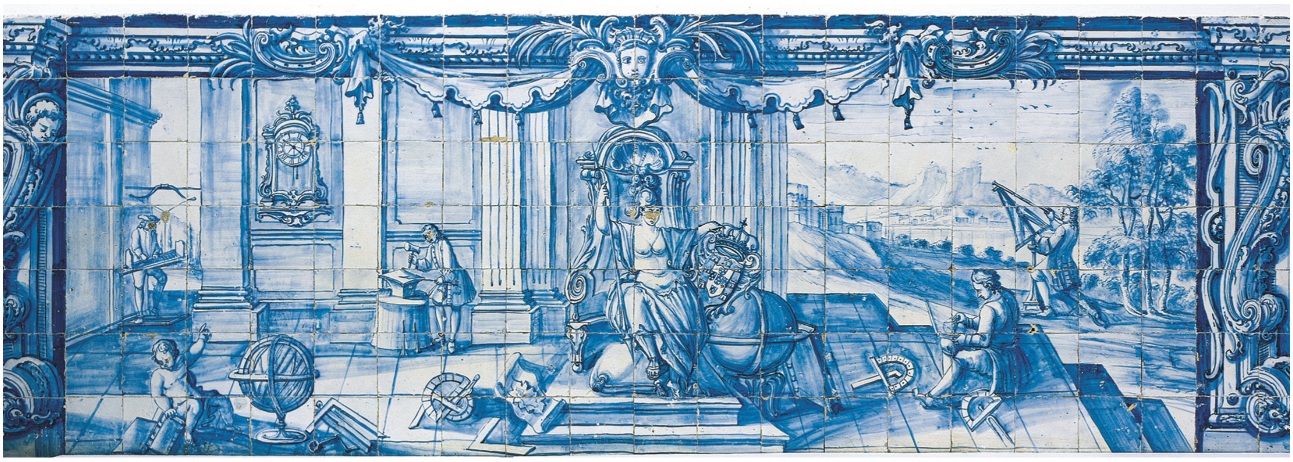
FISH ASSEMBLAGES AS BIOLOGICAL INDICATORS OF ECOLOGICAL QUALITY IN PORTUGUESE RIVERS

Paula Rute Pereira Matono Alves

Tese apresentada à Universidade de Évora
para obtenção do Grau de Doutor em
Ciências do Ambiente

ORIENTAÇÃO: *Doutora Maria Ilhéu, Universidade de Évora*
CO-ORIENTAÇÃO: *Doutor Thierry Oberdorff, Muséum National d'Histoire Naturelle, Paris*

ÉVORA, 2012



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Esta tese beneficiou do apoio financeiro da Fundação para a Ciência e Tecnologia (Bolsa de doutoramento SFRH/BD/23435/2005) e do Instituto de Investigação e Formação Avançada da Universidade de Évora.

Aos meus pais

AGRADECIMENTOS / ACKNOWLEDGEMENTS

À Doutora Maria Ilhéu, minha orientadora, por me ter proporcionado a realização desta tese, pelo constante entusiasmo, dinamismo e perseverança, mesmo nos momentos de maior desânimo e nas longas horas, mesmo muito longas, de discussão de dados. O meu reconhecimento também pela amizade resultante de treze anos de trabalho em conjunto.

Ao Doutor João Bernardo, por todo o acompanhamento e revisão da tese, pela inspiradora mente inquieta, pelas sessões de trabalho únicas e por sempre me ter transmitido o valor de nos pautarmos por elevados critérios de exigência e rigor.

To Dr. Thierry Oberdorff, my co-advisor, for giving me the opportunity to share his knowledge. For all the kindness and invaluable comments and insightful reviews of the thesis.

À Doutora Ana Costa, pelo apoio e enorme pragmatismo quando as sessões de trabalho em conjunto se afiguravam desesperantes e mesmo dramáticas!

Aos meus colegas Luísa Sousa, João Matos, Ana Curto e Santiago Suarez, pelos fantásticos anos de trabalho e aprendizagem em conjunto, com muitas aventuras e desventuras partilhadas no gabinete, laboratório e campo. Um agradecimento especial à Luísa, pela amizade, cumplicidade e longas horas de luta em frente ao computador com tratamentos estatísticos, redacção de textos e afins.

Ao Dárcio Sousa, por todo o companheirismo, esforço e dedicação com que enfrentou comigo alguns dos momentos mais árduos do trabalho de campo, e pela ajuda nas questões de cartografia.

Ao Luís Santa Maria, pela infindável amabilidade e disponibilidade, pelas maravilhosas estórias e deliciosas romãs, e claro, pelo inestimável empenho em me ensinar a manobrar o Jeep com o reboque do barco!

Ao Paulo Alves, Rebecca Hale, António Vareia, Sofia Ramalho, Cleyton Calderan, Luís Mexia de Almeida e todos os voluntários que se dispuseram a ajudar nas muitas campanhas de campo.

Ao Pedro Guilherme, que fez parte da equipa durante os entusiastas anos de recolha dos dados anteriores a 2004 utilizados nesta tese.

À Rute Caraça, pela excelente companhia, boa energia e motivação em alguns momentos difíceis, pela mãozinha na pesca eléctrica e pelo pouco que sei sobre macrófitas!

À Márcia Barbosa, pela disponibilidade para ajudar nas questões de cartografia e esclarecimento de algumas dúvidas estatísticas.

A todas as pessoas que integraram equipas a nível nacional e participaram na recolha de dados de diferente natureza entre 2004 e 2006 durante a fase de implementação da Directiva Quadro da Água, designadamente Universidade de Trás-os-Montes e Alto Douro, Universidade do Porto, Universidade de Coimbra, Universidade de Aveiro, Universidade Nova de Lisboa, Universidade Técnica de Lisboa e Universidade de Évora.

À Fátima Mendes e Maria José Barão, por assegurarem toda a logística do Laboratório de Ecologia Aquática e a realização de parte das análises da água. Uma palavra especial para a Fátima, pela enorme simpatia, disponibilidade e apoio nas tarefas de laboratório ao longo de todos estes anos.

Às meninas do Secretariado do Departamento de Paisagem, Ambiente e Ordenamento, Rita Menezes, Vanda Prazeres e Arlete Sousa, por toda a simpatia e boa disposição.

Aos amigos que foram esperando (e desesperando!) pela entrega da tese, pelo apoio demostrado, mesmo sem perceberem muito bem o que ando para aqui a fazer!

Aos amigos de sempre, Carla, Luís, Tátá, Nuno, Catarina, Filipa e Raquel, que também viveram as agruras de um doutoramento, ou simplesmente sofreram os danos colaterais! À Tátá, e especialmente à Carla, por aturarem neuras, desabafos e crises existenciais! Ainda à Carla tenho que agradecer a preciosa ajuda na maratona final de revisões e artes gráficas.

À minha família Tomarense, os meus sogros, cunhados e belíssimo ramalhete de sobrinhos, pelo carinho e preocupação.

Aos meus pais, meu eterno porto seguro e melhor exemplo de carácter, a quem devo absolutamente tudo, pela admiração e apoio incondicionais em todos os momentos e por serem os melhores avós do mundo.

Ao Francisco, meu *wing man* a quem confiei o meu percurso de vida, por todo o carinho, estímulo e dedicação enquanto marido, amigo e pai, que me permitiram manter o equilíbrio e a

sanidade mental. Pela paciência para lidar com os meus stresses e toda a ajuda em muitos dias de trabalho de campo.

Ás minhas filhas, Margarida e Catarina, a melhor coisa que alguma vez fiz, por generosamente me concederem o maior dos privilégios: ser mãe!

RESUMO

No contexto da aplicação da Directiva-Quadro da Água, a ictiofauna é considerada como um relevante elemento biológico. Pela primeira vez foi desenvolvido um sistema de classificação para avaliação do estado ecológico dos ecossistemas aquáticos em Portugal, apesar dos constrangimentos impostos pela variedade e ubiquidade das pressões humanas e pelas peculiaridades dos agrupamentos piscícolas. Foram definidos grupos piscícolas para posterior estabelecimento de uma tipologia nacional de rios, foram seleccionadas métricas responsivas ás pressões humanas e foi desenvolvido um índice biótico piscícola. Os resultados evidenciam a necessidade de diferenciar correctamente a resposta da ictiofauna ás perturbações naturais e antropogénicas, considerando quer a variabilidade espacial, quer temporal dos agrupamentos piscícolas, típica dos cursos de tipo Mediterrânico. O uso de solo tem fortes consequências na degradação dos rios e na integridade da ictiofauna, sendo importante que a avaliação dos impactos considere a interacção entre diferentes pressões. A compreensão da influência relativa dos factores ambientais e humanos na ocorrência e abundância das espécies não nativas permitiu evidenciar o potencial papel das mesmas como indicadores biológicos. Esta tese contribuiu para o desenvolvimento e aperfeiçoamento de ferramentas de avaliação ecológica em Portugal, permitindo igualmente um aumento do conhecimento sobre a ecologia das comunidades piscícolas e funcionamento dos ecossistemas em rios de tipo Mediterrânico, com vista à sua conservação e reabilitação.

Palavras-chave: Fauna piscícola; avaliação ecológica; tipos de rios; índice biótico; perturbação natural e antropogénica.

ABSTRACT

Under the implementation of the Water Framework Directive fish fauna is considered a useful biological element. For the first time a Portuguese classification system to assess the ecological status of aquatic ecosystems was established, despite several constrains posed by the variety and ubiquity of human pressures and by the peculiarities of the fish assemblages. It was possible to define fish-based geographical groups for the subsequent establishment of a national river typology, to select metrics responsive to human pressures and to develop a fish biotic index. Results underline the needs for accurately differentiate between fish responses to natural and anthropogenic disturbances by accounting for the spatial and temporal variability of fish assemblages. Land use may lead to strong impacts on stream degradation and fish assemblages integrity and this evaluation must consider the interaction of different pressures. Understanding the influence of environmental and human factors in the occurrence and abundance of non-native species enlightened their role as a reliable tool in the ecological assessment of rivers. This thesis represents an important contribution to improve the ecological assessment tools in Portugal, while increasing knowledge on the ecology of fish communities and ecosystem functioning in Mediterranean climate rivers in view of their urgent conservation.

Keywords: Freshwater fish; ecological assessment; river-types; biotic index; natural and anthropogenic disturbance.

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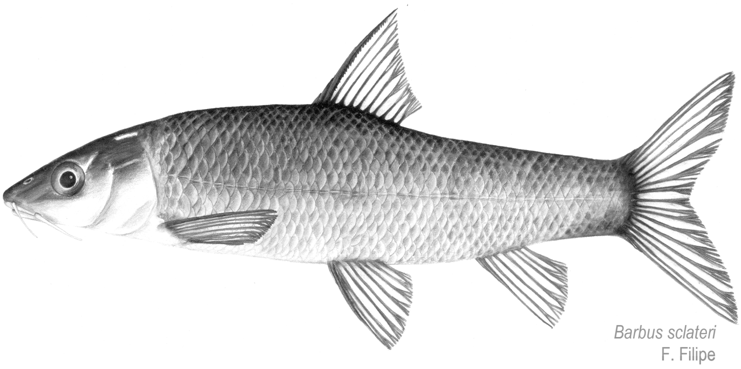
Chapter 1

General introduction



Barbus sclateri
F. Filipe

Mediterranean climate rivers: general characteristics, constrains and threats

Mediterranean ecosystems are limited to five relatively small areas around the world: the region bordering the Mediterranean Sea (Mediterranean basin), central Chile, the Cape region of South Africa, southwestern and southern Australia, and California south to northern Baja California. These ecosystems occur mainly along the western edges of continents between the 30° and 40° parallels in both northern and southern hemispheres.

The Mediterranean climate, moderated by cold ocean currents offshore, is characterized by mild, rainy winters and warm, dry summers. Seasonality and variability in rainfall is the principal attribute of the Mediterranean climate (Gasith and Resh 1999). Mediterranean climate rivers are strongly influenced by climate and river discharge depends on the rainfall pattern, thus these rivers are shaped by predictable seasonal events of flooding and drying over an annual cycle, but also presenting a strong interannual flow variation (Gasith and Resh 1999). Accordingly, aquatic communities undergo a yearly cycle whereby abiotic (environmental) controls that dominate during floods are reduced when the discharge declines, which is also a time when biotic controls (e.g. predation, competition) can become important. As the dry season progresses, habitat conditions become harsher and environmental pressures may again become the most important regulators of stream populations and communities structure (Gasith and Resh 1999).

Portugal is located in the largest area of Mediterranean climate in the world, presenting a complex climatic and topographic diversity. A decrease in precipitation from North to South and from West (littoral) to East (interior) is observed, whereas temperature shows an opposite pattern. Rainfall is mostly concentrated between October and March, and the dry summer period extends from June to September. The South is quite homogeneous, mostly represented by lowland streams and rivers (flat, less rainy, with long hot dry summers), while the North presents a much more complex climatic and geomorphological patchiness, with higher average altitudes, higher annual precipitation, and milder summers in general. Consequently, most rivers are permanent in the North and intermittent in the South.

Under this scenario, intermittent rivers are particularly affected by natural hydrological variability, as during the dry period they are completely dry or reduced to scattered and isolated pools, which serve as refugia for fish until fluvial connectivity is restored (Schlosser 1991; Magalhães et al. 2002a). Flow intermittence is an extreme form of flow variation and one that has major pervasive ecological effects (Larned et al. 2010), thereby strongly conditioning community patterns and ecological processes in rivers (Poff and Ward 1989). In the future, this situation can be aggravated in regions that predictably experience drying trends due to climate change and by water abstraction for socio-economic uses (Larned et al. 2010) as expected in Mediterranean regions.

The environmental variability generated by natural disturbances subjects the native fish fauna to natural harsh conditions, promoting the development of a variety of species adaptations such as short lifespans, rapid growth rates and high fecundity (e.g. Vila-Gispert and Moreno-Amich 2002; Clavero et al. 2004), as well as behavioural adaptations (Magalhães et al. 2002a,b; Magoulick and Kobza 2003; Ilhéu 2004) to cope with the environmental stressors and associated changes in habitat conditions. In that sense, resistance (the capacity to withstand) and resilience (the capacity to recover) are common attributes found in the elements of the biota subjected to highly variable flow regimes, as observed in Mediterranean climate rivers (Gasith and Resh 1999; Lake 2011).

In addition to the harsh natural conditions, Mediterranean climate areas have been exposed since many years ago to human disturbances. In nowhere else in the world the endemic fauna is declining as rapidly as in Mediterranean regions because facing important threats (Smith and Darwall 2006; Ribeiro et al. 2009) due to non-native introductions, anthropogenic impacts (such as damming, water abstraction and land use changes and intensification) or limited availability of water and unsatisfactory management (Smith and Darwall 2006; Ribeiro et al. 2009; Hermoso and Clavero 2011).

Patterns of fish assemblages in rivers

The organization of biotic assemblages in rivers is influenced by a variety of abiotic and biotic factors operating at a range of spatial and temporal scales (Poff 1997; Whittaker et al. 2001; Hoeinghaus et al. 2007; Hugueny et al. 2010). At larger spatial scales, factors related to energy availability, climate, habitat availability and history determine the composition of available species from which local communities are assembled (Oberdorff et al. 2011). Local factors, both biotic interactions and abiotic constrains, often dictate which of the species from the regional pool will occupy a particular community and in what abundance. Additionally, regional factors can interact with local factors altering the degree to which biotic or abiotic determinants influence local community structure, and their relative importance may be dependent on the scale of the study (Jackson et al. 2001).

The identification of environmental gradients structuring fish assemblages in rivers constitutes a major challenge for ecologists and is fundamental to understand the functioning of these systems. Several conceptual models analysed the ecological process operating in rivers and have led to progressive insights into the relative importance of abiotic and biotic factors along spatial and temporal scales in driving aquatic ecosystems structure and function (e.g. Vannote et al. 1980; Elwood et al. 1983; Frissel et al. 1986; Junk et al. 1989; Townsend 1989; Ward 1989).

Flow variability is one of the most important determinants of ecological patterns and processes in rivers (Poff and Ward 1989; Poff and Allan 1995; Richter et al. 2003), playing an important role in structuring fish assemblages in Mediterranean streams (Marchetti and Moyle 2001; Magalhães et al. 2002b; Magalhães et al 2007) and completely shapes their ecological features, as well as habitat patchiness and availability (Bernardo et al. 2003; Ilhéu 2004). Because streamflow conditions exerts control over many important structural attributes in streams (e.g. habitat volume, current velocity, channel geomorphology, and substratum stability), flow measures represent an integration of complex environmental conditions (Poff and Ward 1989).

Both taxonomic and functional approaches can be used to study fish community organization, in a fruitful complementary way (Hoeinghaus et al. 2007). This knowledge is fundamental to allow detecting and measuring deviations from the natural condition resulting from responses to anthropogenic disturbances, and hence to develop and improve management tools and rehabilitation programs in streams.

Introduction of new species and other human interventions can also influence assemblage composition in streams (Vila-Gispert et al. 2002), promoting faunal homogeneization and constituting possible confounding factors (Scott and Helfman 2001), therefore highlighting the need to accurately distinguish natural environmental variability and drivers from anthropogenic disturbances, especially in the context of ecological assessment.

Human impacts on freshwater ecosystems

Society relies on freshwater systems for drinking water, food, commerce and recreation, as well as waste removal, decomposition and aesthetics (Karr and Chu 1999). Consequently, these systems, belonging to the most intensively used and altered ecosystems of the world (Saunders et al. 2002), have been broadly impaired and continue to deteriorate as a result of human activities. The conservation of the biological elements of freshwater systems, as well as the processes sustaining them, is crucial to maintaining the goods and services fresh water provides (Karr and Chu 1999).

Human activities can disturb relationships between fish and the environment by direct action on the communities (e.g. introduction of alien species, chemical and organic pollutions) or indirect action by modification of the ecosystem (e.g. channel and bank modifications, flow regulation and fragmentation) (Karr and Chu 1999).

Rivers are strongly influenced by their surrounding landscape at multiple scales (Schlosser 1991; Allan et al. 1997; Fausch et al. 2002; Townsend et al. 2003), thus land use is emphasized as a major factor affecting the ecological integrity of fluvial ecosystems (e.g. Roth et al. 1996; Lammert and Allan 1999; Allan 2004). Allan (2004) highlighted the importance of land use impacts and identified six main factors of influences on aquatic ecosystems: sedimentation, nutrient enrichment, contaminant pollution, hydrologic alteration, riparian clearing/canopy opening and loss of large woody debris.

Land uses can be roughly summarised in three categories: agricultural, urban and natural (usually forested). Agriculture occupies by far the largest fraction of land area in many developed catchments (Allan 2004) like in Mediterranean regions. This situation is also observed in Portugal, but the agricultural practices have been changing over the last decades towards progressively more intensive and irrigated agriculture, due to the perspective of high profits promoted by the European Common Agriculture Policy (Pinto-Correia and Vos 2004). Olive production is a good example, as hyper-intensive olive groves are increasing exponentially, and incorporating new approaches of production with high uptakes of energy and water at the natural resources expenses.

In Mediterranean climate regions human settlements rely extensively on streams and rivers to meet their water demands for agriculture, industry and domestic consumption (Gasith and Resh

1999), withdrawal of water for agricultural irrigation representing the largest fraction. Moreover, most of the human water demands in Mediterranean climate areas occur in the dry summer months, when water is required for agricultural irrigation and yet precipitation is rare. This asynchrony between water availability and demands, together with high interannual variability of Mediterranean river flows makes water management critical to the conservation of these freshwater ecosystems (Grantham et al. 2010).

With a predominant intermittent character, southern Portuguese streams are particularly vulnerable to these practices, as many river basins impacted by olive intensive/irrigated farming already suffer from excessive water abstraction, morphological degradation and poor water quality. Balancing freshwater ecosystems integrity with intensive agricultural practices under a scenario of water deficit constitutes a major challenge for southern European countries, including the Portuguese streams.

Ecological assessment of rivers

The assessment of the biotic or ecological integrity of water bodies is a central issue to water policies and to nature conservation in general, being defined as “the ability to support and maintain a balanced, integrated, adaptative community of organisms having a species composition, diversity and functional organization comparable to that of the natural habitat of the region” (Karr and Dudley 1981). In Europe, the implementation of the Water Framework Directive (WFD) (European Commission 2000) underlined the central role of biological indicators to assess the ecological status of rivers, requiring its evaluation based on four biotic elements (diatoms, macroinvertebrates, macrophytes and freshwater fish). According to the WFD, all Member States have to assess, monitor, and where necessary, improve the ecological status of surface waters, seeking to achieve at least ‘good ecological status’ by 2015.

Regarding the assessment methods, two different approaches can be distinguished: spatially based or type-specific methods (Schmutz et al. 2007) and site-specific methods (Oberdorff et al. 2002; Pont et al. 2007). Both approaches are based on the reference condition approach (Reynoldson et al. 1997; Bailey et al. 2004), i.e. deviations in ecological quality are established as the difference between expected (reference conditions) and observed conditions. Under this perspective, reference condition is defined as the expected background condition with no or minimal anthropogenic stress. Type-specific and site-specific methods differ in the way they establish the reference conditions for a given site under evaluation. Type-specific approaches rely on grouping techniques (Roset et al. 2007) to cluster reference sites in a set of homogeneous landscape groups environmentally discriminated and biologically meaningful. The site-specific approach does not require any classification and it simply finds specific reference conditions for every new given site according to its environmental characteristics (Roset et al. 2007).

The spatially based approach is the underlying methodological principle in WFD for assessing the ecological status of running waters. Accordingly, the definition of river-types to classify rivers and streams is the first and fundamental step for the ecological assessment, allowing comparisons between groups of streams, and constituting classes for which assessment procedures can be developed and applied. In this sense, accurate estimation of reference assemblage characteristics is therefore crucial and obviously depending both on accurate site selection criteria and on standardised sampling protocols.

The Portuguese continental territory includes a diverse array of lotic ecosystems (permanent, intermittent and ephemeral) and a high spatial complexity regarding climatic, geomorphological and hydrological features. A type-specific approach is then a way to stratify the spatial variability in streams and rivers monitoring and assessment.

In Portugal, before the implementation of the WFD no attempts had been made to establish a river typology or a classification system to assess the ecological status of aquatic ecosystems. Indeed, the process faced considerable constrains, namely the absence of biological monitoring programs, the lack of available databases and the lack of standardised sampling protocols for biological elements.

Fish as biological indicators

The use of biological indicators has gained increasing importance in the quality assessment of inland waters. In this context, fish fauna did not receive as much attention as other biological quality elements (BQE), such as invertebrates or diatoms. The reasons for this situation are related to the particular difficulties of that taxonomic group, such as limited knowledge on the tolerance of some species, differences on the fish fauna occurring in the river basins of a country or region, and several sampling difficulties.

In spite of some undeniable difficulties, fish fauna is considered today as a quite promising BQE. Fish fauna is sensitive to different kinds of pressures and, as some fish species are long lived, they respond to larger time scales than the other BQE, making them a potentially useful element in the biotic assessment. Moreover, some fish species represent the upper trophic levels and thus show an integrated view of the ecosystem, revealing possible problems occurring at lower levels (Barbour et al. 1999).

Regarding fish indexes, the importance of Karr’s (1981) multimetric Index of Biotic Integrity should be mentioned as a benchmark and many multimetric indexes developed afterwards in quite different ecological conditions and for different faunas are adaptations of this pioneer fish index (e.g. Oberdorff and Hughes 1992; Oberdorff et al. 2002; Angermeier and Davideanu 2004; Pont et al. 2006). In southern Europe countries, particularly in Portugal, this topic is still relatively undeveloped. The existing approaches are regional and/or mainly based on expert knowledge and not on sensitivity analysis (Ferreira et al. 1996; Oliveira and Ferreira 2002; Bernardo et al. 2004; Magalhães et al. 2008).

Due to zoogeographical isolation, fish assemblages from Iberian Peninsula show high degree of endemism, low number of species per site, high dissimilarity in assemblages composition among river basins, and high ecological plasticity of species (Almaça 1995). Moreover, they are naturally adapted to constraining environmental conditions and present high seasonal and inter-annual variability (e.g. Magalhães et al. 2002b; Bernardo et al. 2003; Ilhéu 2004), determined by the profound influence of the Mediterranean climate. All these aspects hamper the use of fish fauna as evaluators of anthropogenic pressures exerted on the systems (namely the hydrological ones), and consequently the development of tools to assess the ecological status of Mediterranean climate streams. Furthermore, under human pressure circumstances fish fauna may be more vulnerable to the natural harsh conditions and disturbances, with possible implications on the ecological assessment. Therefore, to use fish as reliable indicators it is crucial to distinguish between the effects of anthropogenic and natural disturbance (Oberdorff et al. 2002; Pont et al. 2006), as well as evaluating the possible interaction of both factors on fish assemblages structure and function.

Whether or not non-native fish species should be considered in the ecological assessment of rivers is a very debated question. Non-native species are considered one of the major threats to the conservation of native fish communities (e.g. Clavero et al. 2004). The impact of non-native fish species seems to be related to human disturbance, as their populations may benefit from a certain level of ecosystem degradation, and they have negative direct or indirect effects on biodiversity, causing decline of native population with risk of extinction of endangered species (Manchester and Bullock 2000).

Aims and structure of the thesis

This thesis was partially developed under the implementation of the Water Framework Directive (WFD) in continental Portugal and aims to:

1. Determine the environmental factors that, in the absence of anthropogenic pressures, influence the structure and distribution of fish assemblages both at a regional (basin) and local scale (stream reach and habitat);

2. Define fish-based river-types and characterize fish assemblages under reference conditions for each one, therefore contributing to the establishment of the national river typology in compliance with the WFD goals;

3. Select the fish metrics (structural and functional) that best reflect the changes in fish communities subjected to anthropogenic pressures and develop a fish-based biotic index to evaluate the ecological quality in Portuguese rivers;

4. Investigate the influences of environmental variability on fish response to anthropogenic pressure and the consequent effects on the ecological assessment;

5. Assess the biological response to the most important anthropogenic pressures identified in rivers, i.e. the way fish assemblages structure and function change along a gradient of human pressure;

6. Examine the relationship between non-native fish species and anthropogenic disturbance and their potential relevance on the ecological assessment of Portuguese rivers.

The thesis comprises 7 chapters, five of them corresponding to papers published, in review or submitted to scientific journals. For that reason, these chapters are organized into introduction, methods, results and discussion. References used are listed for each chapter separately.

Chapter 1 is a general introduction and includes a synthesis of current knowledge on the issues in order to contextualize the objectives.

Chapter 2 identifies fish-based geographical groups for continental Portugal using taxonomic and functional traits and their relationship with environmental and geographical descriptors. A characterization of fish assemblages in each group is made, constituting the reference condition under the absence of significant anthropogenic pressures.

Chapter 3 presents the selection procedure of biological metrics responsive to different types of anthropogenic pressures and the development of a biotic index to assess the ecological status of Portuguese rivers based on fish fauna.

Chapter 4 addresses the effects of hydrological variability on the responses of fish assemblages to anthropogenic pressures in small intermittent streams, where this environmental factor constitutes the major source of natural disturbance and plays a key role structuring fish assemblages, therefore leading to possible implications on the accuracy of the ecological assessment.

Chapter 5 stresses the effects of land use intensification on fish assemblages integrity in Mediterranean climate streams of southern Portugal, specifically focusing on the main environmental disturbances resulting from the accelerated change in land use towards intensive farming systems, as olive production.

Chapter 6 investigates the possible relevance of non-native fish species in the ecological assessment of rivers, namely through evaluation of the environmental drivers that favour their success and of the relative importance of environmental variables and human-induced pressures for their occurrence and abundance in Portuguese rivers.

Chapter 7 summarises and integrates the main findings arising from the results obtained in each chapter, and identifies areas of future research that should further strengthen the conclusions.

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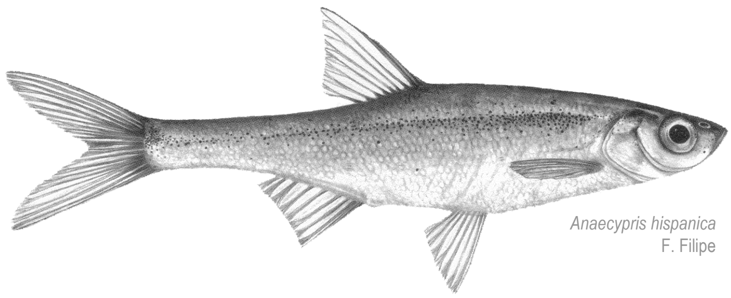
Chapter 2

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



Published as

Matono P, Bernardo JM, Ferreira MT, Formigo N, Raposo de Almeida P, Cortes R, Ilhéu M. (2012) Fish-based groups for ecological assessment in rivers: the importance of environmental drivers on taxonomic and functional traits of fish assemblages. Knowledge and Management of Aquatic Ecosystems 405, 04 DOI: 10.1051/kmae/2012010.

Abstract

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.

Keywords: Freshwater fish; Water Framework Directive; river-types; environmental drivers; Portugal.

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 2.1). 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.

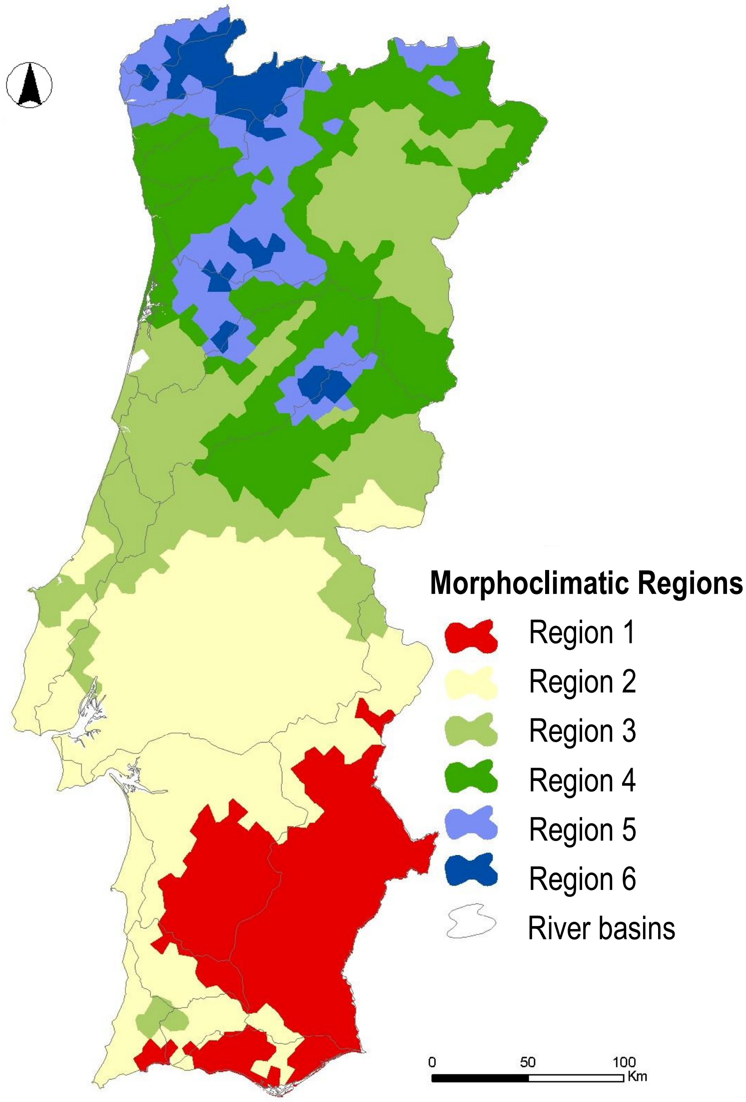


Figure 2.1 Major morphoclimatic regions identified for continental Portugal (Alves et al., 2004; INAG, 2008a).

Sampling

Sampling was carried out during spring between 2004 and 2006 in 155 undisturbed sites in the main Portuguese river basins (Figure 2.2). 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 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).

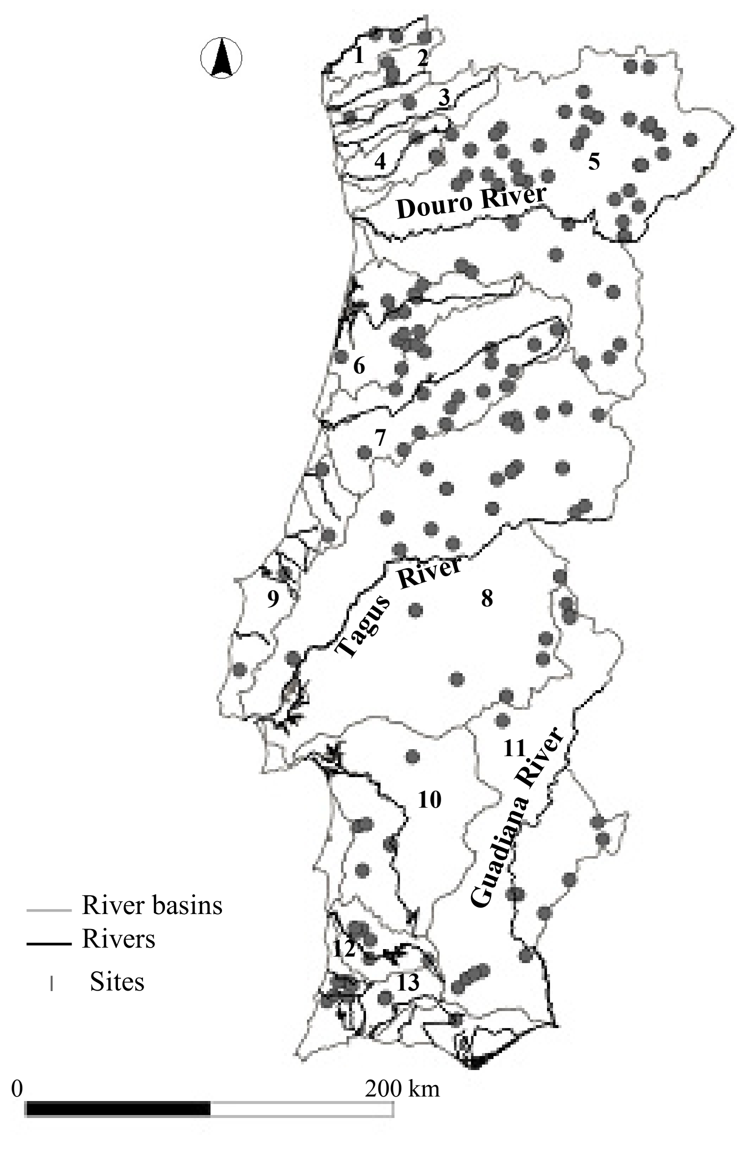


Figure 2.2 Map of the sampled sites in the principal continental Portuguese river basins: 1-Minho, 2-Lima, 3-Cávado, 4-Ave, 5-Douro, 6-Vouga, 7-Mondego, 8-Tagus, 9-West streams, 10-Sado, 11-Guadiana, 12-Mira, 13-Algarve streams.

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/L), chemical oxygen demand - COD (mg/L), oxidability (mg/L), total suspended solids - TSS (mg/L), total dissolved phosphorous – P (mg/L), nitrite - NO_2- (mg/L), nitrate - NO_3- (mg/L), ammonium - NH_4+ (mg/L) and total dissolved nitrogen - N (mg/L).

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 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 (μS/cm), pH, dissolved oxygen (mg/L), mean stream wetted width (m), maximum and mean water depth (m), mean current velocity (m/s), 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) (Appendix 2). The 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 2.3), further characterized concerning fish metrics and guilds (Table 2.1).

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 = 37.7ind/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.

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*.

