology for Portuguese rivers, constituting a fundamental tool for planning and managing water resources.

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RÉSUMÉ

Groupes ichtyologiques pour l'évaluation écologique des rivières : importance des facteurs environnementaux sur la taxonomie et les caractéristiques fonctionnelles des peuplements de poissons

Mots-clés : poissons d'eau douce, directive cadre sur l'eau, rivières types, facteurs environnementaux, évaluation de la qualité écologique

L'utilisation de rivière-types est un moyen pratique, fournissant des groupes pour lesquels des procédures d'évaluation peuvent être développées et appliquées. Une typologie abiotique a été fixée par l'Agence de l'Eau Portugaise, principalement basée sur 6 principales régions morphoclimatiques. Toutefois, pour être biologiquement significative, cette typologie devrait correspondre aux schémas de distribution des communautés, éléments de qualité biologique, proposés dans la directive cadre sur l'eau sous la pression anthropique la plus basse possible. Cette étude visait à identifier et à caractériser des groupes géographiques reposant sur les communautés ichtyologiques pour le Portugal continental et leurs descripteurs environnementaux et géographiques, à l'aide de traits taxonomiques et fonctionnels. L'échantillonnage a eu lieu entre 2004 et 2006 au cours du printemps. La faune de poissons provenant de 155 sites de référence a été analysée en utilisant une approche multivariée. L'analyse de clusters sur la composition en poissons a identifié 10 groupes ichtyologiques, exprimant une correspondance claire à l'échelle du bassin versant, en raison de la distribution limitée à un bassin de nombreuses espèces. Les groupes ont montré une plus large agrégation dans 4 régions avec une plus grande correspondance géographique, confirmée statistiquement par l'analyse de similarité, à la fois sur la composition en poisson et surtout sur les métriques ichtyologiques ou les guildes. L'analyse en Composantes Principales a révélé les principaux facteurs environnementaux associés aux groupes et régions ichtyologiques. Les groupes ichtyologiques ont été regroupés hiérarchiquement sur des régions locales et plus grandes, exprimant une réponse à grande échelle à un gradient Nord-Sud de l'environnement défini par la température, les précipitations, la minéralisation et l'altitude, et une réponse à l'échelle régionale principalement à l'aire de drainage et au débit. Du Nord au Sud, les régions ichtyologiques étaient liées aux zones morphoclimatiques. Les résultats contribuent à réduire la redondance dans les types abiotiques de rivières et définissent la typologie finale pour les rivières portugaises, constituant un outil fondamental pour la planification et la gestion des ressources en eau.

INTRODUCTION

Running waters belong to the most intensively used and altered ecosystems of the world (Saunders *et al*., 2002; Clavero *et al*., 2004; Levin *et al*., 2009). In Europe, about 80% of rivers are affected by water pollution, water removal for hydropower and irrigation, structural alterations and the impact of dams (Schinegger *et al*., 2011).

Over the last few decades tremendous efforts have been undertaken to develop management strategies to improve the ecological status of these ecosystems. The European Union has taken a new course in water policy towards an integrated management of water bodies by enacting a new legislation, the Water Framework Directive (WFD) (European Commission, 2000). The WFD requires Member States of the European Union to assess, monitor and, where necessary, improve the ecological quality status of surface waters, seeking to achieve at least 'good ecological status' by 2015. This landmark piece of environmental legislation incorporates for the first time the importance of the aquatic biota to determine the quality of fresh and marine waters (Sweeting, 2001; Logan and Furse, 2002), and the importance of biogeographical drivers of species distribution patterns (*e.g.* Illies, 1978).

According to WFD, the definition of a typology to classify rivers and streams is the first and fundamental step for the ecological assessment. Differences between climate, hydrology, geomorphology, geology, soil composition, land use and vegetation make comparison of communities in running waters difficult. Thus, a typological approach is a way to stratify the spatial variability in stream and river monitoring and assessment, *i.e.* allowing comparing groups of streams, and constituting classes for which assessment procedures can be developed and applied. In Portugal, no attempts to derive a national river typology were made before the implementation of the WFD.

The establishment of a river typology is supported by a set of reference sites, *i.e.*, in the absence of anthropogenic disturbance, as it is assumed that biological communities are optimal developed at undisturbed or reference conditions (Karr and Chu, 1999). Reference conditions are best described at the scale of a river-type (Nijboer *et al*., 2004) and the comparison of conditions at a current site with those of a reference site belonging to the same stream-type allows an ecological quality evaluation. In short, a typology should be simple, intuitively understandable, with a minimum number of river types, whilst reducing natural variation of reference conditions within river-types (Dodkins *et al*., 2005).

Reference sites should be viewed as the least disturbed sites within a river-type, rather than actual pristine conditions, as they rarely exist in most European countries. Although human-induced disturbance occur among all river-types, some areas exhibit higher degradation status. Overall, river-types with higher human density tend to present higher degradation conditions and therefore reference sites can be quite rare (Matono, 2012). The least disturbed river-types are usually located in high altitude regions (and small drainage basins), frequently in isolated areas with difficult human access, far from the main human pathways (Chaves *et al.,* 2006; Schinegger *et al*., 2011).

The WFD defined abiotic descriptors for classifying streams and rivers into types (Annex II, Section 1 of the WFD) according to two alternative systems: (i) "system A", the fixed typology, is defined by ecoregions (according to Illies, 1978), based on the catchment area, catchment geology and altitude; (ii) "system B" uses five obligatory factors (latitude, longitude, altitude, geology and drainage area of the basin), and an additional group of optional factors. The Portuguese Water Agency (INAG) chose system B, as it expressed better the ecological heterogeneity of Portuguese rivers (Alves *et al*., 2004; INAG, 2008a). Therefore, besides the obligatory factors, six more optional factors are also considered: slope, mean annual precipitation, coefficient of variation of precipitation, mean annual discharge, mean annual temperature and mean annual temperature range. These optional factors together with altitude, latitude and longitude allowed identifying 6 major morphoclimatic regions in continental Portugal as the main base for the definition of the abiotic typology (Alves *et al*., 2004; INAG, 2008a). Further interception of these regions with geological and drainage area classes made possible the establishment of the final abiotic river-types (Alves *et al*., 2004; INAG, 2008a).

To be biologically meaningful, the abiotic river-types should fit the distribution patterns of the biological quality elements, namely fish fauna, under the lowest possible human pressure. Though in the past fish fauna deserved much less attention than other biological groups regarding environmental monitoring, it presents several important attributes as a bio-indicator (Barbour *et al*., 1999).

Due to zoogeographical traits, Iberian fish fauna is very rich in endemisms, many restricted to particular basins. Native species richness per site is generally low (Griffiths, 2006; Reyjold *et al*., 2007) and had undergone a steep decline during the last decades (Doadrio, 2002; Cabral *et al*., 2005; Smith and Darwall, 2006; Ribeiro *et al*., 2009). Moreover fish assemblages from Iberain Peninsula may present a high variability both at seasonal and inter-annual scale determined by the influence of Mediterranean climate (Gasith and Resh, 1999; Bernardo *et al*., 2003; Ilhéu, 2004). All these aspects cause considerable difficulties in the development of tools to assess the ecological status of Mediterranean climate streams, namely regarding fish fauna.

This study aimed to identify environmental and geographical discriptors of fish assemblages and characterize fish-based geographical groups for continental Portugal, using taxonomic and functional traits. Furthermore it should contribute to adjust the abiotic types limits and, if possible, to reduce their number, while improving its consistency.

The complementary use of fish composition, structural and functional fish metrics constitutes an added value in the approach of defining fish-based groups to subsequently derive river-types. Indeed, the analysis of functional organization of fish assemblages allows characterizing different aspects of the community structure other than taxonomic organization (Hoeinghaus *et al*., 2007; Higgins and Strauss, 2008) and permit comparisons among broad geographic regions even when communities present different taxa (Simberloff and Dayan, 1991). In the Iberian Peninsula this approach will contribute to avoid problems related to spatially restricted endemisms. Moreover, it provides a means of testing theoretical expectations of changes in species traits along environmental gradients, such as those generated from habitat templates (Southwood, 1977), the river continuum concept (Vannote *et al*., 1980), and landscape filters (Poff, 1997).

