UNIVERSIDADE DE LISBOA
FACULDADE DE CIÊNCIAS
DEPARTAMENTO DE BIOLOGIA ANIMAL

SPATIAL PATTERNS OF SPECIES COMPOSITION AND RICHNESS IN IBERIAN FRESHWATER FISH

A MACROECOLOGICAL APPROACH

Ana Filipa da Silva Pereira Machado Filipe

DOUTORAMENTO EM BIOLOGIA
(Especialidade Ecologia)

2010

UNIVERSIDADE DE LISBOA
FACULDADE DE CIÊNCIAS
DEPARTAMENTO DE BIOLOGIA ANIMAL

SPATIAL PATTERNS OF SPECIES COMPOSITION AND RICHNESS IN IBERIAN FRESHWATER FISH

A MACROECOLOGICAL APPROACH

Ana Filipa da Silva Pereira Machado Filipe

Tese orientada por:

Professora Maria João Collares-Pereira (Univ. Lisboa)
Professor Paul Angermeier (Univ. Virgínia)

DOUTORAMENTO EM BIOLOGIA
(Especialidade Ecologia)

2010

This dissertation should be cited as:

Filipe, A.F. (2010) *Spatial patterns of species composition and richness in Iberian freshwater fish*. PhD Thesis, Universidade de Lisboa, Portugal.

N’ba N’ga, o teimoso celacanto que ainda hoje se
recusa a viver em terra,

e à minha família.

To N'ba N'ga, the stubborn freshwater Coelacanthus
that still refuses to live on land, and

to my family.

(N'ba N'ga primeiramente citado por Italo Calvino, Cosmicomicas, 1965)

(N'ba N'ga was firstly cited by Italo Calvino, Cosmicomics, 1965)

Preliminary note

The present dissertation includes papers published or submitted in co-authorship. Papers are the output of collective works reflected in the list of co-authors and in the author's contribution section of each paper.

As doctoral candidate presenting the current dissertation and leading author of all papers, I was responsible for the specific scientific design and scheduling, datasets, statistical analyses, and writing. The advisors of the thesis were deeply involved on the dissertation conceptual framework and on all stages of work development, being also co-authors of the papers.

Whereas the dissertation is a personal synthesis based on the mentioned papers where I benefited directly the co-authors participation, I also gained indirectly from informal discussions on a collaborative basis.

This work was supported by the Science and Technology Foundation and European Funding (SFRH/BD/10330/2002).

Este trabalho foi ralizado com o apoio financeiro da Fundação para a Ciência e Tecnologia e do Fundo Social Europeu (SFRH/BD/10330/2002).

List of papers

Filipe, A.F., Alves, M.J. & Collares-Pereira, M.J. How much data are we loosing? Challenges on building freshwater biodiversity databases to attain data quality. *Bioscience*.

Submitted as Overview Article.

Filipe, A.F., Araújo, M.B., Doadrio, I., Angermeier, P.L. & Collares-Pereira, M.J. (2009) Biogeography of Iberian freshwater fishes revisited: the roles of historical versus contemporary constraints. *Journal of Biogeography,* **36**, 2096-2110

Published as Original Article.

Filipe, A.F., Magalhães, M.F. & Collares-Pereira, M.J. Native and invasive fish species richness in Mediterranean streams: the role of multiple landscape influences. *Diversity and Distributions.*

Submitted as Biodiversity Research.

Filipe A.F., Angermeier P.L. & Collares-Pereira M.J. Spatial gradients of species diversity in freshwater fishes: testing theory with empirical evidence. *Global Ecology and Biogeography*.

Submitted as Research Review.

Index

Acknowledgements / Agradecimentos

A todos aqueles me contribuíram, de alguma forma, para a realização deste trabalho aqui deixo o meu agradecimento. Estou profundamente grata aos meus amigos e família que sempre me encorajaram e apoiaram em todos os momentos deste projecto. Mas também aos meus orientadores, colaboradores e colegas por todas as valiosas sugestões e conhecimento partilhado que, em muito, contribuíram para o desenvolvimento deste trabalho. Assim, expresso aqui os meus agradecimentos a alguns deles.

***To all who contributed to this dissertation in so many different ways and during distinct steps I hereby express my gratitude. I am deeply grateful to friends and family, who inspired and stood me during this project. I am especially thankful to my supervisors, collaborators, and colleagues from all the scientific inputs and knowledge sharing, which allowed developing this investigation. Therefore, here I express my gratitude to some of them:**

Aos meus **orientadores** e **colaboradores** com os quais aprendi e pude ultrapassar dificuldades quotidianas, ganhei conhecimento e noção do trabalho necessário para encontrar as melhores soluções e cumprir os objectivos, mas também partilhei entusiasmo. Estou imensamente grata por terem aceitado os numerosos desafios e também as dificuldades, em particular:

À Professora Maria João Collares-Pereira, por ter aceitado o desafio de orientar este projecto de doutoramento, e pelo seu constante entusiasmo ao longo dos trabalhos desenvolvidos, que me contagiou e incentivou sempre a progredir. Especial relevo merece todo o apoio científico e também logístico dado com uma prontidão inigualável. Estou muito grata pela confiança depositada e partilha em imensos desafios que surgiram ao FFishGUL. Também agradeço ter-me integrado em trabalhos da equipa desde 1997, e que me deram a oportunidade de desenvolver o interesse pelos ecossistemas dulciaquícolas e sua fauna, mas também partilhar ideias e conhecimento com extraordinários colegas de equipa.

*To Professor Paul Angermeier, also for being supervisor of this work. I am particularly thankful of the valuable scientific contribution regarding stream ecology, in personal discussions during my sayings in Blacksburg, but also at the distance, from which I gained immense inputs. I also express thanks for all the welcoming in Virginia Tech Department of Fisheries and Wildlife, between its students, and family.

Ao Professor Miguel Bastos Araújo, que aperar de não ser orientador formal pela instituição de acolhimento, a sua contribuição foi valiosa em muitas das fases da investigação, nomeadamente no que respeita ao meu conhecimento da ecologia teórica e aplicada. Ajudou a tomar decisões baseadas em rigor e acuidade de pensamento, mas também a esboçar e concretizar ideias em ecologia, e que estão presentes em diversos temas aqui apresentados.

À Professora Filomena Magalhães, por ter estado activamente presente em inúmeras fases deste trabalho, e que a sua proximidade e à vontade com a ecologia dos cursos de água e ictiofauna, em particular com os abordados no estudo, permitiram efectivas e produtivas discussões, sugestões, e partilha de ideias e preocupações quotidianas. Muito obrigada pela constante ajuda, que foi fundamental nalguns dos trabalhos efectuados.

Al Profesor Ignácio Doadrio, por me ter recibido en el Museo Nacional de Ciencias Naturales en Madrid, por todo suporte durante la revisión de las ocurrencias de los peces en España e por compartir todo su vastísimo conocimiento de los peces y ríos. Muchas gracias también por ayudar en las dudas que han surgido durante este proyecto. Además, por todo el entusiasmo e amistad.

À Doutora Maria Judite Alves, que além de ter contribuído directamente num dos trabalhos, foi, ao longo destes anos, uma ajuda valiosa e efectiva em questões da filogeografia das espécies e outros temas no que respeita aos peixes de água doce em Portugal. Estou especialmente grata por ter partilhado dados previamente recolhidos de ocorrências das espécies em Portugal.

*I am also thankful to all researchers that helped me to resolve specific problems and doubts. I specially thank some experts who helped in specific questions, namely Pedro Peres-Neto (R software, PCNM analysis), Thiago Rangel (SAM software), Nicolas Gotelli (Ecosim software), Robert Colwell (EstimateS software), Bruce Patterson (Nestedness Calculator software), among others, as several authors of extensions tools for ArcView and ArcGIS software. I also thank to revisers of the papers, who were very useful on all improvements. Special thanks to the editors Robert McDowall and David Currie for editing and revising effort.

Aos meus **colegas**, um especial abraço e reconhecimento. Sem a vossa proximidade este trabalho não teria sido possível, ou pelo menos teria sido mais árduo de concretizar. Em particular agradeço:

Ao Pedro Segurado, pela ajuda no tratamento de dados em em SIG aplicado a rios, e pela amizade.

*To Emmanuel Frimpong, for the help on the GIS analyses, but also for sharing determination, enthusiasm and knowledge, and for being always here, for anything needed.

À Maria Peixe Dias, pela iniciação ao programa R e ajudar em cálculos de autocorrelação espacial em curso de água

*To Than Hitt, for all bibliography, and fantastic discussions, where problems, emerging ideas were always present. I am also thankful because all the shared moments in nature, as canoeing. I keep in my memory on that Thanksgiving, also with Mary Anne.

Ao Luís Costa, pela partilha de problemas e incontáveis sugestões, mas especialmente pelo companheirismo e amizade ao longo destes anos.

A Lluis Benejam, que me hay proporcionado, científica y personalmente, una magnífica convivencia y donde hay nascido una “bonica amistat”.

Ao Filipe Ribeiro, pela partilha, desde bibliografia e dados de ocorrências a muitos outros aspectos das nossas espécies e rios, mas também pela amizade ao longo destes anos.

*To Anita Lahey, for all the support in all discussions about fish, nature, thesis, books, etc, and for all the friendship and good times.

A todos os colegas da Faculdade com quem partilhei muitos e bons momentos e um divertido ambiente de trabalho, em especial:

> Às companheiras de gabinete Vânia Proença, Zé Caramujo e Anabel Perdices, pelas inúmeras conversas, música, e mais umas quantas imensuráveis dimensões quotidianas e metafisicas.
>
> A todos os meus restantes colegas e ex-colegas do FFishGUL, em especial ao Joaquim Reis, Natacha Mesquita, Marta Gromicho, Carina Cunha, Patrícia Salgueiro, Hugo Gante, Irene Pala, e Maria Drago, Joana Martelo, Daniel Pires, Cláudia Mieiro, e outos “peixólogos” de renome... Agradeço em especial àqueles que partilharam os dados de ocorrência de espécies, oriuntos dos seus trabalhos, me ajudaram em campanhas de campo ou nos seus preparativos.
>
> Aos restantes colegas Sofia Lourenço, David Santos, Catarina Miranda, e outros incontáveis “seres voadores”, em especial ao Pedro Lourenço, pelo apoio em importantes fases deste trabalho, e muitos outros bons momentos. Mais ainda aos “terrestres” “Tatá” Teresa Sales, ao Nuno Pedroso , à Dália Freitas, è Carla Marques. Um especial agradecimento ao Joaquim Pedro, pela amizade e entusiasmo partilhados.

*To all the remnant colleagues at the Virginia Tech, specially Jamie Roberts, Robert, David Bishop, Larissa Graham with which I shared moments during the stays in Virginia Tech.

A todos os colegas do ICNB, com quem foram partilhadas mais de dois anos de aventuras, especialmente entre os da “equipa do Livro Vermelho”. Um reconhecimento terno à Leonor Rogado, por tanta partilha, e por me ter desafiado a integrar tal equipa. A tua dedicação à conservação dos cursos de água, em especial as espécies de peixes ameaçadas, bem como o teu riso e simplicidade perdurarão sempre entre nós, colegas do ICNB e da Faculdade.

Por último, aos meus professores da Faculdade de Ciências pelo conhecimentnto transmitido durante a licenciatura, e aos da Virginia University durante estes anos de doutoramento. Também agradeço aos professores do Ar.Co., escola onde aprendi a observar e desenhar, e em especial ao Pintor João Queiroz, pela amizade que permanece.