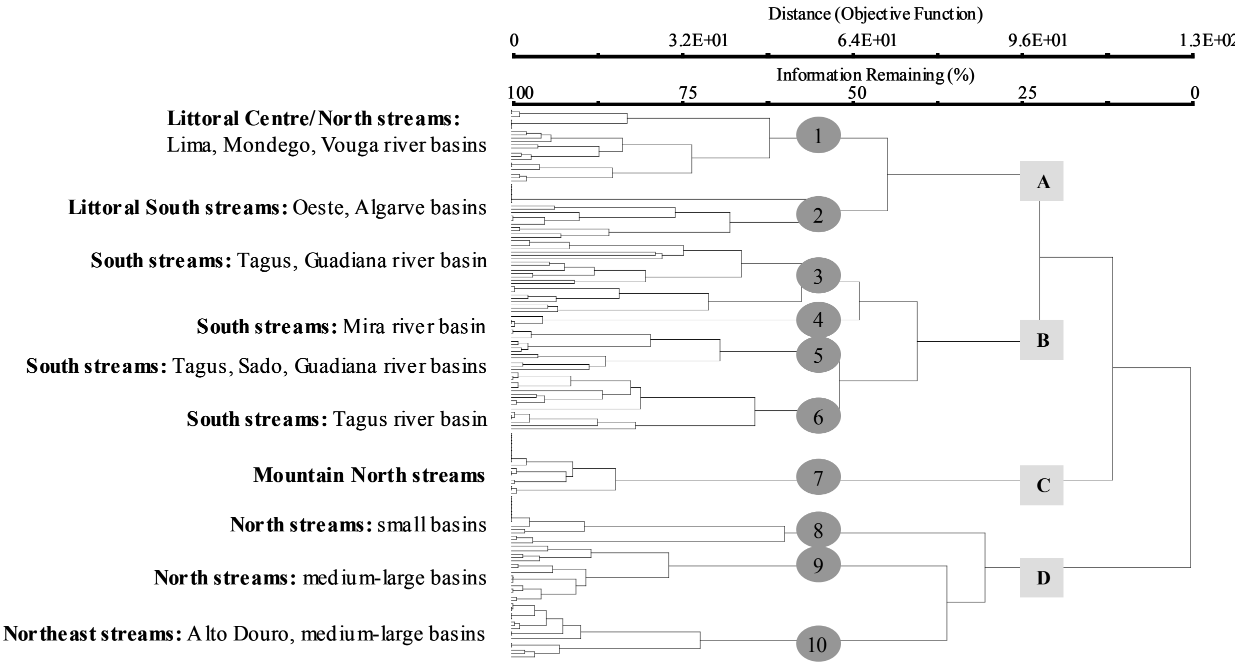


Figure 2.3 Cluster of the sampled sites based on fish species densities identifying 10 fish-groups and 4 fish-regions.

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.

Table 2.1 Characterization of the ten fish-groups based on metrics and guilds (mean ± SD)

		Fish-Groups									
	Metrics and Guilds	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
	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
	Total 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 guild	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 guild	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 guild	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

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).

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 m2; 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 2.4). 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.

ANOSIM based on fish composition

ANOSIM based on fish metrics and guilds

	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 2.4 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).

Concerning metrics and guilds, in general, ANOSIM results revealed lower R values than for fish composition (Figure 2.4). 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 (Figure 2.3 and Figure 2.6): (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 2.5).

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 2.2).

Table 2.2 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 (ms/cm)	**0.69**	**0.65**
Mean annual precipitation (mm)	**-0.65**	-0.29
Mean annual discharge (mm)	**-0.58**	-0.37
Altitude (m)	**-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**

PCA biplot considering fish-groups showed a discrimination along a North-South gradient, mostly defined by the second axis (Figure 2.5). 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 2.5). 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 2.6). Results further suggest including group 2 in the fish-region B, unifying the geographical area of the South (Figure 2.6). General characterization of the 4 fish-regions is given bellow:

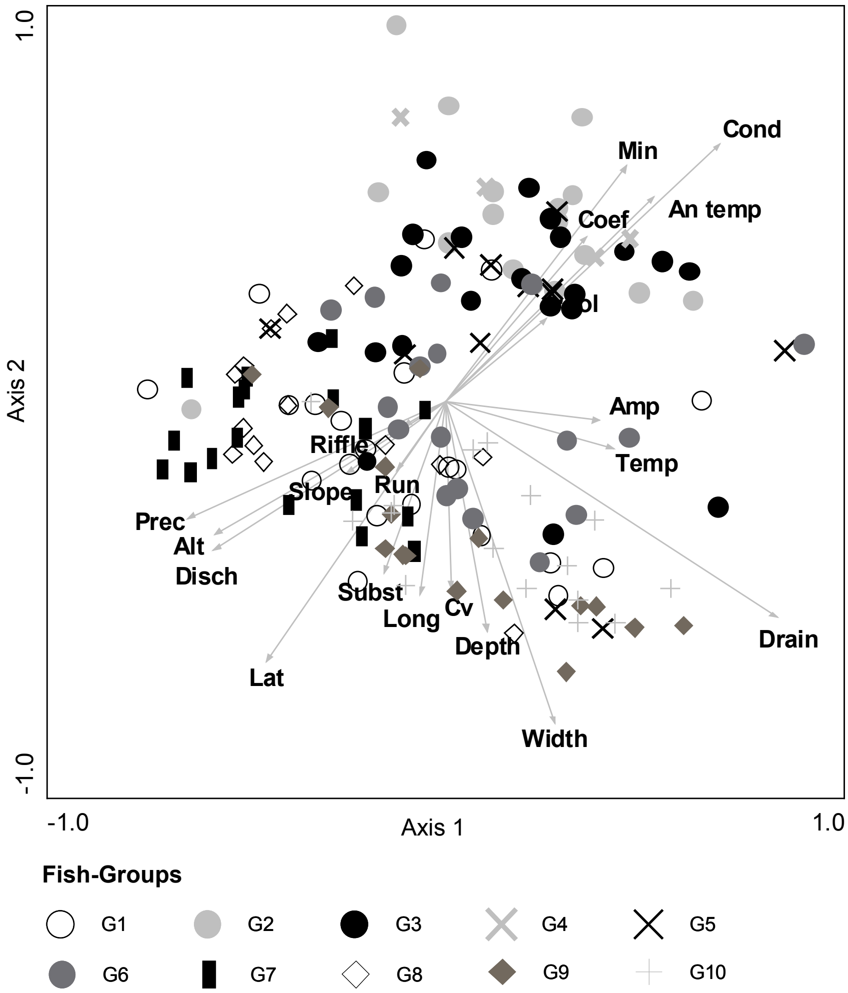


Figure 2.5 Ordination diagram (biplot) of Principal Components Analysis (PCA) based on the environmental variables of sampled sites. Sites are coded according to fish-groups. Variables abbreviations: latitude (Lat), longitude (Long), altitude (Alt), mineralization (Min), conductivity (Cond), mean annual 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).

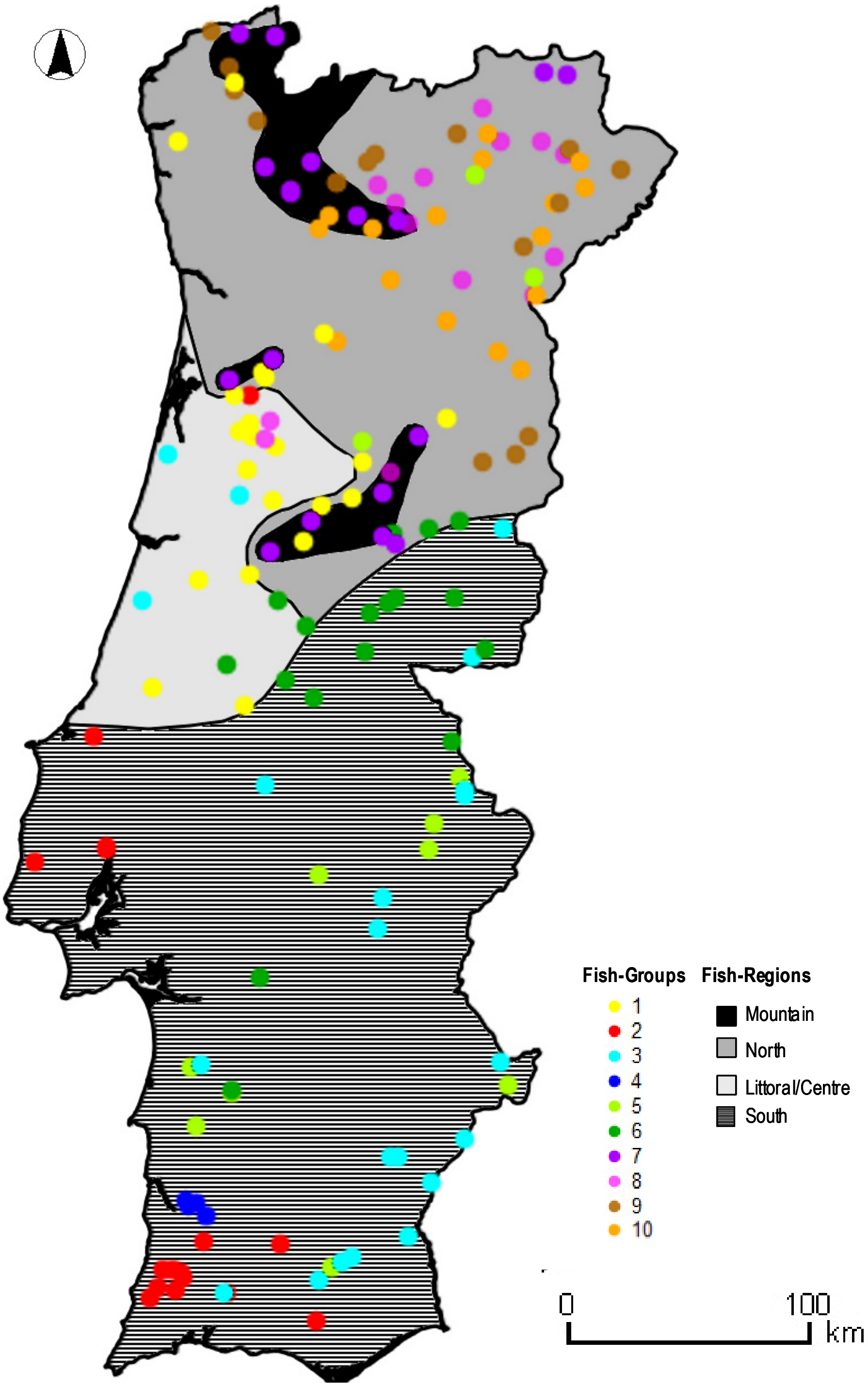


Figure 2.6 Geographical location of the 4 Portuguese fish-regions and the projection of sampled sites according to the 10 fish-groups.

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.

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.

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, 2006). 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 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, b, 2007; Clavero et al., 2004; Ferreira and Oliveira, 2004; Clavero and Garcia-Berthou, 2006; Mesquita et al., 2006; Ferreira et al., 2007a, b). 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) (Figure 2.1 and Figure 2.6). 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 evaluating the changes undergone by the river ecosystem and therefore the effectiveness of mitigation and recovery measures considered necessary to accomplish the WFD objectives.

Acknowledgements

This study was part of a larger program on the implementation of the Water Framework Directive, partially funded by the Portuguese Water Agency (INAG). P. Matono was supported by a PhD grant from FCT (Fundação para a Ciência e Tecnologia). The authors wish to thank all the fieldwork teams involved in the program and Rute Caraça for GIS support. The National Forest Authority provided the necessary fishing permits.

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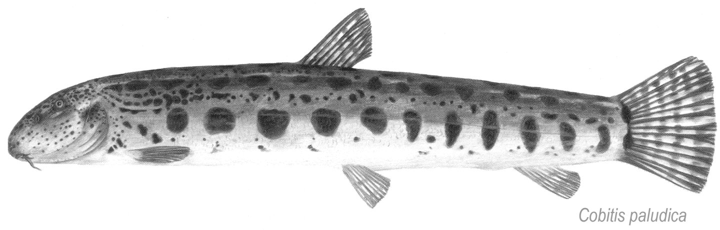
Chapter 3

Development of a Fish Index for the rivers of continental Portugal



Cobitis paludica
F. Filipe

Published as

Matono P, Ilhéu M, Formigo N, Ferreira MT, Raposo de Almeida P, Cortes R, Bernardo JM (2009) Development of a fish index for the rivers of continental Portugal. Recursos Hídricos 3 (2), 75-82.

(This chapter is the basis of the paper published, presenting a further detailed and english version)

Abstract

The Water Framework Directive advocates an assessment of ecological quality of rivers based on hydromorphological and physicochemical variables, and biological elements, including fish fauna. This topic has been poorly developed in Portugal, and the existing approaches are mainly based on expert knowledge and not on sensitivity analysis. This work presents the biological metrics responsive to different types of anthropogenic pressures and the biotic index to assess the ecological status of Portuguese rivers based on fish fauna. Sampling took place between 2004 and 2006, during spring, in 397 sites of the main basins of Portugal. The selection of metrics was based on correlation analysis between fish metrics and human pressure variables. Considering the available data and the obtained results, the assessment of the ecological status using fish fauna is feasible in three out of five Groups of river-types previously identified for this biological element: North Group with medium and large drainage area (N2 and N1>100km^2), Southern mixed Group (N4, S1>100km^2, S2 and S3) and South river-type with medium and large drainage area (S1>100km^2). However, refinement is needed to assess the power and confidence levels of the index. Furthermore, it is essential to continue with sampling programs to extend databases and to perform the necessary adjustments to increase accuracy and reliability in the ecological assessment.

Keywords: Biotic index; fish fauna; Portuguese rivers.

Introduction

The Water Framework Directive (European Commission 2000) establishes a new action plan, considering the possibility of assessing the ecological quality of rivers based on biological and hydromorphological elements. In this context, it is expected the protection, improvement and recovery of European surface water bodies, with the aim of achieving at least good ecological status by 2015.

The use of biological indicators has gained increasing importance in the quality assessment of inland waters, constituting fish fauna one of the most sensitive groups in this context (Barbour et al. 1999): i) longevity provides extensive temporal information; ii) preference for different habitats allows to evaluate their quality; iii) the connectivity of the courses is reflected in the mobility and migratory behaviour of individuals; iv) dependency on primary and secondary productivity reveals disturbances in lower trophic levels.

The Index of Biotic Integrity (Karr 1981) has been the most widely used of the biological indices applied to fish fauna. This index considers different parameters of the communities, or metrics, which reflect the degradation processes, being useful in the identification of anthropogenic pressures. The original version has undergone several adaptations to different regions and habitats (e.g. Faush et al. 1984; Leonard and Orth 1986; Oberdorff and Hughes 1992; Lyons et al. 1996; Harris and Silveira 1999; Oberdorff et al. 2002; Angermeier and Davideanu 2004; Pont et al. 2006, 2007; Hermoso et al 2010). In the countries of southern Europe, particularly in Portugal, this topic is still relatively undeveloped. The existing approaches are regional and/or mainly based on expert knowledge and not on sensitivity analysis (Ferreira et al. 1996; Oliveira and Ferreira 2002; Bernardo et al. 2004; Magalhães et al. 2008).

Mediterranean climate rivers are particular systems (Gasith and Resh 1999), with particular ecological features of fish assemblages (e.g. Ilhéu 2004). In this context, rivers in the Iberian Peninsula are among the most peculiar of Europe regarding ichthyofauna, due to zoogeographical isolation: there is a high number of endemic species, a typically low species richness for local, different assemblages compositions in each river basin and a high ecological plasticity of species (Almaça 1995).

Considering hydrology, Mediterranean rivers, particularly in southern Portugal, show a high variability that leads to great dynamism in the configuration of habitats and in the response of fish communities (Magalhães et al. 2002; Bernardo et al. 2003; Ilhéu 2004). The fact that these communities are naturally adapted to constraining environmental conditions significantly hampers its use as evaluators of anthropogenic pressures exerted on the systems, namely the hydrological ones.

This work aimed to present: i) the selected fish metrics (structural and functional) responsive to different kinds of anthropogenic pressures; and ii) the biotic index developed to assess the ecological quality of rivers based on Portuguese fish fauna.

Methods

Sampling

Sampling took place during spring between 2004 and 2006 in 397 sites from the main Portuguese river basins: Minho, Lima, Leça, Cávado, Ave, Douro, Vouga, Mondego, Lis, Tagus, Sado, Mira and Guadiana, and West and Algarve streams (Figure 3.1).

Anthropogenic pressure was evaluated through 10 semi-quantitative variables assessed at each site (formerly developed within the European 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, toxic and acidification levels, and nutrient/organic load. Each variable was evaluated on a scale from 1 (minimal disturbance) to 5 (maximum disturbance) (Appendix 1). Only sites with scores of 1 and/or 2 and only one variable with a value of 3 were considered undisturbed or least disturbed (references) (following CIS-WFD 2003). According to this criterion, 199 undisturbed sites and 198 disturbed sites were sampled. The sum of the scores of those 10 variables represented the total human pressure in each site.

Additionally, a set of physicochemical parameters were also analysed, in order to assess more accurately the pressures on water quality (namely organic pollution and nutrient enrichment): water temperature (ºC), water transparency (Secchi disk depth, m), conductivity (µS/cm), pH, dissolved oxygen (mg/L), oxidability (mg/L), alkalinity – HCO_3^{2-} (mg/L), hardness - $CaCO_3$ (mg/L), biological oxygen demand - BOD_5 (mg/L), chemical oxygen demand - COD (mg/L), total suspended solids - TSS (mg/L), total dissolved phosphorous – P (mg/L), nitrite - NO_2^- (mg/L), nitrate - NO_3^- (mg/L), ammonium - NH_4^+ (mg/L) and total dissolved nitrogen - N (mg/L).

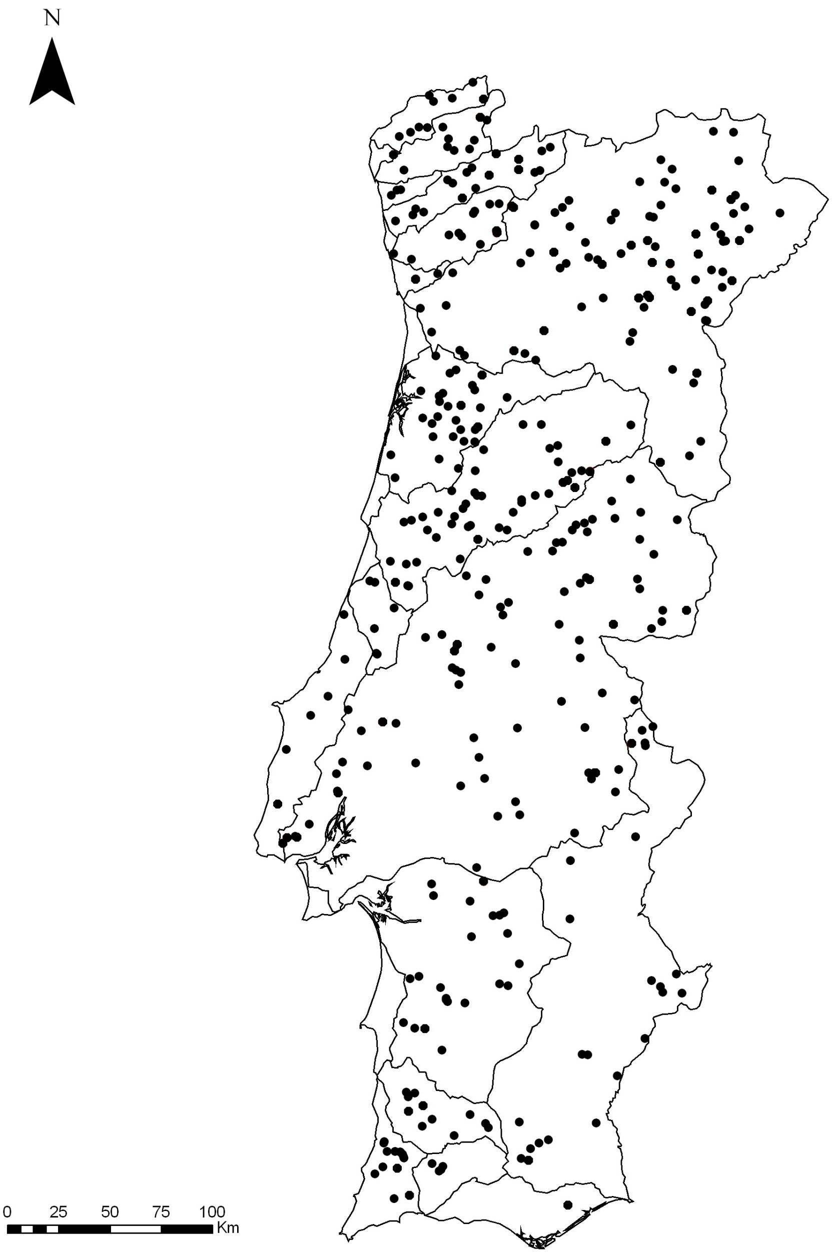


Figure 3.1 Map with the location of sampling sites in the principal Portuguese river basins.

Environmental characterization of sites was mainly based on local variables: mean stream wetted width (m), maximum and mean water depth (m), mean current velocity (m/s), 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). The length of the section sampled was defined as 20 times the average width of the course in a maximum of 150 m sampled.

Fish were collected by electrofishing according to the WFD compliant sampling protocol (INAG 2008a), which follows the CEN standards (CEN 2003). All collected individuals were measured, identified to the species level and immediately returned to the river. Individuals with external, easily observable deformities, eroding fins, lesions and tumors were also registered (e.g. Karr 1981).

Metrics selection

Captures were quantified as density (number of individuals/100 m^2). Species were classified in terms of functional guilds related to habitat, breeding, feeding, migration and tolerance (Table 3.1). The species were assigned to these attributes following information gathered under project FAME (2004), complemented with existing grey or published literature, and expert judgement when necessary.

Community metrics were also used, such as species richness and diversity. The sentinel species considered for each river-type resulted from the analysis of indicator species (Dufréne and Legendre 1997) (Pc-ord 4). Guilds represented by species with very low occurrence were not considered for analysis.

Candidate metrics (Table 3.2) were screened for redundancy (Spearman's rank correlation coefficient $|r| > 0.75$, $P < 0.05$) and variability (graphical analysis with box plots) (Statistica 6). Redundant and low variability metrics were discarded from the analysis.

Final selection of metrics was based on their responsiveness to human pressure variables (Spearman rank correlation coefficient $|r| \geq 0.5$, $P < 0.05$) and on the existence of significant differences between undisturbed and disturbed sites (Mann-Whitney test) (Statistica 6).

Table 3.1 Classification of captured fish species in functional guilds. For each species, only are indicated the guilds included in the Fish Index for the river-types where it is practicable

SPECIES	Native water column	Limnophilic	Eurytopic	Native lithophilic	Tolerant	Endemic
Alburnus alburnus		●			●	
Alosa alosa	●					
Alosa fallax	●					
Ameiurus melas		●			●	
Anaecypris hispanica	●	●				●
Anguilla anguilla			●		●	
Atherina boyeri	●	●				
Barbus bocagei		●		●	●	●
Barbus comizo	●	●		●	●	●
Barbus microcephalus		●		●	●	●
Barbus sclateri		●		●	●	●
Barbus steindachneri	●	●		●	●	●
Barbus spp. (juveniles)	●			●		●
Carassius auratus		●			●	
Achondrostoma arcasii	●					●
Achondrostoma oligolepis	●	●			●	●
Achondrostoma occidentale	●					●
Iberochondrostoma olisiponensis	●					●
Iberochondrostoma lemmingii	●	●		●		●
Iberochondrostoma lusitanicum	●	●		●		●
Iberochondrostoma almacai	●	●		●		●
Pseudochondrostoma polylepis				●		●
Pseudochondrostoma willkommii				●		●
Pseudochondrostoma duriense				●		●
Cobitis calderoni				●		●
Cobitis paludica		●			●	●
Cyprinus carpio		●			●	
Esox lucius		●				
Gambusia holbrooki		●			●	
Gasterosteus gymnurus	●		●			
Herichtys facetum		●			●	
Lampetra fluviatilis				●		
Lampetra planeri				●		
Lepomis gibbosus		●			●	
Liza ramada	●		●		●	
Micropterus salmoides		●			●	
Mugil cephalus	●		●		●	
Oncorhynchus mykiss						
Petromyzon marinus				●		
Platichthys flesus		●				
Salaria fluviatilis				●		
Salmo salar	●					
Salmo trutta	●			●		
Sander lucioperca		●				
Silurus glanis		●				
Squalius alburnoides	●		●	●		●
Squalius aradensis	●		●	●		●
Squalius carolitertii	●		●	●		●
Squalius pyrenaicus	●		●	●		●
Squalius torgalensis	●		●	●		●
Tinca tinca		●			●	

Human pressure variables considered for analysis included physicochemical parameters, dissolved nutrients and human disturbance variables mentioned above. Principal Components Analysis (Canoco 4.5) was additionally used to extract independent axes of human pressure variables, thus resuming them to integrative disturbance gradients.

Prior to analysis, fish metrics and pressure variables were transformed to improve normality: percentages were arcsin [sqrt(x)] and linear measurements were log (x+1) (Legendre and Legendre 1998).

Table 3.2 List of the candidate fish metrics

Fish Metrics	
Composition and Diversity	Total density (ind./100m^2)
	Species richness
	Species diversity (Shannon-Wiener Index)
	Total number of endemic species
	Relative number of endemic species
	Relative abundance of small Iberian cyprinids
	Number of non-native species
	Relative number of non-native species
	Relative abundance of barbels larger than 24 cm
Habitat	Relative abundance of benthic species
	Relative abundance of native water column species
	Relative abundance of rheophilic species
	Relative abundance of limnophilic species
	Relative abundance of eurytopic species
Feeding	Relative abundance of omnivorous species
	Relative abundance of native insectivorous species
Reproduction	Relative abundance of native phytophilic species
	Relative abundance of native lithophilic species
Life cicle	Relative abundance of long-lived species
Migration	Relative abundance of potamodromous species
	Relative number of diadromous species
Tolerance	Relative number of intolerant species
	Relative number of tolerant species
Age structure	Sentinel species (total density and density of each length class)

Fish Index

Under the WFD, the reference condition approach (Reynoldson et al. 1997; Bailey et al. 2004) was used to assess ecological status in Portuguese rivers, assessing the deviation of the present status from the undisturbed river-type-specific conditions.

The final national typology includes 15 Portuguese river-types (Appendix 3) (INAG 2008b). Excluding the large river-types, different formulations of the index were developed for five groups of rivers. Each group was composed by one or more river-types from the final typology with available fish data, that weren't significantly different (R≤0.5, *P*>0.05) (Clarke and Gorley 2001) regarding Similarity Analysis (Primer 6) based on fish fauna (both taxonomic and functional) from undisturbed sites (Ilhéu et al. 2009). This procedure aimed at reducing the number of indexes to propose, facilitating their application:

1. Littoral river-type;
2. North Group with small drainage area (smaller than 100 km^2),

   which includes Mountain, N1<100 km^2 and N3 river-types;
3. North Group with medium and large drainage area (larger than 100 km^2),

   which includes N1>100 km^2 and N2 river-types;
4. Mixed South Group,

   which includes N4, S1 <100 km^2, S2 and S3 river-types;
5. South river-type with medium and large drainage area (larger than 100 km^2).

Selected metrics were standardized between 0 (bad ecological status) and 10 (high ecological status), based on threshold limits and a linear equation - continuous scoring. This method was chosen for having potentially greater ability to express the relationship between metrics and continuous variables of pressure, either statistically or graphically (Minns et al. 1994; Hughes et al. 1998; Ganasan and Hughes 1998; Blocksom 2003; Mebane et al. 2003). Limits depended on the relationship between each metric and human pressure (following the approach from Dauwalter and Jackson 2004):

i) for metrics negatively related to human pressure, the upper limit was the 50th percentile of undisturbed sites and the lower was the 5th percentile of disturbed sites, applying the formula

[(obs - lower limit) / (upper limit - lower limit) x 10]

ii) for metrics positively related to human pressure, the upper limit was the 95th percentile of disturbed sites and the lower limit was the 50th percentile of undisturbed sites, using the formula

[10 - (obs - lower limit) / (upper limit - lower limit) x 10]

Scores below 0 or above 10 were approximated to the nearest limit.

Fish Index varies between 0 (bad ecological status) and 100 (high ecological status) and was calculated according to the mathematical expression

$$\sum Ms_i \times 10 / N$$

where *Ms* is the score of each metric and *N* is the total number of metrics used in that river-type.

The index values were standardised, i.e. transformed into EQRs (Ecological Quality Ratio), dividing each value by the median value of the undisturbed sites from the respective river-type, as required in WFD.

The relationship between the index and total human pressure was assessed using correlation analysis and simple linear regression (SPSS 12.0).

The establishment of 5 ecological quality classes (High, Good, Moderate, Poor and Bad) and their respective boundaries followed the so-called REFCOND methodology (CIS-WFD 2003):

i) the boundary High/Good was defined as the 25th percentile of undisturbed sites;

ii) (ii) the other boundaries were defined by dividing the remaining gradient into 4 classes of equal amplitude.

To analyse index ability to discriminate different levels of anthropogenic pressure, human pressure gradient was divided into 5 abiotic quality classes following the same method presented above for ecological quality classes. Kruskal-Wallis and Mann-Whitney tests were used to detect significant differences in index scores between undisturbed and disturbed sites and among abiotic quality classes. Correlation and Simple Linear Regression analyses were used to evaluate the responsiveness of the Index to the stressor gradient (total human pressure).

Results

Applicability of the Fish Index

During sampling there was a high number of sites with very low fish captures, or even zero. In an attempt to identify these sites, graphical relationships between fish density and drainage area of the basin on the one hand, and mean water depth on the other were evaluated. It was possible to consistently identify a cut off threshold for sites with less than 15 ind/100 m^2 in streams with drainage area smaller than 30 km^2 and mean water depth lower than 0.20 m. In these sites, the small number of catches could derail a consistent response of metrics to human pressure and thus compromising the accuracy of the assessment based on the index developed.

Therefore, the development and application of the Fish Index was not possible for streams where those three conditions were simultaneously verified, regardless of the human disturbance level. Furthermore, due to insufficient data at this stage of the work it was also not possible to select metrics and consequently develop a Fish Index for river-type S4, carsick streams from Algarve (sampled undisturbed sites = 2; sampled disturbed sites = 7).

Littoral river-type

Five fish metrics were selected for the Fish Index: relative abundance of native phytophilic species and omnivorous species, relative number of endemic species and non-native species, and total fish density. These metrics showed significant correlations with organic pollution and nutrient enrichment variables, river connectivity and presence of artificial lentic water bodies. These last morphological/hydrological human pressures were particularly associated to the increase of non-native species (see Ilhéu et al. 2007 for more detailed results).

There was a reasonable separation (P < 0.05) of index values between undisturbed and disturbed sites but the small number of undisturbed sites found and sampled for this river-type (N

= 8) should be taken into consideration interpreting this output (Figure 3.2). Moreover, the discrimination of the abiotic quality classes was not significant, especially under increasing degradation classes. The overall responsiveness of the index to anthropogenic disturbance was not satisfactory (r = -0.41, $P < 0.001$) and the high dispersion of the values along the stressor gradient resulted in a negligible adjustment of the regression model ($r^2 = 0.169$, $P < 0.05$) (Figure 3.2).

North Group with small drainage area – river-types Mountain, N1<100 km^2 and N3.

Four metrics were selected to integrate the Fish Index: relative abundance of limnophilic species, eurytopic species, native lithophilic species and long-lived species. Metrics were mainly related to variables reflecting organic pollution and nutrient enrichment (see Ilhéu et al. 2007 for more detailed results).

An overlap of the index values was observed between undisturbed and disturbed sites and no distinction among abiotic quality classes (Figure 3.2). No clear relationship was detected between the index and total human pressure (r = -0.3, $P < 0.05$; $r^2 = 0.09$, $P < 0.05$) (Figure 3.2), existing high dispersion of index values, especially in undisturbed sites.

North Group with medium and large drainage area – river-types N2 and N1>100 km^2

Five metrics were selected for the Fish Index: relative number of endemic species and tolerant species, relative abundance of native water column species and limnophilic species, and total density of *Pseudochondrostoma duriense* (sentinel species). Organic pollution, nutrient enrichment, morphological and hydrological disturbances constituted the most relevant human pressures to which metrics correlated (see Ilhéu et al. 2007 for more detailed results).

Significant differences in index scores were observed between undisturbed and disturbed sites as well as among abiotic quality classes (Figure 3.2) The responsiveness to total human pressure was good (r = -0.58, $P < 0.001$), despite the poor adjustment of the regression model to data ($r^2 = 0.34$, $P < 0.001$) (Figure 3.2).

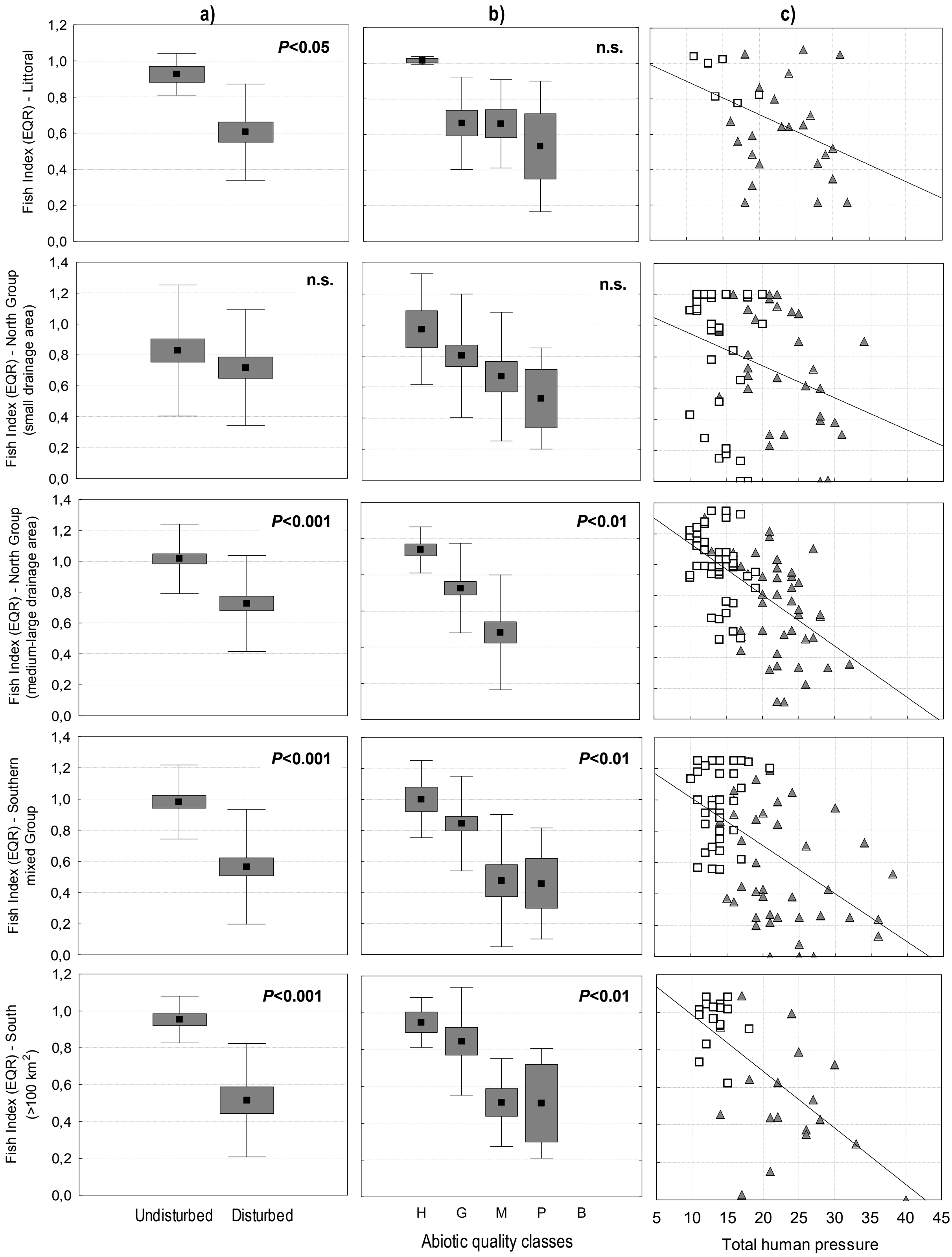


Figure 3.2 Graphical analysis of the Fish Index in each river-type/group: a) and b) box plots of the index scores (EQRs) in undisturbed and disturbed sites and in each abiotic quality class (significance of Kruskal-Wallis and Mann-Whitney tests are shown) (▪): Mean; box: ± SE; whisker: ± SD; c) scatter plots revealing the responsiveness of the index to the human pressure gradient (linear fit trend is shown) - white squares represent undisturbed sites; grey triangles represent disturbed sites.