MATERIALS AND METHODS

> *STUDY AREA*

Continental Portugal is located in the SW extreme of the Iberian Peninsula, covering an area of nearly 90 000 km^2. The geography is dominated by a mixture of Atlantic (in the northern regions) and Mediterranean (in the southern regions) influences.

The climate is Mediterranean, although the influence of factors such as topography and proximity to the Atlantic Ocean cause significant climatic contrasts in a country of a small size as Portugal (Ribeiro, 2011). The general conditions of the atmospheric circulation cause a decrease in precipitation from North to South and from West (littoral) to the East (interior), enhanced by topographical asymmetry. The temperature shows an opposite pattern, increasing from North to South (http://www.igeo.pt/atlas/).

Regarding the relief, the territory is very unequal. The South is a lowland region with few low altitude mountains. To the North of river Tagus the land presents high spatial heterogeneity, including 95% of the areas above 400 m and all the elevations higher than 1000 m. The altitude causes a decrease in temperature and an increase in rainfall (http://www.igeo.pt/atlas/).

The characteristics of the hydrographical network are related to the nature of the rocks, tectonic accidents and climate of the areas traversed. Rivers flow regimes reflect the rainfall variations and are among the most irregular of Europe.

While the South is quite homogeneous, mostly represented by lowland streams and river (flat, less rainy, with long hot dry summers), the North presents a much more complex climatic and geomorphological patchiness, with higher average altitudes, higher annual precipitation, and milder summers in general. Due to these features, most rivers are permanent in the North and intermittent in the South (http://geo.snirh.pt/AtlasAgua; Bernardo and Alves, 1999; INAG, 2008a).

Considering the 6 major morphoclimatic regions identified for Portugal (Alves *et al*., 2004; INAG, 2008a), four are located at the North of river Tagus (regions 3, 4, 5 and 6) and two at the South (regions 1 and 2) (Figure 1). In the South, region 3 is present in small areas with higher altitude and humidity. The numbering of the regions reflects an environmental gradient, with regions 1 and 6 presenting more extreme characteristics. Region 1 is the most arid one, showing higher temperature and less precipitation. Region 6 is located in northern Portugal and includes regions with relatively high altitude and rainfall. The remaining regions form a gradient between these extremes.

> *SAMPLING*

Sampling was carried out during spring between 2004 and 2006 in 155 undisturbed sites in the main Portuguese river basins (Figure 2). For the selection of these undisturbed sites, a preliminary pressure screening using GIS and information on pollution loads was followed. The final selection was based on the human disturbance level, regarding ten semi-quantitative

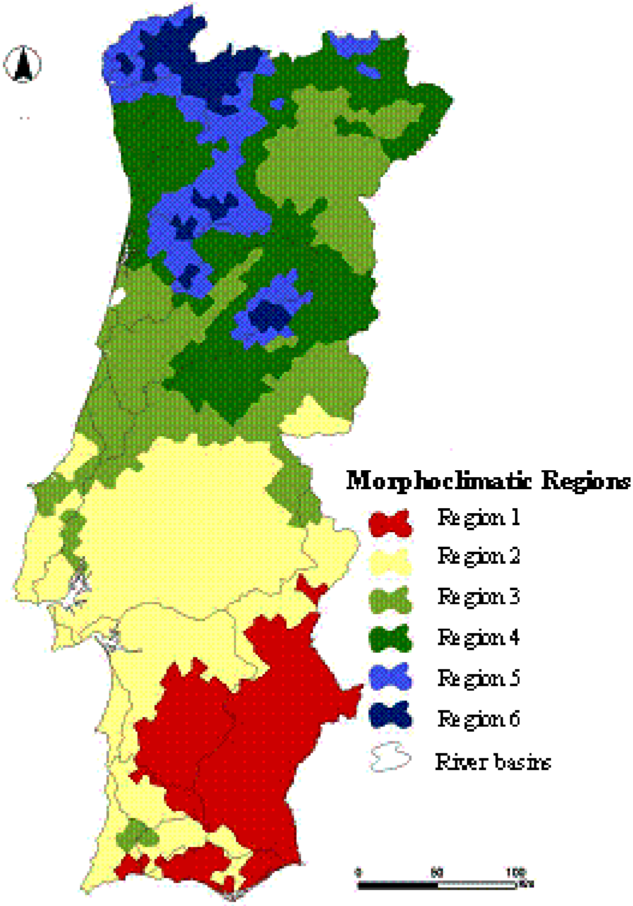

Figure 1
Major morphoclimatic regions identified for continental Portugal (Alves et al.*, 2004; INAG, 2008a).*

variables assessed at each site (formerly developed within EU-project FAME, 2004; available at http://fame.boku.ac.at): land use, urban area, riparian vegetation, longitudinal connectivity of the river segment, sediment load, hydrological regime, morphological condition, presence of artificial lentic water bodies, toxicological and acidification levels, and nutrient/organic load. Each variable was scored from 1 (minimum disturbance) to 5 (maximum disturbance) (Appendix 1) and only sites with scores 1 and/or 2 and only one variable with a 3 were considered as undisturbed or least disturbed (references) (following CIS-WFD, 2003).

Several physicochemical variables complemented and supported the evaluation of human pressure in each site, after laboratory measurements and analyses according to the Standard Methods for the Examination of Water and Wastewater (Clesceri *et al*., 1998): biological oxygen demand – BOD_5 ($mg \cdot L^{-1}$), chemical oxygen demand – COD ($mg \cdot L^{-1}$), oxidability ($mg \cdot L^{-1}$), total suspended solids – TSS ($mg \cdot L^{-1}$), total dissolved phosphorous – *P* ($mg \cdot L^{-1}$), nitrite – NO_{2}- ($mg \cdot L^{-1}$), nitrate – NO_{3}- ($mg \cdot L^{-1}$), ammonium – NH_{4+} ($mg \cdot L^{-1}$) and total dissolved nitrogen – N ($mg \cdot L^{-1}$).

Fish were collected by electrofishing according to the WFD compliant sampling protocol (INAG, 2008b), which follows the CEN standards (CEN, 2003). All collected individuals were measured, identified to the species level and immediately returned to the river.

Landscape and regional variables were obtained from digital cartography with free internet access and included latitude, longitude, mineralization level, drainage area of the basin (km^2), distance from source (km), altitude (m), slope (%), mean annual discharge (mm), mean annual

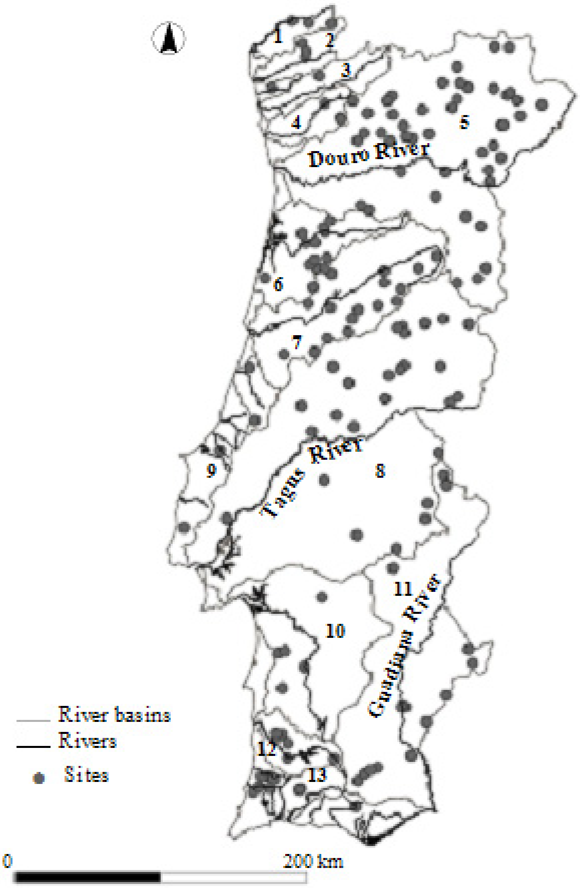

Figure 2
Map of the sampled sites in the principal continental Portuguese river basins: 1-Minho, 2-Lima, 3-Cávado, 4-Ave, 5-Douro, 6-Vouga, 7-Mondego, 8-Tagus, 9-West streams, 10-Sado, 11-Guadiana, 12-Mira, 13-Algarve streams.

air temperature (ºC), mean temperature range, mean annual precipitation (mm) and coefficient of variation of precipitation. Rainfall, temperature and flow variables were described from 30-year data series.