Estou particularmente grata aos restantes **amigos,** e também à minha **familia**, que sempre me acompanharam em inumeráveis momentos. Não posso deixar de referir a alguns, aqui o meu imenso agradecimento:

Aos restantes amigos de Blacksburg, um muito obrigado por me receberem em vossas casas durante as estadias no Virginia Tech, e terem tornado tudo tão leve e divertido, mesmo em momentos menos bons: vocês estiveram lá, para tudo! Anucha, Polanah, Abdul, Renata e bonequinhas lindas: neve ou não, haverá sempre vinho na sexta e “brunch” no sábado... Muito obrigada também à Ana de Nova Iorque: acolheste-me e mostraste e a cidade, entre divertidos momentos de humor.

À Tânia Nobre, Maria Carlos e respectivos companheiros, pela partilha de muitos momentos de amizade inabalável, e também à Patrícia Tiago e ao Tiago Marques com quem partilhei tantos outros momentos, em trabalho de campo, viagens e gargalhadas. O maior abraço, cheio de força, para a Luísa Chaves, a mais corajosa e lutadora de todos nós.

Um abraço enorme por tantos momentos à Inês Alves, Rui Valente, Enrique Sanchez, Gian Luca Bono, Rosário Oliveira e Maria João Amaro. Um com especial carinho à Susana Pereira, pelos “rasgos de genialidade” nos momentos de escrita na Biblioteca Nacional, e também pela amizade, ao longo de tantos anos! E também aos seus pequeninos, por priorizarem o que é mesmo importante.

À Mariana Oliveira, pela amizade inabalável, por todo o apoio e interesse constante durante estes anos. Contigo aprendo a colocar os pés na terra, e a ver a realidade de outras perspectivas. Também à pequena Mia: atravessei o oceano para te ver e valeu a pena...!

Aos meus pais e irmão, mesmo quando não compreendiam muito bem o porquê desta aventura. Mano, aquele grande ecrã foi mesmo útil na revisão e submissão dos documentos! Estou particularmente grata à minha mãe, por ser leve, gostar de rir, e me apoiar, incondicionalmente, com coragem, força e generosidade infinita. Também por aquela visita ao Jardim Botânico em pequena, e me ter contado coisas das flores, insectos, e outros bichos.

A ti, Manel, *gràcies* por compartires dos meus anseios durante a finalização deste projecto, mas também muitos outros momentos, pequenos e simples, de risos, ideias, música, deambulações, e que me deram o alento e energia necessária. Sem ti não teria sido possível estar, aqui e agora, a escrever este texto.

*Finally, I am deeply grateful to all authors who, trough art, from painting and drawing to music and books, contributed to more pleasant daily times, but also to a more parsimonious understanding of nature(s).

Agradecimentos institucionais:

Ao Departamento de Biologia Animal da Faculdade de Ciências da Universidade de Lisboa e ao Centro de Biologia Ambiental, por terem acolhido este doutoramento. Pela mesma razão, agradeço ao *Department of Fisheries and Wildlife* Sciences (*Virginia* Polytechnic Institute and State *University, USA)*.

À Fundação para a Ciência e Tecnologia e ao Fundo Estrutural Europeu no âmbito do III Quadro Comunitário de Apoio pela atribuição da bolsa individual SFRH/BD/10330/2002.

Ao *Museo Nacional de Ciencias Naturales* (CSIC, Madrid) por ter apoiado os trabalhos desencvolvidos no âmbito do programa Synthesys (*European Community Research Infrastructure Action under the FP6*).

Ao Instituto de Conservação da Natureza e Biodiversidade, por me ter dado a oportunidade de ter sido colaboradora e autora da actualização do Livro Vermelho dos Vertebrados de Portugal durante dois anos.

Resumo

Apesar das espécies de peixes continentais ibéricas serem desde há muito alvo de estudos que visam conhecer a sua taxonomia, características biológicas e, mais recentemente, o seu património genético, existe ainda um significativo desconhecimento de muitos aspectos da sua ecologia, nomeadamente, de que forma se distribuem na paisagem, e quais os factores e processos ecológicos envolvidos.

Saber quais e quantas espécies de peixes ocorrem num troco de rio e como se estruturam as comunidades piscícolas é essencial para compreender e proteger os cursos de água e a sua biodiversidade, o que é amplamente reconhecido como urgente face aos desafios ambientais deste início de século. A Península Ibérica tem merecido um destaque especial, tanto a nível mundial, como no contexto europeu e mediterrânico, uma vez que muitas das espécies são endemismos e encontram-se seriamente ameaçadas. Foi partindo desta ideia e do caso da Península Ibérica que se desenvolveu o presente estudo. Para tal, utilizaram-se ferramentas e metodologias de análise de âmbito macroecológico, tentando responder às seguintes questões: Como se distribuem os peixes de água doce na Península Ibérica? Que factores limitam essa distribuição? Quais os processos ecológicos envolvidos? Foi estudando a riqueza e composição das comunidades dos cursos de água existentes em toda a Península, e especialmente os localizados na área a sudoeste, que se desenvolveu este trabalho. Trata-sede uma primeira abordagem aos padrões espaciais da ictiofauna ribeirinha da Península baseada em dados actuais da ocorrência das espécies e recorrendo a ferramentas em Sistemas de Informação Geográfica (SIGs).

Inicialmente, compilaram-se dados georreferenciados de ocorrências de espécies provenientes de amostragens de campo efectuadas em estudos prévios usando pesca eléctrica (de 1990 a 2008). Para tal, foram construídas duas bases de dados com uma componente SIG: a InFISH.PT relativa aos cursos de água portugueses, tendo como unidade de informação os locais de pesca, e a InFISH.IP para os dados ibéricos, onde o grau de informação corresponde a quadrículas 10*10 km. Assim, foi possível descrever a composição e a riqueza específica das comunidades piscícolas locais e cruzar essa informação com variáveis de paisagem (sucintamente, com a variação do ''pool'' de espécies, clima, topografia, rede hidrográfica, a diferentes escalas). A informação sobre a distribuição e taxonomia nestas bases de dados é a mais completa até à data para as espécies dulciaquícolas ibéricas, incluindo espécies primárias (restritas às águas doces), secundárias (que ocasionalmente habitam águas com alguma salinidade) e periféricas (que realizam parte do seu ciclo de vida em águas marinhas),

independentemente de serem nativas ou não (i.e., invasoras). Uma primeira análise à InFISH.PT permitiu identificar potenciais lacunas e, assim, optimizar o processo de compilação, nomeadamente através da realização de amostragens adicionais, quando consideradas necessárias. Permitiu também evidenciar a importância de investir em procedimentos que permitam construir bases de dados de biodiversidade de elevada qualidade no que respeita à icitofauna continental. Deste modo, será potenciada a sua utilidade como ferramenta de base para investigadores, técnicos e gestores do território e educadores, tendo os Museus de História Natural um papel fundamental a desempenhar no processo de validação (Capítulo 2).

Em segundo lugar, analisaram-se os padrões espaciais dos peixes de água doce na Península Ibérica com base em análises estatísticas de semelhança. Dos resultados das análises efectuadas, a variabilidade da composição de espécies das comunidades revelou-se fortemente associada aos limites das redes hidrográficas constituídos há 2,5-1,8 milhões de anos. De facto, desde essa época as bacias hidrográficas mantiveram-se isoladas, apenas com capturas pontuais (por exemplo, conexões temporárias de cursos de água usualmente independentes nas zonas médias ou de cabeceira e conexões hidrológicas na foz de bacias contíguas). Esses limites mostraram-se muito mais correlacionados com a composição das espécies do que com o clima actual, em particular, para as espécies primárias e secundárias. A dispersão e colonização dos organismos parecem ser os processos ecológicos mais relevantes à escala da Península. Partindo deste conhecimento, foram delineadas províncias biogeográficas para esta fauna. As onze províncias propostas constituem uma actualização de estudos biogeográficos prévios, que se basearam em dados considerados actualmente desactualizados relativamente às espécies existentes e às suas distribuições. Estes estudos anteriores também não se basearam nos limites das bacias agora considerados e, como tal, não permitiam examinar adequadamente a permeabilidade das bacias e inferir alguns processos de dispersão e colonização abordados no presente estudo (Capítulo 3).

Seguidamente, analisaram-se os padrões espaciais das espécies piscícolas nos rios da área sudoeste da Península, tipicamente de carácter mediterrânico, com base em métodos estatísticos de regressão. Daí resultou que a riqueza local média encontrada nessas bacias hidrográficas é, em grande parte, explicada pelo total de espécies existente em cada bacia – o ''pool''de espécies –, mesmo quando se distingue entre a riqueza das espécies nativas e invasoras já estabelecidas. Adicionalmente, as bacias com precipitação média anual elevada estão associadas a uma maior riqueza de espécies invasoras. Ao examinar em detalhe os padrões espaciais nas bacias hidrográficas dos Rios Mira e Guadiana, verificou-se que a riqueza local observada para ambos os grupos de espécies (nativas e invasoras) está associada às variáveis de paisagem que caracterizam cada local, como a sazonalidade da precipitação e o declive dos cursos de água. Mas as variáveis que caracterizam a estrutura da rede hidrográfica foram as mais explicativas, particularmente para as espécies nativas. Em geral, as variáveis à escala da paisagem explicaram grande parte da riqueza local das espécies analisadas, o que não implica que outras variáveis não tenham influência. Os resultados permitiram evidenciar que os processos de dispersão e colonização influenciam grandemente as comunidades estudadas (Capítulo 4).

Por último, procedeu-se à análise comparativa de estudos relativos aos padrões espaciais dos peixes de água doce e às variáveis a eles associados que utilizaram dados à escala mundial, continental ou regional. A hipótese mais defendida é a que considera o clima contemporâneo como o factor mais associado às comunidades bióticas, pois estas foram capazes de adequar as suas áreas de distribuição às condições ambientais e, assim, eventuais marcas de eventos históricos poderão já não ser evidentes. Tal não foi confirmado nos resultados das análises efectuadas nesta dissertação e, após esta revisão bibliográfica, foi possível apontar a necessidade de realizar testes mais adequados que integrem, em situação de igualdade, os factores potencialmente mais relevantes, tal como foi agora efectuado. Esses testes poderão ajudar a corroborar, ou não, as hipóteses em debate (Capítulo 5).

Os resultados obtidos apontam que os factores de larga escala têm uma forte influência na biodiversidade local, provavelmente em relação a processos de dispersão e colonização. Na realidade, diferentes factores actuam a níveis hierárquicos distintos. Em cada nível/escala, os factores predominantes divergem consoante os graus de tolerância fisiológica das espécies à salinidade e consoante são ou não oriundas de outras áreas biogeográficas. De realçar a importância da história das paisagens e das espécies, sendo que os ambientes contemporâneos têm também uma forte influência nas comunidades piscícolas actuais. De facto, as redes hidrográficas, tanto no que respeita ao limite das bacias hidrográficas (praticamente mantidas desde tempos geológicos relativamente remotos na Península) como à própria estrutura interna dessas bacias, foram fundamentais para explicar os padrões piscícolas contemporâneos. Também a associação do "pool" de espécies de cada bacia hidrográfica à riquea local reflecte a importância do passado dessas espécies e que levou à sua co-ocorrência nessas áreas. Por seu lado, as condições ambientais actuais revelaram-se também importantes para as comunidades piscícolas que habitam a Península Ibérica. O clima, juntamente com a topografia, está também associado à riqueza encontrada para as espécies invasoras nas bacias dos cursos de água a sudoeste. No interior das bacias hidrográficas, o clima local é relevante para a riqueza observada de espécies nativas e invasoras, nomeadamente, o seu carácter sazonal, embora as variáveis que reflectem a constituição da rede hidrográfica tenham sido mais preponderantes para as bacias analisadas. Assim, apesar da predominância dos factores históricos, o ambiente contemporâneo não deixou de se revelar importante, e os resultados não minimizam as consequências do impacto humano global, já bastante evidentes. Este estudo sugere que as marcas do passado poderão mesmo desaparecer se as alterações ambientais modificarem consideravelmente os padrões piscícolas actuais, pois os organismos, de entre aqueles que persistem, são forçados a dispersar e colonizar áreas menos adversas.