Southern mixed Group - river-types N4, S1<100 km², S2 and S3.

Five metrics were selected for the Fish Index: relative abundance of limnophilic species, native lithophilic species and eurytopic species, relative number of tolerant species and total density of *Squalius pyrenaicus* (sentinel species). Metrics were mainly responsive to organic pollution, nutrient enrichment and land use (see Ilhéu et al. 2007 for more detailed results).

The index allowed distinguishing undisturbed sites from the disturbed ones and also the abiotic quality classes, although some overlap between Moderate and Poor classes was observed (Figure 3.2). Fish index also showed a good response ($r = -0.53$, $P < 0.001$) to the total human pressure, despite the low adjustment of the regression model ($r^2 = 0.28$, $P < 0.001$) (Figure 3.2).

Comparative graphical analysis of index values in undisturbed sites for each river-type constituting the Southern mixed Group proved to be more accurate to set separate boundaries for each of the river-types, although the applicable configuration of the Index should be common to all.

South river-type with medium and large drainage area - river-type S1>100 km²

Six metrics were selected to incorporate de Fish Index: relative abundance of limnophilic species, native lithophilic species and eurytopic species, relative number of tolerant species and endemic species, and total fish density. Metrics were strongly responsive to organic pressure variables and sediment load (see Ilhéu et al. 2007 for more detailed results).

The Index showed a clear significant separation between undisturbed and disturbed sites, but scores in Moderate and Poor abiotic quality classes showed a total overlap (Figure 3.2). The responsiveness of the Fish Index to total human pressure was good ($r = -0.67$, $P < 0.001$), as well as the adjustment of the regression model ($r^2 = 0.44$, $P < 0.001$) (Figure 3.2).

Considering the overall obtained results, uncertainty level associated with the ecological assessment was generally higher in the most degraded classes, being evident in some cases a progressive increase in data dispersion along the pressure gradient (Figure 3.2 b and c). Accordingly, the Index cannot be applied in Littoral river-type and in the North Group with small drainage area. Table 3.3 presents a summary of the information on the Fish Index developed in this study for the river-types where its application is feasible at this stage of the knowledge.

Table 3.3 Summary of relevant information on the Fish Index configurations for the river-types where it is practicable: metrics, percentiles and medians to calculate the index values and EQRs, and quality classes' boundaries. Response to degradation: (+) means that the metric value increases with increasing pressure; (-) means that the metric value decreases with increasing pressure. Boundaries: H - High, G - Good, M - Moderate, P - Poor, B - Bad

Group	River-type	Fish metrics	Response to degradation	50th percentile of undisturbed sites	5th percentile of disturbed sites	95th percentile of disturbed sites	Median of undisturbed sites	Class boundaries (EQRs)
North Group	N1>100 km^2	Rel. number of endemic sp.	-	75.0	33.3	100		HG: 0.91
(medium-large	N2	Rel. abundance of native water column sp.	-	40.2	0	95.2		GM: 0.68
drainage area)		Rel. abundance of limnophilic sp	+	28.3	0	94.5	76	MP: 0.46
		Rel. number of tolerant sp.	+	33.3	0	80.0		PB: 0.23
		P. duriense (total density)	-	0.6	0	5.8		
Southern mixed	N4							HG: 0.96
Group							100	GM: 0.72
								MP: 0.48
		Rel. abundance of limnophilic sp.	+	6.5	0	100		PB: 0.24
	S1<100 km^2	Rel. abundance of eurytopic sp.	-	83.1	0	99.0		HG: 0.93
	S2	Rel. abundance of native litophilic sp.	-	99.5	0	100	80	GM: 0.70
		Rel. number of tolerant sp.	+	50.0	0	100		MP: 0.46
		S. pyrenaicus (total density)	-	0.8	0	9.0		PB: 0.23
	S3							HG: 0.82
							53.1	GM: 0.62
								MP: 0.41
								PB: 0.21
South Group	S1>100 km^2	Total fish density (ind./100m^2)	-	25.5	0.6	97.3		
(medium-large		Rel. number of endemic sp.	-	66.7	0.04	100		HG: 0.94
drainage area)		Rel. abundance of limnophilic sp.	+	17.0	0	100	90.8	GM: 0.71
		Rel. abundance of eurytopic sp.	-	59.4	0	94.6		MP: 0.47
		Rel abundance of native lithophilic sp.	-	90.3	0	100		PB: 0.24
		Rel. number of tolerant sp.	+	50.0	25.4	100		

Discussion

Successful bioassessment requires accurate measurement of biological attributes that maximise the detection of human degradation while minimising the noise of natural variability (Hughes et al. 1998). The river-type approach adopted in this study allowed stratifying spatial variability, considering several regional factors, namely drainage area, which, together with other surrogates of river size, is increasingly recognised as a major determinant of fish assemblage structure in Mediterranean streams, over a wide range of spatial scales (e.g. Pires et al. 1999; Filipe et al 2002; Magalhães et al. 2002; Mesquita et al. 2006). This procedure guaranteed a reduction of the natural variation of reference conditions within river-types (Dodkins et al. 2005).

Accordingly, the ability to define fish assemblages in the absence of anthropogenic disturbance is critical, and requires that relationships between natural environmental conditions and biota are sufficiently strong (Kennard et al. 2006a). However, these relationships may be more difficult to evaluate in systems characterised by high environmental variability, such as that associated with high flow variability (Kennard et al. 2006a), observed in many Portuguese steams.

The index was not developed or applied in sites with less than 15 ind./100 m^2 within streams with drainage area smaller than 30 km^2 and mean water depth lower than 0.20 m, regardless of the human disturbance level. Indeed, the low density may not necessarily be a consequence of environmental degradation, but rather the result of lack of capacity to sustain fish in these streams (Lyons 2006), hampering a reliable development of the index.

Five different formulations of the Fish Index were developed for Portuguese river-types established in the national typology (Appendix 3) (INAG 2008b): Littoral river-type, North Group with small drainage area, North Group with medium and large drainage area, Southern mixed Group and South river-type with medium and large drainage area. The application of the index proved lack of accuracy and reliability in Littoral river-type and in North Group with small drainage area. Based on the obtained results, the responsiveness of these index formulations to total human pressure was low or even negligible. On the contrary, the other three formulations of the index showed strong significant responsiveness to anthropogenic degradation.

The linear relationship between the index and total human pressure was mainly expressed by correlations, as regression analysis revealed less significant results. Nonetheless, more than expressing the strength of the relationship between the index and human pressure, regression analysis was useful evaluating the uncertainty level associated to the index by observation of data dispersion, i.e. graphical analysis of residuals along the stressor gradient. For example, the large data dispersion in undisturbed sites observed in the North Group with small drainage area has serious consequences in the establishment of boundaries, lowering the values, thus resulting in ecological quality classes with small amplitude. Under this scenario, the ecological assessment will be less demanding, leading to a better ecological status than it should be in some situations. It should be noted, however, that the broader implications of the index uncertainty in the fulfilment of the goals of WFD are mainly associated with cases of under assessment (i.e. when index scores and ecological status are lower than they should in good environmental conditions).

An important complementary analysis was to evaluate index ability to actually differentiate between undisturbed and disturbed sites and to discriminate different abiotic quality classes (e.g. Fausch et al. 1990; Norris and Hawkins 2000). Once again, results showed better performances in North Group with medium-large drainage area, Southern mixed Group and South river-type with medium-large drainage area. Particularly, the distinction between Good and Moderate anthropogenic level should be accurately reflected by index scores and has a major practical value to the achievement of WFD goals. Graphical results also suggest some bias towards less sampling effort in very polluted sites, i.e. in bad quality class, which should be filled in order to correctly evaluate index accuracy classifying sites subjected to different levels of anthropogenic degradation.

As mentioned before, one of the most critical steps in ecological assessment is the quality of reference data. The selection of reference sites followed the WFD guidelines, trying not to compromised the characterization of the reference set, even in most disturbed river-types (Littoral and S3 river-types), where some best available sites were chosen, facing the low availability of undisturbed ones. However, this issue surely has to be addressed, since it has direct implications on EQRs calculations, on the establishment of the boundaries of ecological quality classes and consequently on the ecological status assessment. For that reason, class boundaries were set separately for each of the river-types that include Southern mixed Group. Other approaches like predictive reference condition models consider the influence of an array of both regional and local factors (e.g. Joy and Death 2002; Kennard et al. 2006b; Hermoso et al. 2010) and can be preferable in heavily altered regions, where undisturbed reference sets are difficult or impossible to obtain (Hughes et al. 1998; Schmutz et al. 2000).

Screening of metrics and index scoring are fundamental procedures to ensure the objectivity of multi-metric indices as bioassessment tools (Mccormick et al. 2001). In this study, quantitative methods were extensively used in order to select the most non-correlated and sensitive metrics to a human pressure gradient as wider as possible. However, the method of continuous scoring used proved to be not very practical, regardless of the performance of the index evaluating the ecological status. As such, scoring criteria must be improved and other methods should be addressed, keeping in mind a compromise between the objectivity of the method and the need to express as closely as possible the range of variability in selected metrics' values. Furthermore, threshold limits also need better adjustments to the occurrence and abundance of different metrics in disturbed and undisturbed situations, thus increasing the index power reflecting variation in biological integrity.

Fish assemblages characterized by low species richness, as in most Portuguese rivers, offer much reduced guild-type possibilities and ranges, resulting consequently in indexes with few selected metrics (Moyle and Marchetti 1999), as observed especially in North Group with small drainage area. In this study, the list of candidate metrics was mainly based on previous results obtained by Ferreira and Oliveira (2004) and Ferreira et al. (2007a, b) but more alternative metrics can be used and expressed in different possible forms: number, abundance and biomass, in relative or absolute terms. Moreover, each site was sampled only once during the study period, enhancing the possible failure to detect a single species, thus reducing a possible guild-type. This should be a factor to consider in subsequent studies, as it might also result in deviations in expected and observed assemblages.

Selected metrics included feeding and reproductive guilds, species composition, diversity and tolerance. More information and knowledge on fish autoecology would enhance the efficacy of metrics and indices (Norris and Hawkins 2000), as this information is often weak for Mediterranean fish fauna, namely regarding tolerance to degradation (Hermoso et al. 2009). Furthermore, some metrics responded to human pressure considering only native or endemic species. Weather or not non-native species can be used in ecological assessment as reliable biological indicators in Portuguese rivers still needs further research. Non-native species are considered one of the major threats to the conservation of native fish communities (e.g. Clavero et al. 2004) and thus a main issue to consider when assessing the ecological status (Kennard et al. 2005; Pont et al. 2006, 2007).

In Mediterranean streams, species richness and composition generally tend to remain stable over time, whereas abundances fluctuate widely (Matthews and Marsh-Matthews 2003; Magalhães et al. 2007). Most candidate and selected metrics were abundance-based, but the use of proportions tried to overcome this potential problem. Nevertheless, this issue suggests the need to incorporate a temporal component in bioassessment programs, evaluating temporal variability of metrics (Kennard et al. 2006a; Pont et al. 2006) and including the less variable ones in the biotic indices.

Conclusions

The development of fish-based indexes to assess the ecological status with confidence is a complex task. The peculiarities of fish assemblages from many river basins, and the effects of the temporary character of many streams, particularly in southern Portugal, result in increased difficulties or even in the impossibility to develop indexes with actual ability to evaluate the effects of anthropogenic pressures.

In small streams, fish assemblages are less structured and less stable than in larger ones. The development of indexes for small courses is thus more problematic and tends to be less reliable. The number of species can be particularly low, and the response of fish fauna after disturbance (human or natural) can be very variable, because recolonization is dependent on the conditions of longitudinal connectivity. In low flow situations, recovery depends on the distance from the recolonization source. On the other hand, late maturity species take longer to recover their populations.

Despite all these problems, justifying why fish fauna is generally considered a biological element more problematic than others, it was possible to identify metrics and to develop an index that responds to anthropogenic pressures, even in rivers with small drainage area, using existing data and a bottom-up approach.

Refinement is needed to assess the power and confidence of the index, namely by using a validation data set. Moreover, using a minimum number of samples (ideally not less than 3) to classify a given site could substantially reduce possible bias in assessing the ecological status. Furthermore, it would be necessary to confirm the index applicability as more data is being produced.

Accomplished this first step, important for the implementation of the WFD, it is essential to continue with sampling programs to extend databases and perform the necessary adjustments to increase accuracy and reliability in the ecological assessment.

Acknowledgements

This study was funded by the National Water Institute (INAG) in the context of the implementation of the Water Framework Directive in Portugal. P. Matono benefited from a grant from the Foundation for Science and Technology. The authors wish to thank all those who collaborated in the collection, validation and preliminary data analysis, thus contributing to this study.

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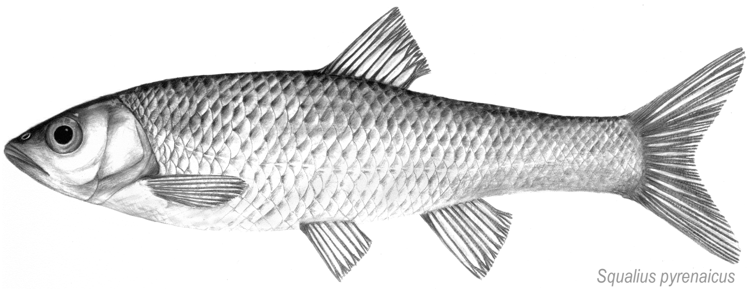
Chapter 4

Effects of natural hydrological variability on fish assemblages in small Mediterranean streams: implications for ecological assessment



Squalius pyrenaicus
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Published as

Matono P, Bernardo JM, Oberdorff T, Ilhéu M. (2012) Effects of natural hydrological variability on fish assemblages in small Mediterranean streams: implications for ecological assessment. Ecological Indicators 23, 467-481.

Abstract

Small Mediterranean streams are shaped by predictable seasonal events of flooding and drying over an annual cycle, and present a strong inter and intra-annual variation in flow regime. Native fish assemblages in these streams are adapted to this natural environmental variability. The distinction of human-induced disturbances from the natural ones is thus a crucial step before assessing the ecological status of these streams. In this aim, the present study evaluates the effects of natural hydrological variability on fish assemblages from disturbed and least disturbed sites in small intermittent streams of south Portugal. Data were collected over the last two decades (1996-2011) in 14 sites located in the Guadiana and Sado river basins. High variability of fish assemblages was strongly dependent on human-induced disturbances, particularly nutrient/organic load and sediment load, and on natural hydrological variability. Natural hydrological variability can act jointly with anthropogenic disturbances, producing changes on fish assemblages structure of small intermittent streams. In least disturbed sites, despite the natural disturbances caused by inter-annual rainfall variations (including drought and flood events), fish assemblages maintained a long-term stability and revealed a high resilience. On the contrary, disturbed sites presented significantly higher variability on fish assemblages and a short and long-term instability, reflecting a decrease on the resistance and resilience of fish assemblages. Under these conditions, fish fauna integrity is particularly vulnerable and the ecological assessment may be influenced by natural hydrological variations. High hydrological variability (especially if it entails high frequency of dryer years and meaningful cumulative water deficit) may affect the impact of the human pressures with significant and consistent consequences on fish assemblage composition and integrity. In this study, fish metrics that maximize the detection of human degradation and minimize the response to natural variability were based on the relative abundance of native species (insectivorous species, eurytopic species, water column species, native lithophilic species), relative abundance of species with intermediate tolerance and relative number of exotic species. Results highlight the importance of assessing temporal variability on stream biomonitoring programs and emphasize the need to improve the assessment tools, accounting for long-term changes in fish assemblages, namely by selecting the most appropriate fish metrics that respond to anthropogenic disturbances but exhibit low natural temporal variability, essential both in the characterization of the biological reference conditions and in the development of fish indexes in intermittent streams.

Keywords: intermittent streams, natural disturbance, human-induced degradation, fish assemblages, ecological assessment, Portugal

Introduction

Mediterranean streams and their endemic fish fauna are among the most threatened ecosystems and biota worldwide (Smith and Darwall, 2006; Hermoso and Clavero, 2011). The Mediterranean region is one of the areas in the world where landscapes have most experienced a simultaneous burst of human activities, such as intensive irrigated agriculture, extensive single cropping, tourism and urbanization. The southern regions (e.g. Portugal and Spain) are particularly vulnerable because of increasing water demands (Gasith and Resh, 1999; Laraus, 2004) and land use changes (e.g. Laraus, 2004; Peña et al., 2007; Symeonakis et al., 2007). In Portugal, besides water quality (e.g. organic and nutrients inputs) and morphological human induced pressures, the hydrological disturbance caused by large impoundments, like Alqueva reservoir in the Guadiana catchment, have considerable impacts on the native biota, namely fish fauna (Santos et al., 2004; Morais, 2008). Flow regulation and other modifications of flow regime and runoff decrease caused by damming and water abstraction are well known human induced hydrological pressures with major consequences in fish species abundance, structure and integrity (e.g. Gorman and Karr, 1978; Orth and Maughan, 1982; Freeman et al., 2001; Murchie et al., 2008; Benejam et al., 2009). Conversely, natural flow regimes are vital to the maintenance of a healthy biota in many river ecosystems (e.g. Poff et al., 1997; Lytle and Poff, 2004; Arthington et al., 2006).

Most rivers in Portugal are strongly influenced by the Mediterranean climate and the hydrological regime is strongly affected by precipitation both at intra and inter-annual scales. As a consequence, especially in southern regions, many rivers are temporary, exhibiting reduced or zero flow during the dry season. This intermittent character strongly shapes the environmental context to which the biota is subjected. The particular characteristics of the Mediterranean climate are also reflected in the irregular and unpredictable annual variation of river discharge. Many lowland Iberian and southern European rivers have huge inter and intra annual flow variations caused by irregular precipitation and frequent events of low flows related to dry periods.

High flows have been regarded as important natural processes maintaining a certain community structure and function in river systems (Cummins and Spengler, 1978; Resh et al., 1988). Many studies reported that an increase in stream flow lead to an increase in fish density and fish assemblage diversity (Pegg and Pierce, 2002; Aarts et al., 2004; Xenopoulos and Lodge, 2006). On the contrary, low flow conditions represent an important negative impact on fish assemblage structure (e.g. Gehrke et al., 1999; Pegg and Pierce, 2002; Sagawa et al., 2007) and some studies reported that reductions in flow result in impoverished fish assemblages often dominated by introduced species (Gehrke and Harris, 2001; Bernardo et al., 2003).

Small Mediterranean intermittent streams are particularly affected by hydrological variability, as available habitats, and their suitability to different life history stages, may fluctuate dramatically. Consequently, fish assemblages tend to present high temporal variability (Magalhães et al., 2002a; Bernardo et al., 2003; Clavero et al., 2005; Mesquita et al., 2006). Moreover, fish fauna exhibit relatively high resistance to harsh environmental conditions as these species evolved in a changeable and sometimes extreme environment (Almaça, 1995). Thus, fish assemblages may be resilient in the short-term to human pressure, whose influence can easily be misinterpreted, but eventually quite vulnerable on the long run to continuous and cumulative human-induced changes (Matthews and Marsh-Matthews, 2003).

The assessment of the ecological integrity in water bodies is a central issue to water policies and to nature conservation in general. In Europe, the implementation of the Water Framework Directive (WFD) (European Commission, 2000) requires the evaluation of ecological integrity of aquatic systems using biota. Accordingly, all Member States have to develop or adopt suitable biotic indexes and demonstrate they respond to human disturbances, i.e. to pressures, in an effective way. To attain this goal, it is important to discriminate between the effects of natural vs. human-induced environmental variability (Oberdorff et al., 2002; Pont et al., 2006).

In the context of Mediterranean rivers, a relevant issue is whether or not the natural environmental disturbance, particularly the hydrological one, can cause an undesirable bias, decreasing the ecological assessment accuracy in small Mediterranean streams. The crucial question is then *Does the natural hydrological variability affect fish assemblages response to human pressure leading to less accurate assessment of the ecological status?*

This paper presents data from least disturbed and disturbed sites during several years, evaluating the effects of hydrological variability on fish assemblages structure and discussing its possible implications on streams ecological assessment. Specifically, the main objectives are: (i) to compare the effect of natural hydrological variability on fish assemblages in disturbed and least disturbed sites; (ii) to evaluate the relationship between hydrological variability and the impact of human-induced pressures; (iii) to identify fish metrics that maximize the detection of human induced degradation and minimize the response to natural variability.

Methods

Study area

Study sites were located in Guadiana and Sado river basins, two of the main rivers in the South of Portugal. This region is characterized by a Mediterranean climate, presenting high susceptibility to drought events (e.g. Pereira et al., 2006). The precipitation regime is highly irregular in the spatial and temporal domains, namely regarding the amount and distribution of rainfall (Daveau, 1977). Flow is strongly dependent on the seasonal distribution of rain, mainly concentrated in October-March. The hydrological regimes of sampled sites are very variable, with severe droughts and floods. These small streams are particularly affected during the summer dry season (June-September), when they are completely dry or reduced to isolated pools (in this case, over 50% of the streambed may dry up). Based on the existing hydrologic records for the South of Portugal, the inter-annual variation of discharge reaches a ratio of approximately 100 to 1 (ARHA, 2011).

During the last decades, severe drought events with a return period of 25–50 years occurred in 1994/95, 2004/05 and 2008/09 (Figure 4.1). Other droughts were registered in 1991/92, 1992/93, 1998/99 and 2003/04, all with a return period of 10–25 years. Important events with above the average rainfall occurred in 1995/96–1997/98 and 2000/01. Over the last 50-year records, frequency and intensity of wetter and dryer periods (deviations from the long-term average rainfall) were quite similar. However, the duration of dry events was clearly higher, resulting in extended drought periods (cumulative deficit) (e.g. 1983/84, 1994/95, 2008/09). Therefore, in this study, sampling period (1996 – 2011) encompassed a wide range of hydrological conditions.

Owing to the relatively low mean annual precipitation (Figure 4.1) and the constant increase on water demand for public supply and agriculture, numerous impoundments were built in this southern region: 67 in Sado catchment and 1643 in Guadiana catchment (ARHA, 2011). Other human impacts on rivers are mainly agriculture diffuse pollution and organic loading, channelization, sand extraction and water abstraction. All these factors have been responsible for major changes in aquatic ecosystems, threatening the native fish fauna.

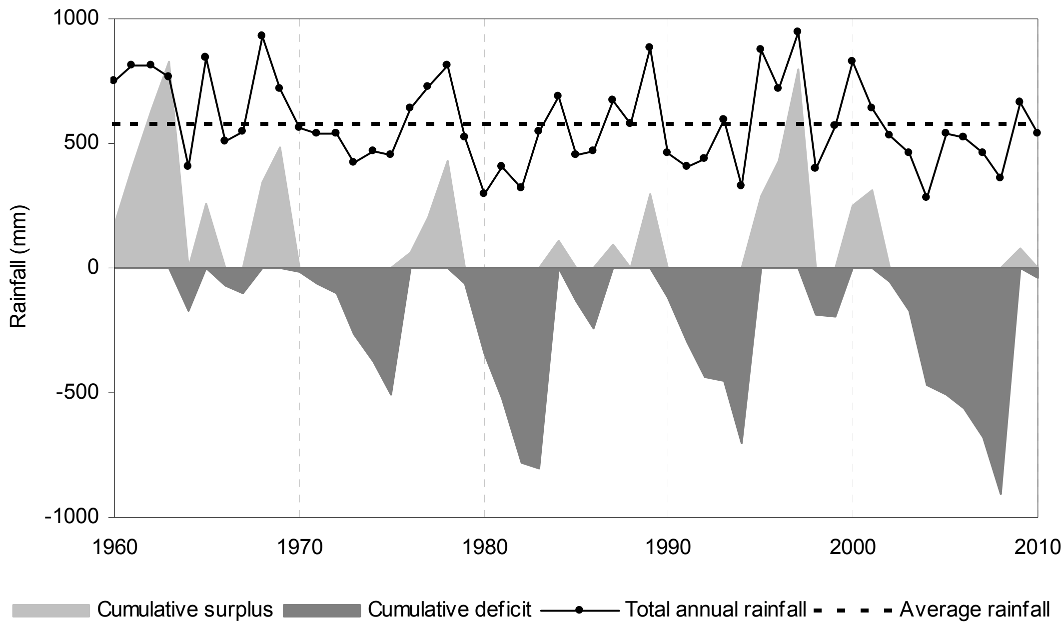

Figure 4.1 Time series of total annual rainfall, cumulative rainfall surplus and deficit (deviations from the long-term average rainfall) between the hydrologic years of 1960/61 and 2010/11 for a gauging station located in Degebe river (Guadiana basin). This pattern is roughly representative of the general rainfall pattern observed in all the gauging stations used, although absolute values may differ.

Fish assemblages generally present low species richness and include many endemic species with high conservation status, particularly in Guadiana river (Cabral et al., 2005). In this basin, the most abundant and frequent species is roach (*Squalius alburnoides* Steindachner), followed by barbel species (*Barbus microcephalus* Almaça, *Barbus comizo* Steindachner, *Barbus steindachneri* Almaça) and Guadiana nase (*Pseudochondrostoma willkommii* Steindachner), all endemic species.

The Sado river basin is comparatively poorer, presenting high abundance of non-native species, namely pumpkinseed (*Lepomis gibbosus* L.). The most significant endemic species are roach (*Squalius alburnoides*), common barbel (*Barbus bocagei* Steindachner), Iberian nase (*Pseudochondrostoma polylepis* Steindachner) and Portuguese nase (*Iberochondrostoma lusitanicum* Collares-Pereira).

Sampling

Data were collected in 14 least disturbed and disturbed sites located in southern Portuguese small intermittent streams (Figure 4.2). Sites were sampled over a 4 to 11 years period between 1996 and 2011, though not always in consecutive years. Site selection criteria privileged the availability of long-term data for each site, in order to allow significant temporal analysis. Only one site (Val) was located in the Sado river basin, being all the other sites located in the Guadiana river basin.

Sampling took place in early spring, following the protocol developed and adopted by the Portuguese Water Agency (INAG) for Portuguese rivers (INAG, 2008) under the implementation of the WFD, following also CEN protocol (CEN, 2003). Surveys were carried out always in flowing water conditions, immediately after the floods and previously to the strong reduction of flow during the summer period, in order to ensure high habitat diversity in the streams. At each site/year one stream section was sampled, encompassing all the existing physically homogeneous units (mesohabitats) – pool, run and riffle. The length of the sampled section was defined as 20 times the mean width of the stream, with a maximum of 150 m long. Fish were collected using backpack battery-powered electrofishing equipment (IG 200/2B, PDC Hans-Grassl GmbH, Schönau am Königssee, Germany), wading in shallow reaches (< 1.2 m) or from a boat in deeper areas. Captured fishes were identified to the species level, measured and returned alive to the stream. The sampling method was maintained along the study sites/years, although the official protocols were only published in recent years. Indeed, the protocol developed under the WFD (INAG, 2008) incorporated exactly these sampling procedures and CEN (CEN, 2003).

Regional variables were obtained from digital cartography with free Internet access and included altitude of the site (m), drainage area of the basin upstream of the site (km^2) and distance from source (km). Local variables were assessed during the field sampling procedure: water temperature (ºC), conductivity (μS/cm), pH, dissolved oxygen (mg/L), mean water depth (m), mean current velocity (m/s) and 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).

In each sampling, human disturbance level was evaluated using 10 semi-quantitative variables (formerly developed within the 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, toxicity and acidification levels, and nutrient/organic load. Each variable was scored from 1 (minimum disturbance) to 5 (maximum disturbance) (Appendix 1) and the sum of these scores represented the total human pressure in each site. Sites with scores 1 and/or 2 and only one variable with a 3 were considered least disturbed.

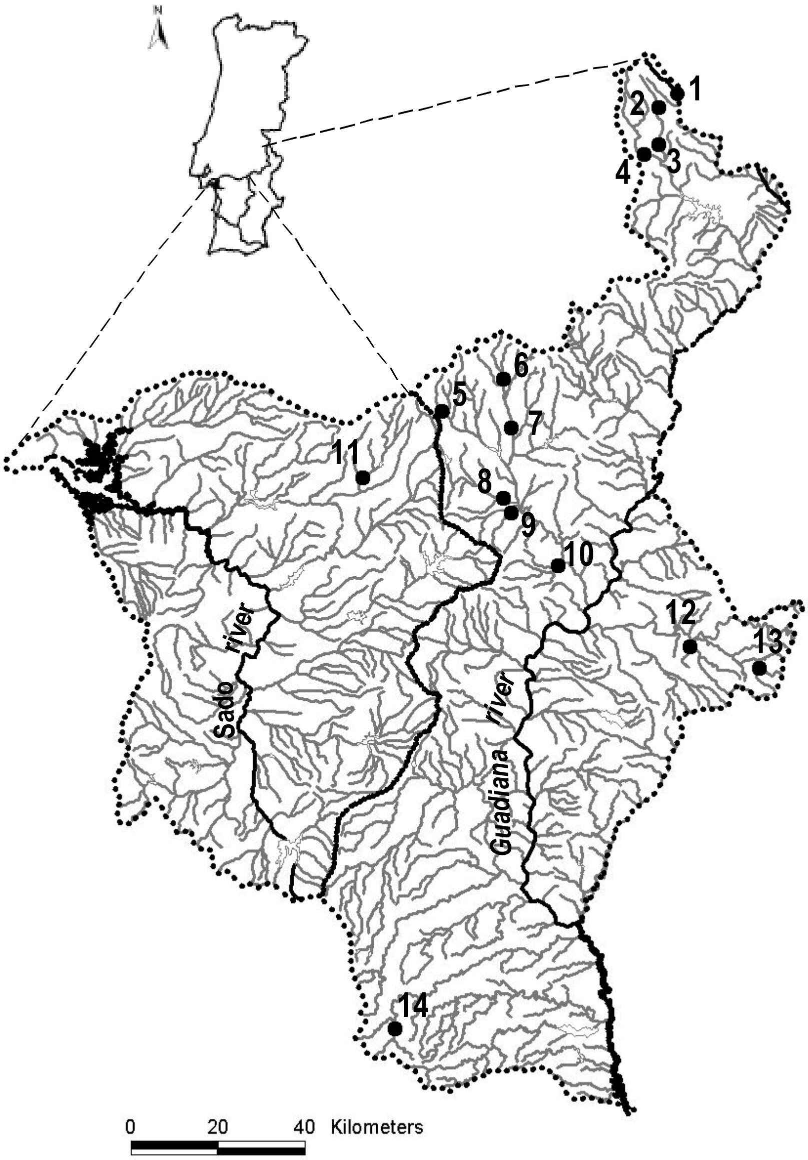

Figure 4.2 Location of sampling sites in small southern Portuguese streams form Guadiana and Sado river basins: 1 – Cbx, 2 – Alg, 3 – Mos, 4 – Asm, 5 – Gar, 6 – Fqm, 7 – Sdg, 8 – Azb, 9 – Pec, 10 – Ami, 11 – Val, 12 – Saf, 13 – Mtg, 14 - Vas.

Several additional physicochemical parameters complemented and supported the evaluation of human-induced disturbance in each site (mainly organic/nutrient enrichment – Appendix 1) after laboratory measurements and analyses according to the Standard Methods for the Examination of Water and Wastewater (Clesceri et al., 1998): five day biological oxygen demand - BOD_5 (mg/L), chemical oxygen demand - COD (mg/L), phosphate – P_2O_5 (mg/L), total dissolved phosphorous – P (mg/L), nitrite - NO_2- (mg/L), nitrate - NO_3- (mg/L), ammonium - NH_4+ (mg/L) and total dissolved nitrogen - N (mg/L). These parameters also reflect the water quality of the sampled sites, as their values interpretation was based on the water features for multiple uses, according to the Portuguese Water Agency guidelines (available at http://snirh.pt/snirh/_dadossintese/qualidadeanuario/boletim/tabela_classes.php).

For samples prior to 2004, the human pressure variables were scored using all the information exhaustively gathered during sampling in all the sites (e.g. field records, photos, physicochemical parameters), and also from digital cartography and online information (e.g. SNIRH, available at http://snirh.pt).

Data analysis

In order to evaluate hydrological variability of sites, records from eleven gauging stations, geographically close to the sampling sites, were used (SNIRH, available at http://snirh.pt/). Rainfall was used instead of flow data because there were few gauging stations with long time series. Furthermore, as in small intermittent streams flow is more dependent on rainfall patterns than in any other streams, the use of rainfall data ensures a correct assessment of hydrological variability. Missing periods of records were filled using predictions of regression models. This procedure involved developing a linear equation to predict monthly rainfall for the missing period from nearby stations.

Using data from meteorological stations, and in situ measurements several variables were derived: mean total annual rainfall, Coefficient of Variation (CV - Standard Deviation/Mean x 100) of total annual rainfall, relative frequency of dryer and wetter years, mean cumulative rainfall surplus and deficit, CV of mean water depth, CV of mean current velocity and CV of dominant substrate class. Variability of water depth, current velocity and dominant substrate were used as a measure of habitat modifications following hydrological disturbances, as these variables have already shown ecological relevance in fish assemblages patterns and distribution in most of the sampled streams (Ilhéu, 2004). Dryer and wetter years were identified based on annual rainfall deviations from the long-term average value (50-year records). Cumulative rainfall surplus and deficit were calculated using the theory of runs (Yevjevich, 1967). A run is defined as a portion of a time series of variable xi, in which all values are either below or above a chosen critical level, yc. Considering a discrete time series, x1, x2, …, xt …, xn, a negative run occurs when xt is less than yc consecutively, during one or more time intervals. Negative runs in rainfall time series are related to drought characteristics and the difference between yc and xt is referred as deficit. Accordingly, positive runs are referred to as rainfall surplus. Cumulative values were then calculated as the run-sums of consecutive negative (deficit) or positive (surplus) deviations from the long-term average rainfall, with changes in the signal of deviations reset to zero. These cumulative values were standardized by the total annual rainfall in each site, to allow comparisons between sites.

Individual human pressure variables and total pressure were averaged for each site. The increase in human pressure along the study period was also quantified for each site, calculating the difference in total pressure between the first and the last sampling occasions. According to the increase in human disturbance level, some sites changed their classification from least disturbed to disturbed during the study period. In these cases, sites were analysed according to their classification, depending on the sampling occasion.

Fish captures were standardized to an area of 100 m^2 in all samples and expressed as density (number of fish/ 100 m^2). The study period was long enough to encompass at least one mean generation time for all the species captured, thus allowing meaningful judgments concerning patterns of fish assemblage variability (Grossman et al., 1990).