Local variables were assessed during the sampling procedure: water temperature (ºC), conductivity ($\mu S \cdot cm^{-1}$), pH, dissolved oxygen ($mg \cdot L^{-1}$), mean stream wetted width (m), maximum and mean water depth (m), mean current velocity ($m \cdot s^{-1}$), dominant substrate class (adapted from Wentworth scale (Giller and Malmqvist, 1998): 1-mud and sand; 2-gravel; 3-pebble; 4-cobble; 5-boulders; 6-boulders larger than 50 cm), riparian vegetation (%), shadow (%) and proportion of different habitat types (pool, run, riffle).

> DATA ANALYSIS

Captures were quantified as density (ind./100 m^2). Besides taxonomic data, the community structure was also considered: fish density (number of fish/100 m^2), number of species (S), species diversity (Shannon-Wiener Index – H), relative abundance of potamodromous individuals, habitat guilds (proportion of rheophilic, limnophilic, eurytopic, benthic and water column individuals), trophic guilds (proportion of omnivorous and insectivorous individuals) and reproductive guilds (proportion of lithophilic and phytophilic individuals) (Table I). The

Table I
Eclogical classification of fish species (according to FAME, 2004; Ilhéu, 2004; Cabral et al. 2005; Holzer, 2008; Magalhães et al., 2008): B (benthic), WC (water column), EUR (eurytopic), RF (rheophilic), LIM (limnophilic), PHY (phythophilic), LIT (lithophilic), OMNI (omnivorous), INSV (insectivorous), POTAD (potamodromous), DIAD (diadromous), NAT (native), END (endemic), EX (exotic/non-native).

Species	Common name	Classification
***Anaecypris hispanica* (Steindachner, 1866)**	Spanish Minnow Carp	WC; LIM; END
***Anguilla anguilla* (Linnaeus)**	Eel	B; EUR; DIAD; NAT
***Barbus* spp. juveniles**	juvenile Barbels	WC; RF; LIT; OMNI; POTAD; END
***Barbus bocagei (*Steindachner, 1864)**	Common Barbel	B; LIM; LIT; OMNI; POTAD; END
***Barbus comizo* (Steindachner, 1864)**	Iberian Gudgeon	WC; LIM; LIT; OMNI; POTAD; END
***Barbus microcephalus* (Almaça, 1967)**	Small-head Barbel	B; LIM; LIT; OMNI; POTAD; END
***Barbus sclateri (*Gunther, 1868)**	South Barbel	B; LIM; LIT; OMNI; POTAD; END
***Carassius auratus* (Linnaeus)**	Goldfish	B; LIM; PHY; OMNI; EX
***Cobitis calderoni (*Bacescu, 1962)**	Stone Loach	B; RF; LIT; INSV; END
***Cobitis paludica* (de Buen, 1930)**	South Stone Loach	B; LIM; INSV; END
***Cyprinus carpio* (Linnaeus)**	Common Carp	B; LIM; PHY; OMNI; EX
***Achondrostoma arcasii* (Steindachner,1866)**	Iberian Roach	WC; RF; PHY; OMNI; END
***Achondrostoma oligolepis* (Robalo *et al.*, 2005)**	Portuguese Roach	WC; LIM; PHY; INSV; END
***Iberochondrostoma lemmingii* (Steindachner, 1866)**	Arched-mouth Nase	WC; LIM; LIT; OMNI; END
***Iberochondrostoma lusitanicum* (Collares-Pereira, 1980)**	Portuguese Nase	WC; LIM; LIT; OMNI; END
***Pseudochondrostoma polylepis (*Steindachner, 1865)**	Iberian Nase	B; RF; LIT; OMNI; POTAD; END
***Pseudochondrostoma duriense* (Coelho, 1985)**	Douro Nase	B; RF; LIT; OMNI; POTAD; END
***Pseudochondrostoma willkommii* (Steindachner, 1866)**	Guadiana Nase	B; RF; LIT; OMNI; POTAD; END
***Gambusia holbrooki* (Girard, 1859)**	Mosquitofish	WC; LIM; INSV; EX
***Gasterosteus gymnurus* (Cuvier, 1829)**	Three-Spined Stickleback	WC; EUR; OMNI; NAT
***Gobio lozanoi (*Doadrio and Madeira, 2004)**	Gudgeon	B; RF; INSV; EX
***Herichtys facetum* (Jenyns, 1842)**	Chamaleon Cichlid	WC; LIM; OMNI; EX
***Lampetra planeri* (Bloch, 1784)**	Brook Lamprey	B; RF; LIT; OMNI; POTAD; NAT
***Lepomis gibbosus* (Linnaeus)**	Pumpkinseed	WC; LIM; INSV; EX
***Micropterus salmoides* (Lacépède, 1802)**	Largemouth Bass	WC; LIM; PHY; EX
***Petromyzon marinus* (Linnaeus)**	Sea Lamprey	B; RF; LIT; DIAD; NAT
***Salaria fluviatilis* (Asso, 1801)**	Freshwater blenny	B; RF; LIT; INSV; NAT
***Salmo trutta* (Linnaeus)**	Brown Trout	WC; RF; LIT; INSV; NAT
***Squalius alburnoides (*Steindachner, 1866)**	Roach	WC; EUR; LIT; INSV; END
***Squalius aradensis* (Coelho *et al.,* 1998)**	Arade Chub	WC; EUR; LIT; INSV; END
***Squalius carolitertii* (Doadrio, 1988)**	North Chub	WC; EUR; LIT; INSV; END
***Squalius pyrenaicus* (Günther, 1868)**	Iberian Chub	WC; EUR; LIT; INSV; END
***Squalius torgalensis* (Coelho *et al.*, 1998)**	Torgal Chub	WC; EUR; LIT; INSV; END

species were assigned and classified into guilds according to published literature (FAME, 2004; Ilhéu, 2004; Cabral *et al*. 2005; Holzer, 2008; Magalhães *et al*., 2008) and expert judgement on their life history traits, when necessary.

Hierarchical Cluster Analysis using Euclidean distance and Ward's method was used to identify groups of sites based on fish composition (density).

Similarity Analysis (ANOSIM) for 1-way layout (Clarke and Warwick, 1994) was performed on fish composition and metrics/guilds to determine if there were significant differences between fish-groups. The test value (R) varies between 1, when samples are completely separated, and 0 if there is no separation on the averages between and among samples. As a rule of thumb, Clarke and Gorley (2001) consider pairs of samples which display an $R \geq 0.75$ as being well separated from each other, those with $0.5 \leq R \leq 0.75$ as overlapping but still clearly different and those with $R \leq 0.5$ as barely separable at all.

Reference sites include undisturbed (all human pressure variables scored with 1 and/or 2) and least disturbed sites (one human pressure variable scored with 3 and the remaining ones with 1 and/or 2), when these were the only available ones for sampling. As such, Similarity Analysis (ANOSIM) was also performed on fish assemblages composition (density) between undisturbed and least disturbed sites in order to understand if the influence of some anthropogenic pressure (score 3 in one of the variables) in least disturbed sites could result in significant differences in the occurrence of some species, thus hampering the consistency of the reference sites.

Environmental drivers of fish-groups were explored with Principal Components Analysis (PCA), based on large, regional and local scale variables of sampling sites. Most intercorrelated variables (Spearman's rank correlations $|r| > 0.8$; $P < 0.05$) were excluded.

Indicator Species Analysis (Dufréne and Legendre, 1977) allowed recognizing species that identified each group.

For statistical analysis data were transformed to improve normality: percentages were arcsin [sqrt(x)] and linear measurements were log (x + 1) (Legendre and Legendre, 1998). Species with very low frequency of occurrence (smaller than 0.02) were excluded from the analysis.

Data analysis was performed with statistical programs Pc-ord 4, Primer 6 and Canoco 4.5. For geographical delimitation of fish-groups/regions the image processing program Gimp 2.6 was used.

RESULTS

> *FISH-BASED GEOGRAPHICAL GROUPS*

A total of 32 fish species from 10 families were captured, including freshwater and 2 diadromous species. Although 7 non-native species were captured, freshwater native species ($N = 23$) represented 72% of the total sampled species and 98% of the fish density in the captures.