Em síntese, a presente investigação, sendo inovadora nas metodologias e escalas utilizadas, contribuiu para o conhecimento dos padrões espaciais das comunidades actuais da ictiofauna dos cursos de água da Península Ibérica. Também enriqueceu debates científicos em torno dos temas abordados. A eventual fragilidade do estudo prende-se com a qualidade dos dados, a intercorrelação de alguns dos factores de paisagem e, por último, com a possível influência de factores não estudados, nomeadamente, dos relativos aos impactos humanos nos habitats. No entanto, não foram encontradas evidências de que estes aspectos possam ter afectado os resultados obtidos.

As conclusões deste estudo têm consideráveis implicações para os investigadores destas temáticas, mas também para gestores do território e educadores. Nomeadamente, os resultados obtidos permitem uma melhor compreensão e consciencialização dos factores e processos ecológicos a larga escala, que influenciam as comunidades locais de peixes que habitam os cursos de água. No que respeita à conservação e gestão da ictiofauna, os desafios são inúmeros, pois este grupo faunístico é muito dependente do meio ribeirinho e, por consequência, os indivíduos encontram-se fortemente constrangidos pela conectividade das redes hidrográficas para dispersar e colonizar novas áreas. O sucesso destes processos é cada vez mais complexo, pois nessas áreas, se alcançadas, os indivíduos necessitam de condições climáticas adequadas e habitats disponíveis, o que é dificultado pela crescente destruição dos mesmos ou ocupação por espécies invasoras. Implicações detalhadas no contexto dos desafios ambientais prementes no âmbito europeu e global são mencionadas ao longo da dissertação, nomeadamente no que respeita: (1) à implementação de directivas europeias, como é o caso da Directiva Quadro da Água (DQA); (2) às alterações climáticas; e (3) à crescente homogeneização da fauna.

As perspectivas de investigação mais pertinentes que emergem deste estudo prendem-se com a necessidade de (a) cruzar dados de biodiversidade (dados evolutivos, de riqueza e composição das comunidades, atributos biológicos e interacção de espécies) com a história das paisagens, ambientes contemporâneos e alterações ambientais (como as despoletadas pelas alterações climáticas), (b) examinar, nas comunidades contemporâneas, a influência de

possíveis vias de dispersão no passado devido a alterações ambientais, (c) analisar o papel da pressão humana através da introdução de espécies nas comunidades. E, por último, (d) estudar processos ecológicos locais associados, por exemplo, às características biológicas e às interacções bióticas.

Os desafios ambientais actuais exigem um maior conhecimento da biodiversidade dos cursos de água e a sua divulgação aos vários sectores da sociedade, de modo a fomentar a conservação das comunidades e dos seus habitats. Espera-se que a presente investigação tenha contribuído para tal, além de ter evidenciado a necessidade de abordagens mais integradas de investigação e acção. Por último, à medida que se encontaram respostas para as questões colocadas, novas questões, hipóteses, e problemas foram levantados para estudos futuros, os quais permitirão, certamente, avançar no conhecimento ecológico, a várias escalas, da biodiversidade ribeirinha.

Palavras-chave: comunidades piscícolas; conservação; cursos de água; influências da paisagem; Península Ibérica.

Summary

Despite the threatened status of many freshwater fish species requiring attention, many questions about their biogeography and ecology remain unsolved. Modern analytical methodologies can help to solve this problem. The aim of this work was to examine the factors driving spatial patterns in Iberian freshwater fish species and the ecological processes involved. The focus was on investigating the composition and richness of stream assemblages across both Iberian Peninsula and south-western streams.

In a first step, databases coupled with a Geographical Information System (GIS) were built with data from field observations (1997-2008) of freshwater fish species (Portugal and Spain). Databases allowed portraying local assemblages' composition and richness, and crossing them with landscape describers. Attaining data voids helped obtaining complete and consistent data.

The spatial variability of assemblage's composition in the Iberian Peninsula was found to be strongly correlated with the hydrological basin boundaries, more than with current climate, especially for primary species; thus biogeographical provinces were delineated based on the former factor. Local richness for native and invasive species across south-western Mediterranean-type river basins was highly constrained by the regional pool of species. Within basins, stream network explained a large amount species variability patterns for both species groups but especially for the native ones, followed by climate and topography for both groups.

Distinguishing among species, particularly concerning dispersal ability and alien origin clarified the patterns and processes involved. The literature review helped to compare findings with previous studies: our results did not support current climate as the main driver of current assemblages. More stringent tests are needed in future studies, as performed here.

Results achieved have especially interest to researchers and managers, particularly to those focused on Iberian or Mediterranean-type fresh waters, and can constitute a knowledge basis, especially regarding the Water Framework Directive (WFD) context and for predicting future scenarios.

Keywords: assemblage composition and richness; conservation; Iberian Peninsula; landscape-scale; stream fish

Chapter I.

Introduction

Children's Song

It was an alder.
After a watermill.
And nearby
a stream.

Everything so still.
What could I do?
I put everything in my pocket
To not miss them.

"Canção Infantil

Era um amieiro.
Depois uma azenha.
E junto
um ribeiro.

Tudo tão parado.
Que devia fazer?
Meti tudo no bolso
para os não perder."

Eugénio de Andrade, 1977, *in* "Primeiros Poemas"

I.

Introduction

Here, after describing the general problem statement and the pertinence of the work, both focal ecological topics and particulars of the study case – the freshwater fish in Iberian streams – are presented. At last, the study aims and the dissertation outline are described.

1.1 Why study stream fish ecological patterns?

How biodiversity patterns are generated in nature has long intrigued biologists. Currently, the impoverishment of biota is happening at unprecedented rates (e.g. Pimm, *et al*. 1995; Chapin *et al*., 2000; Vié *et al*., 2009), and the idea that we are now facing the sixth mass extinction on the earth has become generalized (see Eldredge, 1998). The biodiversity crisis has been widely noticed among the scientific community, and considerable efforts are being devoted to increase knowledge regarding the biodiversity patterns and the drivers related with biodiversity change (e.g. Gaston, 2000; Chapin, *et al*., 2000; Mace *et al*., 2005). Therefore, apart from the interest regarding biological research*,* acquiring a better knowledge of biodiversity patterns, their constraining factors, and biotic processes involved is crucial if we are committed to diminish the prevailing trend of the biodiversity loss and enhance the sustainable use of ecosystems to meet human needs (e.g. Millennium Ecosystem Assessment, 2005).

The biodiversity of fresh waters is experiencing greater declines than the majority of terrestrial ecosystems, thus making prospects for biodiversity and services they provide alarming, especially in Mediterranean regions (e.g. Sala *et al*., 2000; Vié *et al*., 2009). From headwaters to large rivers, watercourses are currently threatened by landscape changes which lead to a loss, fragmentation, or degradation of habitats. The most recognized biodiversity threats include urbanization, land use and land cover changes, water impoundments (for water supply or electric power), as well as the introduction of non-native species and climate changes (e.g. Sala *et al*., 2000; Abell, 2001; Revenga *et al*., 2005; Dudgeon *et al*., 2006), for which recent efforts are being made to predict their effects (e.g. Buisson *et al*., 2008). The loss of freshwater biodiversity affects a broad cross-section of society since it has consequences at a range of highly valued ecosystem services, including fisheries, water supply, nutrient regulation, and recreation (Vörösmarty *et al*., 2005).

In fact, there is a risk of depletion of the freshwater native faunas before we even know what has been lost, where it was located, or how the loss could have been prevented and freshwater services maintained (see Revenga *et al*., 2005). The world's species diversity inhabiting fresh waters is disproportionately high as compared to other ecosystems (Vié *et al*., 2009): occupying only 0.8% of Earth's surface, total species estimates on fresh waters are at least 100,000, nearly 6% of all described species. But a lack of knowledge regarding current and already extinct biodiversity components inhabiting fresh waters remains *(*Bailan *et al*., 2008). Among the freshwater taxa, fish are the best documented faunal group (Bailan *et al*., 2008; Lévêque *et al*., 2008), being, at the same time, seriously threatened: in the last updates of the worldwide IUCN Red List, freshwater fish amounted a high proportion of threatened species among the vertebrate species evaluated, registering alarming percentages: 37% worldwide (Vié *et al.,* 2009), and 56% when only the Mediterranean endemic species are considered (see also Smith & Darwall, 2006).

In the context of the current biodiversity crisis, improving knowledge on freshwater biodiversity and making it available is crucial for developing tools and actions for freshwater faunal conservation and ecosystem services maintenance (Bailan *et al.,* 2008). The freshwater fish fauna might constitute a starting point to examine what drives current freshwater biodiversity patterns and, ultimately, to understand the biodiversity patterns and processes observed in freshwater ecosystems and predict future changes.

1.2 Stream ecosystems and ecological communities

Among freshwater ecosystems, running waters - river and stream systems, here mentioned simply as streams - have a unique directional and hierarchical structure, where the environmental characteristics of surrounding and upstream landscapes have direct effects on the stream reaches downstream (Allan & Johnson, 1997; Allan *et al*., 1997), and complex geomorphic and environmental processes operate at distinct scales (Frissell *et al.,* 1986).

The structure of streams comprises stream reaches within a stream segment, which is part of a watercourse (tributary or main stream) itself contained at a sub-basin of the whole river basin. In each stream reach, pool-riffle sequences can be found, being highly seasonal among Mediterranean-type streams, and ephemeral among temperate streams. Geomorphic and environmental characteristics at one level of the hierarchy influence successively lower levels, from broader scales variations to microhabitats found at the pool-riffle scale (Box 1.1, Fig. 1.1). For example, the topography and climate characteristics have a direct and indirect influence on catchment physiography, types of vegetation and soils, and ultimately, human uses characteristics; human uses by their turn influence sediment and water discharge regimes, setting the morphology and dynamics of the watercourses (e.g. Frissell *et al*., 1986; Naiman *et al*., 1992; Montgomery, 1999).

BOX 1.1 Stream spatial structures

A range of terms and definitions regarding stream structures found across scales have been used in ecological and biogeographical studies, resulting in a difficult or inaccurate interpretation of data and results in spite of the stream structures constituting topographic and hydrological well defined units (Allan *et al.,* 1997).

In this dissertation we followed the definitions of the hierarchical framework of Frissell *et al.* (1986) for scales and stream structures (see table below). Other authors classified the stream structures (Allan *et al.,* 1997; Matthews, 1998). Terms are the ones used by Matthews (1998).

Stream hierarchical spatial structures commonly referred to in the literature. Terms typically used in this dissertation are shown in bold.

Spatial structure	Description
Watercourses	
Stream reach	**Part of a stream segment constituted by pools, rifles, and runs**
Stream segment	Watercourse between confluences
Stream system	Watercourse composed of stream segments
Tributary	Watercourse flowing into main stream
Main stream	Watercourse flowed by tributaries
Hydrological basin network	Watercourses of a river basin constituted by tributaries and the main stream
Watercourses and draining areas	
Sub-basin	**Area draining a tributary**
Basin	**Area draining into sea or ocean, with natural geographical barriers; also called river basin**
Sub-catchment	General term for a draining area of small extent
Catchment	General term for a draining are a constituted by sub-catchments (correspond to sub-basins)
Watershed	General term for a draining area of large extent (correspond to basin or sub-basin)
Larger areas	
Biogeographical provinces	**Area holding ecologically defined biotic communities (fauna, flora and/or ecosystems); also called ecoregion, biogeographic unit**
Ecozone [1]	Area holding distinct biogeographical units. Also called realm

[1] for recent delineation of the Freshwater Ecoregions worldwide see (http://www.feow.org; Abell *et al.,* 2008).