Different approaches were used to access variability in fish assemblages composition over time:

i) Assemblage persistence (P) describes the repeated extinction and immigration of populations in ecological assemblages (Oberdorff et al., 2001) and was quantified as (P = 1 – T) where T is the species turnover rate. Following Eby et al. (2003), Magalhães et al. (2007) and Oberdorff et al. (2001), turnover rate was defined as T = (C + E) / (S1 + S2), where C and E are the number of species that colonized or were extirpated between two sampling occasions and S1 and S2 are the number of species present in each occasion. T ranges from 0 (no turnover) to 1 (complete turnover), so persistence ranges from 0 (no persistence) to 1 (complete persistence). For each site, sequential P values along the sampled years were averaged.

ii) Stability of fish assemblages composition considering species relative abundance was quantified with Bray-Curtis similarity coefficient (Clarke and Warwick, 1994) between each possible pair of sampled years in each site. Mean similarity was subsequently calculated for each site.

iii) Nonmetric Multidimensional Scaling (MDS) (Clarke and Warwick, 1994) of samples, based on the Bray-Curtis similarity matrices calculated in (ii) for each site with the temporal trajectory overlaid. The displacement pattern of points in the plots allowed identifying changes in fish assemblages along the years (Clarke and Warwick, 1994). Fish assemblages could show changes followed by a return to an earlier state (indicating a cyclic pattern of variability with no long-term directional shift) or progressive displacements further away from the original position (indicating a directional shift in composition).

iv) Time lag regression analysis (see Collins, 2000; Eby et al., 2003; Magalhães et al., 2007) complemented the MDS and was used to examine whether fish assemblages were undergoing a long-term directional change (linear regression). The method involves the calculation of the Euclidean Distance (ED) between each possible pair of annual samples. Regression models where then conducted between ED and the square root of the time lag separating the samples. The square root transformation reduces possible bias in the analysis resulting from few data points at larger time lags.

Possible changes in ecological integrity of fish assemblages along the study period in each site were evaluated through variability in structural and functional features of fish assemblages. These community attributes, or fish metrics, included fish density, species richness and diversity (Shannon-Wiener Index), relative abundance of non-native, potamodromous and long lived species and functional guilds related to habitat (relative abundance of rheophilic, limnophilic, eurytopic, benthic and water column species), breeding (relative abundance of lithophilic and phytophilic species), feeding (relative abundance of omnivorous and insectivorous species) and tolerance (relative abundance of intolerant, tolerant and intermediate tolerance species) to which captured species were assigned according to published literature (FAME, 2004; Ilhéu, 2004; Cabral et al., 2005; Holzer, 2008; Magalhães et al., 2008) and expert judgment based on the available knowledge.

A total of 117 metrics was initially considered for analysis, expressed in abundance and number of species, in relative and absolute terms and calculated for total fish assemblages and for native species assemblages. Individual fish metrics were then screened for natural variability by calculating their CV in least disturbed and disturbed sites, and for their response to anthropogenic disturbances using Spearman rank correlation coefficient ($|r| \geq 0.5$; $P < 0.05$) between fish metrics and human pressure variables. Mann-Whitney test was used to detect significant differences in fish metrics CVs between least disturbed and disturbed sites (Siegel and Castellan, 1988). Only non-redundant metrics, presenting significantly low natural variability (CV < 50%) (Grossman et al., 1990) under least disturbed conditions over time compared to disturbed sites, and significant responses to human pressure variables were selected. As for assemblages composition, variability in fish metrics was quantified with Bray-Curtis similarity coefficients for each site.

Relationships between fish assemblages variability, human disturbances and hydrological variability were assessed with Spearman rank correlation coefficient ($|r| \geq 0.5$; $P < 0.05$) and using Redundancy Analysis (RDA) (Jongman et al., 1987). The model was tested with Monte Carlo test (999 permutations). Correlations larger than |0.4| were used in gradients interpretation. To account for multicollinearity, variables were maintained in the models only if their addition did not cause any Variation Inflation Factor (VIF) exceeding 3. A linear ordination method was selected as a preliminary Detrended Correspondence Analysis has shown a gradient length smaller than 3SD (ter Braak and Smilauer, 1998). The possible influence of species richness, total fish density and landscape gradients in fish assemblages variability was taken into account in these analyses.

For sequential Spearman rank correlations and Mann-Whitney tests the obtained *P*–values were adjusted using the Bonferroni correction (see Wright, 1992) and the significance level was set at 0.05.

All data were either log (x+1) (linear measurements) or arcsin [sqrt (x)] (percentages) transformed (Legendre and Legendre, 1998) before calculating Bray-Curtis similarities and performing MDS, Spearman rank correlations and RDA. Statistical analyses were performed using the software Statistica 6.0 (StatSoft Inc., 2001), Primer 6.0 (Clarke and Gorley, 2006) and Canoco 4.5 (ter Braak and Smilauer, 2002).

Results

Environmental and anthropogenic characterization of sites

Sampled streams are representative of small southern Portuguese streams, showing a strong intermittent character. Table 4.1 summarizes the environmental and anthropogenic characterization of sampled sites considering the period and years of sampling.

Drainage area of streams ranged between 10.2 km^2 and 192.9 km^2. Alg, Asm, Cbx, Fqm, Gar and Mos, with drainage areas lower than 60 km^2, represented the smallest streams. Mtg, Ami, Pec, Saf and Vas have drainage areas around 100 km^2. Finally, Azb, Sdg and Val presented the larger drainage areas, with values between 150 km^2 and 200 km^2. Distance from source was highly correlated with drainage area and was therefore excluded from the analyses. As expected, altitude of sites showed a negative relation with drainage area ($r = -0.74$; $P < 0.01$), with Alg, Cbx and Mos located in the highest altitudes, followed by Asm and Vas. Lowest altitudes were registered for Saf and Val.

Considering the hydrological variables, annual rainfall was strongly correlated with altitude ($r = 0.71$; adj $P < 0.01$), with particularly high values occurring in Alg and Cbx (higher than 900 mm). Asm and Vas showed slightly lower values (around 700 and 800 mm) and Saf registered the lowest annual rainfall. In all the other sites values ranged somewhere between 500 mm and 600 mm rainfall. Cumulative rainfall surplus was also correlated with altitude ($r = 0.64$; adj $P < 0.05$) and cumulative rainfall deficit was related to the relative frequency of dryer years ($r = 0.79$; adj $P < 0.01$) and wetter years ($r = –0.79$; adj $P < 0.01$) and maximum values were observed in Cbx, Fqm, Sdg and Vas. Overall hydrological variability, i.e. CV of annual rainfall was highest in Gar (> 50%), followed by Fqm, Mos, Azb, Pec, Sdg and Val (between 30% and 50%). The lowest hydrological variability was observed in Mtg and Vas (< 20%).

Regarding local variables, current velocity showed high variability in general and water depth presented a moderate variability in most sites. Sites with thinner substrate tended to show higher substrate variability over time (e.g. Azb and Ami) than sites with coarser substrate (e.g. Val and Vas).

Table 4.1 Summary of the main environmental and anthropogenic characteristics of sites during the sampling years

Sites	Alg	Asm	Cbx	Fqm	Gar	Mos	Mtg	Ami	Azb	Pec	Sdg	Saf	Val	Vas
No. years	5	7	6	8	9	11	5	6	8	7	10	6	6	4
Altitude (m)	460	282	365	230	249	329	220	180	168	175	225	135	157	270
Drainage area of the basin (km^2)	10.24	47.20	30.62	42.30	59.99	46.44	112.40	100.00	192.93	98.50	167.17	99.61	178.11	107.68
Total annual rainfall (mm) (mean ± SD)	922.00 ± 23.17	715.62 ± 26.77	922.56 ± 28.17	556.63 ± 39.35	517.54 ± 51.62	615.27 ± 34.14	509.55 ± 11.28	626.21 ± 26.18	520.21 ± 33.34	523.79 ± 35.70	529.89 ± 38.40	438.48 ± 24.62	570.86 ± 34.59	816.89 ± 16.49
Total annual rainfall (CV)	23.17	26.77	28.17	39.35	51.62	34.14	11.28	26.18	33.34	35.70	38.40	24.62	34.59	16.49
Cumulative rainfall surplus (%) (mean ± SD)	29.61 ± 37.05	36.34 ± 41.68	3.31 ± 6.53	26.25 ± 39.11	26.29 ± 39.85	23.12 ± 37.13	22.22 ± 28.23	17.57 ± 24.51	15.07 ± 25.49	17.22 ± 26.74	21.00 ± 36.22	1.17 ± 2.87	4.47 ± 7.82	25.63 ± 51.25
Cumulative rainfall deficit (%) (mean ± SD)	17.03 ± 32.16	6.06 ± 11.53	69.21 ± 91.32	146.31 ± 167.03	97.12 ± 153.76	25.74 ± 34.99	38.13 ± 52.33	14.77 ± 20.46	62.92 ± 76.67	65.95 ± 82.29	127.07 ± 152.79	69.50 ± 60.77	80.26 ± 99.97	152.02 ± 142.62
Relative frequency of wet years	0.60	0.71	0.33	0.38	0.44	0.45	0.60	0.50	0.38	0.43	0.30	0.17	0.33	0.25
Relative frequency of dry years	0.40	0.29	0.67	0.63	0.56	0.55	0.40	0.50	0.63	0.57	0.70	0.83	0.67	0.75
Water depth (CV)	24.42	24.72	36.26	34.71	35.41	40.38	62.87	17.03	37.30	8.04	47.33	72.18	31.62	17.82
Current velocity (CV)	62.47	59.43	27.03	84.07	157.95	36.54	39.56	87.76	51.13	95.59	56.44	92.97	56.53	67.09
Dominant substrate	gravel - pebble	cobble - boulders	pebble - cobble	sand - gravel	mud - sand	pebble - cobble	cobble - boulders	gravel - pebble	mud - sand	sand	sand - gravel	sand - gravel	cobble - boulders	boulders
Dominant substrate (CV)	24.64	10.50	9.21	23.82	15.95	14.67	12.79	33.45	59.31	20.42	24.22	22.37	5.12	7.52
Total human pressure (mean (min - max))	11.8 (11 - 13)	18.6 (14 - 20)	14.3 (14 - 15)	18.4 (16 - 21)	24 (21 - 29)	12.7 (11 - 14)	15 (13 - 18)	13 (11 - 17)	16.9 (12 - 24)	19.6 (17 - 22)	21 (20 - 22)	18.7 (18 - 21)	18	15 (14 - 16)
Abiotic classification	Least disturbed	Least disturbed/ Disturbed	Least disturbed	Least disturbed/ Disturbed	Disturbed	Least disturbed	Least disturbed/ Disturbed	Least disturbed/ Disturbed	Least disturbed/ Disturbed	Disturbed	Disturbed	Disturbed	Disturbed	Least disturbed

During the sampling years, almost all sites registered an increase in human pressure, except Val. Accordingly, most of the least disturbed sites have changed their abiotic classification along the years (Asm, Fqm, Mtg, Ami, Azb). All these sites suffered a marked increase in human pressure and two even became some of the most disturbed ones (Azb and Fqm). Only Alg, Cbx, Mos and Vas remained least disturbed. As these sites are located at high altitudes, a negative correlation between total pressure and altitude of sites was observed ($r = -0.5$; adj $P < 0.05$). On the contrary, Gar, Pec, Sdg and Saf were always subjected to meaningful anthropogenic disturbances, which continued to increase along the years, especially in Gar.

Total human pressure and its increase were mainly due to land use/land use changes ($r = 0.78$; adj $P < 0.01$), namely throughout agriculture and livestock intensification in the last years, degradation of riparian vegetation ($r = 0.94$; adj $P < 0.001$), nutrient/organic input ($r = 0.74$; adj $P < 0.05$) and sediment load ($r = 0.85$; adj $P < 0.001$). Considering that these variables were all significantly intercorrelated ($r > 0.5$; adj $P < 0.01$), riparian degradation may occur independently or as a consequence of land use practices, leading all together to increases in nutrient/organic input and sediment loads in sampled streams. The remaining pressure variables were fairly stable and ranged mostly between high (score 1) and good (score 2) condition.

Composition, persistence and stability of fish assemblages

Eleven native species and eight non-native species were captured during the study (Table 4.2). *S. alburnoides* was the most abundant and frequent species in most of the sites. *Squalius pyrenaicus* was particularly abundant and frequent in Alg, Cbx and Vas. *Iberochondrostoma lemmingii* and *Cobitis paludica* tended to occur in sites with finer substrate, especially in Fqm, Gar and Saf, which were also some of the most human disturbed sites. Nevertheless, *C. paludica* also occurred with high relative abundance in Asm, Mos and Vas. Barbels were all widespread species, though higher abundances and frequencies of occurrence were observed for *B. microcephalus* and juvenile individuals (*Barbus* spp.). *B. bocagei* only occurred in Val, the only site sampled in the Sado river basin, due to different and restricted distribution areas of barbel species. *P. willkommii* showed higher occurrence and abundance in Mos, Ami and Cbx. The occurrence of *Anaecypris hispanica*, a small endemic and endangered species was reduced to three sites and only showed meaningful values in Asm. Overall, fish assemblages were dominated by native species in the least disturbed sites. Non-native species were mainly present in human disturbed sites, particularly in Gar, Pec, Sdg, Saf and Val, where they represented a large percentage of the total fish species or even dominated the assemblages. Non-natives were mostly represented by *L. gibbosus* and *Gambusia holbrooki*.

Table 4.2 List of captured species (scientific and common names), occurrence type (End Ib – Iberian endemism; End Bas – Basin endemism) in Guadiana and/or Sado river basins and respective frequency of occurrence

		Occurrence			
Species	**Common name**	**Type**	**Guadiana basin**	**Sado basin**	**Frequency**
Anaecypris hispanica	Spanish Minnow Carp	End Bas	x		0.05
Barbus spp. (juveniles)	juvenile Barbels	End Ib	x		0.52
Barbus bocagei	Common Barbel	End Ib		x	0.05
Barbus steindachneri	Steindachneri Barbel	End Ib	x		0.12
Barbus comizo	Iberian Gudgeon	End Ib	x		0.17
Barbus sclateri	South Barbel	End Ib	x		0.02
Barbus microcephalus	Small-head Barbel	End Bas	x		0.52
Squalius pyrenaicus	Iberian Chub	End Ib	x	x	0.51
Squalius alburnoides	Roach	End Ib	x	x	0.86
Pseudochondrostoma willkommii	Guadiana Nase	End Ib	x		0.38
Iberochondrostoma lemmingii	Arched-mouth Nase	End Ib	x		0.41
Cobitis paludica	South Stone Loach	End Ib	x	x	0.58
Lepomis gibbosus	Pumpkinseed	Nnat	x	x	0.57
Cyprinus carpio	Common Carp	Nnat	x	x	0.11
Carassius auratus	Goldfish	Nnat	x	x	0.01
Micropterus salmoides	Largemouth Bass	Nnat	x	x	0.08
Herichtys facetum	Chamaleon Cichlid	Nnat	x	x	0.01
Ameiurus melas	Black Bullhead	Nnat	x	x	0.02
Alburnus alburnus	Bleak	Nnat	x	x	0.08
Gambusia holbrooki	Mosquitofish	Nnat	x	x	0.32

Total number of fish species within sites ranged from 2 to 9, with lower mean values occurring in Alg and Val. In the remaining sites, mean species richness was very similar. As such, this metric was fairly stable along the years in least disturbed sites (mean CV= 21.8%) and moderately stable in disturbed ones (mean CV= 40.4%). Fish density showed high variability amongst sampled sites (mean CV between 61% and 188%) with no significant differences between disturbed and least disturbed sites (adj $P > 0.05$) (Table 4.3).

Persistence and stability of fish assemblages composition followed a similar pattern in sampled sites ($r = 0.57$; $P < 0.05$), yet persistence values were less variable than stability between sites. Persistence registered the highest values in Alg, Vas and Cbx, and the lowest in Fqm and Mtg. Assemblage stability was highest in Alg and Cbx, and lowest in Fqm and Pec. Neither persistence nor variability in fish assemblages composition revealed significant correlations (adj $P > 0.05$) with species richness or with total fish density (Table 4.3).

Table 4.3 Species richness (mean, min-max), total fish density (ind/100 m^2), assemblage persistence (*P*) and stability (mean Bray-Curtis similarity coefficient) in each site

	Speies richness (mean (min - max))	**Total fish density (ind/100 m2)**	**Assemblage persistence (P)**	**Assemblage stability (mean similarity)**
Alg	2	68.85 $\pm$ 129.45	1	93.4
Asm	6 (3 - 9)	100.78 $\pm$ 96.05	0.75	66.00
Cbx	4.8 (3 - 6)	129.20 $\pm$ 100.97	0.80	81.40
Fqm	4.6 (1 - 7)	16.84 $\pm$ 13.95	0.44	60.00
Gar	5.1 (4 - 7)	60.36 $\pm$ 50.99	0.83	71.00
Mos	4.7 (3 - 6)	87.34 $\pm$ 118.04	0.72	75.60
Mtg	3.8 (1 - 9)	9.80 $\pm$ 13.17	0.46	71.60
Ami	5.3 (4 - 7)	24.18 $\pm$ 14.82	0.70	64.30
Azb	5.1 (4 - 8)	64.04 $\pm$ 106.62	0.61	65.10
Pec	5.2 (3 - 8)	43.16 $\pm$ 50.64	0.63	57.90
Sdg	6.8 (3 - 9)	28.74 $\pm$ 20.16	0.73	63.60
Saf	5.3 (4 - 7)	71.80 $\pm$ 65.94	0.60	60.90
Val	3 (2 - 5)	11.69 $\pm$ 11.22	0.54	72.40
Vas	5.8 (5 - 6)	20.35 $\pm$ 31.26	0.88	77.60

Multidimensional Scaling and time lag regression analysis provided complementary results, showing different trends in fish assemblages variability for sampling sites (Table 4.4, Figure 4.3 and Figure 4.4). Fqm, Gar, Ami, Azb, Pec, Sdg, Saf and Val showed significant linear patterns of changes in fish assemblages considering both MDS and time lag regression results. Despite the low values obtained for the determination coefficient (R^2) in several sites, particularly in Fqm and Azb, the regression models and all the equations coefficients were significant, confirming the existence of a linear pattern underlying the data. Alg, Cbx, Mos and Vas did not show significant results for time lag regression, but MDS plots revealed the existence of cyclic patterns in fish assemblages. For Asm and Mtg results suggest a random pattern, with no evident trend in fish assemblages changes. Overall, results suggest a trend away from cyclic patterns towards linear patterns with the increase in total human pressure.

Table 4.4 Results from Multidimensional Scaling (MDS) and time lag regression analysis in each site

Site	Time lag regression (linear)		MDS
	R^2	Trend	Trajectory
Alg	$P > 0.05$	-	Cyclic
Asm	$P > 0.05$	-	Random
Cbx	$P > 0.05$	-	Cyclic
Fqm	0.10; $P < 0.05$	Directional	Directional
Gar	0.24; $P < 0.01$	Directional	Directional
Mos	$P > 0.05$	-	Cyclic
Mtg	$P > 0.05$	-	Random
Ami	0.52; $P < 0.01$	Directional	Directional
Azb	0.17; $P < 0.05$	Directional	Directional
Pec	0.29; $P < 0.01$	Directional	Directional
Sdg	0.23; $P < 0.001$	Directional	Directional
Saf	0.29; $P < 0.05$	Directional	Directional
Val	0.31; $P < 0.05$	Directional	Directional
Vas	$P > 0.05$	-	Cyclic

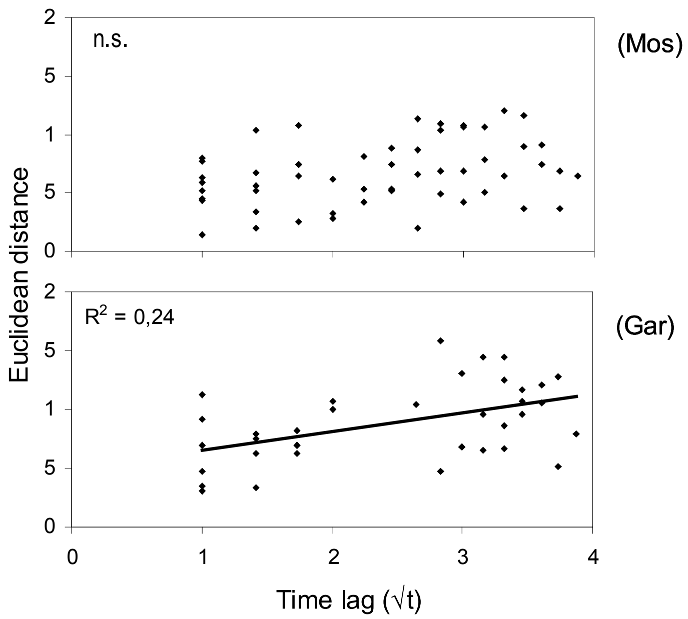

Figure 4.3 Time lag regression analysis for sampled sites Mos and Gar, showing examples of absent and directional (linear regression) trends in fish assemblages over the study period.

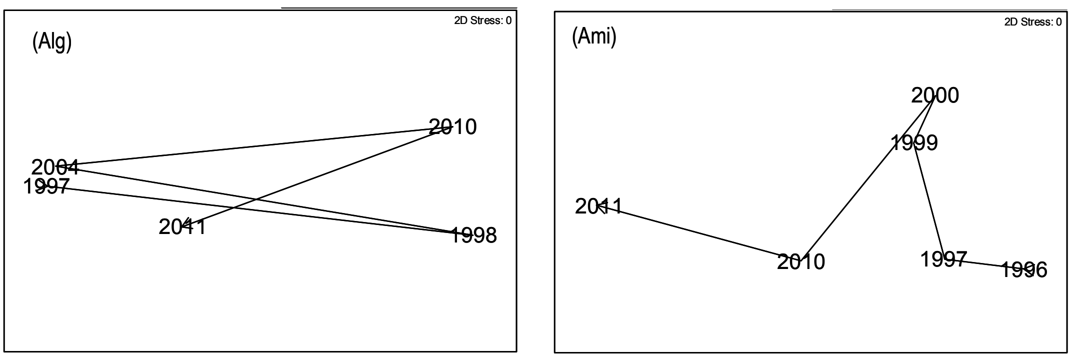

Figure 4.4 2-dimensional MDS configuration plots for sampled sites Alg and Ami with superimposed temporal trajectory, showing examples of directional (progressive move further away from the initial position) and cyclic (displacement followed by a return to an earlier position) changes in fish assemblages over the study period. The stress values obtained (< 0.05) indicate an excellent ordination diagram, with no prospect of misinterpretation (Clarke and Warwick, 1994).

Structural and functional changes in fish assemblages

The mean CVs of fish metrics expressed in terms of absolute abundance were extremely high (> 100%). On the contrary, fish metrics based on relative abundance, absolute and relative number of species revealed lower variability, particularly in undisturbed sites (< 50%). 32 fish metrics with low temporal variability revealed significant differences between least disturbed and disturbed sites. After further screening for significant responses to human pressure variables, seven metrics were selected to evaluate possible ecological changes in fish assemblages (Table 4.5): relative abundance of native species, relative abundance of native insectivorous species, relative abundance of native eurytopic species, relative abundance of native water column species, relative abundance of native lithophilic species, relative abundance of species with intermediate tolerance and relative number of non-native species. Despite the observed redundancy between metrics calculated for total fish assemblages and for native assemblages, native metrics behaved better, regarding the selection criteria.

All the selected metrics showed significant correlations ($|r| > 0.5$; adj $P < 0.001$) mainly with total human pressure, sediment load and organic/nutrient enrichment, although meaningful relations were also observed with land use, degradation of riparian vegetation, and morphological condition (Table 4.5). The non-native metric was positively related with human pressure variables, whereas the native metrics showed and inverse response.

Mean values of fish metrics in each site reflected the observed relationship with human disturbance (Table 4.5). As such, least disturbed sites presented a trend towards high proportion of native metrics and low proportion of non-native species, whilst disturbed sites showed an opposite trend along the human pressure gradient. Accordingly, Alg, Cbx, Mos, Vas and Mtg should present higher fish assemblages integrity over the study period than the other sites, particularly Gar, Pec, Sdg, Saf and Val.

Mean similarities of fish metrics in sampled sites followed the pattern already observed for fish assemblages composition, though with higher values. Furthermore, metrics similarities tended to present higher differences between least disturbed and disturbed sites than similarities of fish composition. As for fish composition, fish metrics did not present significant correlations with species richness and total fish density (adj $P > 0.05$).

Table 4.5 Fish metrics selected to evaluate structural and functional variability of fish assemblages in sites. Differences in the coefficients of variation (mean ± SD) between least disturbed and disturbed sites are significant at $P < 0.001$, and significant correlations ($|r|$? 0.5; $P < 0.05$) with human pressure variables are listed

Fish metrics	Coefficient of Variation: Least disturbed	Coefficient of Variation: Disturbed	Significant correlations with human pressure		Alg	Asm	Cbx	Fqm	Gar	Mos	Mtg	Ami	Azb	Pec	Sdg	Saf	Val	Vas
Relative abundance of native species	1.5 ± 2.3	54.8 ± 30.1	Total human pressure Land use Sediment load Organic/nutrient load Riparian degradation	-0.65 -0.54 -0.64 -0.60 -0.59	100	84.42 ± 17.76	100	74.59 ± 32.90	61.79 ± 32.10	99.28 ± 1.67	98.55 ± 3.24	82.28 ± 33.51	76.14 ± 17.52	67.05 ± 27.97	49.93 ± 37.11	47.46 ± 43.08	27.60 ± 22.26	100
Relative abundance of native insectivorous species	21.0 ± 14.1	81.4 ± 35.7	Total human pressure Land use Sediment load	-0.50 -0.50 -0.50	100	49.77 ± 27.26	85.61 ± 9.17	46.42 ± 33.67	41.34 ± 27.17	71.74 ± 24.38	87.14 ± 14.24	44.82 ± 30.11	52.75 ± 28.37	58.53 ± 29.89	38.90 ± 33.88	21.72 ± 23.32	7.55 ± 11.14	70.44 ± 16.52
Relative abundance of native eurytopic species	24.7 ± 9.2	81.1 ± 41.3	Total human pressure Land use Sediment load Organic/nutrient load Riparian degradation	-0.55 -0.56 -0.50 -0.51 -0.50	79.27 ± 11.49	59.81 ± 30.64	76.63 ± 13.79	39.93 ± 34.17	29.32 ± 23.68	75.84 ± 19.14	83.47 ± 16.80	62.25 ± 27.52	62.77 ± 18.97	34.89 ± 34.06	42.45 ± 32.46	28.90 ± 27.49	6.76 ± 10.49	59.47 ± 23.87
Relative abundance of native water column species	12.3 ± 9.7	76.2 ± 39.3	Land use Sediment load Organic/nutrient load	-0.50 -0.50 -0.50	100	65.96 ± 30.33	87.16 ± 8.49	65.36 ± 33.70	54.01 ± 33.10	74.33 ± 17.58	92.99 ± 7.42	51.58 ± 24.89	65.09 ± 25.27	36.34 ± 36.54	44.35 ± 34.70	26.77 ± 27.12	7.30 ± 11.32	75.78 ± 10.85
Relative abundance of native lithophilic species	5.9 ± 3.6	65.7 ± 29.7	Total human pressure Land use Sediment load Organic/nutrient load Riparian degradation	-0.63 -0.57 -0.61 -0.62 -0.54	100	63.51 ± 31.91	95.79 ± 6.01	67.26 ± 34.42	54.11 ± 33.18	92.64 ± 8.82	96.62 ± 4.64	80.42 ± 32.66	76.14 ± 17.52	39.61 ± 37.84	49.38 ± 36.69	37.32 ± 35.59	27.35 ± 22.62	86.48 ± 10.09
Relative abundance of species with intermediate tolerance	5.9 ± 3.6	73.0 ± 40.9	Total human pressure Land use Sediment load Organic/nutrient load Riparian degradation	-0.62 -0.55 -0.60 -0.61 -0.52	100	63.51 ± 31.91	95.79 ± 6.01	67.26 ± 34.42	54.11 ± 33.18	92.64 ± 8.82	96.62 ± 4.64	80.42 ± 32.66	76.14 ± 17.52	39.61 ± 37.84	49.38 ± 36.69	37.32 ± 35.59	7.30 ± 11.32	86.48 ± 10.09
Relative number of non-native species	27.7 ± 60.5	62.4 ± 37.1	Total human pressure Land use Sediment load Organic/nutrient load Riparian degradation Morphological condition	0.68 0.58 0.65 0.62 0.55 0.50	0	19.40 ± 11.69	0	16.61 ± 11.97	35.58 ± 10.42	5.61 ± 9.78	4.44 ± 9.94	14.29 ± 23.90	29.11 ± 11.08	42.26 ± 22.10	44.90 ± 26.15	31.75 ± 25.90	51.11 ± 8.61	0
Structural and functional stability (mean similarity)					99.14	80.67	94.92	67.95	77.87	90.92	91.39	77.73	87.46	68.99	67.88	63.75	77.6	92.34

Relationships between fish assemblages variability, human pressure and hydrological variability

Variability in fish assemblages was associated to human pressure and hydrological variability. Fish composition similarities showed significant correlations with altitude (r = 0.64; adj $P < 0.05$), annual rainfall (r = 0.61; adj $P < 0.05$) and CV of current velocity (r = -0.51; adj $P < 0.05$), but also with total pressure (r = -0.66; corrected $P < 0.01$), riparian degradation (r = -0.59; adj $P < 0.05$), sediment load (r = -0.7; adj $P < 0.05$) and organic/nutrient enrichment (r = -0.5; adj $P < 0.05$) (Table 4.6). Likewise, fish metrics similarities revealed significant correlations with altitude (r = 0.69; adj $P < 0.01$), annual rainfall (r = 0.58; adj $P < 0.05$), CV of annual rainfall (r = -0.53; adj $P < 0.05$), total pressure (r = -0.72; adj $P < 0.01$), land use (r = -0.59; adj $P < 0.05$), riparian degradation (r = -0.62; adj $P < 0.01$), sediment load (r = -0.76; adj $P < 0.01$) and organic/nutrient input (r = -0.50; adj $P < 0.05$) (Table 4.6). On the other hand, assemblage persistence was only dependent on altitude (r = 0.71; adj $P < 0.01$) and annual rainfall (r = 0.60; adj $P < 0.05$) of sites. Despite the absence of significant results, a trend towards lower values was observed in most degraded sites.

However, total human pressure was also related to hydrological variability, showing significant correlations with annual rainfall (r = -0.61; adj $P < 0.05$) and CV of annual rainfall (r = 0.59; adj $P < 0.05$). Moreover, sediment load was related with the CV of annual rainfall (r = 0.72; adj $P < 0.01$) and nutrient/organic enrichment decreased with the relative frequency of wetter years (r = -0.64; adj $P < 0.05$) and increased with the relative frequency of dryer years (r = 0.64; adj $P < 0.05$) and cumulative rainfall deficit (r = 0.61; adj $P < 0.05$).

RDA results were in accordance with the observed correlations, further contributing to the general interpretation of major influences in assemblages variability and possible changes in ecological integrity. The first two axes ($\lambda_1 = 0.71$; $\lambda_2 = 0.07$) accounted for 78% of the total variance in fish assemblages variability (considering both composition and structural and functional features) and nine significant ($P < 0.05$) variables were included in the ordination model. According to canonical coefficients and inter-set correlations, axis 1 was mainly defined by annual rainfall (r = -0.76) and total human pressure (r = 0.70), and to a less extent by CV of current velocity (r = 0.54) and CV of annual rainfall (r = 0.41), while axis 2 was more related to the increase in human pressure (r = 0.63).

Table 4.6 Most relevant correlations (|r| ≥ 0.5; adj P < 0.05) between fish assemblages variability (fish composition similarities, fish metrics similarities and assemblage persistence) and hydrological and anthropogenic variables.

Variables (hydrological and anthropogenic)	Correlations		
	Fish composition similarities	Fish metrics similarities	Assemblage Persistence
Altitude	r = 0.64; P < 0.05	r = 0.69; P < 0.01	r = 0.71; P < 0.01
Annual rainfall	r = 0.61; P < 0.05	r = 0.58; P < 0.05	r = 0.60; P < 0.05
CV of annual rainfall	n.s.	r = -0.53; P < 0.05	n.s.
CV of current velocity	r = -0.51; P < 0.05	n.s.	n.s.
Total human pressure	r = -0.66; P < 0.01	r = -0.72; P < 0.01	n.s.
Land use	n.s.	r = -0.59; P < 0.05	n.s.
Riparian degradation	r = -0.59; P < 0.05	r = -0.62; P < 0.01	n.s.
Sediment load	r = -0.70; P < 0.05	r = -0.76; P < 0.01	n.s.
Organic/nutrient load	r = -0.50; P < 0.05	r = 0.50; P < 0.05	n.s.

Ordination diagram (biplot of sites x human pressure and hydrological variables) (Figure 4.5) showed a good spatial dispersion of sites, especially along the first axis. Least disturbed sites (Alg, Cbx, Mos and Vas) showed the highest fish assemblages stability and were mainly associated to high annual rainfall and cumulative surplus. On the contrary, most disturbed sites, especially Gar, Sdg, Saf and Pec, experienced meaningful changes in fish assemblages and simultaneously suffered the influence of high hydrological variability represented by CV of annual rainfall and habitat disturbance (CV of current velocity and substrate). Cumulative rainfall deficit was one of the least important variables. However, this variable was more associated to assemblages changes in the most disturbed sites, even though high values have been also registered in least disturbed ones.

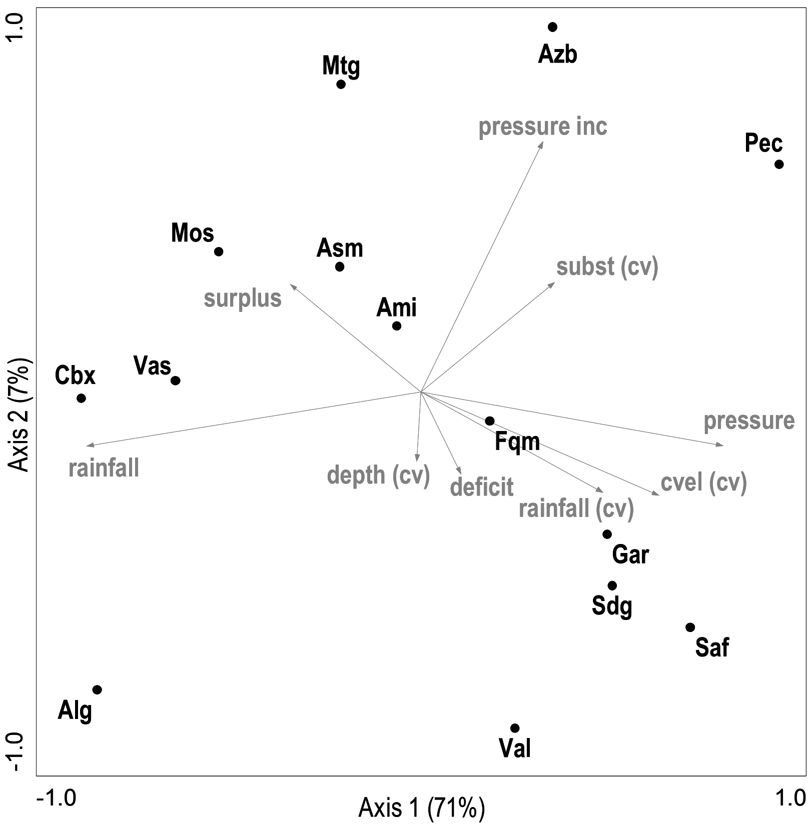

Figure 4.5 Ordination diagram of Redundancy Analysis (RDA) between fish assemblages variability (Bray-Curtis similarities of sites along the years using fish composition and metrics), human pressure variables and hydrological variables.

Discussion

Under the need for developing reliable biological indicators based on fish assemblages in a hydrologically variable environment as Mediterranean climate streams, this study analysed the role of hydrological variability on the responses of fish assemblages to human disturbance in small intermittent streams.