No fish was captured in 13% of the sampling sites. These sites were mainly located in small drainage areas (smaller than 30 km^2), in upstream reaches (mean water depth lower than 0.2 m) with low mineralization level. Northern river basins represented 44% of fishless sites (Minho, Lima, Cávado and Douro river basins).

Hierarchical Cluster Analysis performed on fish species composition allowed identifying 10 fish-groups (Figure 3), further characterized concerning fish metrics and guilds (Table II).

Group 1 included streams from the Littoral, both in the Centre and North regions with a clear dominance of *Achondrostoma oligolepis*. The most representative guilds were omnivorous, limnophilic and phytophilic individuals. Nevertheless, salmonids also presented some expression, accounted for the significant presence of *Salmo trutta*, comparatively to other groups, making this group a mixed cyprinid-salmonid one. This group presented high fish density (mean = 37.7 ind./100 m^2, SD = 43.5), species richness (mean S = 5.2; SD = 2.3) and diversity (mean H = 1.1; SD = 0.5) per site.

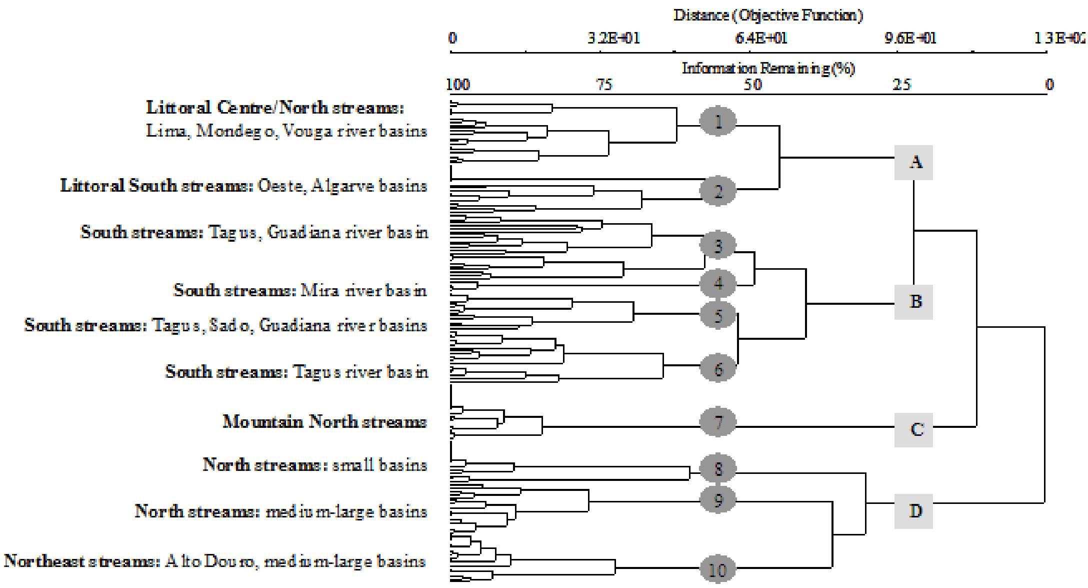

Figure 3
Cluster of the sampled sites based on fish species densities identifying 10 fish-groups and 4 fish-regions.

Groups 2, 3, 4, 5 and 6 belonged to the South region of Portugal. Group 2 included the southern Littoral river basins from Algarve region. The dominant species were *Squalius aradensis* and *Anguilla anguilla*. Group 3 was mostly represented by streams from Guadiana river basin. The dominant species were *Squalius alburnoides* and the endemic barbels from Guadiana basin, especially *Barbus microcephalus*. Group 4 was represented by streams from Mira river basin. Fish assemblages were dominated by *Squalius torgalensis*, endemic from this basin. Group 5 was represented by fish assemblages common to Guadiana, Sado and Tagus river basins. *Squalius alburnoides* was the most representative and abundant species, followed by *Squalius pyrenaicus.* Group 6 was mostly represented by streams from Tagus river basin, therefore located in a transitional region between the North and the South of Portugal. Fish assemblages were dominated by *Barbus bocagei*, *Pseudochondrostoma polylepis* and *Squalius pyrenaicus*.

Overall, compared to the other fish-groups, southern ones showed high abundance of *Squalius* spp, followed by barbel species. Fish assemblages were dominated for eurytopic, water column, lithophilic and insectivorous individuals. Most of these groups showed high fish density, including the group with the highest values of total density (mean = 73.7 ind./100 m^2; SD = 97.2) and total captured species (N = 18). Nevertheless, mean species richness and mean diversity were high only in groups 3, 5 and 6.

It was possible to identify two main functional units in the southern fish-groups: groups 2, 4 and 5 were clearly dominated by eurytopic and water column insectivorous cyprinids, while in groups 3 and 6 this pattern was less obvious, because they also showed high percentages of omnivorous individuals, due to the marked presence of barbel and nase species. Moreover, these last two groups presented higher percentages of rheophilic individuals than the other southern fish-groups and the highest values of species richness and diversity within the South.

Group 7 included small mountain streams from the North and was mostly represented by *Salmo trutta.* Therefore, was highly discriminated by salmonid, rheophilic and insectivorous guilds. Fish assemblages presented low density (mean = 5.84 ind./100 m^2; SD = 6.6), diversity (mean H = 0.5; SD = 0.5) and species richness (mean S = 2.1; SD = 1.2).

Table II
Characterization of the ten fish-groups based on metrics and guilds (mean ± SD).

Metrics and Guilds		Fish-groups 1	2	3	4	5	6	7	8	9	10
General metrics	Species richness	5.2 ± 2.3	2.3 ± 1.6	4.2 ± 2.1	2.0 ± 1.2	3.5 ± 1.5	4.5 ± 1.3	2.1 ± 1.2	2.2 ± 1.5	4.8 ± 1.9	4.0 ± 1.6
	Species diversity (Shannon-Wiener)	1.1 ± 0.5	0.3 ± 0.4	0.9 ± 0.5	0.3 ± 0.4	0.6 ± 0.3	0.9 ± 0.4	0.5 ± 0.5	0.4 ± 0.5	1.1 ± 0.4	0.8 ± 0.4
	Fish density (ind./100 m^2)	37.7 ± 43.5	44.2 ± 67.2	56.2 ± 64.0	25.4 ± 18.9	73.7 ± 97.2	61.0 ± 45.6	5.8 ± 6.6	26.7 ± 36.2	11.2 ± 8.4	24.6 ± 35.7
	Salmonids (% ind./100 m^2)	2.9 ± 5.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 1.1	75.7 ± 26.1	0.3 ± 0.8	1.8 ± 4.7	1.1 ± 2.7
	Potamodromous (% ind./100 m^2)	36.6 ± 26.8	54.3 ± 45.4	33.1 ± 32.7	91.1 ± 10.4	22.3 ± 26.5	79.7 ± 17.2	17.0 ± 22.4	87.8 ± 24.0	47.4 ± 25.3	93.0 ± 8.5
Habitat guilds	Rheophilic (% ind./100 m^2)	9.9 ± 12.3	0.0 ± 0.0	22.1 ± 30.6	0.0 ± 0.0	1.3 ± 2.2	18.0 ± 21.0	88.6 ± 18.6	13.8 ± 21.9	60.8 ± 25.0	45.0 ± 33.4
	Eurytopic (% ind./100 m^2)	36.3 ± 26.4	86.8 ± 27.3	45.6 ± 35.3	89.5 ± 13.3	74.7 ± 31.8	47.2 ± 34.1	7.6 ± 13.6	81.3 ± 27.4	19.3 ± 18.4	9.7 ± 8.9
	Limnophilic (% ind./100 m^2)	53.8 ± 31.5	13.2 ± 27.3	31.6 ± 32.2	10.5 ± 13.3	23.9 ± 30.9	34.7 ± 30.3	3.8 ± 12.7	4.8 ± 10.1	20.0 ± 18.3	45.3 ± 32.3
	Benthic (% ind./100 m^2)	31.7 ± 26.4	50.7 ± 46.0	29.3 ± 31.2	8.9 ± 10.4	18.9 ± 27.3	47.1 ± 32.9	16.1 ± 19.4	17.1 ± 25.1	33.1 ± 24.1	86.3 ± 13.5
	Water column (% ind./100 m^2)	68.3 ± 26.5	47.6 ± 45.2	68.0 ± 30.1	91.1 ± 10.4	75.0 ± 31.9	51.0 ± 32.8	83.9 ± 19.4	81.6 ± 27.3	64.4 ± 24.0	13.6 ± 13.6
Trophic guilds	Omnivorous (% ind./100 m^2)	57.2 ± 28.6	11.2 ± 26.5	41.6 ± 33.3	2.8 ± 5.6	16.6 ± 28.3	48.7 ± 35.4	16.7 ± 20.2	8.9 ± 15.5	73.5 ± 18.0	87.8 ± 9.7
	Insectivorous (% ind./100 m^2)	31.6 ± 29.1	44.5 ± 44.4	54.6 ± 34.8	96.0 ± 5.3	76.8 ± 32.7	49.4 ± 35.1	80.5 ± 21.4	89.6 ± 15.4	20.0 ± 17.5	10.9 ± 10.1
Reproductive guilds	Lithophilic (% ind./100 m^2)	47.8 ± 34.1	55.4 ± 45.5	71.5 ± 38.5	91.1 ± 10.4	91.2 ± 5.8	95.9 ± 7.5	93.8 ± 15.6	98.5 ± 5.0	49.2 ± 23.9	95.9 ± 6.9
	Phytophilic (% ind./100 m^2)	38.8 ± 35.0	0.0 ± 0.0	5.4 ± 16.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 1.8	3.4 ± 12.7	0.1 ± 0.2	44.4 ± 24.7	2.7 ± 6.3