Considering streams as hierarchical systems constituted the basis for adopting a heuristic framework of stream communities, which was firstly based on the faunal ‘filters’ concept proposed by Simpson (1953): the species constituting regional pool are ‘filtered’ (or ‘screened’) according with factors operating at distinct scales, resulting in a subset of species coexisting locally (Fig. 1.2). How a hierarchy of filters can affect stream fauna was firstly formalized by Smith & Powell (1971), and later on adopted by other researchers (e.g. Tonn *et al.*, 1990; Poff, 1997), stating that the species are subjected to the existence of faunal filters progressively acting at finer spatial and temporal scales. The dynamic nature of filters through space and time was later on evidenced by Angermeier and Winson (1998).

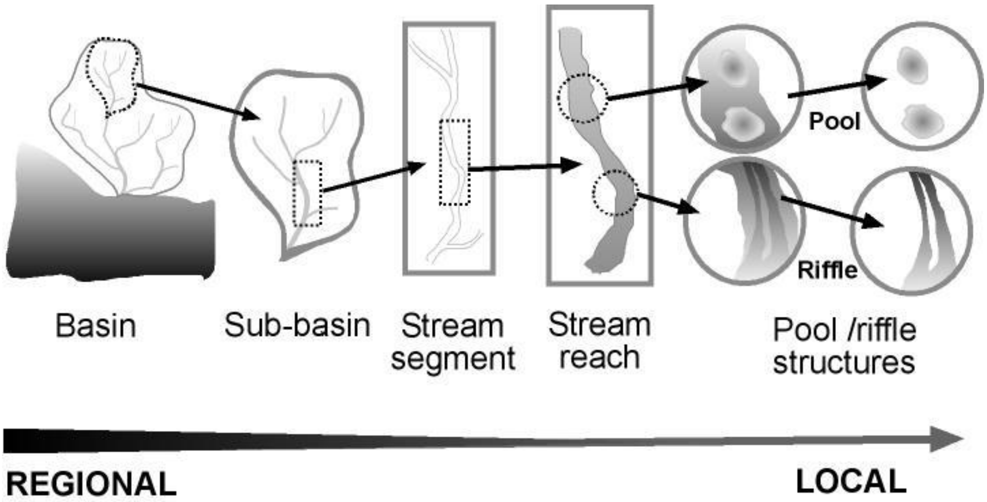


Figure 1.1 Representation of the hierarchical spatial structures (landscape and habitats) found at streams across spatial scales. A stream reach is comprised by multiple pool-riffle units where multiple microhabitats are found. Reaches are contained within stream segments, forming a sub-basin, which is a tributary or the main stream of a river basin [adapted from Frissell *et al.* (1986b) to the range of scales mentioned in the present dissertation].

This framework - the hierarchical faunal filters – has been helpful to explain the processes that might be determining, at each scale, the ecological communities, by comparing the differently filtered species regarding their biological attributes (fecundity, maximum age, body size, and physiological tolerance; Tonn *et al*., 1990; Poff, 1997; Jackson *et al*., 2001 Quist *et al*., 2005). For example, at the basin scale, history and climate (by controlling the potential range that any given species can occupy) may act on biogeographical processes such as species evolution and dispersal, thus shaping their distribution ranges. At the reach scale, contemporary factors as stream size (which affects habitat volume, food resources and stability) may filter species by the body size trait; biotic interactions are usually associated with the most local filter. If filters are minimal (as dispersal barriers, habitat variability, disturbance and biotic interactions), species occurring in a site are similar to the species pool (Angermeier & Winston, 1998).

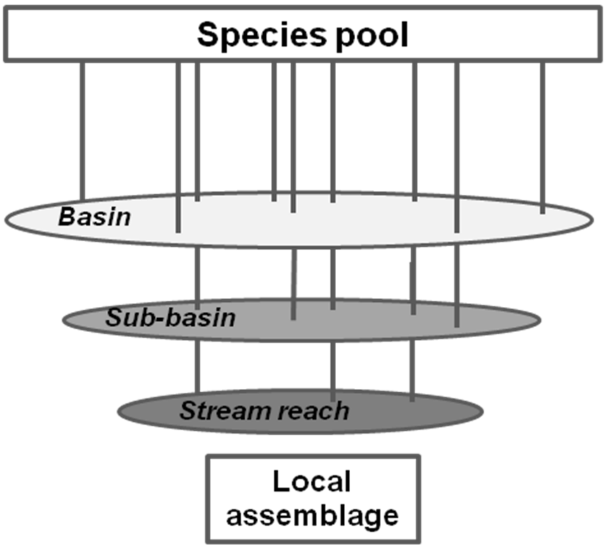


Figure 1.2 The 'filtering' of species with certain attributes by landscape constraints, from broader to finer scales, resulting into the subset of species comprising the local assemblage. The environment or historical filters at regions or river basins restrict, at the sub-basin scale, the occurrence of species lacking certain traits, whereas the hydrological regime, among others, restrict the stream reach fauna, and so on to the microhabitat scale. Life-history and biological traits, and physiological limits of species define the filtering, as portrayed by the truncation of the vertical lines [adapted from Smith & Powell (1971) to the filters focused on the present dissertation].

The adoption of the hierarchical faunal filters as a general organization framework resulted as being useful in examining and understanding the drivers of species composition and richness, and particularly in the development of multiscale approaches, as previously refereed as by various stream ecologists (see Poff, 1997 and references therein).

1.3 What drives the variability of patterns in stream fish communities?

To begin with, some matters regarding the factors driving variations of species diversity and the involved processes are introduced, before particularising to the patterns found in stream. fish communities.

Detecting drivers and examining processes

Patterns in ecological communities - the distribution, richness and diversity of species - depend on a complex interplay between processes acting at distinct temporal and spatial scales (Menge & Olson, 1990). At local-scale, processes as predation and competition, and adaptation to resources, disturbance or stochastic variations influence communities. At larger scales processes of speciation, dispersal, and colonization play their role (Ricklefs, 1987; Godfray & Lawton, 2001), and factors such as latitude, water availability, and long-range dispersal pathways act on shaping biological patterns (e.g. Menge & Olson, 1990; Willis & Whittaker, 2002). Moreover, ecological patterns and processes have been often documented as being scale dependent, and scale is now largely recognized as a critical component when examining ecological patterns in nature (Wiens, 1989, 2002a; Box 1.2).

Among the factors to incorporate when investigating ecological communities, evidences have been given to the necessity of accommodate spatial characteristics of landscapes in spatial explicit approaches, whether these factors can constitute a statistical nuisance or a characteristic of interest (e.g. Dutilleul, 1993). Particularly, it has been proposed checking for the presence of spatial autocorrelation in species or environmental data (Legendre & Legendre, 1998) should be a preliminary step in ecological studies dealing with large-scale patterns (Legendre, 1993; Diniz-Filho *et al*., 2003). An important consequence of dealing with spatially autocorrelated data is the potential statistical problem it brings with it, as important assumptions of standard statistical tests are violated, namely that the values of studied variables are independent among data points (Cliff and Ord, 1981; Legendre, 1993; Araújo & Guisan, 2006). This can affect the outcome of analyses regarding the relationships between the dependent biotic and the explanatory variables, particularly when the dependent variable exhibits some autocorrelation (see Diniz Filho *et al*., 2003); Lennon 2000). But positive spatial autocorrelation can indicate local communities are driven by biotic confounding processes operating at larger scales, as dispersal ability of organisms and colonization events; conversely, no spatial autocorrelation can suggest that biotic confounding processes might not be playing an important role (e.g. Selmi & Boulinier, 2001).

BOX 1.2 Scale dependency and components

Scale dependency occurs if a relationship (form and/or parameterization) changes with grain or extent (Wiens, 1989, 2002a; Willig *et al*., 2003). Therefore the scale of investigation studies determines the range of patterns and processes that can be detected, and any inferences on scale dependency in a system are constrained by the scale of investigation (Wiens, 1989).

When referring to spatial or temporal scale, two components are involved which define the upper and lower limits of resolution of a study (O'Neill, 1988; Wiens, 1989; Whittaker *et al*., 2001): grain is the size of the unit of analysis, and extent is the area or time scope of the study.

The spatial grain is normally coincident with the sampling resolution (or larger), while the spatial extent refers to the size of the study area.

In studies on stream fish, spatial grain can vary along the stream hierarchical structure, ranging between >10 m^2 to >10,000 m^2, depending on sampling method, effort performed, and/or dataset used, but usually corresponds to stream reaches. The study area can encompass a wide range of stream structures, from microhabitat to river basins or the world surface (Matthews, 1998).

One solution to obtain reliable outputs is to perform statistical procedures that incorporate the spatial structure of landscapes in alternative to spatially independent approaches, specially when the considered variables, or the residuals of the analyses, are likely to be spatially autocorrelated (Segurado & Araújo, 2004; Segurado *et al*., 2006; Dormann *et al*., 2007). As expected, the spatial variance can be also dependent on the scale of the investigation (Wiens, 1989).

BOX 1.2 Spatial autocorrelation

Spatial autocorrelation occur when locations close to each other exhibit more similar values of certain attribute - positive autocorrelation - or less similar - negative autocorrelation - than those further apart (Cliff & Ord, 1973).

Significance of spatial autocorrelation is calculated by comparing observed data with the expected values if the variable was not dependent of geographic location (Legendre & Legendre, 1998).

The fact we are now facing a biodiversity crisis has lead to a special concern on identifying the factors that determine biodiversity patterns and understanding the ecological processes behind (Godfray & Lawton, 2001; Willis & Wittaker, 2002). Despite the increasing number of empirical studies on patterns of biodiversity at larger scales (e.g. Fraser & Currie 1996; Saab, 1999; Valone & Hoffman, 2002), most studies have been conducted for terrestrial systems, thus enhancing a demand of studies conducted for freshwater biodiversity components (Abell, 2002; Olson *et al*., 2002).

The stream fish ecological communities

Stream fish communities can be influenced by landscape factors at several scales (see Bain & Finn, 1988; Angermeier & Schossler, 1989; Rahel & Hubert, 1991; Schlosser, 1991, 1995). Some factors such as latitude, geological history, but also climate and topography (e.g. Tonn *et al.,* 1990; Ricklefs & Schulter, 1993) operate on stream fish diversity over large spatial and temporal scales across and within river basins on processes such as speciation and dispersal (Livingston *et al*., 1982; Hughes *et al*., 1987; Hugueny, 1989; see Chapter 5). At finer spatial and temporal scales, the water flow across a fluvial landscape is thought to be the key factor describing fish species diversity, as it can have a strong effect on many other local abiotic factors (e.g. Johnson *et al*., 1995; Poff & Allan, 1995; Sparks, 1995; Pegg & Pierce, 2002). Interactions among species, as competition, may structure the coexistence of species, but empirical evidences are not consensual about their relevance (e.g. Peres-Neto, 2004). Environmental disturbances (as climate change and other anthropogenic influences) are leading to species extirpation, and community recovering will depend on colonization events more than local processes (e.g. Angermeier & Winston, 1998;Buisson *et al*., 2008).