In this context, spatial and inter-annual variability of fish assemblages was analysed based on relative abundance of fish species and structural and functional assemblage attributes (i.e. fish metrics). Fish assemblage attributes, which respond to anthropogenic disturbances but exhibit low natural temporal variability, are potentially the most sensitive indicators of human pressures to use in bioassessment (Paller, 2002). Seven metrics fulfilled those criteria and were thus selected to indicate possible changes in fish assembles integrity in each site: relative abundance of native species, relative abundance of native insectivorous species, relative abundance of native eurytopic species, relative abundance of native water column species, relative abundance of native lithophilic species, relative abundance of species with intermediate tolerance and relative number of exotic species. These metrics are then the most appropriate to include in a possible multimetric index based on fish fauna in small intermittent Mediterranean streams.

High variability of fish assemblages was mainly associated with the human-induced disturbance, particularly nutrient/organic load and sediment load. Both correlations and RDA results showed strong associations of Bray-Curtis similarity coefficients based on fish composition and metrics with human pressure variables. Diffuse pollution from agriculture is possibly the major human impact on the aquatic systems of southern Portugal, though livestock may have an important contribution to the organic loading. The absence of fences along the streams is very common, allowing cattle to invade the streams, often with destruction of riparian vegetation, factors that together lead to an increase in water eutrophication and ecosystem degradation (e.g. Vidon et al., 2008).

Fish assemblages variability was also related to hydrological variability, mainly expressed by CV of mean annual rainfall and alterations in habitat conditions following hydrological disturbances, particularly current velocity and substrate type. These results agree with previous studies on Mediterranean-type streams, presenting a wide inter-annual variation on fish assemblages associated to the typical hydrological fluctuations observed (Filipe et al., 2002; Magalhães et al., 2002a, 2007; Bernardo et al., 2003; Clavero et al., 2005; Mesquita et al., 2006; Ferreira et al., 2007). In least disturbed sites, despite the natural disturbances caused by inter-annual rainfall variations (including drought and flood events), long-term assemblage stability seems to be maintained. This was further confirmed by time lag regression and MDS analyses, as all least disturbed sites showed cyclic temporal patterns of changes in fish assemblages that underpin the existence of a drift-and-recovery pattern and a high resilience to natural disturbances (Magalhães et al., 2007). On the contrary, in disturbed sites, a directional trend pattern was observed, reflecting a decrease on the resistance and resilience of fish assemblages. In moderate disturbance conditions, no evident trend in fish assemblages changes was observed. The highly variable floods and drying events in Mediterranean streams may have acted as selective environmental filters over very long time scales, thereby reducing the subset of species to those evolutionarily adapted to cope with the prevailing harsh environmental patterns (sensu Poff, 1997). On the contrary, disturbed sites presented much higher variability of fish composition and metrics, and directional temporal changes, hence a short and long-term instability possibly entailing changes in assemblages integrity. Similar results were found in other studies (e.g. Karr et al., 1987; Schlosser, 1990; Taylor et al., 1996; Paller, 2002) showing that fish assemblages exhibit higher temporal variability in disturbed environments.

In dryer years, low flow conditions directly affect the availability of and access to habitats, namely the reproductive ones, and, decreasing the water volume and wetted area, affect to some extent the life of fish, as well. As a result, reproduction and recruitment of the native fish are negatively affected and susceptibility to predation increases caused by confinement in smaller water bodies. For non-native species with benthic affinities, e.g. centrarchids, carp, or mosquitofish, stream conditions in dryer years become more suitable. But in human disturbed systems, especially in nutrient/organic enriched streams, during dry periods the aquatic biota face additional pressures. Low flow causes the intensification of eutrophication, as no washing out of finer and organic sediments and no dilution of nutrients and biomass take place and slower running waters are more favourable to primary production. Under these circumstances, the fish assemblages are subject to the natural hydrological disturbance and to its consequences on the water quality level, as well.

In the least disturbed sites, fish assemblages have to cope with the habitat changes caused by low flows and subsequently recover, which causes the observed cyclic pattern (alternating some loss of integrity and recovery). In the more disturbed sites, low flow conditions enhance the pressures exerted upon fish assemblages and no similar recovery is observed, originating a quite different pattern of response over time.

In this study neither the number of species nor the total fish density were shown to influence fish assemblages variability, leading to possible confounding results, as sites with higher species richness and abundance are likely to be less susceptible to environmental disturbances than sites with impoverished fish fauna, which are more prone to local extinctions (Tilman et al., 1998).

Along the study years, most sampled streams were subjected to an increase in human pressure, especially those located in streams nearby more populated areas. On the contrary, streams located at higher altitudes, in isolated areas with limited or difficult access, suffered the lowest increase in human disturbance and maintained least disturbed abiotic classification over the study period. This fact was determinant in the observed negative relation between human pressure and altitude, and consequently with total annual rainfall, which normally present high values in altitude areas. Therefore, the existence of a landscape gradient in fish assemblages variability defined by altitude actually reflects a prevailing human pressure gradient. Regarding other possible confounding landscape factors in interpreting the importance of human pressure and hydrological variability in fish assemblages variability, results also did not show any direct influence of drainage area (Horwitz, 1978; Schlosser, 1982).

Considering that inter-annual hydrological variability tended to be low in higher altitude streams, the discussed increase in anthropogenic disturbance observed in many lowland sites was also partially responsible for the positive relation observed between total human pressure and hydrological variability. Nevertheless, results also suggest that hydrological variability may aggravate the impact of human pressures (and to a certain extent their evaluation) in fish assemblages, as reflected by the relations between sediment load and the CV of annual rainfall, and also between nutrient/organic load and the relative frequency of wetter and dryer years and cumulative rainfall deficit.

Accordingly, in sites with high annual rainfall and lower inter-annual hydrological variability high flows are more frequent, leading to habitat rearrangement and flush out of fine sediments and organic material. In fact, the mentioned negative correlation between the relative frequency of wetter years and organic/nutrient pressure is related to the fact that high flow conditions may promote nutrient dilution, improving the water quality and reducing the impact of organic/nutrient load on the biota. Moreover, wetter years favour the reproduction of native species (Hill et al., 1991; Bernardo et al., 2003) as well as promoting post-summer recolonizations and spawning migrations up the river systems (Bernardo and Alves, 1999; Magalhães et al., 2002b; Ilhéu, 2004). Several studies reported increased recruitment of native juveniles following flow disturbance, causing rapid recovery of fish assemblages (e.g. Bohnsack, 1983; Closs and Lake, 1996; Lobón-Cerviá, 1996).

On the contrary, in sites with lower annual rainfall and high hydrological variability, the frequent and cumulative absence of floods can act at two levels (Bernardo et al., 2003): (i) decreasing habitat complexity and diversity, promoting siltation and degradation of the water quality, negatively affecting the native fish habitats including the reproductive ones and those required for early life-history stages; (ii) favouring conditions for the recruitment and no flush out of the alien limnophilic species, thus increasing the pressure on the native species through competition and predation.

Results from the sites where abiotic classification changed during the study period (Mtg, Asm, Ami, Azb and Fqm) further reinforced these conclusions. In fact, changes in fish assemblages were lower when hydrological variability was also lower (Asm and Mtg), despite the more or less similar increase in human pressure registered in these sites.

In sum, results suggest that hydrological variability can act jointly with anthropogenic disturbances, producing both direct and indirect effects on fish assemblages in small intermittent streams (Figure 4.6). In human disturbed situations, high hydrological variability (especially if it entails high frequency of dryer years and meaningful cumulative water deficit) may affect the impact of the human pressures both on habitat and on water quality, with significant and consistent variations of fish assemblages composition and integrity that may influence the ecological assessment. Indeed, in sites with variable hydrological regimes and disturbance histories, substantially different conclusions from bioassessment of a site can be obtained from different years, even if data was collected identically on each sampling occasion and no change in human disturbance was registered (Wilcox et al., 2002).

The synergetic effects of hydrological variability and human pressures on fish assemblages, acquires particular importance under a climatic change scenario (see Santos and Miranda, 2006) that foresees significant impacts not only towards an increase in temperature, but also towards reductions in rainfall and increases in inter-annual flow variability in Mediterranean streams. Consequently, these aquatic ecosystems are likely to be highly vulnerable to long-term changes in hydrologic regime in the future. The selection of metrics for the fish multi-metric indexes should avoid possible bias related to metrics, which might respond to natural hydrological variations

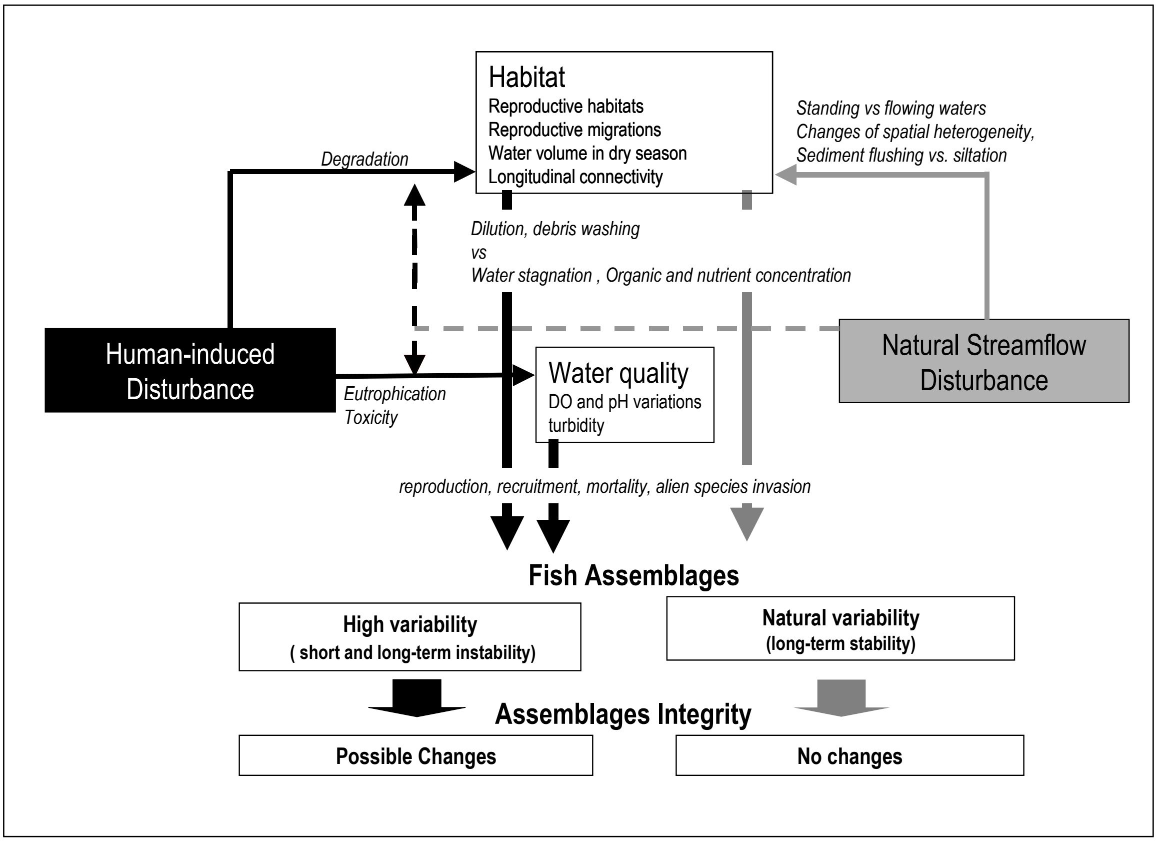

Figure 4.6 Potential anthropogenic and hydrological mechanisms determining variation in fish assemblages.

Conclusions

High hydrological variability may affect fish assemblages composition, but native fish species seem to exhibit high resilience to natural disturbance when hydrological and habitat conditions resemble the pre-disturbed state. Despite the relatively small number of sites analysed, this study contributes to highlight the importance of assessing temporal variability on stream biomonitoring programs. This approach makes possible to distinguish substantive ecological changes from natural fluctuations of fish assemblage structure. In this study, the fish metrics selected to assess changes in assemblages integrity are based on the relative abundance of native species (insectivorous species, eurytopic species, water column species, native lithophilic species), relative abundance of species with intermediate tolerance and relative number of exotic species. These metrics are the most appropriate for inclusion in a multimetric index to be developed for these streams because they respond to anthropogenic disturbances but exhibit low natural temporal variability. Nevertheless, re-evaluation of this issue with larger data sets from different river types and longer time series including extreme natural events would help to improve and expand the present results. Moreover, a correction of class boundaries for the developed biotic indexes may also be a possible solution in order to improve the assessment accuracy in small Mediterranean climate streams.

Acknowledgements

This study gathered data collected under several different programmes: i) assessment of the ecological flow in Guadana river basin, partially funded by the National Water Agency (INAG, Instituto Nacional da Água); ii) the implementation of the Water Framework Directive, partially funded by the National Water agency; and iii) climate change and fish communities of Mediterranean-type streams - potential impact on the bio-integrity and implications on the ecological status assessment, funded by FCT (Fundação para a Ciência e Tecnologia). P. Matono was supported by a grant from FCT and later by a grant from IIFA (Instituto de Investigação e Formação Avançada, Universidade de Évora). Special thanks are due to all the colleagues and volunteers from the University of Évora who collaborated in the fieldwork along the study years. We also thank Eng. Manuela Correia for helping with the production of the study area map. The National Forest Authority provided the necessary fishing permits.

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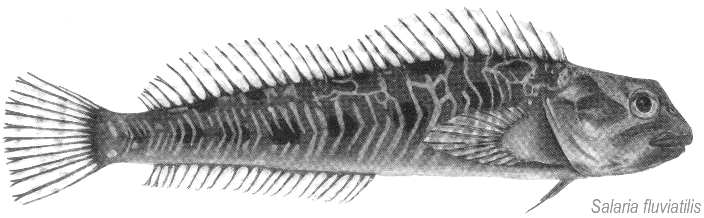
Chapter 5

Effects of land use intensification on fish assemblages in Mediterranean climate streams



Salaria fluviatilis
F. Filipe

In review as

Matono P, Sousa D, Ilhéu M. Effects of land use intensification on fish assemblages in Mediterranean climate streams. Environmental Management.

Abstract

The South of Portugal is experiencing a rather accelerated change in land use towards intensive farming systems, namely olive production. These systems have strong negative environmental impacts and can affect the ecological integrity of aquatic ecosystems. This study aimed to identify the main environmental disturbances related with olive grove intensification on Mediterranean climate streams in Southern Portugal and to evaluate their effects on fish assemblages structure and integrity. 26 streams were sampled within the direct influence of traditional, intensive and hyper-intensive olive groves. Human-induced disturbances were analysed along the olive grove intensification gradient. Integrity of fish assemblages was evaluated through deviation from an independent set of undisturbed/least disturbed sites (references) considering metrics and guilds, based on multivariate analyses. Disturbance variables and physicochemical parameters showed an overall increase along the gradient of olive intensification, mostly organic/nutrient enrichment, sediment load and riparian degradation. Animal load showed an opposite pattern, due to high livestock production nearby traditional olive groves. Olive grove sites were dominated by non-native and tolerant fish species while references sites presented higher fish richness, density and were mainly composed by native and intolerant species. Fish assemblages structure was significantly different from the reference set but not among olive grove types. Bray-Curtis Similarities with reference sites showed a trend decrease in fish assemblages' integrity along the olive grove gradient. Olive production led to multiple in-stream disturbances, whose cumulative effects promoted the loss of the biota integrity, even in traditional olive groves. The impact of these low intensity practices on the aquatic ecosystems can be dramatically different when they are coupled with livestock production. Although preliminary, this study may contribute to guide policy decision-makers in agriculture and water management.

Keywords: Land use intensification; olive grove; river degradation; water quality; fish assemblages; Portugal.

Introduction

Land use and land cover change play an important role on the global change phenomena (Foley et al. 2005; Lambin and Geist 2006), and is an important factor affecting the ecological integrity and status of fluvial ecosystems (e.g. Roth et al. 1996; Lammert and Allan 1999; Allan 2004).

The Mediterranean region has been shaped by human activity and maintained by traditional practices of land use for centuries. The South of Portugal has long been characterized by the maintenance of evergreen oak agroforestry systems, including olive agroecosystems (traditional olive groves) but also large extensive non-irrigated farming (Pinto-Correia 1993; Vos et al. 1993). However, in the last decades, farming systems experienced a rather accelerated change, due to the perspective of a profitable irrigated agriculture, namely through olive hyper intensive systems (Pinto-Correia and Vos 2004). Despite the growing recognition of intensive agriculture systems impacts on water resources and ecosystems, the European Common Agriculture Policy launched strong production incentives to the intensification of several cultures, namely olive farming. Intensive olive systems are characterized by a high-density of trees, systematic irrigation and mechanized harvesting. Recently, hyper-intensive olive groves have also been increasing, and incorporating new approaches of production with high uptakes of energy and water at the natural resources expenses.

The intensive agrosystems have strong negative environmental impacts, particularly on soil erosion, run-off to water bodies, degradation of habitats and exploitation and contamination of scarce water resources (Beaufoy and Pienkowski 2000; Beaufoy 2001). High fertilisation and irrigation associated with intensive agriculture, may result in degradation of aquatic ecosystems and depletion of superficial and groundwater resources (e.g. Zalidis et al. 2002). Intensive agriculture/forestry can result in several types of stress, which alone or together affect the structure and functioning of inland waters. For instance, the decrease of river discharge due to water over-exploitation (both underground and surface) may change river hydrology, increasing siltation and reducing habitat heterogeneity, with negative effects on aquatic biota, namely on fish bio-integrity (e.g. Bernardo et al. 2003; Meador and Carlisle 2011). On the other hand, downstream effects of run-off from irrigated cultures, as topsoil, fertiliser and herbicides are washed into the water bodies leading to the water contamination and loss of biodiversity (Beaufoy 2001).

The integrity of inland waters is a central issue to water policies and to nature conservation. In Europe, with the implementation of Water Framework Directive (WFD) (European Commission 2000), all Member States must assess, monitor and improve, if necessary, the ecological quality status of water bodies. The identification of human-induced disturbances and the evaluation of their effects on the aquatic ecosystems are therefore crucial to support the implementation of effective environmental policies and will contribute to support scientifically the recommendations to the farmers, technicians and agriculture/forestry organizations to minimize the identified impacts, integrating science with practice.

Fish assemblages are generally recognized as indicators of aquatic ecosystem health. Under this context, the assessment of water quality coupled with fish guilds is useful to evaluate the status of the aquatic biota integrity and its relationship with human activities.

The objective of this study was to identify the main environmental disturbances related with olive grove intensification on Mediterranean climate streams in southern Portugal, namely regarding water quality and morphological condition, and to evaluate their effects on fish assemblages' structure and integrity.

Methods

Study area

Study sites were located in the South of Portugal, distributed through Tagus (19% of sites), Sado (27%) and Guadiana (54%) river basins. All the sites were located within a relatively homogeneous environmental area, comprising three South river-types (see INAG 2008a) as defined under the implementation of WFD in Portugal. The South of Portugal is a lowland region with few low altitude mountains. The climate is typically Mediterranean, with high intra and inter-annual precipitation and discharge variation, with severe and unpredictable floods between autumn and spring and persistent summer droughts (Miranda et al. 2002). Mean annual air temperature is high (16ºC) and mean annual precipitation ranges from 350 to 1200 mm (APA, available at http://sniamb.apambiente.pt/webatlas). Although it is not an overpopulated area, the landscape has been deeply transformed during the last century by agricultural activity. Most of the land use is currently under agricultural practices and a marked trend towards intensive irrigated agriculture is observed (e.g. olive groves, vineyards and irrigation crops). As a consequence of the growing water demands, numerous reservoirs have been built in this region, being one of them the largest artificial lake in Europe (Alqueva reservoir). Besides diffuse pollution caused by agriculture, livestock production impacts on water quality and water abstraction, other common human-induced disturbances include river channelisation and depletion of the riparian vegetation.

Site selection and sampling

Sampling took place during the spring of 2010 in 26 streams within the direct influence of traditional (N = 9), intensive (N = 11) and hyper-intensive (N = 6) olive groves. Olive groves areas ranged between 2 and 10 hectares and were within a maximum of 100 m distance from the sampled streams.

The classification of olive grove types was based on tree density: (i) traditional olive grove with a density of less than 200 trees/ha; (ii) intensive olive grove with a density between 200 and 1000 trees/ha; and (iii) hyper-intensive olive grove with more than 1000 trees/ha (Fontanazza et al. 1998). Unlike traditional olive groves, all the intensive and hyper-intensive systems were irrigated. Pre-selection of sites followed a GIS screen, based on digital cartography with information on land cover (Caetano et al. 2009). Afterwards, sites were selected based on field surveys, because many areas are undergoing a continuous change in land use and consulted cartography was not updated.

Fish were captured 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 distance from source (km), altitude (m), mean annual discharge (mm) and mean annual air temperature (ºC). Local variables were assessed during the field sampling procedure: water temperature (ºC), water transparency (Secchi disk depth, m), conductivity (μS/cm), pH, dissolved oxygen (mg/L), mean stream wetted width (m), maximum and mean water depth (m), mean current velocity (m/s), 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 meso-habitat types (pool, run, riffle).

Human disturbance level was evaluated regarding ten semi-quantitative 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 the sum of these scores represented the total human pressure in each site. Livestock production is an important activity in the study area with strong potential impacts on the streams health. Pasture areas are usually located near water bodies. Moreover, absence of fences along the streams is very common, making possible the access of cattle into the water. As such, this disturbance was considered separately from agricultural land use, designated as animal load and scored following the same criteria as the other human disturbance variables. The assessment of several physicochemical water parameters complemented and supported the evaluation of human-induced disturbance and water quality in each site: Phosphate – P_2O_5 (mg/L), total dissolved phosphorous – P (mg/L), nitrite - NO_2^- (mg/L), nitrate - NO_3^- (mg/L), ammonium - NH_4^+ (mg/L) and total dissolved nitrogen - N (mg/L).

Data analysis

Captures were quantified as density (Nb. individuals/100 m^2). Since several sites presented very low number of captures, the use of an index of biotic integrity would reduce overall ecological quality assessment certainty and accuracy. Therefore, integrity of fish assemblages was evaluated with multivariate approaches through deviation from the reference scenario considering metrics and guilds: species diversity (Shannon-Wiener Index), total number of species, total number of native, non-native, intolerant and tolerant species, total fish density (Nb. individuals/100 m^2), proportion of potamodromous and long lived 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).

References included a set of independent undisturbed or least disturbed sites (N = 29) representative of each river-type where sampling took place. These reference sites were previously defined and characterised, under the implementation of WFD (Ilhéu et al. 2009).

Correspondence Analysis (CA) of reference and sampled sites based on metrics and guilds allowed to identify the main patterns of variation in fish assemblage structure.

To face the problem related with the effect of co-variation along natural gradients when interpreting biotic responses to human disturbances a Principal Components Analysis (PCA) was carried out on environmental and human pressure variables of reference and olive grove sites, trying to extract independent and synthetic environmental and perturbation gradients. The environmental axis was then correlated with CA axes in order to account for natural variability in the analysis. No significant correlation would be expected if community structure patterns were completely independent of environmental gradient effects. On the contrary, this environmental gradient should be incorporated as co-variable when analysing responses to olive grove intensification practices.

Permutational Multivariate Anova (Permanova) evaluated the existence of differences in fish community structure among olive grove types and the reference set (Anderson 2001). Model was tested with Monte Carlo test under 9999 permutations.

Bray-Curtis similarities quantified the distances between each site and the reference set. Kruskal-Wallis test was used to find significant differences in Bray-Curtis similarities along the olive grove intensification gradient.

Univariate analysis was conducted to analyse human-induced disturbance variables along olive grove intensification gradient. Significant differences were detected with Kruskal-Wallis test.

Water quality of sites was classified into five classes, in compliance with the characteristics for multiple uses of water and according to the National Water Agency guidelines (SNIRH, available at http://snirh.pt). Classes ranged from A (Excellent quality) to E (Very Bad quality).

Prior to analysis, data were transformed to improve normality: percentages were arcsin [sqrt(x)] and linear measurements were log (x+1). Data analysis was performed using statistical programs Canoco 4.5, Primer 6 and Statistica 6.0.

Results

Anthropogenic disturbance and water quality

In order to explore the maximum gradient range of land use intensification, the reference sites were also considered in the analysis of human-induced disturbance. Results are shown for the most relevant and significant variables and parameters.

Olive production reflected a significant intensification of land use ($P < 0.001$) as well as a general increase in total human pressure ($P < 0.001$) (Figure 5.1). Several other disturbance variables showed significant differences along the land use intensification gradient. Organic/nutrient enrichment and sediment load showed a linear and gradual increasing ($P < 0.001$), whereas animal load and degradation of riparian vegetation followed different patterns ($P < 0.001$). When considering only the olive grove gradient, lower significant differences were observed for most of the disturbance variables. The degradation of riparian vegetation was not significantly different ($P > 0.05$) between olive grove types, and animal loads decreased significantly along the olive grove intensification gradient ($P < 0.01$).

Except for water transparency, physicochemical parameters were not significantly different ($P > 0.05$) among olive grove types, although conductivity ($P < 0.05$), total phosphorous ($P < 0.001$) phosphate ($P < 0.001$), nitrite ($P < 0.001$) and ammonium ($P < 0.001$) concentrations tended to increase along the total gradient of land use (Figure 5.2). Water temperature and nitrate concentrations presented the highest values both on traditional and hyper-intensive systems.

Regarding water quality status (Figure 5.3), no sampled sites presented “Very Good” quality and sites with “Very Bad” water quality were observed only in intensive and hyper-intensive olive groves. Traditional olive grove sites showed similar percentages of “Good”, “Moderate” and “Bad” water quality, while intensive olive grove sites presented a higher percentage of “Moderate” and “Bad” water quality classes. Sites under the influence of hyper-intensive olive groves showed exclusively “Bad” and “Very Bad” water quality.

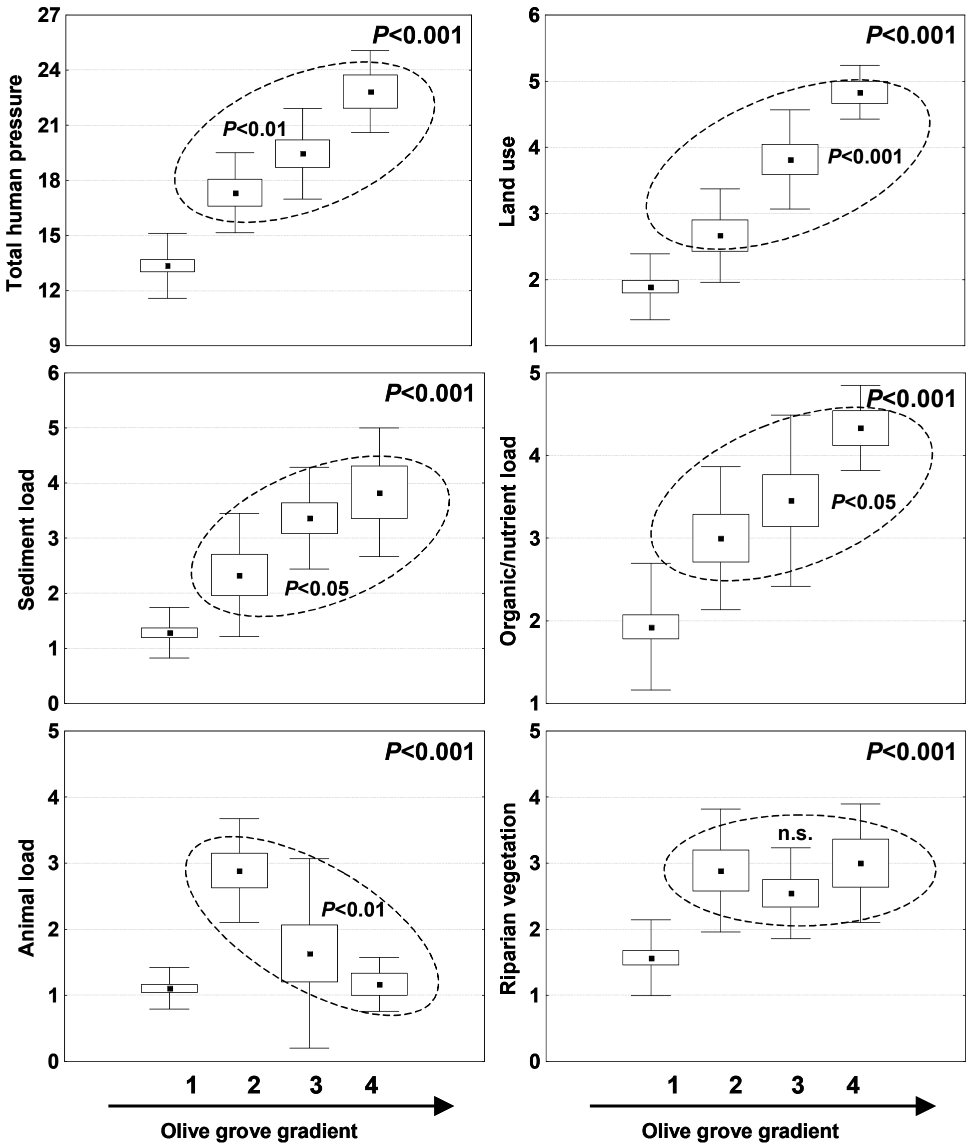


Figure 5.1 Box plots of the most significant results for human-induced disturbances along olive grove intensification gradient. Results from Kruskal-Wallis tests are shown. Olive grove gradient follows the graphical sequence: reference (1), traditional olive grove (2), intensive olive grove (3), hyper-intensive olive grove (4). (▪): Mean; box: $\pm$ SE; whisker: $\pm$ SD.

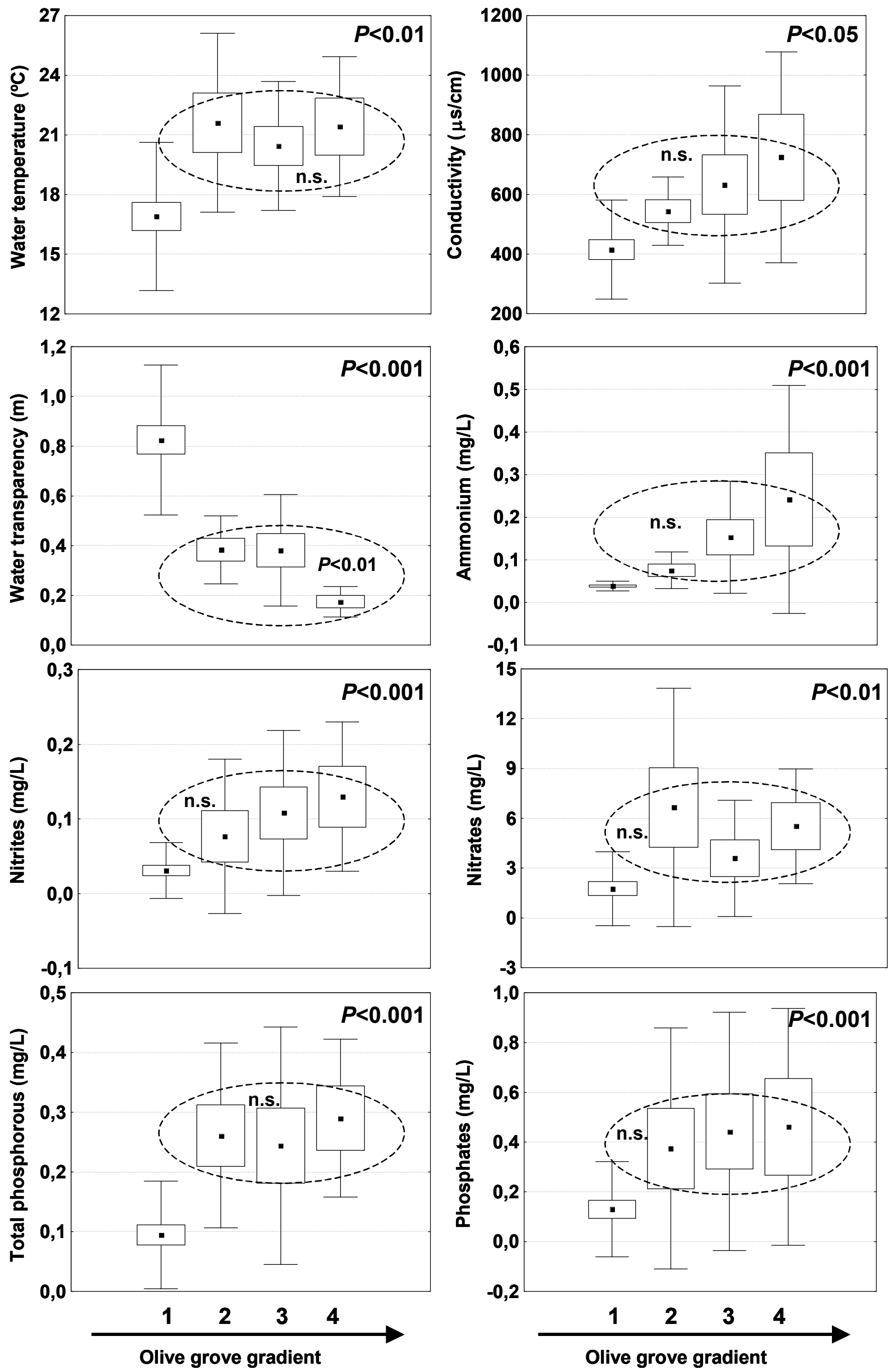


Figure 5.2 Box plots of the most significant results for physicochemical parameters along olive grove intensification gradient. Results from Kruskal-Wallis tests are shown. Olive grove gradient follows the graphical sequence: reference (1), traditional olive grove (2), intensive olive grove (3), hyper-intensive olive grove (4). (▪): Mean; box: ± SE; whisker: ± SD.

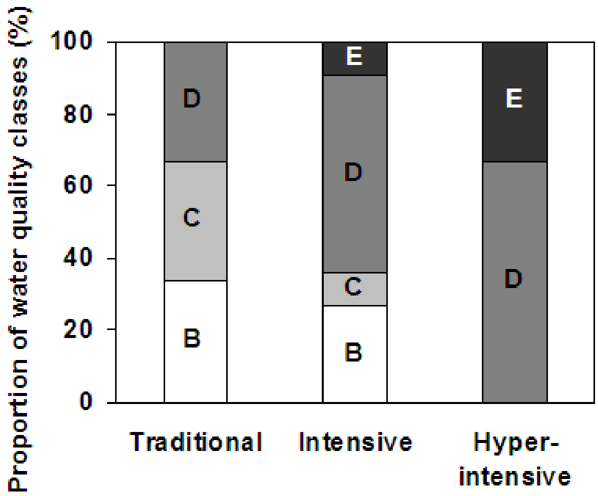


Figure 5.3 Proportion of different water quality classes in each olive grove type.

Changes in fish assemblages structure

A total of 14 fish species were captured: (i) 8 native species - *Barbus bocagei* Steindachner, 1864, *Barbus steindachneri* Almaça, 1967, *Barbus microcephalus* Almaça, 1967, *Squalius pyrenaicus* (Günther, 1868), *Squalius alburnoides* Steindachner, 1866, *Pseudochondrostoma willkommii* Steindachner, 1866, *Iberochondrostoma lemmingii* (Steindachner, 1866), *Cobitis paludica* (de Buen, 1930); and (ii) 6 non-native species - *Lepomis gibbosus* (Linnaeus, 1758), *Cyprinus carpio* Linnaeus, 1758, *Carassius auratus* (Linnaeus, 1758), *Micropterus salmoides* (Lacépède, 1802), *Alburnus alburnus* (Linnaeus, 1758), *Gambusia holbrooki* Girard, 1859. Overall, there was a high occurrence of non-native species in all sites under the influence of olive groves, particularly *G. holbrooki* and *L. gibbosus*. In average, non-native species represented more than 50% of the mean density and species richness per site and were the only species captured in 17% of the sampled sites. All native species are Iberian endemisms (in many cases basin endemisms) and present high conservation status, including two endangered species: *S. pyrenaicus* and *I. lemmingii* (Cabral et al. 2005).