ANOSIM based on fish composition (upper triangle); ANOSIM based on fish metrics and guilds (lower triangle)

	1	2	3	4	5	6	7	8	9	10
1		0.67 ***	0.6 ***	0.94 ***	0.79 ***	0.73 ***	0.83 ***	0.62 ***	0.66 ***	0.73 ***
2	0.5 ***		0.53 ***	0.58 ***	0.76 ***	0.85 ***	0.87 ***	0.84 ***	0.85 ***	0.88 ***
3	0.10 **	0.25 ***		0.49 ***	ns	0.39 ***	0.77 ***	0.72 ***	0.74 ***	0.74 ***
4	0.5 **	ns	ns		0.96 **	1 ***	1 ***	0.99 **	1 ***	1 ***
5	0.33 ***	ns	ns	ns		0.41 ***	0.99 ***	0.88 ***	0.88 ***	0.82 ***
6	0.20 ***	0.32 ***	ns	0.38 **	0.18 *		0.97 ***	0.95 ***	0.88 ***	0.81 ***
7	0.69 ***	0.67 ***	0.59 ***	0.65 **	0.70 ***	0.71 ***		0.92 ***	0.93 ***	0.92 ***
8	0.52 ***	0.18 **	0.25 ***	ns	ns	0.34 ***	0.52 ***		0.55 ***	0.53 ***
9	0.16 **	0.58 ***	0.28 ***	0.80 ***	0.61 ***	0.40 ***	0.64 ***	0.65 ***		0.34 ***
10	0.43 ***	0.59 ***	0.35 ***	0.90 ***	0.64 ***	0.26 ***	0.76 ***	0.72 ***	0.43 ***	

Figure 4
Results from Similarity Analysis (ANOSIM) between fish-groups, based on fish composition and on fish metrics and guilds. Significance levels for P < 0.001 (***), P < 0.01 (**), P < 0.05 (*) and P > 0.05 (ns).

Group 8 included small permanent streams from the North. The most abundant species was *Squalius carolitertii* and fish assemblages were mainly composed by water column, insectivorous, eurytopic, and lythophilic species. Relatively low values of total density (mean = 26.7 ind./100 m^2; SD = 36.2), species richness (mean S = 2.2; SD = 1.5) and diversity (mean H = 0.4; SD = 0.5) were also observed per site.

Group 9 was represented by North streams with medium and large drainage area. The most abundant species was *Achondrostoma arcasii*, but *Squalius carolitertii* and *Barbus bocagei* were represented as well. Fish assemblages were dominated by omnivorous, phytophilic, water column and rheophilic individuals. Although mean total density was low (mean = 11.2 ind./100 m^2; SD = 8.4), species richness (mean S = 4.8; SD =1.9) and diversity (mean H = 1.1; SD = 0.4) showed high values per site.

Group 10 represented a particular area of Douro river basin named Alto Douro in the Northeast of Portugal. Though *Squalius carolitertii* occured with relatively high density, fish assemblages were dominated by *Barbus bocagei* and *Pseudochondrostoma duriense*. Therefore, high values of potamodromous, omnivorous and rheophilic individuals were registered. Density per site was relatively low (mean = 24.6 ind./100 m^2; SD = 35.7), but number of species (mean S = 4; SD = 1.6) and diversity (mean H = 0.8; SD = 0.4) were high.

ANOSIM results considering fish composition showed significant differences ($P < 0.05$) and a good separation ($R > 0.75$ or near) between most of the groups, particularly for groups 4 and 7 (Figure 4). Significant, though lower R values (<0.5 or near) were obtained among southern fish-groups (2, 3, 4, 5 and 6) and also between northern groups 8, 9 and 10, reflecting an overlapping or even a close taxonomic similarity between some of these groups.

Concerning metrics and guilds, in general, ANOSIM results revealed lower R values than for fish composition (Figure 4). Significant similarities ($R < 0.5$ and $P < 0.05$ or $P > 0.05$ – n.s.) were verified between several groups, especially among the southern ones (2, 3, 4, 5 and 6), emphasizing taxonomic results. North groups 8, 9 and 10 showed less functional similarities among them than it has been observed for fish composition. These three fish-groups also presented functional similarities with some South and Littoral groups, particularly fish-group 8. In fact, this group was dominated by *Squalius carolitertii*, thereby presenting high proportions of insectivorous, eurytopic, water column and lithophilic individuals, which also characterized South fish-groups. Group 7 was the most functionally distinct.

There was a clear correspondence of the 10 fish-groups to the river basin level, due to the restrict basin distribution of many species, and a trend division between North and South. Fish-groups showed a wider aggregation in 4 regions (Figures 3 and 6): (i) Region A including sites nearby Littoral areas; (ii) Region B including sites from the South region, namely Tagus, Sado, Mira and Guadiana river basins; (iii) Region C including sites from the Mountain North region; and (iv) group D including sites from the North region. These regions showed a broader geographical correspondence than fish-groups, though region A seemed rather fragmented. ANOSIM results supported this major division but suggested more taxonomic and functional similarities between group 2 and the South fish-groups (3, 4, 5 and 6) than with group 1 from the same Littoral region.

> ENVIRONMENTAL DRIVERS

Principal Components Analysis (PCA) revealed a good segregation of sites along the first two ordination axes, which together accounted for 70% of total variation (Figure 5).

Overall, large-scale and regional variables assumed more relevance discriminating species and sites than the local ones. According to the correlations between environmental variables and PCA axes, drainage area ($r = 0.83$), conductivity ($r = 0.69$), precipitation ($r = –0.65$), annual discharge ($r = –0.58$), altitude ($r = –0.58$) and mean annual temperature ($r = 0.52$) were the most important variables for the first axis, whereas mean stream width ($r = –0.81$), latitude ($r = –0.66$), conductivity ($r = 0.65$), mineralization ($r = 0.59$), mean water deph ($r = –0.58$), drainage area ($r = –0.54$) and mean annual temperature ($r = 0.52$) were the variables with the highest contributions to the second axis (Table III).

PCA biplot considering fish-groups showed a discrimination along a North-South gradient, mostly defined by the second axis (Figure 5). North fish-groups were associated with low annual temperature, mineralization and conductivity, high altitude, annual discharge and local variables reflecting high flow conditions and permanent flow regime: high current velocity, coarser dominant substrate and high percentage of turbulent habitats. South fish-groups exhibited less diverse environmental features and were mainly associated with high temperature, mineralization and conductivity, low alitude, annual discharge and high percentage of slow current habitats. The first axis mainly discriminated the North fish-groups (Figure 5). Groups 7 and 8 were associated with higher altitude and annual discharge and small drainage area, compared to fish-groups 9 and 10. South groups were quite dispersed along this axis, particularly group 6. This group represented the majority of Tagus river basin, located in the transition area between the North and the South regions, thus sharing environmental influences with both. Littoral fish-groups showed a scattered distribution along both axes, though group 1 seemed closer to North groups and group 2 to the South ones.