Although the importance of multiple constraints across scales was recognized regarding the patterns and processes of biota inhabiting streams (see previous chapter section), current knowledge remains restricted to few taxa and ecosystems, for which datasets are available (Matthews, 1998; Bailan *et al*., 2008; see also Chapter 2). Also, few studies have examined the patterns across scales and their likely landscape constraints simultaneously and on an equal footing, and distinguishing species with distinct life-history traits [but see Labbe & Fausch (2000); see also Chapter 3 and 4]. Finally, as previously referred, progresses on stream ecology are far from being properly considered and referenced by ecologists from other fields, and thus to integrate holistic ecologic debates (Menge *et al*., 2009; see also Chapter 5).

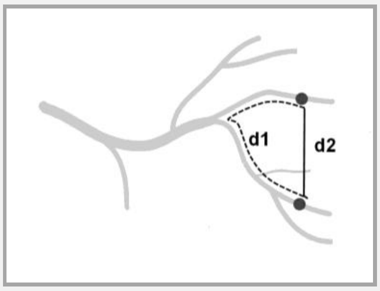
Therefore, there is a wide range of questions that remain unexplored. This is not surprising, since traditional stream ecology was dedicated to matters measured at small spatial scales (Matthews, 1998), and it was not until the 1990s that more generalized patterns across large scales started to be examined (see Lawton, 1999). In fact, the traditional view of stream ecologists before the 1990s was focused mainly on local population trends and habitat quality, but the improvement of computer capabilities, data available at larger scales and new and complex analytical methods triggered the recognition that freshwater populations persistence may depend on processes operating at broader temporal and spatial scales, namely at the basin and catchment scale (see Box 1.1 and Chapters 3 and 4). The importance of landscape characteristics was already implicit at previous works as the ‘river continuum’ concept (Vannote *et al*., 1980), but the emphasis was given later on with the development of the landscape ecology approach extended to freshwater systems (Wiens, 2002b), where the spatial characteristics of landscapes were highlighted. New technology, namely on Geographic Information Systems (GIS) and analytical methods, are opening new possibilities for developing innovative ecological studies at broad scales and addressing some largely unanswered questions, and simultaneously providing new knowledge to meet management challenges.

Studies have been showing that stream biotic and abiotic processes are complex, as they may operate simultaneously and be scale dependent (e.g. Palmer & Poff, 1997; Box 1.2). Scale dependency on patterns of stream biodiversity has been documented regarding a number of factors, from environmental variables, as habitat heterogeneity to spatial structure of streams (Lancaster & Belyea, 1997; Palmer *et al.*, 2000; Fagan *et al.*, 2002, 2005; Pegg & Taylor, 2007).Because of the noticeable spatial nature of streams, the spatial structure of data was considered at landscape-scales since the early times of stream ecology (e.g. Hugueny & Lévêque, 1994; Matthews & Robinson, 1998; Wilkinson & Edds, 2001). For example, some traditional geomorphic variables used to describe stream network configurations were adopted in studies to examine biotic variation along the upstream-downstream gradient and referred as determinant on shaping stream communities (see Mathews, 1998), and continue being used (e.g. Filipe *et al.*, 2002, 2004; Grenouillet *et al.*, 2004). These measures reflect the stream size at each site and describe upstream characteristics relative to the entire drainage upstream [e.g. the drainage area, stream order (Strahler, 1964; Shreve, 1967), and tributary spatial position (Osborne & Wiley, 1992)]. Currently, notable advances in the GIS treatment of stream networks allow building, accurately and at large-scale extents, spatial and geomorphic variables for streams, and also calculating distances between sampling locations along the stream paths (Box 1.3).

BOX 1.3 Geographical distance measures in streams

Spatial distances between sampling sites of organisms that are exclusively depend on watercourse as fish are better addressed by network distances than by the Euclidian distances commonly used for overland distances (Box 1.3; e.g. Olden *et al*., 2001; Beisner *et al*., 2006).

New tools for stream ecologists to calculate distances among sampling sites at Geographic Information Systems (GIS) allow calculating stream distances. Those distance measures may include directional characteristics of the network, from upstream to downstream reaches (e.g. Ganio *et al*. 2005, Peterson *et al*., 2006).



Distance between sampling sites at a stream network can be measured either as the shortest distance between two points (Euclidian distance, full line d2) or as the distance along the network pathway (dashed line d1).

Analytical techniques are able to statistically reduce the stream distances calculated on a set of spatial predictors (e.g. Principal Coordinates of Neighbour Matrices approach PCNM; Borcard & Legendre, 2002).

As a synthesis, an array of patterns and processes has been examined in freshwater biota, particularly regarding assemblages variability. Although some studies provided multiscale approaches (Downes *et al*., 2000; Labbe & Fausch, 2000), or considered explicitly the spatial structure of data (e.g. Heino *et al*., 2002; Grenouillet *et al*., 2004), advances in the species datasets and tools available are opening new opportunities of research.

1.4 The case study: the Iberian stream fish

The freshwater fish assemblages inhabiting the Iberian Peninsula streams offer an excellent model system to study the biodiversity patterns across space because of the highly diverse stream habitats and hydrological networks with well defined boundaries and pathways, which embrace an endemic and threatened fish community with a diversification history of species rather complex but considerably well known. Moreover, there is an absence of studies regarding this fauna, or the remnant freshwater faunal groups, aiming to explore the observed patterns across the peninsular landscape and driving factors involved. Studying this stream fauna allows performing specific empirical tests that can illuminate the ecological processes acting in face of the strong spatial constraints of dispersal to aquatic pathways. A particular emphasis was given to the Mediterranean-type south western streams because they comprise a highly endemic fish fauna~~,~~ for which analyses across the region are scarce, but a considerable amount of data is available (but see some studies on within-basin extents, as Collares-Pereira *et al*., 1999; Magalhães *et al*., 2002; Filipe *et al*., 2002).

Next, the characteristics of the Iberian Peninsula landscapes and of the fish fauna inhabiting these streams are presented. A more detailed characterization of the specific study-cases considered - the Iberian Peninsula (IP) and the south-western Iberia (SW) and their fish species - i s given in Chapter 3 and 4, respectively.

Iberian Peninsula

Despite its size (581,000 km^2, Fig. 1.3), the Iberian Peninsula (IP) has a remarkable landscape diversity as a consequence of its biogeographical location, climate transition between the Eurosiberia and the Mediterranean, and topography, with large mountain ranges primarily oriented east-west and a high central plateau, called the “Meseta” (Rivas-Martinez, 1987). The highly unique biodiversity of the Peninsula is attributed to the past events of (1) speciation enhancement and population’s differentiation, (2) preservation of the existent fauna during glacial periods on refuge areas, or (3) inhibition of North-South biotic migrations (e.g. Puente *et al*., 1998; Vargas *et al*., 1998; Doadrio, 2002).

Located at the western part of the Palaearctic Realm facing the Atlantic Ocean, the IP is geographically isolated from North Africa by the Atlantic Ocean and Mediterranean Sea (Strait of Gibraltar), and from Europe by the Pyrenees Mountain Chain. A wide range of microclimates can be found across the landscape, including the Alpine Climate in the high mountains, but mostly there are two major climatic zones, the Mediterranean and the Atlantic (Font Tullot, 1983). Most of IP has a Mediterranean climate, which is typically highly seasonal, with warm and dry summers, and cool and wet winters. In contrast, the Atlantic climate, mostly located along the western and northern coasts and the Pyrenees Mountains, is wetter, cooler and less seasonal (Fig. 1.3). Across IP mean annual temperatures oscillate between 2.2 ºC and 19 ºC, and total annual precipitation between 203 and 2990 mm (Font Tullot, 1983). These climatic zones host an enormous diversity of vegetation types, from deciduous and coniferous forests to sclerophyllous woodlands or annual steppe grasslands (Rey-Benayas & Scheiner, 2002).

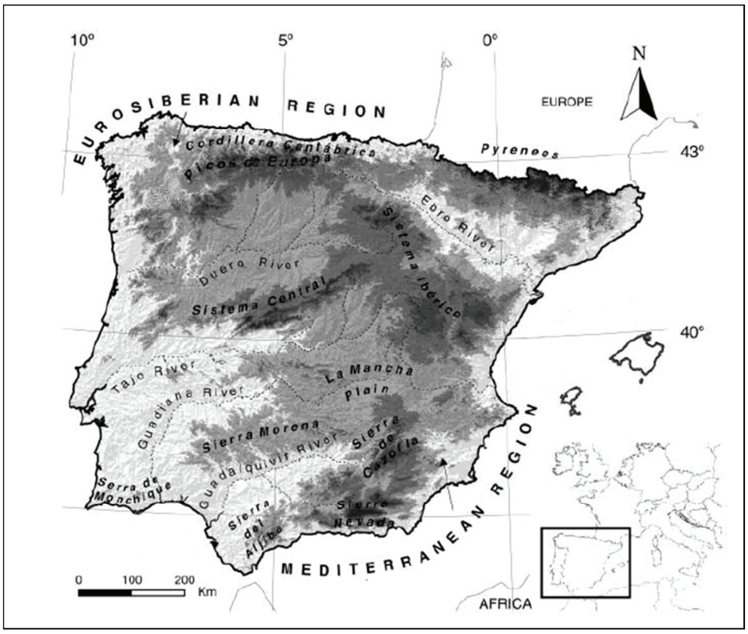


Figure 1.3 Main geographical features of the Iberian Peninsula (adapted from Alcaraz *et al*., 2006).

The stream network that can be presently found at IP date from the Pliocene-Peistocene (2.5-1.8 Ma ago) (Calvo *et al*., 1993; Box 1.4) and are mostly of temperate and Mediterranean-type regimes following the landscape gradients referred above (Fig. 1.4). Mediterranean streams are normally found at the southern regions of IP, but also at some northern areas, whereas temperate streams are mostly located at the northern Atlantic areas. Temperate streams are mostly permanent with a flow regime dominated by flooding events, whereas Mediterranean streams are temporary, dominated by seasonal events, mostly climate driven by floods and dry periods (Gasith & Resh, 1999; Pardo & Álvarez, 2006). The riparian corridors of temperate streams are dominated by deciduous species with pulses of litter fall coupled with the vegetative seasonal activity, while riparian vegetation of Mediterranean streams is dominated by evergreen plants (Rey-Benayas *&* Scheiner*,* 2002).

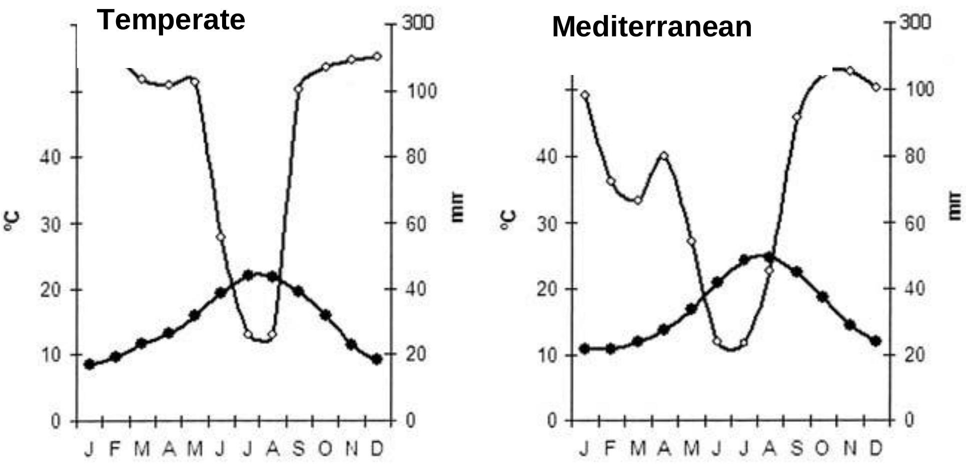


Figure 1.4. Climatic diagrams for the Atlantic coast (Ponte Areas; Vigo, Spain) and for the Mediterranean coast (Can Serra, Pollensa; Spain). Stream regimes follow these climatic gradients and therefore they have a temperate or Mediterranean-type regime (adapted from Pardo & Alvarez, 2006).