Ordination diagram from Correspondence Analysis (CA) based on fish metrics and guilds showed a good segregation of the reference sites, particularly along the 2nd axis, which together with 1st axis explained 58% of data variability (Figure 5.4). Olive grove sites presented a wide overlap among them. Considering overall fish assemblage structure, olive grove sites were particularly associated with non-native, tolerant, phytophilic and limnophilic species. Reference sites were related to higher fish richness and density and were mainly composed by native species. These sites were also represented by a larger diversity of functional guilds, including intolerant species.

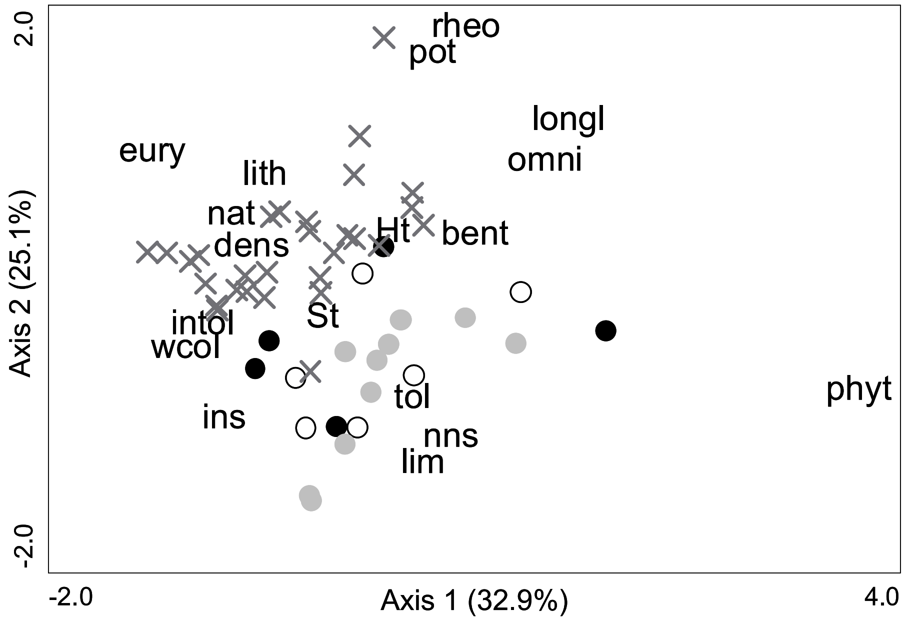


Figure 5.4 Ordination diagram from Correspondence Analysis (CA) of reference and sampled sites based on fish community structure. Sites' symbols: references (grey cross), traditional olive grove (white dots), intensive olive grove (grey dots) and hyper-intensive olive grove (black dots). Metrics and guilds abbreviations: species diversity (Ht), total number of species (St), number of native (nat), non-native (nns), intolerant (intol) and tolerant (tol) species, total density (dens), proportion of potamodromous (pot), long lived (longl), rheophilic (rheo), limnophilic (lim), eurytopic (eury), benthic (ben), water column (wcol), omnivorous (omni), insectivorous (ins), lithophilic (lith) and phytophilic (phyt) individuals.

Considering the possible co-variation of the environmental gradient in the biological analysis, results from PCA allowed to define two main axes, which together accounted for 54% of total variation in data. Ordination diagram didn't reveal any discrimination among olive grove types. According to the correlations between variables and PCA axes, PC1 was mainly related to human disturbance variables (e.g. organic/nutrient load ($r = 0.63$), sediment load ($r = 0.61$), riparian vegetation ($r = 0.57$), ammonium ($r = 0.66$), nitrate ($r = 0.81$)), while PC2 was highly correlated with environmental variables reflecting river size (e.g. distance to source ($r = -0.60$), mean stream width ($r = -0.53$) and water depth (-0.52), habitat diversity, as proportion of pools ($r = -0.74$) and runs ($r = 0.55$), thus the longitudinal environmental gradient. Spearman rank correlations between

PC2 and CA axes didn't reveal any significant (*P* > 0.05) influence of environmental gradients on the variation of fish community structure among sampled sites.

According to previous correlations, Permanova did not include environmental gradient as a co-variable in the analysis. Significant differences in community structure were observed between the reference set and all olive grove types (*P* < 0.001). However, no differences were detected among olive grove types (*P* > 0.05).

Bray-Curtis Similarities between community features of each sampled site and the reference set showed a trend with olive grove intensification (Figure 5.5), suggesting a correspondent decrease in fish assemblages integrity along the stressor gradient, although no significant differences (*P* > 0.05) were observed.

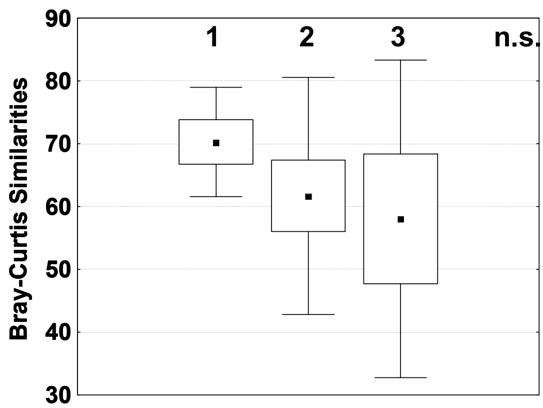


Figure 5.5 Box plot of Bray-Curtis similarities between references and each olive grove type: traditional (1), intensive (2) and hyper-intensive (3). Results from Kruskal-Wallis test is shown. (▪): Mean; box: $\pm$ SE; whisker: $\pm$ SD.

Discussion

In this study olive grove intensification was related to increases in overall human-induced disturbance. Along the intensification gradient there was a major significant increase in organic/nutrient and sediment loads in sampled streams. Conductivity, ammonium, nitrite, total phosphorous and phosphate concentrations showed increasing trends, but differences only were significant when reference sites were considered. Water transparency decreased along the analysed gradient, also confirming this trend towards disturbance. These types of disturbance are strongly related to soil erosion and high levels of fertilisation and irrigation commonly associated with intensive agriculture practices. Soil erosion is cited as one of the principal environmental problems associated with olive farming in Mediterranean regions (e.g. Graaff and Eppink 1999). In intensive olive plantations, farmers usually keep the soil bare of vegetation all the year; hence severe erosion takes place with heavy rains. Soil erosion and water runoff into nearby streams can be a major source of suspended sediments (and consequent turbidity), nutrients and pesticides in watersheds dominated by agricultural land (e.g. Kuhnle et al. 2000; Vanni et al. 2001). This problem can be further enhanced by the removal of riparian vegetation and channelisation of streams, frequent in farmlands. Riparian zones have the ability to prevent sediment run-off and to hold excess nutrients and modify their inputs to the stream (e.g. Muenz et al. 2006). Because of their position at the interface between terrestrial and aquatic ecosystems, they play a crucial role in controlling the flow of nutrients from watersheds (Roth et al. 1996). Furthermore, removal of streamside vegetation can increase mean water temperature (e.g. Wohl and Carline, 1996) and promote changes in stream morphology as streams typically widen and become shallower (Roth et al. 1996).

The main pollutants of water from fertiliser use are nitrate and phosphate (e.g. Carpenter et al. 1998). Nitrate is highly mobile, leached with water and reaching both surface water and the groundwater. Phosphate is less soluble in water and travels associated with the sediments they drag. The excess of these nutrients causes eutrophication, which results in depletion of dissolved oxygen (Mallin et al. 2006). Stream bank deterioration has also been linked to high phosphorus sediment losses and poor overall water quality (Sekely et al. 2002). Like fertilisers, pesticides may have strong negative impacts in surface and groundwater and aquatic ecosystems (e.g. Liess and von der Ohe 2005).

The physicochemical parameters of water showed significant differences along the land use gradient only when the reference sites were considered, and were not significantly different among olive grove types. This result suggested a high level of disturbance in all olive grove sites, even though a general increasing trend was observed along the olive grove intensification gradient.

Animal load, however, presented a decreasing pattern with olive grove intensification. Traditional olive groves presented the highest animal loads as a result of livestock associated to these systems. Traditional olive tree farming used to be founded on the principles of organic economy, together with the systematic use of human and animal labour. Livestock constituted a basic element, entering the system not only as work force but also as producer of manure. Nonetheless, over the years, livestock production, mainly cattle, has undergone a huge increment in the study area. This was probably the cause of the pattern observed in water temperature, nitrate concentration and degradation of streamside riparian vegetation. These parameters presented the lowest values in the reference sites and the highest ones both in traditional and hyper-intensive olive groves. Literature clearly demonstrates that livestock grazing with unrestricted access to streams have negative impacts on the aquatic ecosystem. This practice increases in-stream trampling, habitat disturbance and erosion from overgrazed stream banks, reducing sediment trapping by riparian and in-stream vegetation and decreasing bank stability, therefore leading to an increase on turbidity, nutrients and suspended solids concentrations in streams (e.g. Kauffman and Krueger 1984; Nagels et al. 2002; Vidon et al. 2008). Streams contamination occurs both by water runoff from adjacent land during and immediately after irrigation/precipitation events and/or by direct excretion of faecal material into streams. In the current study, sampling was conducted during spring, immediately after heavy rains and flash floods typical of the Mediterranean climate, thus favouring the observed results. This effect is further enhanced by the absence or degradation of riparian vegetation, due to its role on stream protection, discussed above.

These results emphasized the fact that, despite the low intensity of agricultural practices, stream sites influenced by traditional olive groves sites are subjected to a considerable level of disturbance as a consequence of high animal loads.

In a general way, stream sites subject to olive farming presented problems in water quality, due to crops fertilization, sediment run-off or animal loads. Water quality is actually a growing problem in the three sampled river basins (SNIRH, available at http://snirh.pt/). In the present study there was a trend towards deterioration along the sampled gradient, suggesting the negative effects of more intensive farming.

There was a strong shift in fish assemblages structure between references and the olive grove sites. Reference sites presented higher fish density and a richer, diverse and native community with intolerant species. On the contrary, olive grove sites were strongly associated with non-native, tolerant, phytophilic and limnophilic fish species. The influence of land use on aquatic organisms is made through interrelated impacts on water quality, hydrology, and habitat (Paul and Meyer 2001; Allan 2004), which were already discussed above. These impacts have been shown to change substantially fish assemblages (Argent and Carline 2004), decrease species richness/diversity and sensitive species, whereas increase tolerant and introduced species and ultimately influence the integrity of fish assemblages (e.g. Roth et al. 1996; Fischer et al. 2009; Sullivan and Watzin 2010) corroborating the observed results. A loss of intolerant species accompanied by a gain in the tolerant ones has been identified as a potential factor contributing to the homogenization of biotic assemblages in freshwater systems (Scott and Helfman 2001; Olden and Poff 2004). On the other hand, less disturbed streams tend to support more trophic, reproductive, and habitat specialist species (Poff and Allan 1995).

No differences were observed in fish assemblages structure among olive grove types, but major differences existed between each olive grove group and the reference set. Furthermore, community similarities between references and each olive grove type showed a decreasing trend along the olive grove gradient, though without significant differences. Results from the present study are in accordance with numerous studies (e.g. Roth et al. 1996; Allan et al. 1997; Lammert and Allan 1999; Nerbonne and Vondracek 2001; Sawyer et al. 2004; Heitke et al. 2006), documenting the deviation of biological assemblages from reference conditions with increasing land use within the watershed.

One important outcome from this study is that, although fish assemblages under the influence of traditional olive groves tended to show higher similarities with reference sites than the other olive grove types, they are far from being communities with high integrity.

Conclusions

Although preliminary, this study illustrates the relationships between land use and the ecological integrity of Southern Portuguese streams regarding water quality, stream morphology and fish community structure. Intensive agriculture, such as olive production can result in several different types of stress, which alone or together affect the structure and functioning of streams. In the current study, olive production led to multiple in-stream disturbances, with emphasis on sediment, nutrient and organic loads, as well as degradation of riparian vegetation. The cumulative effects of these multiple disturbances promoted the loss of the biota integrity, making it highly vulnerable. Even when traditional farming practices seems to inherently support highest natural value, as is the case of traditional olive groves, the impact of these systems on the aquatic ecosystems can be dramatically different when they are coupled with livestock production. Under this perspective it is important to promote stream rehabilitation and develop more stringent guidelines limiting cattle access to streams. Management plans, legislation and restoration programs will need to consider all these aspects simultaneously to protect the aquatic ecosystems health.

Making olive production practices compatible with “good status” of ground and surface waters required for the WFD represents a major challenge in Mediterranean regions. Many river basins impacted by olive intensive farming already suffer from excessive water abstraction, morphological degradation and poor water quality. Therefore, evaluate and planning whether intensive and hyper-intensive agriculture practices can continue to expand, is crucial to ensure the achievement of WFD goals and promote a sustainable agricultural development.

Acknowledgements

We are grateful to Institute of Agrarian and Environmental Mediterranean Sciences (ICAAM) for funding the study. P. Matono was supported by a PhD grant from FCT (SFRH/BD/23435/2005). Special thanks are due to Eng. Rute Caraça for all the GIS procedures and helping in the fieldwork. We are also grateful to Vânia Silva and several volunteers for valuable help in the fieldwork. The National Forest Authority provided the necessary fishing permits.

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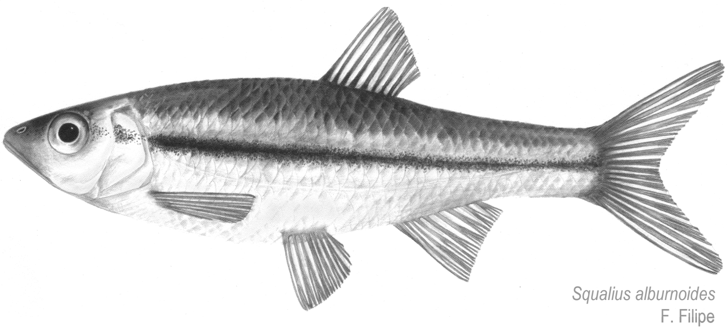
Chapter 6

Non-native fishes in Mediterranean river-types: the importance of environmental drivers and human pressure on the invasibility



Squalius alburnoides
F. Filipe

In review as

Ilhéu M, Matono P, Bernardo JM. Non-native fishes in Mediterranean river-types: the importance of environmental drivers and human pressure on the invasibility. Biological Invasions.

Abstract

Invasive species are considered as a biological pressure on the good ecological status of water bodies. Understanding the factors promoting successful biological invasions is of great conceptual and practical importance. Fish communities were sampled in 180 undisturbed sites and 198 sites disturbed by human activities, considering 12 river-types defined for continental Portugal under the implementation of the Water Framework Directive. Pumpkinseed sunfish, *Lepomis gibbosus* (L.), and mosquitofish, *Gambusia holbrooki* (Girard), were the most abundant non-native species (NNS) in the southern river-types and Iberian gudgeon, *Gobio lozanoi* Doadrio and Madeira, was the dominant species in the North/Centre. Small northern streams with high slope showed null or low occurrence and an abundance of NNS, while southern river-types with medium and large drainage areas presented the highest values. The occurrence of NNS was significantly lower in undisturbed sites and the highest density of NNS was associated with high human pressure. Results from variance partitioning showed that natural environmental factors determine the distribution of the most abundant NNS while the increase in their abundance and success is mainly explained by human-induced disturbance factors. This study stresses the high vulnerability of the warm water lowland river types to non-native fish invasions, which is amplified by human-induced degradation.

Keywords: Environmental degradation; invasive species; *Lepomis gibbosus*; *Gobio lozanoi; Gambusia holbrooki*; Portugal.

Introduction

The rate and extent of invasions in freshwater ecosystems are particularly alarming in the Mediterranean region, which is among the most heavily invaded ecosystems in the world (Clavero and García-Berthou 2006; Leprieur et al. 2008). The Iberian freshwater habitats are one of the most paradigmatic examples where there are constant reports of new invading fish species and colonization of new areas (see e.g. Caiola and Sostoa 2002; Ribeiro et al. 2006; Benejam et al. 2007; Comesaña and Ayres 2009; Pérez-Bote and Romero 2009; Ribeiro et al. 2009b; Miranda et al. 2010).

The success of biological invasions can be explained by several factors, with environmental drivers being among the most important ones (Davis et al. 2000; Ruesink 2005). One of the most frequently stated hypotheses in the biological invasion literature is that species should have a greater chance of success if they are introduced to an area with a climate that closely matches that of their original range (Brown 1989; Williamson 1996; Duncan et al. 2001; Bomford et al. 2009). Other environmental drivers, such as spatial heterogeneity and environmental variability, may also be important (e.g. Moyle and Light 1996; Fausch et al. 2001; Davies et al. 2005). According to Moyle and Light (1996), if the abiotic factors are appropriate for an introduced species, then it is likely to invade successfully regardless of the recipient biota. Thus, the success of an invading fish may be predicted with reference to environmental conditions. The features of the recipient ecosystem and the success of an introduced species may be determined by human-induced pressure. The ‘‘human activity’’ hypothesis argues that human activities facilitate the establishment of non-native species (NNS) by disturbing natural landscapes and by increasing propagule pressure (Leprieur et al. 2008; Blanchet et al. 2009; Chiron et al. 2009). In Mediterranean climate rivers, both landscape and human disturbance factors are expected to play a major role in the biological invasions, as these systems are largely governed by stochastic processes (*sensu* Connell 1978; Bernardo et al. 2003) and have suffered a long history of human-induced pressures (e.g. Gasith and Resh 1999; Smith and Darwall 2006).

Understanding the factors promoting successful invasions is of great conceptual and practical importance. From a practical point of view, it should help to prevent future invasions and to mitigate the effects of recent invaders through early detection and prioritisation of management measures. Thus, the identification of the environmental determinants of fish invasions is relevant in forecasting the overall impact of invasions on a global scale and a prerequisite to help management authorities to adopt sound conservation policies.

Although non-native species are not mentioned specifically in the Water Framework Directive (WFD, European Commission 2000), in the context of the Directive's objectives NNS represent an important pressure (listed in the WFD annexes), since they can modify the structure of native biota and the ecological functioning of aquatic systems. NNS affect the ecological quality of natural environments in multiple ways and may represent a serious threat to native communities. Potential effects include genetic alterations within populations, spreading of pathogens and parasites, competition with and replacement of native species, and habitat deterioration or modification (see Leunda 2010 for Iberian freshwater ichthyofauna). All the combined effects may result in changes in ecosystem function and global homogenization (Rahel 2000; Olden and Poff 2003; Clavero and Garcia-Berthou 2006), with profound impact at ecological, evolutionary, genetic, and economic levels (e.g. Leonardos et al. 2008; Leprieur et al. 2009; Gozlan 2010).

The relative importance of environmental factors in explaining non-native invasibility could depend upon both the spatial scale and the historical context of the area surveyed. Approaching invasibility in environmentally homogeneous river groups, that is, in river-types, facilitates comparability and the identification of drivers, as acknowledged in the WFD.

The objectives of the present study were to determine: 1) the patterns of non-native fish richness and abundance in Mediterranean climate river-types; 2) the environmental drivers that favour non-native fish success within the morphoclimatic gradients; and 3) the relative importance of environmental variables and human-induced pressures for the occurrence and abundance of non-native fish species.

Methods

Study area

The study considered 12 river-types defined for continental Portugal under the implementation of the Water Framework Directive (WFD), based on hydromorphological and biological elements, including fish fauna (INAG 2008a) (Fig. 6.1). The climate is typically Mediterranean, with high intra- and inter-annual precipitation and discharge variation, with severe and unpredictable floods between autumn and spring and persistent summer droughts (Miranda et al. 2002), although the influence of factors such as topography and proximity to the Atlantic Ocean causes significant climatic contrasts. The general conditions of the atmospheric circulation cause a decrease in precipitation from North to South and from West (coast) to East (interior), enhanced by orographic asymmetry. Indeed, the mountain barrier in the North and away from the coast causes less rainfall in the interior. The temperature shows an opposite pattern, increasing from North to South. The altitude causes a decrease in temperature and an increase in rainfall.

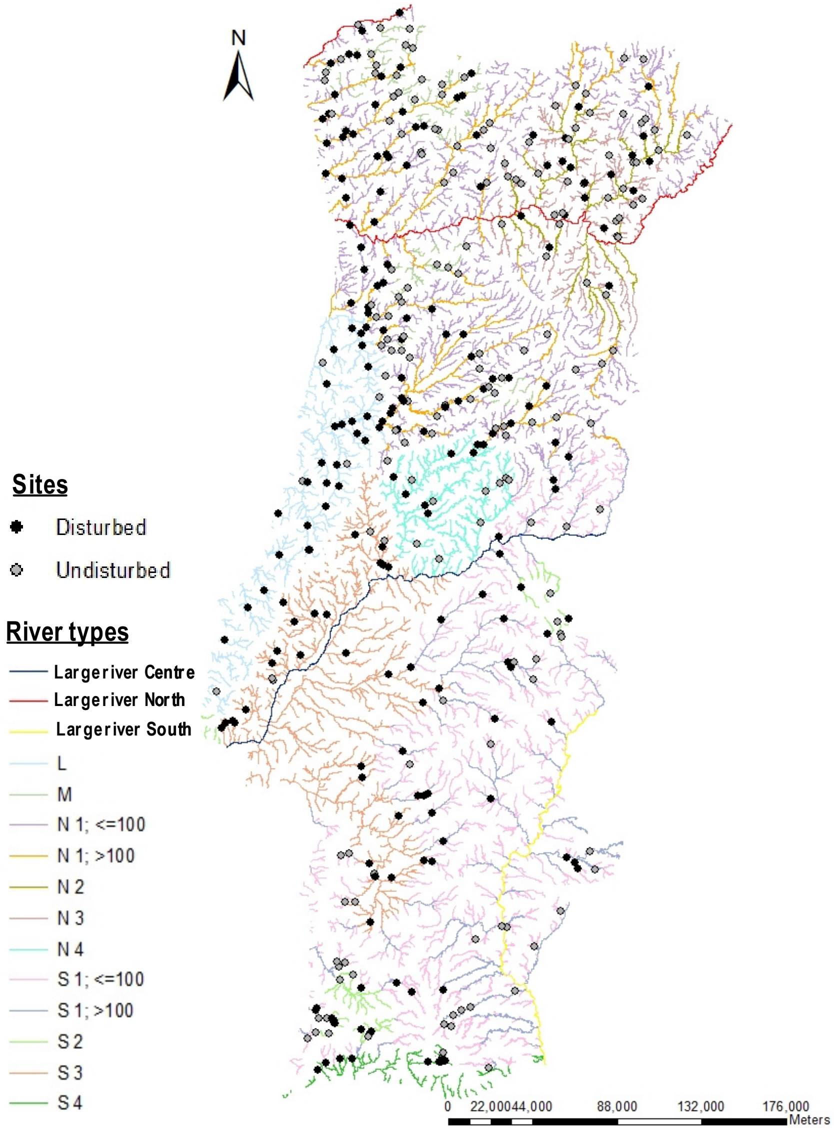


Figure 6.1 Map of the river-types defined to continental Portugal showing undisturbed (grey dots) and disturbed (black dots) sampling sites.

Portuguese river-types reflect two main geographical and climatic gradients: North–South and West–East. The North–South gradient is associated with a decrease in altitude, precipitation, annual discharge, and increasing temperature. The West–East gradient is related to a continental effect, with a precipitation decrease and an increase in temperature extremes. Owing to these features, most rivers are permanent in the North and intermittent in the South.

The most relevant climatic and morphological characteristics of each river-type are presented in Table 6.1 (see INAG 2008a).

Table 6.1 Main morphoclimatic features of river-types in continental Portugal (mean ± SD)

River-Types	Mean Annual Temperature (ºC)	Mean Annual Precipitation (mm)	Altitude (m)	Drainage Area (km^2)
M Northern mountain streams	11.0 ± 1.5	1944 ± 379	506 ± 300	24.8 ± 17
N1<100 km^2 North streams with small drainage area	12.4 ± 1.3	1190 ± 358	413 ± 242	33 ± 23
N1>100 km^2 North streams with large drainage area	12.6 ± 1.2	1196 ± 347	274 ± 205	549 ± 65
N2 Streams from Alto Douro with large drainage area	13.1 ± 1.0	596 ± 81	300 ± 141	960 ± 1115
N3 Streams from Alto Douro with small drainage area	13.0 ± 0.8	671 ± 134	432 ± 160	32 ± 23
N4 Transition streams between north and south	14.1 ± 0.7	1065 ± 168	280 ± 122	151 ± 361
L Littoral streams of west/center region	14.8 ± 0.3	941 ± 118	44 ± 44	180 ± 671
S1<100 km^2 South streams with small drainage area	15.7 ± 0.9	628 ± 86	183 ± 75	30 ± 21
S1>100 km^2 South streams with large drainage area	15.8 ± 0.9	587 ± 84	137 ± 68	439 ± 579
S2 Southern mountain streams	15.4 ± 0.3	743 ± 85	175 ± 147	60 ± 87
S3 Streams of sedimentary deposits in Tagus and Sado basins	15.6 ± 0.4	730 ± 118	54 ± 46	388 ± 1081
S4 Southern carsick streams of Algarve	16.9 ± 0.5	632 ± 60	54 ± 57	67 ± 89

Native freshwater fish fauna of Portuguese rivers present relatively low species richness, being dominated by Cyprinids. Most of the fish species are endemic, with high conservation status, particularly in the south; nevertheless many of them are threatened with extinction (Cabral et al. 2005; Smith and Darwall 2006).

Sampling and data collection

Sampling was carried out between 2004 and 2006 during spring at 380 sites in the main Portuguese river basins (Fig. 6.1). The number of sites of each river-type was sampled in proportion to the area concerned: M = 28 sites; N1<100 km^2 = 60 sites; N1>100 km^2 = 68 sites; N2 = 20 sites; N3 = 28 sites; N4 = 16 sites; L = 33 sites; S1<100 km^2 = 26 sites; S1>100 km^2 = 33 sites; S2 = 22 sites; S3 = 37 sites; S4 = 9 sites.

Fish were collected by electrofishing following the WFD-compliant sampling protocol (INAG 2008b) and also CEN protocol (CEN 2003). All collected individuals were measured, weighted, identified to species level, and immediately returned to the river.

Environmental characterization of sites was based on regional and local variables. Regional variables were obtained from digital cartography with free Internet access and included the drainage area of the basin (km^2), distance from source (km), altitude (m), slope (%), mean annual discharge (mm), mean annual air temperature (ºC), and mean annual rainfall (mm). Rainfall, temperature, and flow variables were described from 30-year data series. Topographical variables were derived from a Digital Elevation Model (DEM), with a 90-m grid cell resolution (CGIAR-CSI 2005), using ArcMap 9.1. Local variables were assessed during the sampling procedure: water temperature (ºC), conductivity ($\mu S\ cm^{-1}$), pH, dissolved oxygen ($mg\ L^{-1}$), mean stream wetted width (m), maximum and mean water depth (m), mean current velocity ($m\ s^{-1}$), dominant substratum class [adapted from the Wentworth scale (Giller and Malmqvist 1998): 1 – mud and sand; 2 – gravel; 3 – pebble; 4 – cobble; 5 – boulders; 6 – boulders larger than 0.50 m], riparian vegetation (%), shadow (%), and proportion of different habitat types (pool, run, riffle).

Human disturbance level was evaluated with regard to ten locally evaluated variables (FAME 2004): land use, urban area, riparian zone, longitudinal connectivity of the river segment, sediment load, hydrological regime, morphological condition, presence of artificial lentic water bodies, toxicological and acidification level, and nutrient/organic load. Each variable was scored from 1 (minimum disturbance) to 5 (maximum disturbance) (Appendix 1) and only sites with scores of 1 and/or 2 and only one variable with a 3 were considered as undisturbed or least disturbed (references). The sum of these scores represents the total human pressure. A total of 182 undisturbed and 198 disturbed sites were sampled. Sampling was carried out in order to cover the entire pressure gradient in each river-type. Several physicochemical variables complemented the evaluation of human pressure in each site, after laboratory measurements and analyses: biological oxygen demand – BOD_5, (mg L^{-1}), chemical oxygen demand – COD (mg L^{-1}), total suspended solids – TSS (mg L^{-1}), total dissolved phosphorous – P (mg L^{-1}), nitrite – NO_2^- (mg L^{-1}), nitrate – NO_3^- (mg L^{-1}), ammonium – NH_4^+ (mg L^{-1}), and total inorganic dissolved nitrogen – N (mg L^{-1}).

Data analysis

Captures were quantified as density (individuals per 100 m^2) and biomass (grammes per 100 m^2). The degree of invasibility was measured using NNS richness and abundance. The relationship between richness (total number of species per site) and abundance (mean density and biomass per site) and each environmental variable was initially explored using Spearman's rank correlations ($|r| \geq 0.5$; $P < 0.05$) considering only undisturbed sites, in order to exclude the effects of human pressure in the analysis.

Principal Components Analysis (PCA) of sampled sites based on environmental variables allowed relationships to be established and compared between river-types and these variables. Most intercorrelated variables (Spearman's rank correlations $|r| > 0.75$; $P < 0.05$) were excluded. The biplot of this PCA was also projected based on the occurrence of the most abundant NNS in order to associate NNS to river-types and their environmental characteristics.

Differences in total human pressure between river-types were tested with the Kruskal-Wallis test. The Mann-Whitney test was used to identify significant differences in the number and density of NNS between undisturbed and disturbed sites. The Z test of proportions (Daniel 1987) evaluated the existence of significant differences in frequency of occurrence of non-indigenous species between undisturbed and disturbed sites. The response pattern of the most abundant NNS to pressure gradient was tested with a Kruskal-Wallis test and involved the establishment of five quality classes and boundaries (High, Good, Moderate, Poor, and Bad) for the stressor gradient (total human pressure) following CIS-WFD (2003): (i) the High–Good boundary was defined as the 25th percentile of the undisturbed sites; (ii) the other boundaries were defined by dividing the remaining gradient into four classes of equal width.

Variance partitioning (Borcard et al. 1992) was used to determine the relative importance of three sets of predictor variables [environmental (A), human-induced pressure (B), and spatial (C)] and their shared effects on the occurrence and density of the most abundant NNS: pumpkinseed sunfish *Lepomis gibbosus* (L.), Iberian gudgeon *Gobio lozanoi* Doadrio and Madeira, and mosquitofish *Gambusia holbrooki* (Girard). The analysis of each species included only the dataset from river-types in which the species occurred. Seven univariate Generalized Linear Models (GLMs) using forward selection were performed for each response variable: A, B, C, A × B, A × C, B × C, and A × B × C. The best models (minimal adequate) were estimated according to the lowest Akaike Information Criterion (AIC). Plots of residuals (not shown) were examined to complement AIC values and to confirm goodness-of-fit (see Zuur et al. 2007). For the occurrence of species (presence–absence data), GLMs were performed using the binomial distribution and logit link function. In order to overcome the problems of distribution fitting resulting from the high number of absences in the density matrix, this continuous response variable was standardized to the maximum value for the species and converted into four classes: 0 (0% of individuals), 1 (between 0% and 10% of individuals), 2 (between 10% and 50% of individuals) and 3 (more than 50% of individuals). For this transformed density response variable (count data), GLMs were performed using the Poisson distribution and log link function. The existence of over-dispersion in data (variance higher than the mean; dispersion parameter > 1) was checked during the analysis. If the value observed was higher than the threshold limit then quasi-binomial and quasi-Poisson distributions should be used, respectively.

Multicollinearity among environmental variables may result in the exclusion of ecologically meaningful variables from the models if another intercorrelated variable or variables happen to explain the variation better in statistical terms (MacNally 2000). Therefore, as for PCA, some of the most clearly intercorrelated variables (Spearman's rank correlations $|r| > 0.75$; $P < 0.05$) were excluded. The exclusion decision also took into account the potential relevance of the variable in the occurrence and distribution of NNS. Furthermore, also to account for multicollinearity, variables were maintained in the models only if their addition did not cause any Variation Inflation Factor (VIF) to exceed 3.0. The spatial structure of data was incorporated into the analyses to prevent misinterpretation of relations between and within the spatially arranged datasets (Hinch et al. 1994). Owing to spatial autocorrelation, values of particular variables in neighbouring sites are more or less similar than they would be in a random set of observations (Legendre 1993). Autocorrelation is a frequently observed feature in spatially sampled biological data (Diniz-Filho et al. 2003) that may make the identification of plausible relationships between biota and the environment difficult (Legendre and Fortin 1989). The spatial structure of data was explored, including geographical coordinates of sites and their higher and cross product terms, in the modelling procedure ($x, y, xy, x^2, y^2, x^2y, xy^2, x^3$ and y^3) (Legendre 1993). The x and y coordinates were centred to zero mean before computing the matrix of spatial descriptors, to reduce collinearity between successive terms when fitting the polynomial (Legendre and Legendre 1998).

Prior to the analyses, all data were either log (x + 1) (linear measurements) or arcsin [sqrt(x)] (percentages) transformed to improve normality (Legendre and Legendre 1998). Statistical analysis was performed using CANOCO 4.5, STATISTICA 6, and BRODGAR 2.6 software applications.

Results

Abundance and distribution of non-native species

A total of 41 fish species were captured, including 6 diadromous species and 10 NNS: *L. gibbosus*, carp *Cyprinus carpio* L.; goldfish *Carassius auratus* (L.), *G. lozanoi*, largemouth bass *Micropterus salmoides* (Lacépède), chameleon cichlid *Herichthys facetum* (Jenyns), black bullhead *Ameiurus melas* (Rafinesque), *G. holbrooki*, pike-perch *Sander lucioperca* (L.), and bleak *Alburnus alburnus* (L.) (Table 6.2). With the exception of pike *Esox lucius* L., all introduced fish species recorded for Portugal were collected in the present study.

NNS occurred in 33% of the sites, representing nearly 11% of both the total mean density (3.58, SD = 12.54) and number of species per site. Although absolute values may seem low, they represented an important percentage of the total density and number of species, as Mediterranean streams usually show low species richness and density per site. For this reason, percentage values for density and number of NNS were also considered when interpreting results. Biomass values were not shown or included in the analysis, as they followed the density pattern and were therefore redundant.