Together with ANOSIM, PCA results supported the existence of 4 fish-based major regions in continental Portugal with a meaningful biological and environmental correspondence (Figure 6). Results further suggest including group 2 in the fish-region B, unifying the geographical area of the South (Figure 6). General characterization of the 4 fish-regions is given bellow:

Mountain fish-region (fish-group 7) corresponded to small headwater streams of northern mountain basins. These sites presented low mineralization, conductivity and annual temperature and were located in the highest altitude areas, with high slope, permanent flow and cold water. *Salmo trutta* was the most abundant and indicator species.

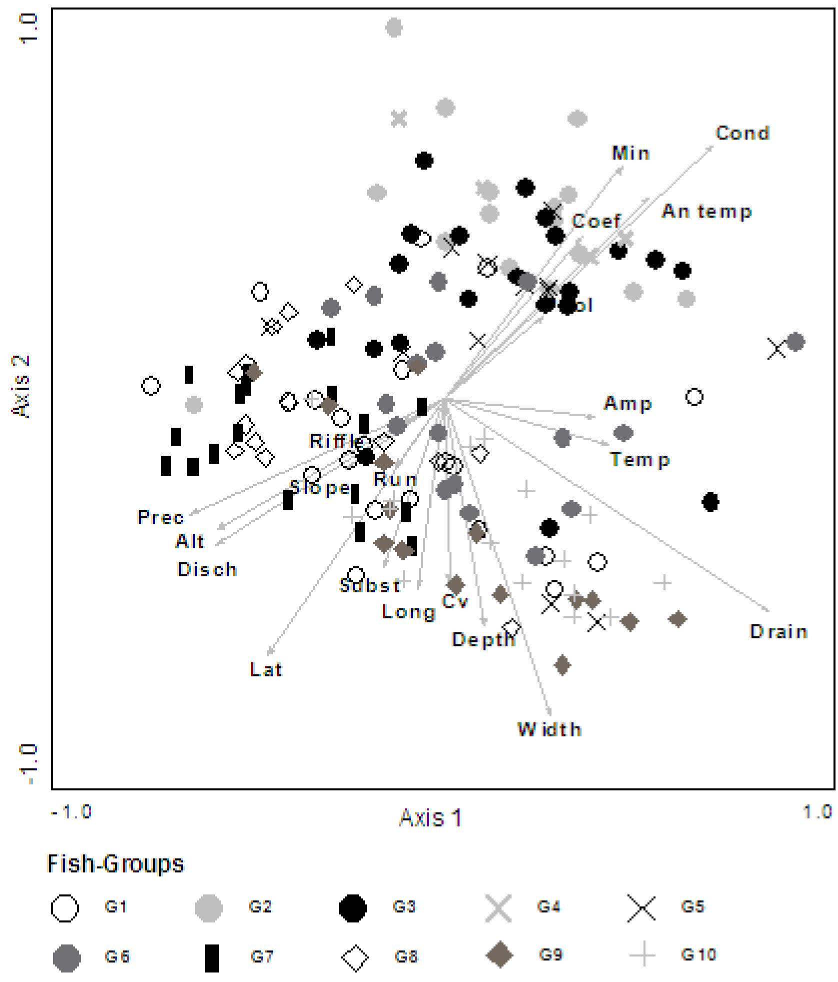

Figure 5
Ordination diagram (biplot) of Principal Components Analysis (PCA) based on the environmental variables of sampled sites. Sites are coded according to fish-groups. Variables abbreviations: latitude (Lat), longitude (Long), altitude (Alt), mineralization (Min), conductivity (Cond), mean annual air temperature (An temp), thermal amplitude (Amp), water temperature (Temp), precipitation (Prec), coefficient of variation of precipitation (Coef), drainage area (Drain), mean annual discharge (Disch), mean stream width (width), mean water depth (Depth), current velocity (Cv), dominant substrate class (Subst).

North fish-region (fish-groups 8, 9 and 10) included most of the North river basins and presented rather similar characteristics, though less marked, to the Mountain fish-region. As such, sites generally showed low mineralization, conductivity and annual temperature, but variable altitude, flow and drainage area. *Squalius carolitertii* and *Pseudochondrostoma duriense* were the indicator species.

Littoral/Centre fish-region (fish-group 1) included streams from the Littoral area, both in the Centre and North, therefore tend to have environmental characteristics closer to the North fish-region. *Achondrostoma oligolepis* was the most abundant and indicator species.

South fish-region (fish-groups 2, 3, 4, 5 and 6) corresponded to lowland streams and included the South river basins. These streams were mainly discriminated by high annual temperature, mineralization and conductivity and low annual flow. *Squalius alburnoides* and *Squalius pyrenaicus* were the indicator species.

Table III
Largest absolute correlations between each environmental variable and the first two ordination axes of Principal Components Analysis.

	Correlation coefficients	
	Axis 1	Axis 2
Drainage area (km^2)	**0.83**	**–0.54**
Conductivity (µs·cm^{-1})	**0.69**	**0.65**
Mean annual precipitation (mm)	**–0.65**	–0.29
Mean annual discharge (mm)	**–0.58**	–0.37
Altitude	**–0.58**	–0.33
Mean annual temperature (ºC)	**0.52**	**0.52**
Mean stream width (m)	0.27	**–0.81**
Mean water depth (m)	0.10	**–0.58**
Latitude	–0.45	**–0.66**
Mineralization	0.45	**0.59**

DISCUSSION

The Iberian Peninsula was considered by Illies (1978) as an ecoregion. However, the Portuguese continental territory includes a diverse array of lotic ecosystems (permanent, intermittent and ephemeral). A large environmental gradient from North to South exists with a high spatial complexity from climatic, geomorphological and hydrological points of view. This heterogeneity causes considerable difficulties in the development of a river typology, either biotic or abiotic, and is further amplified by a long history of human-induced pressures. Indeed, Mediterranean rivers are especially susceptible to degradation due to high human settlement and progressively more intensive agricultural production. In some regions the variety and ubiquity of human pressures hampers to attain a set of reference sites completely absent from anthropogenic stress, and consequently researchers settle for 'least disturbed' or 'best available' sites. This can make reference condition less consistent, which may not correctly represent biological potential in the absence of anthropogenic disturbance (Chessman, 2001). This issue is of major importance when evaluating ecological quality through the deviation from reference condition (Hawkins *et al*., 2010), being fundamental to consider changes that these modifications entail and evaluate their ecological influence (Monaghan *et al*., 2008). In this study, reference sites were selected in compliance with the WFD normative and objectives. Even when least disturbed sites were considered, this didn't compromise the set of a reference condition, as no significant differences were detect between undisturbed and least disturbed sites ($R < 0.5$ and $P > 0.05$).

Introduction of new species and other human interventions can influence assemblage composition in streams (Vila-Gispert *et al*., 2002). Homogenization of regional fish faunas by widespread species introductions or extinction of endemic species may blur the roles played by historical factors such as colonization and speciation in shaping local assemblages (Rahel, 2000; Olden and Poff, 2003; Clavero and Garcia-Berthou, 2006; Leprieur *et al*., 2008;). In the present study, the definition of fish-groups/regions were not influenced by this effect, as collected data showed low occurrence and abundance of non-native species (about 2% of fish density in all captures).