Iberian stream fish

The Iberian freshwater fish fauna has relatively few species and most are endemic and highly threatened (Doadrio, 2002; Rogado *et al*., 2005; Smith & Darwall, 2006; Fig. 1.5).

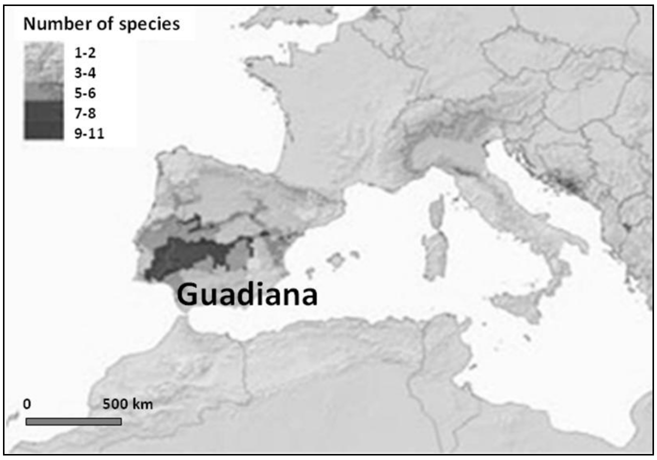


Figure 1.5 Species richness of threatened endemic freshwater fish in the western Mediterranean. The greatest concentration in whole western Mediterranean area of threatened endemic freshwater fish is located in the Lower Guadiana River in southern Iberian Peninsula (adapted from Smith & Darwall, 2006).

Regarding the potential ability to disperse, according with Darlington's classification (1957), most of native fish inhabiting Iberian freshwaters (Cyprinids family) are unable to disperse throughout salt waters – 'primary' species. A fewer number of species are seawater-tolerant – the 'peripheral' species – and probable migrate back and forth from sea to freshwaters during their life-cycle or simply have occupied freshwaters during recent evolutionary times. An even smaller number of species inhabiting fresh waters is able of making limited incursions into seawaters, because of a partial tolerance to this environment – the 'secondary' species.

Phylogenetic studies of some primary freshwater fish species indicated speciation was related to the formation of the current hydrographical network (namely for the genus

Squalius, *Barbus*, *Achondrostoma*, *Iberochondostoma*, *Parachondrostoma*, and *Pseudochondrostoma*; Doadrio *et al*., 2002; Cunha *et al*., 2004; Mesquita *et al*., 2007; Robalo *et al*., 2006a,b; Box 1.5). By contrast, the current distributions of peripheral fish species, tolerant to salt waters, are likely limited by other factors beyond terrestrial boundaries (Lucas *et al*., 2001). River basin boundaries have been used to describe the current distribution of freshwater fish species in the Iberian Peninsula, but such descriptions did not include the current amount of data available on species taxonomy and distribution, or phylogeny (see for references Chapter 3 and also Appendix I).

BOX 1.5 The Iberian Peninsula geological history

Some geological evens might have influenced the current biodiversity patterns of freshwater fish, and therefore some of the history of the Iberian Peninsula is here described. Cyprinids colonized the Iberian Peninsula at the end of the Oligocene Epoch (Tertiary Period), 28 Ma[1] ago. At this time this region had a quite distinct icthyofauna from the current one, as Characiforms, Siluriforms and other tropical fish fauna (De la Pena, 1995).

During the following Miocene and main part of Pliocene Epoch (11–5 Ma), endorrheic drainages were formed along active geological faults (Calvo *et al*., 1993), and ~5.59 Ma the African and Iberian continental margins joined by a land corridor for about 260,000 years and lead to the partial evaporation of the Mediterranean Sea (the Messinian Salinity Crisis; Duggen *et al*., 2003). During the latter Pliocene and early Pleistocene (2.5-1.8 Ma), the current hydrographic network was formed (Calvo *et al*., 1993).

At the Quaternary Period, during the Pleistocene, a succession of glaciations occurred (1.80 – 0.11 Ma) which resulted on partial ice coverage's and consequent ice retreats across the Peninsula, accompanied by fluctuations of the sea level. These environmental changes are likely to have provoked alterations in inland fish species distributions, dispersal, colonization, and genetic subdivision or admixture of species (e.g. Sousa-Santos *et al*., 2007). Particularly, in the last glaciation (Würm, 16,000-18,000 years ago), the sea level along the Atlantic cost of Iberian Peninsula was estimated to be 130-140 m below the contemporary level, which might have allowed interconnection between river mouths of some adjacent rivers (Dias *et al*., 2000).

In summary, since ~1.8 Ma ago the river basin boundaries constituted the main land barrier to dispersal of freshwater biota across the Peninsula, with few punctual stream captures and formation of pathways between neighbour river mouths likely to have occurred afterwards.

[1] Ma =10^6= one million years

Regarding the current assemblage patterns of stream fish in the Iberian Peninsula, examining within and across river basins at some regions reported a strong heterogeneity of the patterns observed, and also that such heterogeneity is highly correlated with current environmental attributes (e.g. Carmona *et al*., 1999; Corbacho & Sánchez, 2001; Filipe *et al*., 2004; Mesquita *et al*., 2007). Particularly, those patterns have been associated with the typical ecological fluctuations across time and space in Mediterranean-type streams (Filipe *et al*., 2002; Magalhães *et al*., 2008). Among the environmental attributes associated with stream fish assemblage variation, variables of landscape context seem to have a determinant role and, at least in some Mediterranean-type streams, a substantial proportion of the assemblage composition may be explained by such large-scale factors, irrespective of microhabitats and local biotic interactions (Magalhães *et al*., 2002; see Chapter 4), whereas local abundances seem more driven by the habitat attributes (Mesquita *et al*., 2007). For temperate-type streams, assemblage composition of fish has been commonly associated with local habitat attributes, but there are evidences that landscape features might be determinant on shaping those patterns (e.g. Godinho *et al*., 1997, 2000; Santos *et al.,* 2004; Ferreira *et al*., 2008).

The majority of the established non-native species are piscivore amd belong to a wide spectrum of families which were introduced in Iberian inland waters (Centrarchidae, Cichlidae, Esocidae, Fundilidae, Ictaluridae, Percidae, Poeciliidae and Siluridae). They were introduced mainly because of recreational fisheries interests (Alcaraz *et al*., 2005; Ribeiro *et al*., 2009a). Analysis of their spatial distribution is being used to detect introduction and invasion routes, and to predict future invaders (see Chapter 4); for example, most non-native species have been dispersing by pathways from France to Spain and from Spain to Portugal (García-Berthou *et al*., 2005; Ribeiro *et al*., 2009b)

1.5 Study aims and approach

As previously introduced, examining and understanding stream fish biodiversity patterns is scientifically interesting, but also imperative to the conservation and management of freshwater communities. Particularly, increasing knowledge of Iberian freshwater fish is required to management and conservation strategies and actions in the Peninsula since : 1) freshwater fish species are heavily endangered (Smith & Darwall 2006; section 1.4); 2) freshwater faunas are likely to be heavily affected by climate change, and predictions indicate freshwater fish biota will have difficulties on tracking with the subsequent environmental changes (e.g. Magalhães *et al*., 2008; Buisson *et al*., 2008); and 3) there is the compromise established by the Water Directive Framework (WDF) between European Community state-members of achieving and maintaining good water status by 2015 (see www.euwfd.com/ website). Improving scientific knowledge on stream fish will help on building problem solving tools for freshwater conservation and management, which is a necessary investment in face of the threats to these ecosystems, their biodiversity, and ultimately to the services they provide (Millennium Ecosystem Assessment, 2005).

performing the current dissertation aims to contribute to a better knowledge of the spatial patterns freshwater fish.

In this context, the general objective of this dissertation was to examine the ***spatial patterns*** *of species composition and richness in Iberian freshwater fish stream communities, and the* ***ecological processes*** *involved.*

In this context, some particular questions were formulated:

(I) *How complete, congruent and accurate are the data available for the native fish species of Portuguese mainland freshwaters? Which uncertainties and voids can be identified? How improvements on data collecting and gathering at biodiversity databases can be achieved?*

(II) *How does the diversity of native fish species vary across the Iberian Peninsula? Is there any distinctness among groups of species with different ability to disperse? What influence most the spatial distribution of these communities: the current environment or the historical constraints? What biogeographical regions can be defined based on this fauna?*

(III) *Does stream fish species richness vary among the South-western river basins? How much distinct landscape factors (as basin's species pool, area, and environmental attributes) contribute to the local species richness found between basins? And within basins, how much environmental gradients and spatial structure account the species richness variability? Are the answers different for native and for invasive species?*

(IV) *What gaps in knowledge exist regarding the freshwater fish patterns found in nature? What are the possible ways to improve that knowledge? Which empirical tests on freshwater biodiversity might be the more useful?*

To address these questions we used large-scale datasets of species occurrences to perform empirical tests. Achievements were framed on the current scientific knowledge by making a literature review.

The study was based on a macroecological approach, which encompasses the traditional ecology and biogeography disciplines, and offers the possibility of answering some central questions regarding the spatial species patterns, while taking advantage of the current technological advances (Brown, 1995; Brown & Lomolino, 1998; Gaston & Blackburn, 2000). The focus was directed to ecological matters currently in debate on general and/or fresh waters specialized literature, and analytical procedures were based on correlations and multivariate statistical techniques to examine the variation of community attributes using large datasets. It explicitly mentions data uncertainties and biases, scales used (extent and grain of analyses), and spatial structure of landscapes.

Species-based measures of biodiversity were adopted to study the fundamental structural properties of community assemblages, particularly the species number (richness) and species members (composition). The exclusive focus on fish was considered a basis for attaining a better knowledge on freshwater patterns and processes and, ultimately to enhance debates on freshwater biodiversity matters.

Obviously, to answer the four sets of questions formulated, specific methods were used (see Methods section at Chapters 2, 3, 4, and 5). Particularly, regarding the scales assessed on the empirical tests performed, distinct extents and grains were used: at the Iberian Peninsula extent (581,000 km^2), data had 20-km grid cell resolution (Chapter 3), while at south-western extent (87,000 km^2), stream sites of approximately 300-600 m^2 of wetted area were used as scale grain (Chapter 4).

This research used data of freshwater fish species occurrence and up-to-date taxonomical information from both published and unpublished studies and field surveys performed by the candidate. Databases with a spatial component coulpled with a Geographic Information System (GIS) component were built to facilitate data storage and queries performance (Chapters 2, 3 and 4). Also, the databases allowed identifying the information gaps, and to consequently focus efforts on improving data completeness and accuracy for particular species and regions (Chapter 2).

In detail, the PORTUGUESE INLAND FISH SPECIES DATABASE (thereafter InFISH.PT) was built with data samplings performed in stream reaches during the period of 1990 to 2008 in Portuguese mainland streams, mostly from previous regional and national projects (Chapter 2). Data used combined a multitude of field surveys of distinct sources, which were merged and filtered according with data quality; supplementary data were obtained by additional samplings performed by the candidate (Chapters 2 and 4). Also, a new and wider database, the

IBERIAN INLAND FISH SPECIES DATABASE (InFISH.IP), was built at the grain of 10x10 km grid cells with additional data of occurrence and taxonomy of Spanish streams and published at the Spanish Red Data Book (Doadrio, 2002). Supplementary data were gathered at the Natural Museum of Madrid, particularly those regarding the distribution of new species in Spanish inland waters (Chapter 3).