Table 6.2 Number (%), mean density (± SD) (ind/100 m²) and frequency of occurrence (f.oc.) of non-native fish species (NNS)

	Total	NNS f.oc.	M	N1 <100 km²	N1 >100 km²	N2	N3	N4	L	S1 <100 km²	S1 >100 km²	S2	S3	S4
Nbr. NNS (%)	12.0 ± 21.0	-	0	3.0 ± 9.2	13.1 ± 19.3	19.3 ± 14.2	2.9 ± 11.2	4.6 ± 10.0	14.8 ± 19.6	13.1 ± 23.2	31.1 ± 30.1	16.3 ± 31.1	19.6 ± 25.9	0
NNS Mean density (ind/100 m²)	3.6 ± 12.5	-	0	2.3 ± 9.1	4.8 ± 12.8	1.2 ± 1.6	0.07 ± 0.3	0.3 ± 0.8	9.32 ± 30.1	3.6 ± 8.6	6.4 ± 11.5	1.1 ± 2.1	6.3 ± 12.5	0
NNS Mean density (%)	10.8 ± 23.9	-	0	5.2 ± 17.8	14.9 ± 26.1	7.5 ± 8.1	0.77 ± 3.2	0.4 ± 0.8	11.6 ± 19.1	11.1 ± 25.0	28.6 ± 38.4	13.5 ± 31.9	18.5 ± 28.6	0
NNS f.oc.	-	0.30	0	0.1	0.4	0.7	0.07	0.2	0.5	0.4	0.7	0.3	0.5	0
Lepomis gibbosus	0.9 ± 3.3	0.21	0	0.1 ± 0.9	1.1 ± 3.7	0.6 ± 0.9	0.01 ± 0.02	0.3 ± 0.8	0.8 ± 2.1	2.3 ± 6.7	2.1 ± 4.4	0.06 ± 0.3	2.3 ± 5.4	0
Cyprinus carpio	0.1 ± 1.6	0.03	0	0	0	0.01 ± 0.01	0	0	0.09 ± 0.4	0.01 ± 0.06	0.9 ± 5.5	0	0.08 ± 0.4	0
Carassius auratus	0.001 ± 0.03	0.01	0	0	0.01 ± 0.06	0.01 ± 0.01	0	0	0	0	0	0	0.01 ± 0.02	0
Gobio lozanoi	1.8 ± 10.9	0.14	0	2.15 ± 9.1	3.4 ± 10.6	0.6 ± 1.2	0.07 ± 0.3	0	7.5 ± 29.9	0	0	0	1.6 ± 6.7	0
Micropterus salmoides	0.07 ± 0.7	0.02	0	0	0.2 ± 1.5	0	0	0	0.2 ± 0.6	0	0.1 ± 0.6	0.07 ± 0.3	0.08 ± 0.5	0
Herichtys facetum	0.03 ± 0.4	0.01	0	0	0	0	0	0	0	0.3 ± 1.3	0.1 ± 0.3	0	0	0
Ameiurus melas	0.1 ± 2.2	0.003	0	0	0	0	0	0	0	0	0	0	1.2 ± 7.1	0
Gambusia holbrooki	0.5± 3.1	0.11	0	0	0.03 ± 0.2	0	0	0.01 ± 0.06	0.7 ± 2.0	1.1 ± 2.5	2.7 ± 9.0	0.9 ± 2.1	1.1 ± 3.2	0
Sander lucioperca	0.01 ± 0.1	0.003	0	0	0.04 ± 0.3	0	0	0	0	0	0	0	0	0
Alburnus alburnus	0.03 ± 0.5	0.01	0	0	0	0	0	0	0	0	0.4 ± 1.6	0	0	0

Considering river-types, NNS did not occur in the Mountain river-type or in the Southern Carsick river-type. The small northern stream types N1<100 km^2, N3, and N4 registered very low densities, frequency of occurrence, and percentage of NNS. N2 and S2 also showed low densities of NNS, but represented a high proportion of the fish assemblages (larger than 15% per site) and registered high values of frequency of occurrence (f. oc.); N2 even presented the highest value: f. oc. = 0.7 (Table 6.2). For S2, although the density of NNS was low, it represented more than 10% of the total fish density. N1>100 km^2 and S1<100 km^2 presented high values of occurrence and density of NNS, also representing more than 10% of total density and species richness. Littoral and southern river-types S1>100 km^2 and S3 showed the highest occurrence and abundance of NNS. However, whereas in Littoral type NNS represented almost 12% of the total density and 15% of the total species richness, S1>100 km^2 and S3 registered percentages between 20% and 30%. Therefore, NNS represented, on average, more than 10% of the total density and/or mean number of species captured in seven river-types: N1>100 km^2, N2, L, S1<100 km^2, S1>100 km^2, S2, and S3. Particularly in medium/large southern rivers they were dominant (> 50% of total fish assemblages) in a considerable number of sites.

The most frequent and abundant NNS were *L. gibbosus* (mean density = 0.89, SD = 3.3; f. oc. = 0.21), *G. lozanoi* (mean density = 1.79, SD = 10.9; f. oc. = 0.14), and *G. holbrooki* (mean density = 0.54, SD = 3.1; F. oc. = 0.11) (Table 6.2). The remaining species registered low occurrences and abundances.

L. gibbosus and *G. holbrooki* were the most abundant species in the southern river-types and *G. lozanoi* was the dominant species in North/Centre river-types. *C. carpio* and *M. salmoides* occurred in several river-types, but showed higher values in the South. The remaining NNS, *H. facetum*, *A. melas*, and *A. alburnus*, only occurred in the southern river-types S1<100 km^2, S1>100 km^2, and S3 and *S. lucioperca* only occurred in N1>100 km^2, all presenting very low values of occurrence and abundance. *C. auratus* registered nearly vestigial occurrence in N1>100 km^2, N2, and S3 (Table 6.2).

Environmental variables

For undisturbed sites, the abundance and number of total NNS were significantly correlated with drainage area and distance from source (r = 0.5; $P < 0.05$).

PCA based on environmental variables revealed a good segregation of sites along the first two ordination axes, which together accounted for 67.2% of total variation. According to the correlations between environmental variables and PCA axes, altitude (r = –0.71, $P < 0.001$) and drainage area (r = 0.87, $P < 0.001$) were the most important variables for the first axis, whereas latitude (r = –0.64, $P < 0.001$), mean annual discharge (r = –0.54, $P < 0.001$), mean stream width (r = –0.7, $P < 0.001$), mean annual temperature (r = 0.57, $P < 0.001$), pH (r = 0.51, $P < 0.001$), and conductivity (r = 0.79, $P < 0.001$) were the variables with the highest contributions to the second axis.

PCA biplot considering river-types showed a good association with different environmental variables and discrimination along a North–South gradient, mostly defined by the second axis (Fig. 6.2). Northern river-types were associated with low annual temperature and conductivity, high annual discharge, and local variables reflecting high flow conditions and a permanent flow regime: high current velocity and dissolved oxygen, coarser dominant substrate, and a high percentage of turbulent habitats. Southern river-types exhibited less diverse environmental features and were mainly associated with high temperature and conductivity, low annual discharge, and a high percentage of slow current habitats. The first axis discriminated mainly the northern river-types (Fig. 6.2). Mountain, N1<100 km^2, and N3 river-types were associated with high altitude and small drainage area. Southern types were quite dispersed along this axis, although a narrow separation of S1<100 km^2 and S2 from S1>100 km^2 and S3 is observed. N4 and Littoral showed a scattered distribution along both axes, with N4 being closer to northern types and the Littoral type closer to southern ones.

The results of PCA biplots considering the most abundant NNS complemented the previous results (Fig. 6.3). *L. gibbosus* showed a wide distribution in both northern and southern river-types but its occurrence was particularly associated with high annual temperature and conductivity, large drainage area, and low altitude (corresponding to N1>100 km^2, N2, L, and mostly southern river-types). *G. lozanoi* exhibited a higher occurrence in river-types with large drainage area and low altitude, especially in the North/Centre region (N1>100 km^2, N2, and L). *G. holbrooki* had the narrowest distribution, almost limited to the southern river-types with low altitude, high temperature and conductivity, and low annual discharge.

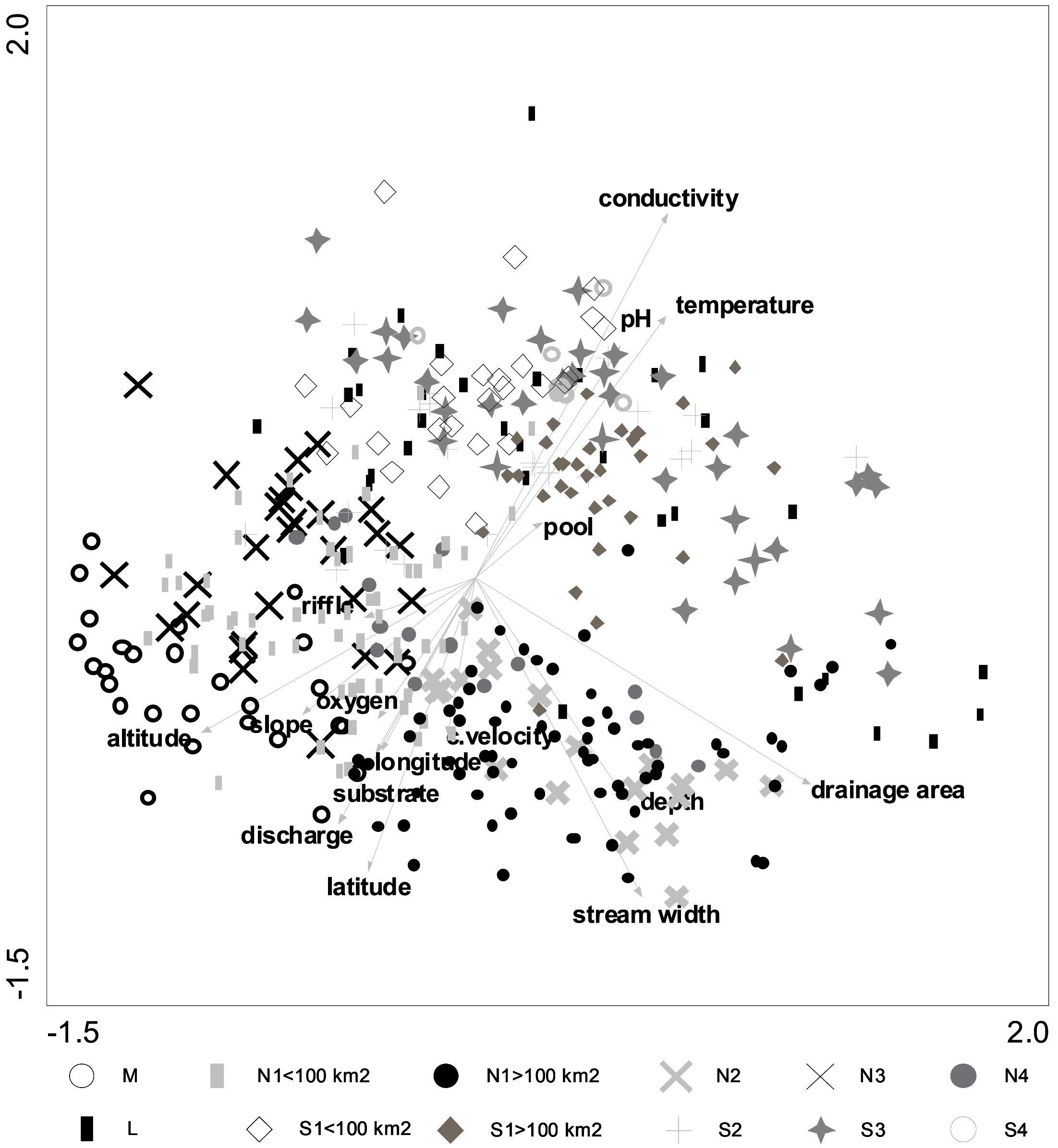


Figure 6.2 Biplot of Principal Components Analysis of sampled sites based on environmental variables. Sites are coded according to river-types.

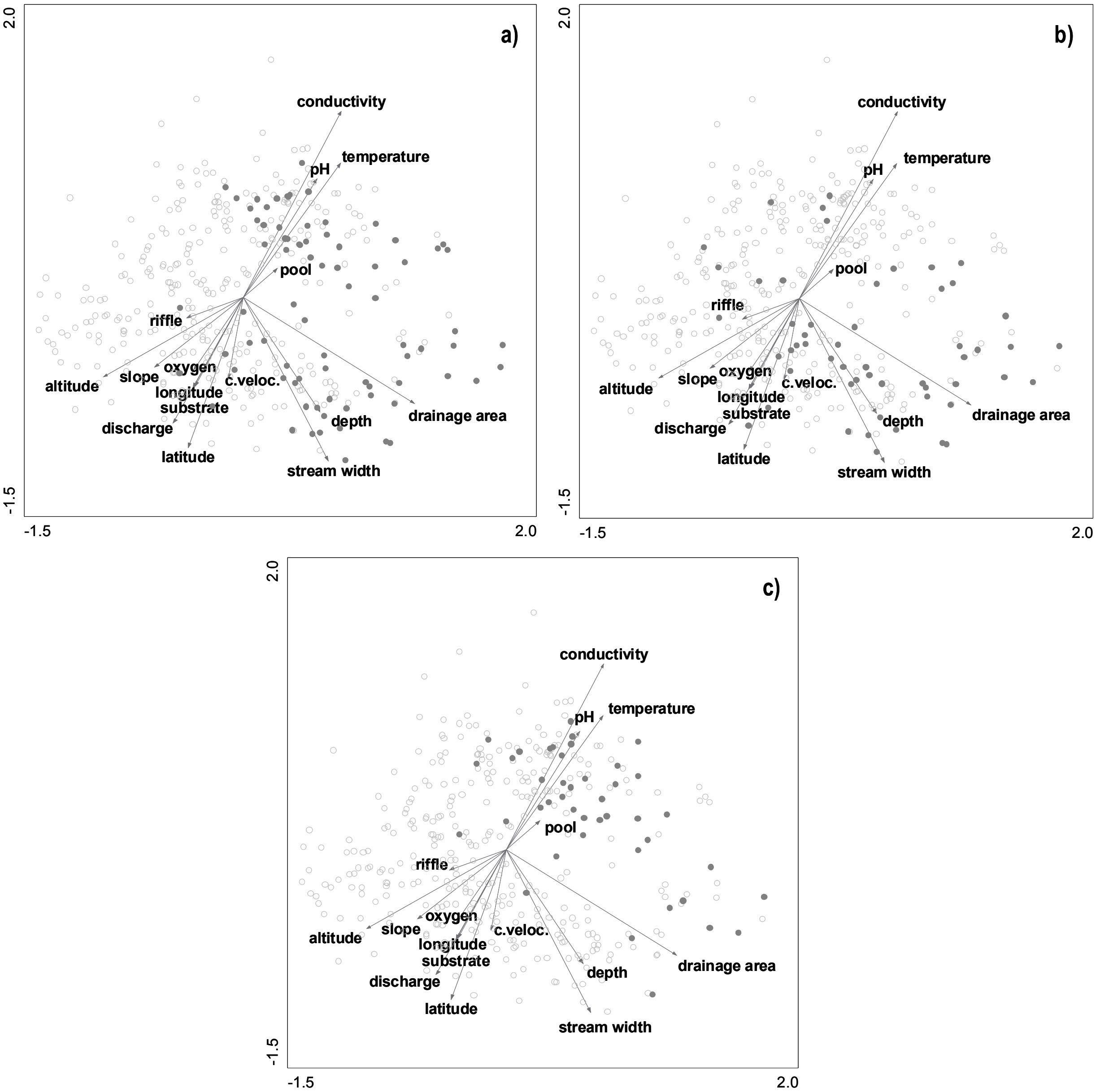


Fig. 6.3 Biplot of Principal Components Analysis of sampled sites based on environmental variables. Sites are coded according to absence (white dots) or presence (grey dots) of NNS **a)** *L. gibbosus*; **b)** *G. lozanoi*; **c)** *G. holbrooki.*

Total human pressure

Most river-types presented wide ranges of total human pressure and some variability in mean total human pressure (Fig. 6.4). Mean values of human-induced disturbance were higher in Littoral, N4, and S3, and lower values were observed in N2, M, S1<100 km^2, and S2, followed by N3. On average, higher values of total pressure tended to occur in the centre and southern river-types and/or with large drainage area.

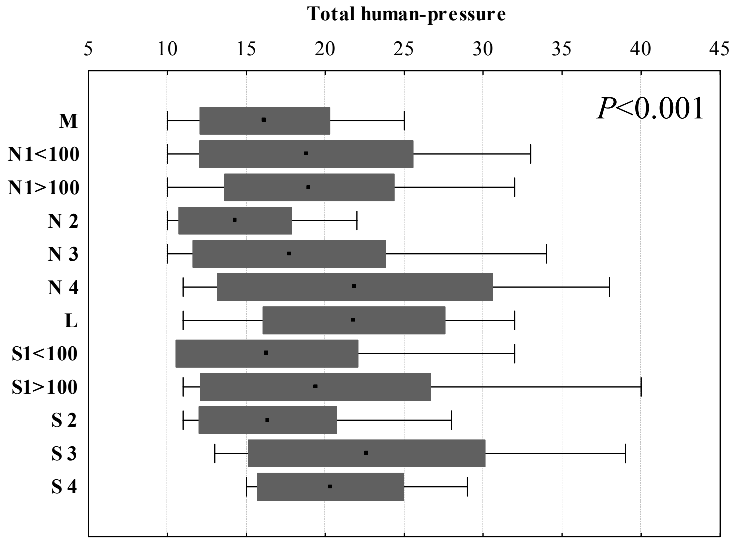


Figure 6.4 Total human-induced pressure in each river-type. (▪): Mean; box: ± SD; whisker: Min-Max. Significance of Kruskal-Wallis test is showed.

Overall, disturbed sites showed significantly higher occurrence (f. oc. = 0.46) (P < 0.001) and abundance (mean = 6.41, SD = 16.8) (U_{380} = 12155.00, Z = -6.56, P < 0.001) of NNS than undisturbed ones (f. oc. = 0.18; mean = 0.5, SD = 1.7). *M. salmoides*, *A. melas*, *S. lucioperca*, and *A. alburnus* only occurred in disturbed sites (Fig. 6.5). Except for *H. facetum*, all NNS exhibited significantly higher occurrence (P < 0.01) in disturbed sites. Fish density was significantly higher in disturbed sites only for the most abundant species, *L. gibbosus* (U_{380} = 15029.50, Z = 3.94, P < 0.001), *G. lozanoi* (U_{380} = 15594.50, Z = 3.73, P < 0.001), *G. holbrooki* (U_{380} = 14676.50.00, Z = 5.68, P < 0.001), and *M. salmoides* (U_{380} = 17199.00, Z = 2.91, P < 0.01) (Fig. 6.5). The occurrence of NNS was significantly higher in disturbed sites in all river-types (P < 0.01) except for N3. The abundance and percentage of NNS was higher in human-disturbed sites only in the Littoral, N1<100 km2, N1>100 km2, S1<100 km2, and S1>100 km2 (Fig. 6.6). The majority of river-types that did not reveal significant differences between undisturbed and disturbed sites presented null or very low abundance of non-native fishes.

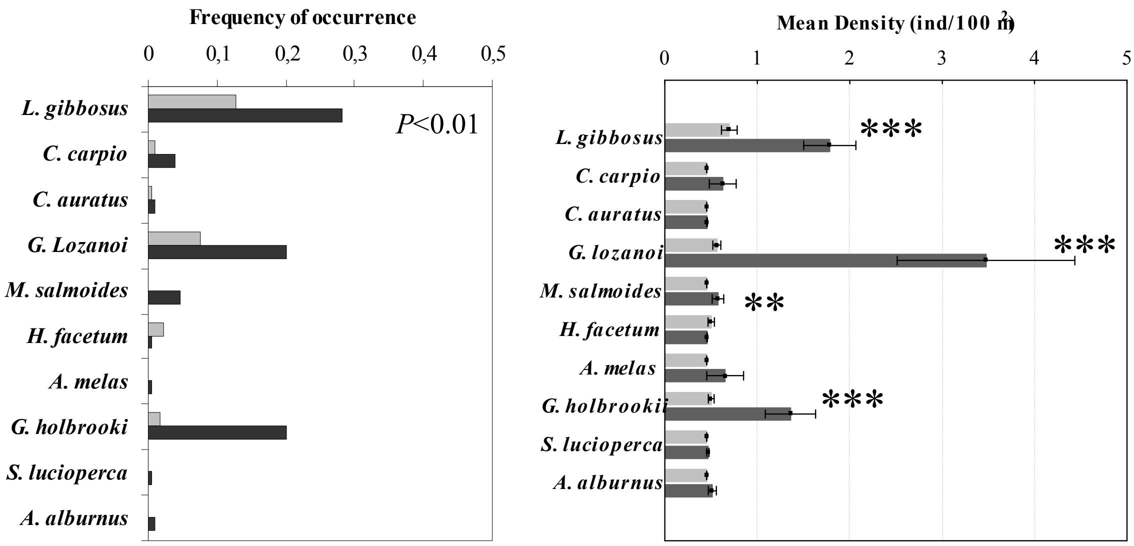


Figure 6.5 Frequency of occurrence and mean density (ind/100 m2) of NNS in undisturbed (grey bars) and disturbed (black bars) sites. Box: Mean; whisker: ± SE. Significances of Z test of proportions (for frequency of occurrence) and Mann-Whitney test (for mean density - * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) are showed.

The response of the most abundant NNS to total pressure gradient showed a clear increase in fish density along the quality classes, that is, with human disturbance, which was particularly noticeable for *G. lozanoi* ($H_{(4,246)} = 21.69$, $P < 0.001$) and *G. holbrooki* ($H_{(4,235)} = 28.32$, $P < 0.001$) (Fig. 6.7). Fish mean densities reached the highest values in the poor quality class and there was a decrease in fish abundance only in the extreme of the gradient, when the pressure was maximal. The increase in fish density was particularly marked for *G. holbrooki* between moderate and poor quality classes. This species showed a comparatively higher mean density in the bad quality class and a smooth decrease between the poor and bad quality classes. *L. gibbosus* densities in each quality class showed a considerable dispersion. Nevertheless, the highest mean density was observed in moderate pressure conditions, decreasing in the poor quality class and presenting very low values in the extreme of the pressure gradient (Fig. 6.7). Overall, the response of this species along the disturbance gradient was less marked ($H_{(4,343)}$ =

4.46, P > 0.05) than was observed for the other species, suggesting a lower tolerance to total pressure.

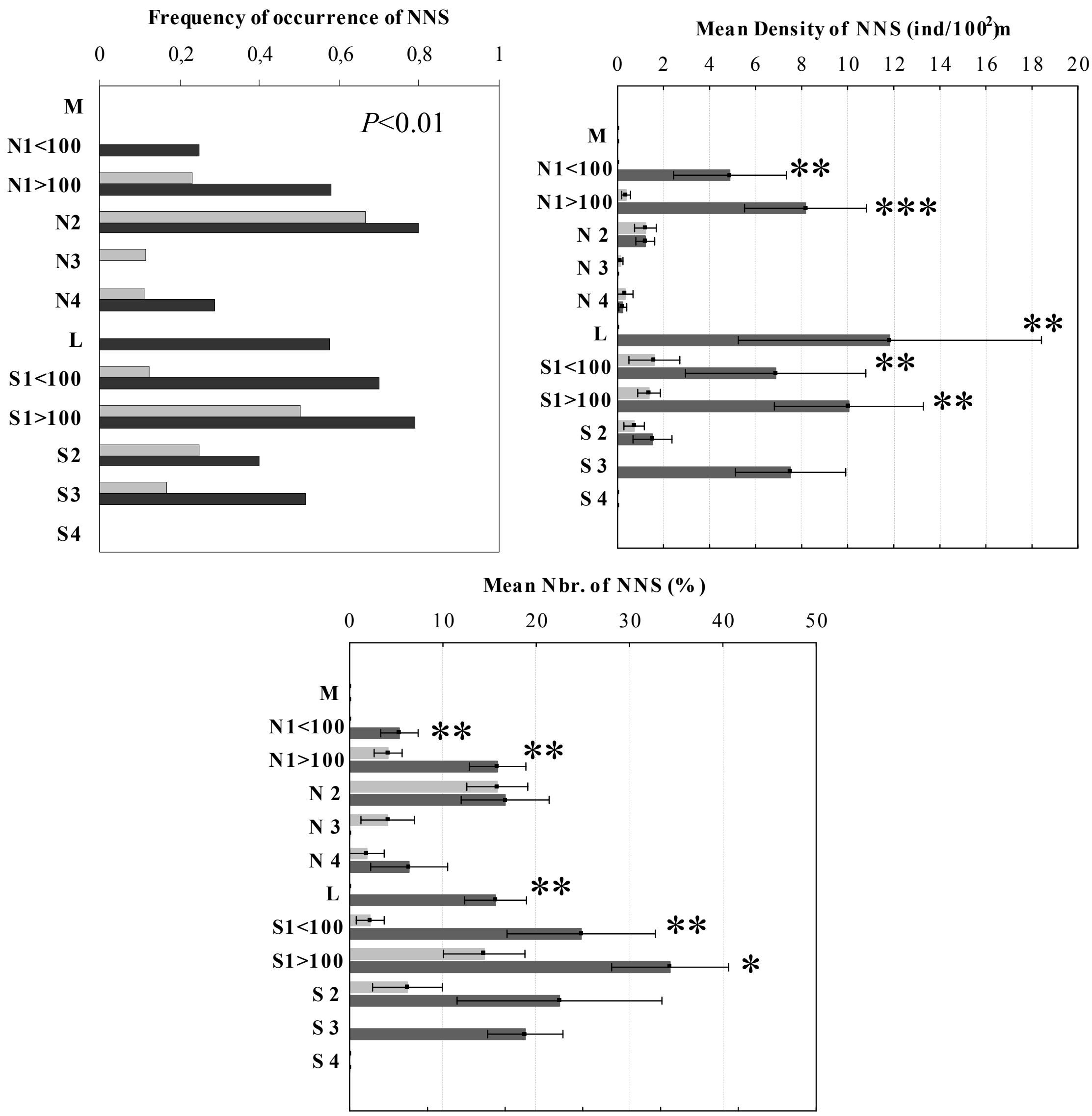


Figure 6.6 Frequency of occurrence, mean density (ind/100 m^2) and mean number (%) of NNS in each river-type, considering undisturbed (grey bars) and disturbed (black bars) sites. Box: Mean; whisker: ± SE. Significances of Z test of proportions (for frequency of occurrence) and Mann-Whitney test (for mean density and number (%) - * P < 0.05, ** P < 0.01, *** P < 0.001) are showed.

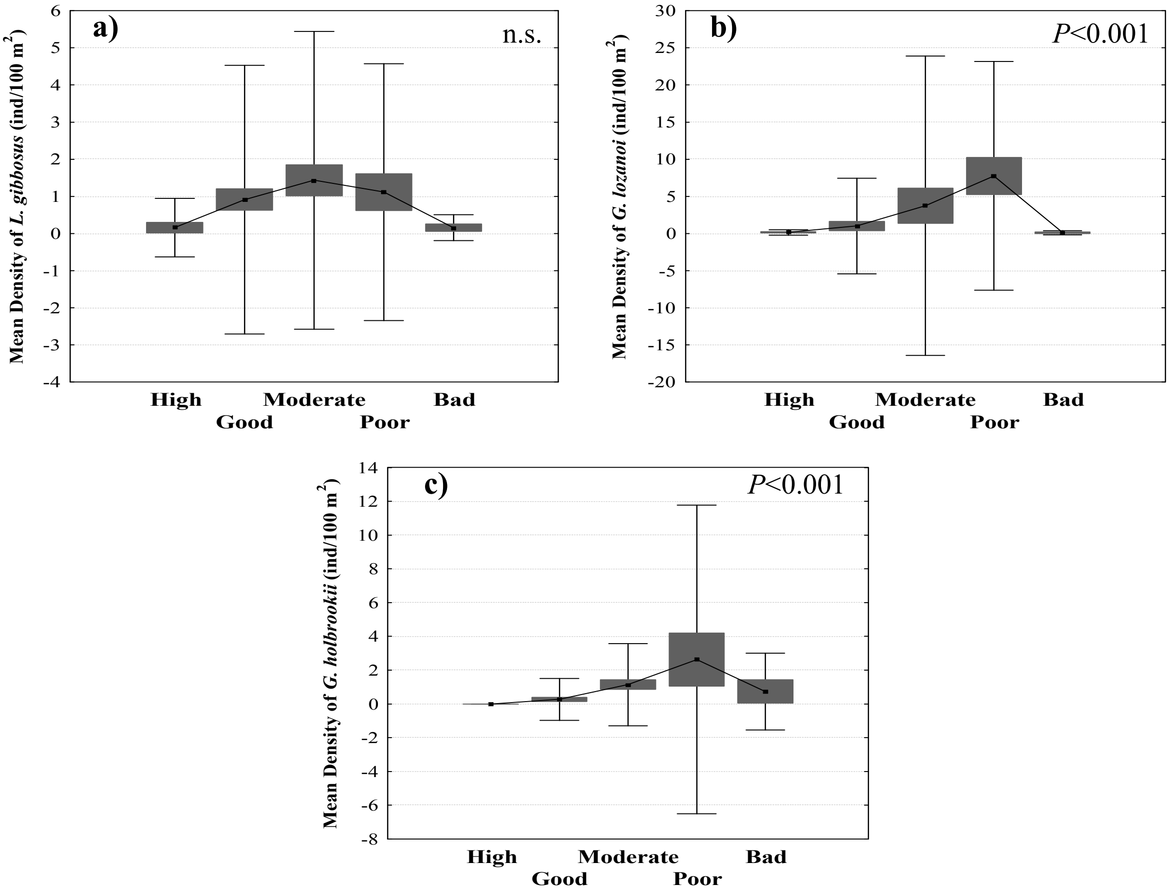


Figure 6.7 Response pattern of the most abundant NNS to human-induced pressure gradient, established according to 5 quality classes: a) Mean density of *L. gibbosus*; b) Mean density of *G. lozanoi*; c) Mean density of *G. holbrooki*. (▪): Mean; box: $\pm$ SE; whisker: $\pm$ SD. Significance of Kruskal-Wallis test is showed.

Variation partition

The above results showed that NNS, especially the most frequent and abundant ones, were particularly associated with specific environmental variables. Although their occurrence in different regions and river-types is obviously dependent on their potential distribution area, it was clear that those types present some common characteristics. Moreover, the results demonstrated that NNS were also associated with the most disturbed sites, being almost absent in undisturbed ones. Therefore, the analysis of the relationship between occurrence and abundance of NNS and the environmental and human pressure gradients was complemented with a GLM approach in order to simultaneously evaluate the relative influence of environmental variables, human-induced pressure, and spatial trends.

All the GLM models showed over-dispersion parameters under the threshold limit of 1, and were therefore performed using binomial and Poisson distributions as initially described. For all the response variables, most of the explained variability was accounted for by unique, or "pure", components. Shared effects of explanatory variables were responsible for a comparatively small proportion of data variability (Fig. 6.8). Together, environmental variables and human pressure accounted for the majority of the explained variation in the occurrence and abundance of the three considered NNS (from 31.7% to 40.2%) (Fig. 6.8). The partition of the variation showed some differences in the relative influence of each variable set among the three NNS. Occurrence of NNS was mainly determined by environmental variables, whereas abundance showed a proportionally higher influence of human pressure variables. *L. gibbosus* occurrence and abundance revealed a high dependence on environmental variables. *G. lozanoi* presented a balanced influence of environmental and pressure variables. *G. holbrooki* showed a strong influence of human pressure on both occurrence and abundance (Fig. 6.8). The results suggest that these three species have different levels of tolerance to human pressure. In all models, spatial variables (autocorrelation) represented the smaller fraction of the explained variation in both occurrence (between 0.8% and 4.8%) and abundance of the three NNS (1.4% to 2.5%) (Fig. 6.8). A higher influence of these variables on the occurrence of *G. lozanoi* and *G. holbrooki* was observed, which is in accordance with a more restricted and concentrated distribution of these two species.

Overall, both regional and local explanatory variables were present in the best ecological models in environmental and human-pressure GLMs (Table 6.3). Among environmental GLMs, drainage area, in particular, showed a significant reduction in deviance, and positively shaped all the response variables. Slope and percentage of riffles ranked as the second most important predictors respectively in *G. lozanoi* and *L. gibbosus* occurrence and abundance. For *G. holbrooki* only mean annual temperature revealed a higher positive influence than drainage area. Concerning human-pressure variables, toxicity and acidification levels were the most important predictors and were negatively related to the occurrence and abundance of the three NNS. For *L. gibbosus*, TSS (total suspended solids) and COD (chemical oxygen demand) also assumed a significant positive influence. Although nutrient/organic loads were retained in both *L. gibbosus* human-pressure GLMs, these variables did not present a relevant decrease of deviance. Total dissolved nitrogen (N) and urban area showed an important relation with *G. lozanoi* occurrence and density. Sediment load revealed a particular influence on *G. holbrooki* occurrence, registering the highest deviance reduction in this model. All values of VIF were very low, indicating the lack of multicollinearity between predictors.

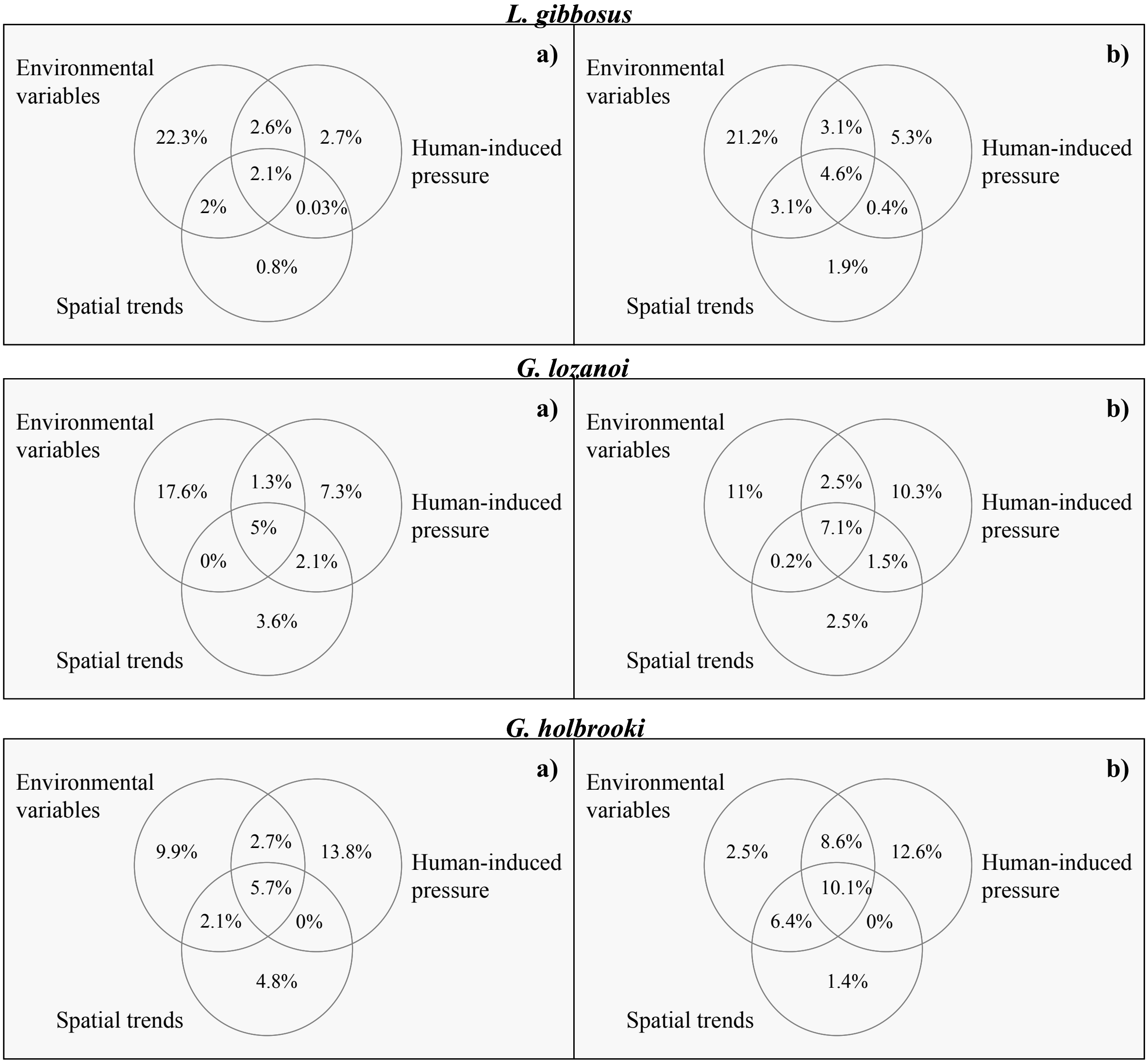


Figure 6.8 Veen diagrams showing the partition of the variation in the occurrence (a) and mean density (b) of the most abundant NNS explained by environmental variables, human-induced pressure and spatial trends.