Due to long-lasting isolation periods punctuated by southward European and northward African movements, Iberian rivers generally present low species richness per site and a high

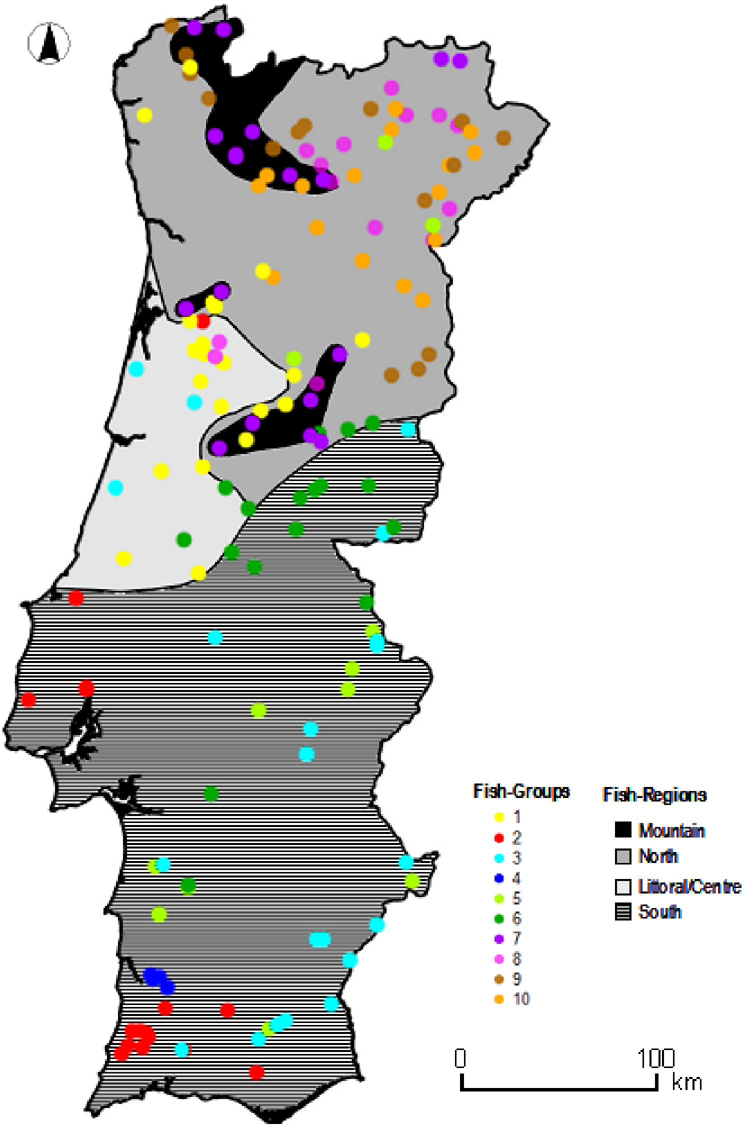

Figure 6
Map with de geographical location of the 4 Portuguese fish-regions and the projection of sampled sites according to the 10 fish-groups.

degree of endemicity (dominated by cyprinids), as many species are exclusive from a particular river basin (Ferreira and Oliveira, 2004). The isolation of the small South basins enabled the speciation of several different species, as is the case of the *Squalius* genus. In the northern part of the territory only one endemism exists – *Pseudochondrostoma duriense*.

To freshwater fish, barriers to dispersal are a major constraint to primary species distribution and strongly influence spatial patterns of assemblage composition. Barriers such as basin boundaries are frequently considered as relevant determinants of the geographical patterns of freshwater fishes and not the current environmental conditions (*e.g.* Williams *et al*., 2003; Smith and Bermingham, 2005; Filipe *et al*., 2009). Results revealed that the first level of fish-groups differentiation was due to fish composition, particularly related to occurrence of endemic species in small basins. It is the case of groups 2 and 4, in the South of Portugal, which were formed owing to the occurrence of the endemic *Squalius aradensis* in Algarve streams and *Squalius torgalensis* in the small basin of Mira river.

Ecologists increasingly recognize that local communities (*i.e.* communities sampled at a small spatial extent) are structured by a combination of historic, regional, and local factors operating at different spatial and temporal scales (Poff, 1997; Whittaker *et al*., 2001; Hoeinghaus *et al*. 2007). Whether are large-scale (Schlosser, 1991; Marsh-Matthews and Matthews, 2000; Magalhães *et al*., 2002a; Hoeinghaus *et al*., 2007) or local environmental factors (Ricklefs and Schluter, 1993; Ricklefs, 2006), the principal determinants of community structure continuous to be a widely debated issue. At larger spatial scales, historic (*e.g.* speciation and dispersal) and regional (*e.g.* geology and climate) factors determine the composition of available species from which local communities are assembled. Local factors, both biotic interactions and abiotic constraints, often dictate which of the species from the regional pool will occupy a particular community and in what abundance. Moreover regional factors can interact with local factors altering the degree to which biotic or abiotic determinants influence local community structure.

Fish assemblages patterns exhibited a strong association with large-scale spatial patterns. Fish-groups were hierarchically grouped over major and local regions, expressing a large-scale response to the North-South environmental gradient defined by temperature, precipitation, mineralization and altitude, and a regional-scale response mainly to drainage area and flow discharge. In streams with strong seasonal and annual environmental variation, fish abundance and distribution probably bear little relationship to local habitat features (Angermeier and Schlosser, 1989). This may be the case in Mediterranean streams, where the prevailing patterns of surface water distribution across stream networks result to a large extent from processes operating over large spatial scales, such as the regional climate patterns, groundwater seepage, geomorphology and catchment geometry (Sabater *et al*., 1995).

The environmental North-South gradient is followed by a considerable shift in the composition and structure of fish communities. There was an increase in species number towards South, particularly endemic cyprinids, reaching the maximum value in Guadiana river basin (Almaça, 1978). Furthermore, the North fish-groups, particularly the Mountain one, presented the lowest fish density (Shannon-Wiener Index) and diversity, while the South and the Littoral/Centre fish-groups showed the highest fish abundances.

Drainage area and flow discharge were also determinant factors in distinguishing fish-based geographical groups. With the increase in river size, fish assemblages shifted both taxonomically and functionally (Vannote *et al*., 1980). Small streams are highly variable according to climate, morphology and flow discharge. As a result, these streams are typically inhabited by low numbers of species, which is in accordance with ecological theory of species–area relationships (*e.g.* MacArthur and Wilson, 1967; Borda de Água *et al*., 2002; Ricotta *et al*., 2002). In this study, 3–5 species were recorded in the small upland streams (within Mountain, North, and South regions), a number which can increased up to 10 species in river sites with medium to large drainage area (particularly in the lowland groups). Accordingly, similar pattern in species diversity was observed. These results are in accordance with numerous previous studies (Godinho *et al*., 1997, 2000; Carmona *et al*., 1999; Pires *et al*., 1999, 2004; Mesquita and Coelho, 2002; Magalhães *et al*., 2002a, 2002b, 2007; Clavero *et al*., 2004; Ferreira and Oliveira, 2004; Clavero and Garcia-Berthou, 2006; Mesquita *et al*., 2006; Ferreira *et al*., 2007a, 2007b). In fact, increasing local species richness along the longitudinal stream gradient is a very well known pattern (*e.g.* Oberdorff *et al*., 1995; Pont *et al.*, 1995; Grenouillet *et al*., 2004) that has been observed on almost all continents (Ibañez *et al*., 2009).

As expected, the well oxygenated, summer-cold rhithral and small permanent upland basins (*e.g.* Mountain fish-region) were inhabited by cold adapted, rheophilic and rheopar species, whereas the lowland rivers, particularly in downstream sectors, were occupied by eurytopic, and rheo-tolerant species (*e.g.* Huet, 1949; Fieseler and Wolter, 2006), clearly dominated by cyprinids. The sampled rhithral streams were generally occupied by insectivorous and sand-or gravel-spawning species, which in the case of the Mountain fish-region (fish-group 7) were mainly represented by trout, *Salmo trutta*, while in the fish-group 8 cyprinid species were dominant, particularly *Squalius carolitertii*. Within the same latitudes (*i.e.* in the northern basins), larger rivers with high flow discharge presented higher number of fish species and fish diversity. In the case of fish-group 9 fish assemblages were represented by reophilic species, both cyprinid and salmonids, with *Achondrostoma arcasii* as the dominant species, while fish-group 10 (mainly Alto-Douro) was mostly represented by potamodromous, limnophilic and omnivorous species, with fish assemblages dominated by *Barbus bocagei* and *Pseudochondrostoma duriense*.

All the southern streams and rivers showed a dominance of eurytopic, lithophilic and insectivorous cyprinids, mainly represented by roach species, *Squalius* spp. The association of these species to lowland, summer-warm, potamal streams have been reported also in other studies (*e.g.* Ferreira and Oliveira, 2004; Fieseler and Wolter, 2006).