1.6 Study outline

The dissertation includes a general introduction (**Chapter 1**), where the pertinence of studying the topics addressed and a short knowledge background are offered, before presenting the study aims and describing the thesis outline.

Chapters 2 to 5 attain the specific questions formulated (section 1.5), being each chapter constituted by a paper published, submitted, or to submit on an internationally peer-reviewed journal. Specifically:

- **Chapter 2** addresses question (I). The Portuguese freshwater fish species database built during the current research (InFISH.PT) is examined regarding its sampling completeness, and the value of high quality biodiversity databases of freshwater biota is considered (paper submitted to *Bioscience*).
- **Chapter 3** tackles on questions (II), by using the most recent data on native fish species assemblage composition (InFISH.IP database) for a biogeographical approach focused on examining the relevance of both historical factors and contemporary constraints to the faunal group in question (paper published in *Journal of Biogeography*).
- **Chapter 4** addresses questions (III). It uses data of the south-western Iberian Mediterranean-type streams dataset extracted from InFISH.PT database. The role of multiple landscape factors on shaping the species patterns of native and invasive species is examined (paper submitted to *Diversity and Distributions*).

- **Chapter 5** focuses on questions (IV) by reviewing the scientific literature on freshwater fish that included empirical tests about the current main hypotheses to explain broad-scale biodiversity patterns (paper submitted to *Global Ecology and Biogeography*).

Chapter 6 presents a general discussion, by integrating the matters previously addressed. Additionally a retrospective of the knowledge gained and of the key strengths and weaknesses is included. Implications of the study performed are highlighted, particularly concerning conservation and management matters. In **Chapter 7**, the main achievements of the investigation are synthesized, as well as some items for future research.

In **Appendix I** previous delineations of Iberian biogeographical provinces based on spatial patterns of the ichthyofauna are presented, which were based on the occurrence or evolutionary history of species.

In Appendix II it is included the paper entitled “Selection of priority areas for fish conservation in the Guadiana River Basin, Iberian Peninsula” published in *Conservation Biology* since it illustrates the questions focused by the candidate prior to this dissertation that led to the questions now formulated. Particularly, this previous study raised the question of how relevant it might be to account broad-scale landscape attributes to understand the current patterns of biodiversity in fresh waters.

1.7 References

Abell, R. (2002) Conservation Biology for the Biodiversity Crisis: a Freshwater Follow-up. *Conservation Biology,* **16**, 1435-1437.

Abell, R., Thieme, M.L., Revenga, C., Bryer, M.T., Kottelat, M., Botutskaya, N.G., Coad, B., Mandrak, N.E., Contreras Balderas, S., Bussing, W., Stiassny, M.L.J., Skelton, P.H., Allen, G.R., Unmack, P.J., Nascka, A.M., Ng, R.; Sindorf, N., Robertson, J., Armijo, E., Higgins, J.V., Heibel, T.J., Wikramanayake, E.D., Olson, D., López, H.L., Reis, R.E., Lundberg, J.G., Sabaj Pérez, M.H. & Petry, P. (2008) Freshwater Ecoregions of the World: A New Map of Biogeographic Units for Freshwater Biodiversity Conservation. *BioScience,* **58**, 403-414.

Alcaraz, C., Vila-Gispert, A. & García-Berthou, E. (2005) Profiling invasive fish species: importance of phylogeny and human use. *Diversity and Distributions*, **11**, 289-298.

Alcaraz, C., Paruelo, J. & Cabello, J. (2006) Identification of current ecosystem functional types in the Iberian Peninsula. *Global Ecology & Biogeography,* **15**, 200-212.

Allan, J.D., Erickson, D.L. & Fay, J. (1997) The influence of catchment land use on stream integrity across multiple spatial scales. *Freshwater Biology,* **37**, 149-161.

Allan, J.D. & Johnson, L.B. (1997) Catchment-scale analysis of aquatic ecosystems. *Freshwater Biology,* **37**, 107-111.

Angermeier, P.L. & Schossler, I.J. (1989) Species-area relationships for stream fishes. *Ecology,* **70**, 1450-1462.

Angermeier, P.L. & Winston, M.R. (1998) Local vs. regional influences on local diversity in stream fish communities of Virginia. *Ecology,* **79**, 911-927.

Araújo, M.B. & Guisan, A. (2006) Five (or so) challenges for species distribution modelling. *Journal of Biogeography,* **33**, 1677-1688.

Bailan, E.V., Segers, C., Lévêque, C. & Martens, K. (2008) The freshwater animal diversity assessment: an overview of the results. *Hydrobiologia,* **595**, 627-637.

Bain, M.B. & Finn, J.T. (1988) Streamflow regulation and fish community structure. *Ecology,* **69**, 382-392.

Beisner, B.E., Peres-Neto, P.R., Lindstrom, E.S., Barnett, A. & Longhi, M.L. (2006) The role of environmental and spatial processes in structuring lake communities from bacteria to fish. *Ecology,* **87**, 2985-2891.

Borcard, D. & Legendre, P. (2002) All scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. *Ecological Modelling,* **153**, 51-68.

Brown, J.H. (1995) *Macroecology* The University of Chicago Press, Chicago.

Brown, J.H. & Lomolino, M.V. (1998) *Biogeography* First edition. Sinauer Associates, Sunderland.

Buisson, L., Thuiller, W., Lek, S., Lim, P. & Grenouillet, G. (2008) Climate change hastens the turnover of stream fish assemblages. *Global Change Biology,* **14**, 2232–2248.

Calvo, J.P., Daams, R., Morales, J., López-Martínez, N., Augustí, J., Anadon, P., Armenteros, I., Cabrera, L., Civis,J., Corrochano,A., Díaz-Molina, M., Elizaga, E., Hoyos, M., Martín-Suárez, E., Martínez, J., Moissenet, E., Muñoz, A., Pérez-García, A., Pírez-González, A., Portero, J., Robles, F., Santistebas, C., Torres, T., Van Der Meulen, A.J., Vera, J.A. & Mein, P. (1993) Up-to-date Spanish continental Neogene Synthesis and paleoclimatic interpretation. *Revista de la Sociedad Geológica de España,* **6**, 29-39.

Carmona, J.A., Doadrio, I., Márquez, A.L., Real, R., Hugueny, B. & Vargas, J.M. (1999) Distribution patterns of indigenous freshwater fishes in the Tagus River basin, Spain. *Environmental Biology of Fishes,* **54**, 371-387.

Chapin, III F.S., Zavaleta, E.S., Eviner, V.T., Naylor, R.L., Vitousek, P.M., Reynolds, H.L., Hooper, D.U., Lavorel, S., Sala, O.E., Hobbie, S.E., Mack, M.C. & Diaz, S. (2000) Consequences of changing biodiversity. *Nature,* **405**, 234-242.

Clavero, M. & García-Berthou, E. (2006) Homogenization dynamics and introduction routes of invasive freshwater fish in the Iberian Peninsula. *Ecological Applications,* **16**, 2313-2324.

Cliff, A.D. & Ord, J.K. (1973) *Spatial autocorrelation*. Pion Ltd, London.

Cliff,A.D. & Ord,J.K. (1981) *Spatial processes: models and applications*. Pion Ltd, London.

Collares-Pereira, M.J., Cowx, I.G., Rodrigues, J.A., Rogado, L. & Costa, L.M. (1999) The status of *Anaecypris hispanica* in Portugal: problems of conserving a highly endangered Iberian species. *Biological Conservation,* **88**, 201-212.

Corbacho, C. & Sánchez, J.M. (2001) Patterns of species richness and introduced species in native freshwater fish faunas of a Mediterranean-type basin: The Guadiana River (Southwest Iberain Peninsula). *Regulated Rivers: Research & Management,* **17**, 699-707.

Cunha, C., Coelho, M.M., Carmona,J.A. & Doadrio,I. (2004) Phylogeographical insights into the origins of the *Squalius alburnoides* complex via multiple hybridization events. *Molecular Ecology,* **13**, 2807-2817.

Darlington, P.J. (1957) *Zogeography: the geographical distribution of animals* Wiley, New York.

De la Pena, A. (1995) Tertiary fishes from the Iberian continental basins: history and fossil record. *Coloquies de Paleontología,* **47**, 25-47.

Dias,J. M.A., Boski, T., Rodrigues, A. & Magalhães, F. (2000) Coast line evolution in Portugal since the Last Glacial Maximum until present - a synthesis. *Marine Geology* **170**, 177-186.

Diniz-Filho, J.A.F., Bini, L.M. & Hawkins, B.A. (2003) Spatial autocorrelation and red herrings in geographical ecology. *Global Ecology and Biogeography,* **12**, 53–64.

Doadrio, I. (2002) *Atlas y Libro Rojo de los Peces Continentales de España*. Dirección General de Conservación de la Naturaleza. Museo Nacional de Ciencias Naturales, Madrid.

Doadrio, I., Carmona, J.A. & Machordom, A. (2002) Haplotype Diversity and Phylogenetic Relationships Among the Iberian *Barbels* (*Barbus*, Cyprinidae) Reveal Two Evolutionary Lineages. *The American Genetic Association,* **93**, 140-147.

Dormann, C.F., McPherson, J.M., Araújo, M.B., Bivand, R., Bolliger, J., Carl, G., Davies, R.G., Hirzel, A., Jetz, W., Kissling, W.D., Kuhn, I., Ohlemüller, R., Peres-Neto, P.R., Reineking, B., Schroder, B., Schurr, F.M. & Wilson, R. (2007) Methods to account for spatial autocorrelation in the analysis of species distributional data: a review. *Ecography,* **30**, 609-628.

Downes, B.J., Hindell, J.S. & Bond, N.R. (2000) What's in a site? Variation in lotic macroinvertebrate density and diversity in a spatially replicated experiment. *Austral Ecology,* **25**, 128-139.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.I., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A.-H., Soto, D., Stiassny, M.L.J. & Sullivan, C.A. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews,* **81**, 163-182.

Duggen, S., Hoernle, K., van den Bogaard, P., Rüpke, L. & Morgan, J.P. (2003) Deep roots of the Messinian salinity crisis. *Nature*, **422**, 602–606.

Dutilleul, P. (1993) Spatial heterogeneity and the design of ecological field experiments. *Ecology,* **74**, 1646-1658.

Eldredge, N. (1998) *Life in the Balance. Humanity and the biodiversity crisis.* Princeton University Press, Princeton.

Fagan,W.F. (2002) Connectivity, fragmentation, and extinction risk in dendritic metapopulations. *Ecology*, **83**, 3243-3249

Fagan, W.F., Aumann, C., Kennedy, C.M. & Unmack, P.J. (2005) Rarity, fragmentation, and the scale dependence of extinction risk in desert fishes. *Ecology,* **86**, 34–41

Ferreira,M.T., Sousa,L., Santos,J.M., Reino,L., Oliveira,J.M., Almeida,P.R., & Cortes,R.V. (2008) Regional and local environmental correlates of native Iberian fish fauna. *Ecology Freshwater Fish*, **16**, 504-514.

Filipe, A.F., Cowx, I.G. & Collares-Pereira, M.J. (2002) Spatial modelling of freshwater fish in semi-arid river systems: a tool for conservation. *River Research and Applications,* **18**, 123-136.

Filipe, A.F., Marques, T., Seabra, S.G., Tiago, P., Ribeiro, F., Moreira da Costa, L., Cowx, I.G. & Collares-Pereira, M.J. (2004) Selection of priority areas for fish conservation in the Guadiana River Basin, Iberian Peninsula. *Conservation Biology,* **18**, 189-200.