Table 6.3 Summary of the environmental and human pressure GLM models for *L. gibbosus*, *G. lozanoi* and *G. holbrooki* (occurrence and density as response variables). Explanatory variables type: environmental (Env) and human-induced pressure (Pres). Significance levels (*P*) at **P* < 0.05, ***P* < 0.01, ****P* < 0.001

Species	Response variables	Models features	Models AIC	Explanatory variables	Type	Deviance reduction	Coef. sign	VIF	*P*
L. gibbosus	Occurrence	Binomial distribution		Drainage area	Env	76.31	+	2.14	***
		"logit" link function	274.7	% Riffles	Env	21.95	-	1.5	*
				TSS	Pres	21.93	+	1.09	*
			356.5	Nutrient/organic loads	Pres	3.06	+	2.09	*
	Density	Poisson distribution		Drainage area	Env	69.53	+	2.36	***
		"log" link function		% Riffles	Env	28.44	-	1.63	**
			430.9	Mean annual discharge	Env	7.66	-	1.78	*
				Toxic/acid levels	Pres	18.99	-	1.44	*
				COD	Pres	13.08	+	1.31	**
				TSS	Pres	5.66	+	1.12	*
			496.1	Nutrient/organic loads	Pres	2.68	+	2.19	*
G. lozanoi	Occurrence	Binomial distribution		Drainage area	Env	38.40	+	2.21	**
		"logit" link function	216.3	Slope	Env	11.22	+	1.61	*
				Dissolved N	Pres	17.62	+	1.42	**
				Toxic/acid levels	Pres	13.53	-	1.92	*
				COD	Pres	5.39	-	2.27	*
			236.1	TSS	Pres	4.31	+	1.29	**
	Density	Poisson distribution		Drainage area	Env	26.26	+	2.73	**
		"log" link function	286.4	Slope	Env	13.73	+	1.69	*
				COD	Pres	15.06	-	2.17	**
				Urban area	Pres	11.41	+	2.62	*
				TSS	Pres	4.94	+	1.22	*
				Toxic/acid levels	Pres	6.16	-	1.69	*
			293.38	Land use	Pres	6.13	-	2.43	*
G. holbrooki	Occurrence	Binomial distribution		Mean annual temperature	Env	22.88	+	2.12	*
		"logit" link function		Drainage area	Env	14.87	+	2.38	**
			186.1	% Riffles	Env	7.86	-	1.44	*
				Sediment load	Pres	22.06	+	2.31	***
			186.7	Toxic/acid levels	Pres	13.21	-	1.98	***
	Density	Poisson distribution		Mean annual temperature	Env	26.06	+	2.25	*
		"log" link function	247.3	Drainage area	Env	9.48	+	2.29	*
				Toxic/acid levels	Pres	42.29	-	1.77	**
				Sediment load	Pres	9.70	+	1.36	*
			240.8	Art. Lentic water bodies	Pres	6.40	+	2.75	*

Discussion

Non-native fish present in the Portuguese rivers have different histories. Although fast spreading is possible in a short period, the timing of introductions is relevant to the distribution expansion. The introduction of *C. carpio* or *C. auratus* dates back to some centuries ago. *G. holbrooki* was introduced in the early twentieth century (the first reference is Boto 1932 in Almaça 1995), while most other species were introduced later (Ribeiro et al. 2009a). *G. lozanoi* was detected in 1925 (Ribeiro et al. 2009a), *M. salmoides* was introduced during the 1950s and *L. gibbosus* in the late

1970s. *A. melas*, *A. alburnus*, and *S. lucioperca* were all introduced more recently (in the late 1990s and beginning of 2000, see Ribeiro et al. 2006, 2009a,b), which explains their relatively narrow distribution and abundance compared with other NNS introduced earlier. In broad terms, the timing of introductions of non-native fishes in the Iberia Peninsula is similar to that of other European countries (Crivelli 1995; Copp et al. 2005).

The most frequent and abundant species in this study were pumpkinseed sunfish, *L. gibbosus*, Iberian gudgeon, *G. lozanoi*, and mosquitofish, *G. holbrooki*. In southern rivers the most common and abundant NNS were *L. gibbosus* and *G. holbrooki*, while *G. gobio* dominated the northern ones. As such, the discussion will mainly focus on these NNS, which presented the higher invasive potential.

Ecological and environmental factors

An important distinction among the non-native fishes is their ecological character. Some species are warm water species and spread mostly and more notoriously in the southern river basins (*M. salmoides, L. gibbosus, H. facetum, G. holbrooki*), while others may be considered as cool water species or intermediate species, such as *G. lozanoi*.

Until recently, *G. lozanoi* was identified as *Gobio gobio* (*L.*) (Doadrio and Madeira 2004) and knowledge on this species ecology is still scarce. Like *G. gobio*, an invasive species, *G. lozanoi* presents a rapid growth and an early reproductive stage, and this *r* selection strategy with high fecundity allowed the successful colonization of Iberian rivers (Coelho 1981; Lobón-Cervia et al. 1991). As both species present morphological similarities, a certain degree of ecological proximity might be expected. *G. gobio* is an opportunistic species, with high dispersal and colonizing capacities (Prenda et al. 1997; Blanck et al. 2007), which enable the establishment of dense populations as observed for *G. lozanoi* in this study. In the Iberian Peninsula *Gobio gobio* populations are particularly abundant in lowland rivers with slow flow (Doadrio and Madeira 2004), which correspond to the general characteristics of river-types with higher abundance of *G. lozanoi* (N1>100 km^2 and Littoral river-types). This species abundance was related to slope and drainage area (which guarantees water flow all year long), being absent from high altitude rivers and warm waters. For this reason *G. lozanoi* did not occur in the warmer southern and central rivers, presenting low or no flow during summer.

G. holbrooki is a widespread fish species mainly present in southern lowland river-types (S1>100 km^2, S1<100 km^2, and S3) with the highest temperature and conductivity. This species abundance was positively related with temperature and drainage area. Although G*. holbrooki* is able to withstand wide temperature ranges, they prefer warm water temperatures conditions (Lloyd 1984; Pyke 2005), which enable this species to persist in critical environments as the small summer pools of the southern watercourses. Conversely, the lower temperatures of the northern rivers could limit the species proliferation, due to the effect of latitude on life-history traits, namely the reproductive ones (Benejam et al. 2009). At the habitat scale, *G. holbrooki* was negatively related with riffles, that is, it displays a preference for standing or slow flowing waters rich in organic detritus and with muddy sediments. Flowing aquatic systems with naturally variable discharge regimes are not suitable for *G. holbrooki* because high river discharges tend to eliminate populations (Meffe 1984; Arthington et al. 1990; Galat and Robertson 1992), as this species has no swimming abilities or no behavioural responses to flowing waters (Minckley and Meffe 1987). Moreover, the mosquito larvae and small pelagic or zooplanktonic prey organisms are washed out by fast flows and *G. holbrooki* is less efficient at preying on mosquito larvae in flowing waters (Reddy and Pandian 1972).

L. gibbosus is unquestionably one of the most widespread NNS in Portugal and a successful invader. This species was particularly frequent and abundant in warm water rivers of southern and central Portugal – Littoral and southern river-types, with the exception of southern mountain rivers – while in the North it occurred mainly in larger rivers (N1>100 km^2 and N2). Its abundance was positively related to drainage area and negatively related with runoff and percentage of riffles, which is consistent with its higher occurrences and abundance in the warm water lowland rivers. Sunfishes exhibit low ability to withstand high water velocities and are often reduced in number during flood events (e.g. Minckley and Meffe 1987; Schultz et al. 2003). Differences in swimming ability or behavioural responses to high water velocities between native and non-native species may be the mechanism responsible for observed patterns of differential removal (Bernardo et al. 2003; Ward et al. 2003). *L. gibbosus* is one of the species that better explains the invasion process in southern European rivers. It tolerates a wide range of environmental conditions (Vila-Gispert et al. 2002) and because of its high ecological plasticity may display an opportunistic strategy (Fox et al. 2007). The physiological response to elevated water temperatures and its consequences for population growth also seem to be a relevant part of the explanation of *L. gibbosus* success. Fast growth and precocious maturity, resulting in a high generation rate, appear to be an adaptive response to high water temperatures, as predicted for most ectotherms (Atkinson 1994). In England, *L. gibbosus* presents slow individual growth and late maturity and the species is non-invasive. On the contrary, populations of southern Europe have a fast growth and early maturity due to the warm waters (Cucherousset et al. 2009) and present an invasive character (Fox et al. 2007). This illustrates the high vulnerability of the warm water rivers of southern Europe, where all environmental conditions favour the establishment and proliferation of species with this type of strategy and where high flows seems to be the major constraint.

A striking fact regarding the distribution of NNS in this study was their absence from some river-types. Mountain rivers or small highland streams, that is, with high slope and energy and low productivity (such as M, N4, S4, and N3) seem to be much less vulnerable to invasions. The upstream reaches of most Mediterranean basins experience strong seasonal patterns in their environmental conditions. In winter and spring, streams present high flows, whereas in summer they are frequently reduced to small isolated pools. This hydrological regime may prevent the invasion of NNS, which are poorly adapted to high discharge events, including flash flows (e.g. Meffe 1984; Bernardo et al. 2003). Conversely, NNS occurrence and abundance were particularly meaningful in river-types with medium and large drainage areas, both in the northern "cool–warm water" and in the South. At the local scale, the high proportion of non-native fishes in the middle and lower reaches of Mediterranean climate rivers was also reported in other studies (e.g. Ilhéu 2004; Vila-Gispert et al. 2005).

Human-disturbance factors

In general, the pattern of NNS distribution and abundance among river-types followed the pattern of human-induced disturbance. Although human-induced disturbance occurred among all river-types, some areas exhibited higher degradation status. Overall, the sites located in littoral regions, both in the Centre-North and in the southern region, which are associated with higher human density, presented higher degradation conditions and higher occurrence and abundance of non-native fishes. The least disturbed river-types were those located in higher altitude regions and with small drainage areas, where NNS were absent or present in very low densities. Those streams are frequently located in isolated areas with difficult human access, far from the main human pathways. Remote areas with less human disturbance receive fewer invasive alien species than areas that are in the middle of trade routes or that host immense human settlement and activity (Drake et al. 1989). Furthermore, heavily populated areas also tend to be located on coasts, and coastal areas and cities are important foci of biological introductions (e.g. Mack 2003; Seabloom et al. 2006).

All river-types, excepting those with null or very low abundance of NNS, exhibited significantly higher occurrence, abundance, and percentage of NNS in sites with higher human pressure. This pattern was observed for all species except for *H. facetum*. Several species occurred exclusively in degraded sites. Non-native freshwater fish have commonly been documented to succeed in degraded aquatic habitats in many areas of the world (e.g. Arthington et al. 1983, 1990; Gido and Brown 1999; Ross et al. 2001; Meador et al. 2003; Kennard et al. 2005). Disturbed systems and communities may attract biological invasions more than pristine ecosystems due to the redistribution of space and energy resources and may promote new vacant niches for the most adaptable and tolerant invaders (Davies et al. 2005; Belote et al. 2008).

One of the most important human pressures in the Mediterranean climate rivers is related to nutrient and organic loads, which may contribute to the increase of aquatic productivity, and therefore lead to higher food availability for fishes, including NNS. The resource availability theory argues that invasibility may be directly influenced by nutrient enrichment and by the amount of available resources (Davis et al. 2000; González et al. 2010), which may facilitate biological contamination by reducing resource limitation and therefore competition. The high proportion of NNS in medium and large lowland rivers where productivity is naturally higher corroborates this hypothesis. Moreover, these river-types are also the most affected by hydrological disturbances (Littoral, North and South > 100 km^2), flow regulation, connectivity loss, and habitat modification due to dam construction. In fact, dams typically create lentic conditions that favour non-native fishes, which exhibit a higher abundance in regulated streams than in unregulated ones (Power et al. 1996; Alexandre and Almeida 2010). Thus, these river-types are also particularly vulnerable to non-native fish invasions, as several of the introduced species, for example *L. gibbosus* and *G. holbrooki*, prefer lentic enriched habitats (e.g. Godinho and Ferreira 2000; Bernardo et al. 2003).

The response of non-native fish species to the human-pressure gradient is shaped by a marked increase in NNS abundance along the quality classes (Fig. 6.7). Only in highly disturbed conditions does NNS density tend to decrease. Although the same general pattern was observed for the three most abundant species, *G. lozanoi* and *G. holbrooki* were particularly responsive to degradation, reaching the highest abundances in the poor quality status class. The establishment and invasibility of *G. holbrooki* seems to be mostly determined by human-induced disturbance factors. The abundance of this species was strongly related to indicators of disturbance describing local in-stream habitat modifications and organic and sediment loads. The response to degradation is partially related to the species tolerance to a wide range of environmental conditions, including pH (from 3.9 to 8.8, see Brown-Peterson and Peterson 1990) and dissolved oxygen (0.28 mg/L, see Lloyd 1984; Pyke 2005). The ability of this species to tolerate low dissolved oxygen enables it to survive in anoxic stagnant eutrophic waters with high organic and nutrient loads, and it is often the only fish species present in these water bodies (Ilhéu pers. obs.). It is also tolerant to a wide range of pollutants, including organic wastes, phenols, pesticides, and heavy metals, due to the species phenotypic plasticity (Andreasen 1985; Saiki et al. 2004).

The occurrence of *G. lozanoi* was mostly explained by environmental variables, such as drainage area and slope, while its abundance was equally determined by landscape/habitat and human-disturbance factors. However, the species abundance was positively correlated with human-induced hydrological disturbance, habitat modification, water quality degradation (TSS, COD, acidification), and surrounding agricultural areas. Other studies also evidenced this species response to anthropogenic hydrological disturbance: i) the occurrence of gobio in several areas followed the construction of reservoirs (Lobón-Cervia et al. 1991); ii) Miranda et al. (2005) reported an increase in gobio after the construction of the compound-gauging weir in Ebro River basin; and iii) Alexandre and Almeida (2010) observed significantly higher populations of this species in sites disturbed by small physical obstacles.

L. gibbosus responded positively to a certain degree of anthropogenic disturbance, namely hydrological and habitat modification, and nutrient and organic loads, probably because these conditions lead to an increase in suitable habitats (Godinho and Ferreira 2000) and also offer feeding advantages (Almeida et al. 2009). However, at a broad scale, this species abundance and distribution was mostly explained by pure environmental variables, such as drainage area, stream flow, and meso-habitat patchiness. The intermediate tolerance of *L. gibbosus* to degradation, also reported by other authors (e.g. Halliwell et al. 1999), supports the idea that human-induced disturbance is not a requisite for successful invasion by all introduced species (Niemelä and Spence 1991; Townsend 1996), and that a particular site may contain introduced organisms simply because they were introduced there and natural environmental conditions were favourable to their establishment.

Conclusions

This study reveals that natural landscape/environmental drivers are major factors determining the distribution of non-indigenous species, while their abundance seems to be largely promoted by human-induced disturbance. The importance and role of different invasion factors are context dependent, because of the interaction between species and environment, regarding habitat, climate match, and human disturbance, and thus the river-type approach seems to be useful to predict NNS invasibility. This study also stresses the high vulnerability of the warm water lowland river-types to invasion by NNS, which is amplified by human-induced degradation. Although some warm water species may be present in most basins (e.g. *L. gibbosus*), the successful invasive character is mainly expressed in lowland rivers. On the contrary, high energy and low productivity rivers, typically at higher altitudes, resist invasion better even under anthropogenic pressures. These are relevant issues that stewardship actions should be aware of in ecological status assessment, river basin management, and river rehabilitation programmes.

Acknowledgements

This study was part of a larger programme on the implementation of the Water Framework Directive, partially funded by INAG (Instituto da Água). P. Matono was supported by a PhD grant from FCT (SFRH/BD/23435/2005). We are grateful to several persons and their teams, who have taken part in data collection and entry: field colleagues from Universidade de Évora, M. T. Ferreira from Instituto Superior de Agronomia, P. Raposo de Almeida from Instituto de Oceanografia/Universidade de Évora, N. Formigo from Universidade do Porto and R. Cortes from Universidade de Trás-os-Montes e Alto Douro. We are also grateful to Dárcio Sousa for GIS help and A. Márcia Barbosa for statistical advice in GLM procedure. Valuable comments and critical review on an earlier draft of this manuscript by Dr. Thierry Oberdorff were deeply acknowledged. The National Forest Authority provided the necessary fishing permits.

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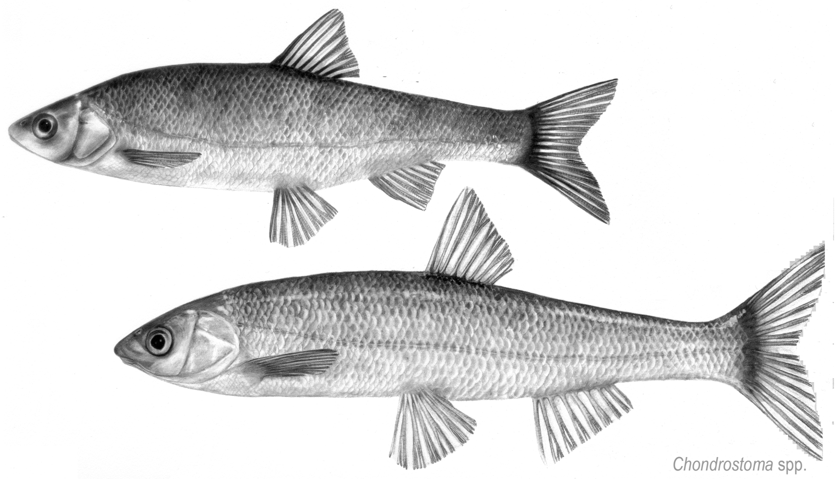
Chapter 7

Conclusions



Chondrostoma spp.
F. Filipe

Fish-based groups for ecological assessment in Portuguese rivers

Using a large sampling network covering all the main Portuguese river basins and a multivariate approach, it was possible to identify the major patterns of distribution of fish assemblages and the underpinning environmental drivers under the lowest possible human pressure, enabling the definition of fish-based geographical groups.

The first level of fish-groups differentiation was related to fish composition and particularly to the occurrence of endemic species in the different basins. Fish assemblages were hierarchically grouped, expressing a large-scale response to a North-South environmental gradient defined by temperature, precipitation, mineralization and altitude, and a regional-scale response mainly due to drainage area and flow discharge. This environmental gradient was followed by a significant shift in the composition, structure and functional attributes of fish communities.

The complementary use of both taxonomic and functional traits of fish was decisive as it allowed a broader interpretation of results, supporting the aggregation of fish-groups into 4 major fish-based geographical regions with a strong biological and environmental correspondence: Mountain region, North region, Littoral/Centre region and South region.

The defined fish-groups/regions were related to the initial abiotic river typology required by the Water Framework Directive, therefore ensuring the reliability of these fish assemblages for subsequent development of ecological assessment tools. These results, together with those from the other biological quality elements, contributed to set the final typology for Portuguese rivers (INAG 2008).

Development of a Fish Index for Portuguese rivers: a methodological challenge

After the definition of 15 national river-types, the selection of fish metrics responsive to anthropogenic pressure and the development of a multimetric Fish Index was undertaken for five groups of river-types that were not significantly different regarding fish assemblages composition, structure and function (INAG 2008): Littoral river-type, North Group with small drainage area (including Mountain, N1<100 km^2 and N3 river-types), North Group with medium and large drainage area (including N1>100 km^2 and N2 river-types), Southern mixed Group (including N4, S1<100 km^2, S2 and S3 river-types), and South river-type with medium and large drainage area.

Metrics were selected using quantitative methods and include feeding and reproductive guilds, species composition, diversity and tolerance, therefore covering a wide range of structural and functional attributes of fish communities and allowing a reliable base for evaluating their integrity.

The application of the index revealed a significant responsiveness to anthropogenic degradation in North Group with medium and large drainage area, Southern mixed Group and South river-type with medium and large drainage area, but low accuracy in the other two groups. The development and application of the index also faced particular limitations when low fish density was observed in small and shallow headwater streams, as this low density may not necessarily be a consequence of environmental degradation, and therefore hampers a reliable development of the index, and an accurate assessment of the ecological status.

The development of the Fish Index faced several problems, constituting a methodological challenge in the context of Mediterranean climate rivers. For instance, the peculiarities of fish assemblages, and the effects of the temporary character of many streams, particularly in southern Portugal, made complicated the establishment of reference conditions, the definition of the quality classes boundaries and the sound evaluation of fish assemblages response to human pressures, thus leading to increased difficulties in developing reliable biotic indexes. Despite these problems, it was possible to develop a classification system to assess the ecological status in Portuguese rivers using the existing data and a bottom-up approach.

Implications of hydrological variability on the ecological assessment

Hydrological variability is one of the key characteristics of Mediterranean climate rivers, and one of the main sources of natural environmental variability influencing the patterns and ecological features of fish assemblages (e.g. Magalhães et al. 2002, 2007; Bernardo et al. 2003; Ilhéu 2004). Small intermittent streams are particularly affected by hydrological variability both at intra and inter-annual scales, which blurs the distinction between the responses of fish assemblages to human-induced and natural disturbances, therefore leading to less accurate assessment of the ecological status.

In least disturbed sites fish assemblages maintained a long-term stability, revealing a high resilience, despite the natural hydrological disturbances. On the contrary, fish assemblages from disturbed sites presented significantly higher variability and a short and long-term instability, reflecting a decrease in the resistance and resilience of fish assemblages. Under human pressure conditions, fish fauna is particularly vulnerable and the ecological assessment may be dependent on the joint action of natural hydrological variations and anthropogenic disturbances: i) frequent high flows in streams with low inter-annual hydrological variability promote habitat rearrangement, flush out of fine sediments and nutrient dilution, improving the water quality and reducing the impact of organic/nutrient load on the biota; ii) frequent and cumulative low flows and absence of floods in streams with high hydrological variability may aggravate the impact of the human pressures both on habitat and on water quality.

Results underline the need to incorporate not only the spatial, but also the temporal variability of fish assemblages in the development of ecological assessment tools. Considering this, several fish metrics were selected to evaluate changes in assemblages integrity, maximising the detection of human degradation and minimising the response to natural variability: relative abundance of native insectivorous, eurytopic, water column and lithophilic species, relative abundance of species with intermediate tolerance and relative number of exotic species. These metrics are then the most appropriate to use in ecological assessment, specifically regarding an improvement of the multimetric index based on fish fauna for small intermittent streams in southern Portugal.

Ecological response of fish assemblages along a gradient of land use intensification

In the South of Portugal land use is changing towards intensive and irrigated farming systems, namely olive production. Considering the Water Framework Directive goals, it is important to evaluate the sustainability of these practices in regions with already over-exploited and scarce water resources, as in southern Portugal.

Disturbance variables and physicochemical parameters showed an overall increase along the gradient of olive intensification, mostly organic/nutrient enrichment, sediment loading, riparian degradation and poor water quality. These types of disturbance are strongly related to soil erosion and high levels of fertilisation and irrigation, commonly associated with intensive agriculture practices (e.g. Beaufoy and Pienkowski 2000). Animal load showed an opposite pattern, due to high livestock production associated with traditional olive groves. This practice increases in-stream trampling, habitat disturbance and erosion from overgrazed stream banks, reducing sediment trapping by riparian and in-stream vegetation and decreasing bank stability, therefore leading to an increase in turbidity, nutrients and suspended solids concentrations in streams (e.g. Kauffman and Krueger 1984; Nagels et al. 2002; Vidon et al. 2008).

Fish assemblages suffered a progressive loss of integrity along the stressor gradient, though no significant differences were observed among olive grove types, but only between these and the reference set. Despite the low intensity of agricultural practices, streams influenced by traditional olive groves were also subjected to a considerable level of disturbance posed by livestock density and fish assemblages were far from pristine. Olive grove sites were dominated by non-native and tolerant fish species, while the reference set presented higher fish richness, density and were mainly composed by native and intolerant species

Olive production led to multiple in-stream disturbances, whose cumulative effects promoted the loss of biota integrity, even in traditional olive groves, as the impact of these low intensive practices on the aquatic ecosystems can be dramatically different when they are coupled with livestock production.

Environmental and anthropogenic drivers of non-native fish species

Weather or not non-native species can be used in ecological assessment, as a reliable biological indicator, is a very debated issue. To clarify this question, it is essential to understand their distribution patterns and to evaluate the relative importance of environmental and anthropogenic drivers.

Small northern streams with high slope showed null or low occurrence and abundance of non-native fish species, while southern river-types with medium and large drainage areas presented the highest values. Moreover, the pattern of distribution and abundance of non-native species through the river-types followed the pattern of human disturbances. The least disturbed river-types were located in high altitudinal regions, with difficult human access, where non-native species were absent or present in very low densities. On the contrary, streams located in littoral regions, both in the Centre-North and in the southern regions, associated with higher human density, presented degraded conditions and higher occurrence and abundance of non-native fishes. This stresses the high vulnerability of warm water lowland river-types to non-native fish invasions, which is further amplified by other human-induced degradation.

Lepomis gibbosus and *Gambusia holbrooki* were the most abundant non-native species captured in the southern river-types, whereas *Gobio lozanoi* was the dominant species in the North/Centre river-types. Occurrence and distribution of these species were mostly determined by natural environmental factors, namely drainage area, slope, mean annual temperature and proportion of riffles. The increase in their abundance and success were mainly explained by human-induced disturbance factors, especially acidification levels, nutrient/organic and sediment loading, total suspended solids and chemical oxygen demand. Furthermore, for these species there was a marked increase in abundance along the abiotic disturbance classes, and only in highly disturbed conditions density tends to decrease.

Final remarks and future needs

The implementation of the Water Framework Directive in Portugal has represented a remarkable and unprecedented national effort, involving an interdisciplinary approach and several research teams. Based on standardised sampling protocols and on a wide network of sampling points it was possible to elaborate robust databases, nearly absent in Portugal until this action plan. For the first time, the integration of physicochemical and hydromorphological information together with data from several biological elements, enabled the development of classification systems for the assessment of ecological status in Portuguese rivers (Bernardo et al. 2009). In this context, the present thesis is an important contribution for the assessment of ecological status based on fish fauna while allowing to improve knowledge on the ecology of fish communities and ecosystem functioning in Mediterranean climate rivers.

The development of fish-based groups for the subsequent establishment of a national river typology presented the added value of being not only based on fish composition but also on structural and functional metrics. The selection of metrics responsive to human pressure and the development of the Fish Index were based on quantitative and objective methods. However, these procedures faced numerous challenges posed by the variety and ubiquity of human pressures and by the peculiarities of the fish assemblages (due to zoogeographical constrains and to the influence of the Mediterranean climate to which Portuguese rivers are subjected). These factors and their interaction have consequences on the establishment of reference conditions and on the accuracy of evaluating fish response to human pressure, therefore compromising the assessment of the rivers ecological status.

The establishment of reference conditions is a crucial step of the ecological assessment. The Water Framework Directive advocates a type-specific approach to define reference conditions, but this was not an easy task, because fish assemblages in Portuguese rivers present high natural variability and respond to continuous environmental gradients at different spatial and temporal scales, hampering an effective partition of the aquatic systems. Accordingly, long time data series including extreme natural events should be considered to improve the accuracy of reference conditions. Moreover, references should be viewed as the least disturbed sites within a river-type, rather than actual pristine conditions, as they rarely exist in most of the European countries. In this perspective, the correct assessment of human pressures through objective criteria independent of the operator is of the outmost importance.

Regarding the evaluation of fish fauna response to human pressure, this thesis contributes to the clarification of important aspects such as i) the possible influence of hydrologic variability on the response of fish assemblages to human pressure, ii) the response of fish assemblages along a gradient of human pressure; iii) the relative influence of environmental and anthropogenic factors in the occurrence and abundance of non-native fish species.

Owing to climatic and environmental constrains, the southern rivers, particularly the intermittent ones, present increased challenges in the context of ecological assessment. In these rivers, the combination of spatial and temporal environmental variability (mainly associated with high flow variability) maximises the natural variability of fish assemblages, therefore presenting added difficulties in the development of ecological assessment tools. On the one hand, it is obviously more difficult to characterise reference conditions. On the other hand, assemblages response to human pressures is affected by hydrological variability (especially if it entails high frequency of dry years and meaningful cumulative water deficit), compromising the distinction between the response to natural and anthropogenic disturbances. Under human pressure conditions, fish assemblages become particularly vulnerable to natural disturbances, revealing low resilience and consistent changes in their structural and functional features, with implications on the ecological assessment. Future climate change scenarios foresee long-term changes in hydrologic regime (Santos and Miranda 2006), further aggravating the vulnerability of these rivers (Larned et al. 2010) and enhancing ecological assessment difficulties. It is therefore necessary to address temporal variability when approaching ecological assessment, namely by using long time series of data and by integrating complementary information from hydrological models, specifically developed under different climate change scenarios.

Intensive and irrigated agricultural practices and livestock production have strong impacts on stream degradation and fish assemblages integrity. Along a gradient of land use intensification, the progressive loss of integrity in fish assemblages does not rely on a single type of pressure, which makes difficult to discriminate responses to individual pressures. The accurate evaluation of fish response to land use should take into account the interaction of different pressures at different spatial and temporal scales (e.g. Roth et al 1996; Allan 2004). Furthermore, results clearly demonstrate the extreme importance of accounting for livestock production when evaluating the impacts of land use on aquatic ecosystems (even under traditional agricultural practices), and the urgent needs to develop more stringent guidelines limiting cattle access to streams. This highlights again the need for proper evaluation of anthropogenic disturbance levels and to invest in improving the knowledge on species tolerance.

Non-native species are strongly related to human disturbance conditions and are seen to represent a serious threat to native fish communities, habitat structure and ecosystem functioning (Leunda 2010), therefore affecting the ecological quality of natural environments in multiple ways. In this sense, they can be considered as a reliable tool in the ecological assessment, either as a response to human pressure or as a biological pressure per se. Nonetheless, under the Water Framework Directive, biotic indices are developed in order to incorporate metrics responsive to environmental disturbances induced by anthropogenic activities. This means that disturbance induced by biological pressures, as non-native species, are possibly being disregarded. Indeed, despite the higher occurrence and abundance of non-native species in most degraded conditions, they can still occur and proliferate in least disturbed sites, possibly with values meaningful enough to produce impacts in aquatic systems. If this is the case, in these situations ecological quality status may not correspond to the good abiotic conditions because of the biological pressure and a Biopollution Index, based on non-native species, could complement the Fish Index in the ecological assessment (Olenin et al. 2007; Ilhéu et al. in prep.). In this perspective, further analyses are needed to clarify the possible structural and functional consequences of non-native species occurrence and abundance on native fish fauna under least disturbed conditions, as well as the characteristics of the native biota receptor that could maximize the impact of non-native species (Ilhéu et al. in prep.).

Mediterranean rivers are among the most threatened ecosystems in the world (Smith and Darwall 2006), requiring urgent measures for their conservation and sustainable management. Improving ecological assessment procedures and tools is essential in this context, and overall this thesis represents an important contribute to the major advances achieved in Portugal. However, there are no perfect and definite classification systems for assessing ecological status. It is thus essential to continue sampling programs and to invest in multimetric indices development and refinement (e.g. testing new metrics with more data, trying different scoring criteria and index formulations, using validation data sets and including temporal variability on stream biomonitoring programs).

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Appendices

Appendix 1

Description, assessment scale and methods, and scoring criteria of the 10 variables used to evaluate the level of anthropogenic disturbance in sampled 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 use 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 (presence of only annual plants)	Local expert assessment
			4	Fragmented vegetation with the presence of bushes or reeds	
			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)	
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	Channelized sector, missing most of the natural habitats	
			3	Channelized 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)	

Appendix 1 (Cont.)

Variables	Description	Assessment scale	Score	Criteria	Methods
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 information from gauging stations(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	
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 information from gauging stations (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 organic 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	Site immediately downstream of a large reservoir or within the influence area of its backwater	SNIRH** and available cartography
			4	Site immediately downstream of a mini-hydro or within the influence area of its backwater	
			3	Site downstream of a massive standing water body or within the influence area of a reservoir	
			2	Site downstream of a mini-hydroelectric installation or within the influence area of its backwaters	
			1	No influence of reservoirs	
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

**available from http://snirh.pt.

**available from http://snirh.pt/snirh/ dadossintese/qualidadeanuario/boletim/tabela classes.php.

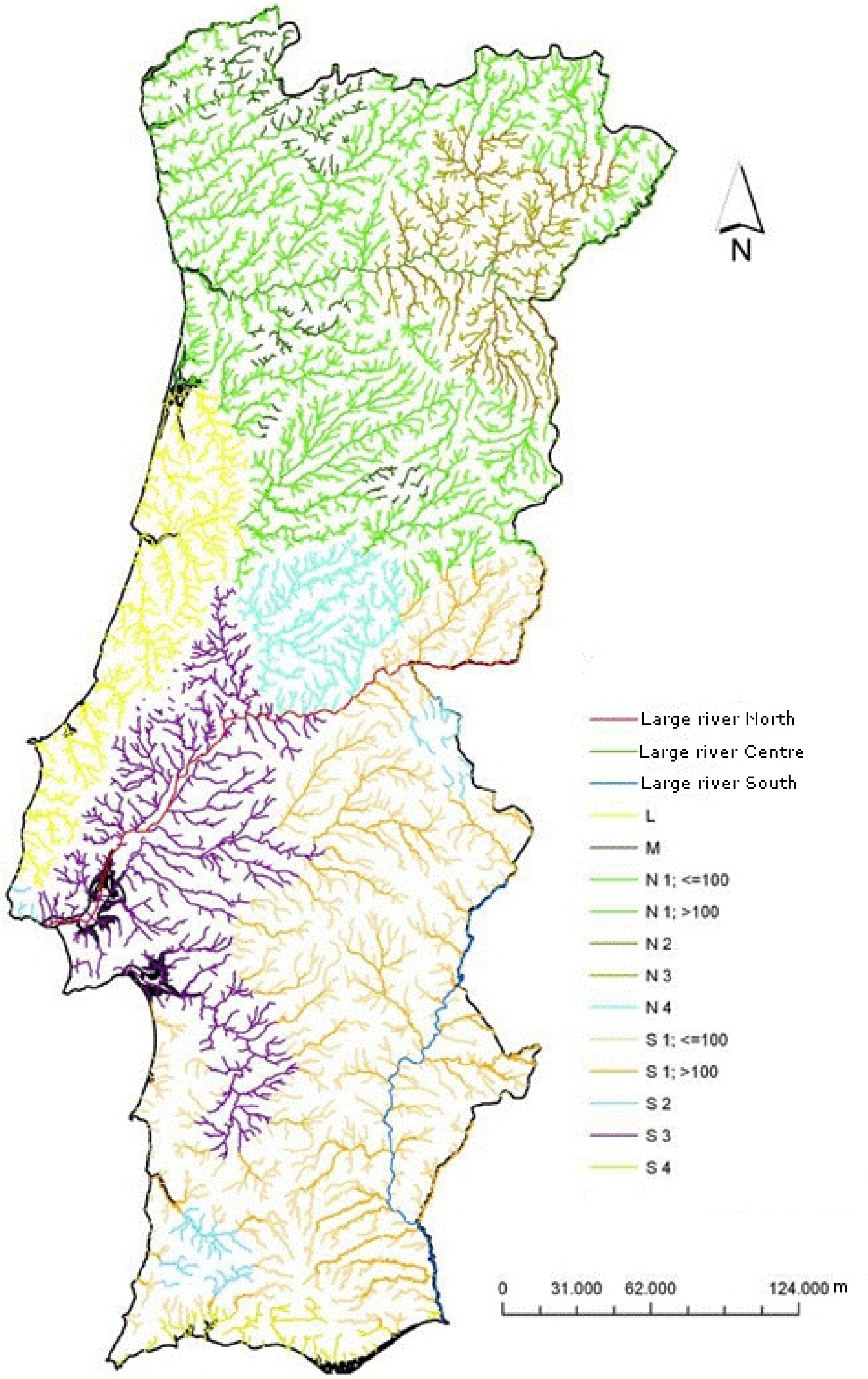
Appendix 2

Eclogical classification of fish species captured in reference sites: 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; EUR; 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, Doadrio, Almada & Kottelat, 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 & 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, Bogutskaya, Rodrigues & Collares-Pereira, 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, Bogutskaya, Rodrigues & Collares-Pereira, 1998	Torgal Chub	WC; EUR; LIT; INSV; END

Appendix 3

National typology defined for Portuguese rivers under the implementation of the Water Framework Directive



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