The aggregation of fish-groups into 4 fish-based regions of continental Portugal presented a strong biological and environmental correspondence. Results further suggested including fish-group 2 in the fish-region B, unifying the geographical area of the South. The complementary use of both taxonomic and functional traits of fish assemblages was fundamental to interpret these results. The composition of traits in lotic fish communities appears to be structured along an environmental gradient (Pont *et al*., 2006, 2007; Hoeinghaus *et al*., 2007; Ibañez *et al*., 2007, 2009; Logez *et al*., 2010), whereas patterns derived from taxonomic composition reflect the role played by geographical and historical factors in species distribution (Hoeinghaus *et al*., 2007).

From North to South, and expressing an altitude and climate gradient, fish-regions related to the morphoclimatic regions (the base of abiotic typology) (Figures 1 and 6). The Mountain fish-region overlaps with the morphoclimatic region 6 of the abiotic typology. In general, this high slope mountain region showed high precipitation and low mineralization, representing the typical small trout streams. The North fish-region included rather different rivers, both lowland and altitude ones, with variable flow and drainage area, and low mineralization. This fish-region corresponds to morphoclimatic regions 3, 4 and 5. Littoral/Centre fish-region was also quite heterogeneous and includes areas both from morphoclimatic region 2 and 3. The South fish-region (merging fish-groups 2, 3, 4, 5 and 6) included intermittent lowland rivers, located in low altitude, with relatively low flow discharge and high conductivity. It corresponds to morphoclimatic regions 1 and 2.

Though no perfect overlap between fish-regions and the morphoclimatic regions exists, a certain agreement is observed mainly in the extremes of the environmental gradient. The validation of the abiotic typology by significantly independent fish groups/regions is important to ensure the reliability of fish assemblages in a certain river-type, and therefore the accuracy of the ecological assessment (as already discussed above).

The fish grouping obtained in this study was in general consistent with previous patterns observed for the Iberian Peninsula (*e.g.* Ferreira and Oliveira, 2004; Ferreira *et al*., 2007a). These results, along with those obtained from the other biological elements considered in the WFD, were used to set the final typology for Portuguese rivers (INAG, 2008a) and constitutes a fundamental tool in planning and managing of water resources. It allows to evaluate the changes undergone by the river ecosystem and therefore the effectiveness of mitigation and recovery measures considered necessary to accomplish the WFD objectives.

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Appendix 1
Description, assessment scale and methods, and scoring criteria of the 10 variables used to evaluate the level of anthropogenic disturbance in each sampled site and to select reference sites.

Variables	Description	Assessment scale	Score	Criteria	Methods
Land use	Impact of farming/ forestry practices	River segment	5	> 40% Agricultural use (intensive agriculture), very severe impact (rice field)	Local expert assessment complemented with Corine Land Cover (2000, 2006)*
			4	> 40% Strong impact (area with strong forestry, including clearcuts)	
			3	<40% Moderate impact (subsistence gardens, pastures)	
			2	<40% Small impact (cork and holm oaks, high-growth forest)	
			1	<10% No significant impacts (natural forest and bush)	
	Land cover and bankface characterization	Local	5	Irrigated crops and/or high stocking	
			4	Horticultural crops, semi-intensive grazing	
			3	Extensive cultures (*e.g.* pastures, cereal crops, pine, eucalyptus), extensive grazing	
			2	Cork and holm oaks	
			1	Natural	
Urban area	Impact of urban areas	River segment	5	Very severe (location near a city with basic sanitation needs)	Local expert assessment complemented with Corine Land Cover (2000, 2006)*
			4	Town	
			3	Village	
			2	Hamlet	
			1	Negligible (isolated dwellings)	
Riparian vegetation	Deviation from the natural state of the riparian zone	River segment	5	Lack of riparian shrubs and trees (only the presence of annual plants)	Local expert assessment
			4	Fragmented vegetation with bushes and/or the presence of reed	
			3	Second replacement step (dominance of dense brushwood)	
			2	First replacement step (presence of shrub or tree strata with some level of preservation).	
			1	Potential vegetation (presence of shrub and tree strata according to the geo-series)	

Appendix 1.
(Cont.).

Variables	Description	Assessment scale	Score	Criteria	Methods
Morphological condition	Deviation from the natural state of the stream bed and banks	Local	5	Transverse and longitudinal profile of the channel completely changed, with very few habitats	Local expert assessment
			4	Channelised sector, missing most of the natural habitats	
			3	Channelised sector, missing some types of natural habitats, but maintaining much of the shape of the natural channel	
			2	Poorly changed sector, close to the natural mosaic of habitats	
			1	Morphological changes absent or negligible	
Sediment load	Deviation from the natural sediment load (both carried in the water column and deposited on the riverbed)	River segment and local	5	>75% of coarse particles of the stream bed are covered with fine sediments (sand, silt, clay)	Local expert assessment
			4	50–75% of coarse particles of the stream bed are covered with fine sediments (sand, silt, clay)	
			3	25–50% of coarse particles of the stream bed are covered with fine sediments (sand, silt, clay)	
			2	5–25% of coarse particles of the bed are covered with fine sediments (sand, silt, clay)	
			1	<5% of coarse particles of the stream bed are covered with fine sediments (sand, silt, clay)	
Hydrological regime	Deviation from the natural hydrological regime (flow pattern and/or quantity). Includes all sources of hydrologic alteration, such as significant water abstraction	Local	5	<50% and strong deviation from the natural variability of the flow regime	Local expert assessment complemented with SNIRH
			4	<50% and moderate deviation from the natural variability of the flow regime	
			3	> 50% and duration of flood periods close to the natural	
			2	> 75% and duration of flood periods close to the natural	
			1	> 90% and normal duration of natural flood periods	
		Local	5	<10% of mean annual discharge	
			4	<15% of mean annual discharge	
			3	> 15% of mean annual discharge	
			2	> 30% of mean annual discharge	
			1	> 90% of mean annual discharge	

Appendix 1.
(Cont.).

Variables	Description	Assessment scale	Score	Criteria	Methods
Toxic and acidification levels	Deviation from the natural state of toxicity conditions, including acidification and oxygen levels	Local	5	Constant for long periods (months) or frequent occurrence of strong deviations from natural conditions (*e.g.* pH < 5.0, DO < 30%)	Local expert assessment complemented with SNIRH
			4	Constant for long periods (months) or frequent occurrence of strong deviations from natural conditions (*e.g.* pH < 5.5, DO < 30–50%)	
			3	Occasional deviations (single measurements or episodic) in relation to natural conditions (*e.g.* pH < 5.5, DO < 30–50%)	
			2	Occasional deviations (single measurements or episodic) in relation to natural conditions (*e.g.* pH <6.0)	
			1	Conditions within the normal range of variation	
Organic and nutrient loads	Deviation from the normal values of BOD, COD, ammonium, nitrate and phosphate concentrations	Local	5	> 20% of values in classes D or E	SNIRH (classification of water quality for multiple uses, according to the guidelines from the Water National Institute**), complemented with local expert assessment
			4	> 10% of values in classes D or E	
			3	> 10% of values in class C	
			2	No obvious or too small signs of eutrophication and organig loading	
			1	No signs of eutrophication and organic loading	
Artificial Lentic water bodies	Impact related to the presence of artificial lentic water bodies upstream and/or downstream of the site (upstream change in thermal and flow regimes; downstream invasion by exotic species of lentic character)	Local	5	Local immediately downstream of a large reservoir or within the influence area of its backwater	SNIRH and available cartography
			4	Local immediately downstream of a mini-hydro or within the influence area of its backwater	
			3	Local downstream of a large reservoir or within the influence area of the reservoir	
			2	Local downstream of a mini-hydro or within the influence area of its backwater	
			1	No influence of reservoirs	

Appendix 1.
(Cont.).

Variables	Description	Assessment scale	Score	Criteria	Methods
Connectivity	Impact of artificial barriers to fish migration	River basin and segment	5	Permanent artificial barrier	SNIRH, available cartography, documental data and local expert assessment
			4	Occasional passage of some species	
			3	Passage of certain species or only in certain years	
			2	Passage of most species in most years	
			1	No barriers or existence of an effective pass-through device	

* Caetano *et al.*, 2009. ** Information available at http://snirh.pt/snirh/_dadossintese/qualidadeanuario/boletim/tabela_classes.php.