Font Tullot, I. (1983) *Climatología de España y Portugal.* Instituto Nacional de Meteorología edn Madrid, Spain.

Fraser, R.H. & Currie, D.J. (1996) The species richness-energy hypothesis in a system where historical factors are thought to prevail: coral reefs. *American Naturalist,* **148**, 138-159.

Frissell, C.A., Liss, W.J., Warren, C.F. & Hurley, M.D. (1986) A hierarchical framework for stream habitat classification - viewing streams in a watershed context. *Environmental Management,* **10,** 199-214.

Ganio, L.M., Torgersen, C.E. & Gresswell, R.E. (2005) A geostatistical approach for describing spatial pattern in stream networks. *Frontiers in Ecology and the Environment,* **3**, 138-144.

García-Berthou, E., Alcaraz, C., Pou-Rovira, Q., Zamora, L., Coenders, G. & Feo, C. (2005) Introduction pathways and establishment rates of invasive aquatic species in Europe. Canadian Journal of Fisheries and Aquatic Sciences, **5**, 453-463.

Gasith, A. & Resh, V.H. (1999) Streams in Mediterranean Climate Regions: abiotic influences and biotic responses to predictable seasonal events. *Annual Review of Ecology and Systematics,* **30**, 51-81.

Gaston, K.J. (2000) Global patterns in biodiversity. *Nature,* **405**, 220-227.

Gaston, K.J. & Blackburn,T.M. (2000) *Pattern and process in macroecology* Blackwell Science, Oxford.

Godfray, H.C.J. & Lawton, J.H. (2001) Scale and species numbers. *Trends in Ecology and Evolution,* **16**, 400-401.

Godinho, F.N., Ferreira, M.T. & Cortes, R.V. (1997) Composition and spatial organisation of fish assemblages in the lower Guadiana basin, southern Iberia. *Ecology of Freshwater Fish*, **6**, 134–143.

Godinho, F.N., Ferreira, M.T., & Santos, J.M. (2000) Variation in fish community composition along an Iberian river basin from low to high discharge: relative contributions of environmental and temporal variables. *Ecology Freshwater Fish* **9**, 22-29.

Grenouillet, G., Pont, D. & Hérissé, C. (2004) Within-basin fish assemblage structure: the relative influence of habitat versus stream spatial position on local species richness. *Canadian Journal of Fisheries and Aquatic Sciences,* **61**, 93-102.

Heino, J., Muotka, T., Paavola, R., Hamalainen, H. & Koskenniemi, E. (2002) Correspondence between regional delineations and spatial patterns in macroinvertebrate assemblages of boreal headwater streams. *Journal of the North American Benthological Society,* **21**, 397-413.

Hughes, R., Rexstad, E. & Bond, C.E. (1987) The relationship of aquatic ecoregions, river basins and physiographic provinces to the ichthyogeographic regions of Oregon. *Copeia,* **1987**, 423-432.

Hugueny, B. (1989) West African rivers as biogeographic islands: species richness of fish communities. *Oecologia* **79**, 236-243.

Hugueny, B. & Lévêque, C. (1994) Freshwater fish zoogeography in west Africa: faunal similarities between river basins *Environmental Biology of Fishes,* **39**, 365-380.

Jackson, D.A., Peres-Neto, P.R. & Olden, J.D. (2001) What controls who is where in freshwater fish communities — the roles of biotic, abiotic, and spatial factors. *Canadian Journal of Fisheries and Aquatic Sciences,* **58**, 157-170

Johnson, B.L., Richardson, W.B. & Naimo, T.J. (1995) Past, present and future concepts in large river ecology. *BioScience,* **45**, 134-141.

Labbe, T.R. & Fausch, K.D. (2000) Dynamics of intermittent stream habitat regulate persistence of a threatened fish at multiple scales. *Ecological Applications,* **10**, 1774-1791.

Lawton, J.H. (1999) Are there general laws in ecology? *Oikos,* **84**, 177-192.

Legendre, P. (1993) Spatial autocorrelation: Trouble or new paradigm? *Ecology,* **74**, 1659-1673.

Lennon, J. J. 2000. Red-shifts and red herrings in geographical ecology. *Ecography* **23**,101–113.

Legendre, P. & Legendre, L. (1998) *Numerical ecology*, 2nd English edn. Elsevier, Amsterdam

Lévêque, C., Oberdorff, T., Paugy, D., Stiassny, M.L.J. & Tedesco, P.A. (2008) Global diversity of fish (Pisces) in freshwater. *Hydrobiologia,* **595**, 545-567.

Livingston, D.A., Rowland, M. & Bailey, P.E. (1982) On the size of African riverine fish faunas. *American Zoologist,* **22**, 361-369.

Lucas, M.C., Baras, E., Thom, T.J., Duncan, A. & Slavik, O. (2001) *Migration of freshwater fishes*. Blackwell Science, Oxford.

Mace, G.M., Masundire, H., Baillie, J., *et al*. (2005) Chapter 4 Biodiversity. In *Ecosystems and Human Well-being: Current State and Trends, Volume 1*. pp. 77-122. Findings of the Condition and Trends Working Group of the Millennium Ecosystem Assessment. Edited by Hassan.R.; Scholes,R.; Ash,N. Island Press. Washington, DC. Available at http://www.millenniumassessment.org.

Magalhães, M.F., Batalha, D.C. & Collares-Pereira, M.J. (2002) Gradients in stream fish assemblages across a Mediterranean landscape: contributions of environmental factors and spatial structure. *Freshwater Biology,* **47**, 1015-1031.

Magalhães, M.F., Beja, P., Schlosser, I.J., Collares-Pereira, M.J. (2008) Effects of multi-year droughts on fish assemblages of seasonally drying Mediterranean streams. *Freshwater Biology,* **52**, 1494-1510.

Matthews, W.J. (1998) *Patterns in freshwater fish ecology*. Chapman & Hall, New York.

Matthews, W.J. & Robinson, H.W. (1998) Influence of drainage connectivity, drainage area and regional species richness on fishes of the interior highlands of Arkansas. *American Midland Naturalist,* **139**, 1-19.

Menge, B. A., Chan, F., Dudas S., Eerkes-Medrano, D., Grorud-Colvert, K., Heiman, K., Hessing-Lewis, M., Iles, A., Milston-Clements, R., Noble, M., Page-Albins, K., Richmond, E., Rilov, G., Rose, J., Tyburczy, J., Vinueza, L. & Zarnetske, P. (2009) Do terrestrial ecologists ignore aquatic literature? *Frontiers in Ecology and the Environment*, **7**, 182-183.

Menge, B.A. & Olson, A.M. (1990) Role of scale and environmental factors in regulation of community structure. *Trends in Ecology & Evolution,* **5**, 52-57.

Mesquita, N., Cunha, C., Carvalho, G.R. & Coelho, M.M. (2007) Comparative phylogeography of endemic cyprinids in the south-west Iberian Peninsula: evidence for a new ichthyogeographic area. *Journal of Fish Biology*, **71**, 45–75.

Millennium Ecosystem Assessment. 2005. Ecosystems and Human Well-being: Biodiversity Synthesis. World Resources Institute. Washington, DC.

Montgomery, D.R. (1999) Process domains and the river continuum. *Journal of the American Water Resources Association,* **35**, 410.

Naiman, R.J., Lonzarich, D.G., Beechie, T.J. & Ralph, S.C. (1992) General principles of classification and the assessment of conservation potential in rivers. *River Conservation and Management* (ed. by P.J.Boon, P.Calow, and G.E.Petts), pp. 93-123. John Wiley and Sons, Chichester.

Olden, J.D., Jackson, D.A. & Peres-Neto, P.R. (2001) Spatial isolation and fish communities in drainage lakes. *Oecologia,* **127**, 572-585.

O'Neill, R.V., Milne, B.T., Turner, M.G. & Gardner, R.H. (1988) Resource utilization scales and landscape patterns. *Landscape Ecology,* **2**, 63-69.

Olson, D.M., Eric, D., Powell, G.V.N. & Wikramanayake, E.D. (2002) Conservation Biology for the biodiversity crisis. *Conservation Biology,* **16**, 1-3.

Osborne, L.L. & Wiley, M.J. (1992) Influence of tributary spatial position on the structure of warmwater fish communities. *Canadian Journal of Fisheries and Aquatic Sciences,* **49**, 671-681.

Palmer M.A. & Poff N.L. (1997) The influence of environmental heterogeneity on patterns and processes in streams. *Journal of the North American Benthological Society,* **16**, 169–173.

Pardo, I. & Álvarez, M. (2006) Comparison of resource and consumer dynamics in Atlantic and Mediterranean streams. *Limnética,* **25**, 271-286.

Pegg, M.A. & Pierce, C.L. (2002) Fish community structure in the Missouri and Lower Yellowstone Rivers in relation to flow characteristics. *Hydrobiologia,* ***479***, 155-167.

Pegg, M.A. & Taylor, R.M. (2007) Fish species diversity among spatial scales of altered temperate rivers. *Journal of Biogeography,* **34**, 549–558.

Peres-Neto, P.R. (2004) Patterns in the co-occurrence of fish species in streams: the role of site suitability, morphology and phylogeny versus species interactions. *Oecologia,* **140**, 352-360.

Peterson, E.E., Merton, A.A., Theobald, D.M. & Urquhart, N.S. (2006) Patterns of spatial autocorrelation in stream water chemistry. *Environmental Monitoring and Assessement*, **121**, 571-596.

Pimm, S.L., Russel, G.J., Gittleman, J.L. & Brooks, T.M. (1995) The future of biodiversity. *Science,* **269**, 347-350.

Poff, N.L. (1997) Landscape filters and species traits: towards mechanistic understanding and prediction in stream ecology. *Journal of North American Benthological Society,* **16**, 391-409.

Poff, N.L. & Allan, J.D. (1995) Functional organization of stream fish assemblages in relation to hydrological variability. *Ecology,* **76**, 606-627.

Puente, A.I., Altonaga, K., Prieto, C.E. & Rallo, A. (1998) Delimitation of Biogeographical Areas in the Iberian Peninsula on the Basis of Helicoidea Species (*Pulmonata: Stylommatophora*). *Global Ecology and Biogeography Letters,* **7**, 97-113.

Quist, M.C.,Rahel, F.J. & Hubert, W-A. (2005) Hierarchical faunal filters: an approach to assessing effects of habitat and non-native species on native fishes. Ecology of Freshwater Fish, **14**, 24-39.

Rahel, F.J. & Hubert, W.A. (1991) Fish Assemblages and Habitat Gradients in a Rocky Mountain-Great Plains Stream: Biotic Zonation and Additive Patterns of Community Change. *Transactions of the American Fisheries Society,* **120**, 319-332.

Revenga, C., Campbell, I., Abell, R., de Villiers, P. & Bryer, M. (2005) Prospects for monitoring freshwater ecosystems towards the 2010 targets. *Philosophical Transactions of the Royal Society B,* **360**, 397-413.

Rey-Benayas, J.M. & Scheiner, S.M. (2002) Plant diversity, biogeography and environment in Iberia: patterns and possible causal factors. *Journal of Vegetation Science* ,**13**, 245-258.

Ribeiro, F., Collares-Pereira, M.J., Moyle, P.B. (2009a) Non-native fish in the fresh waters of Portugal, Azores and Madeira Islands: a growing threat to aquatic biodiversity. Fisheries Management and Ecology, **16**, 255-264.

Ribeiro, F., Gante, H.F., Sousa, G., Filipe, A.F., Alves, M.J., Magalhães, M.F. (2009b) New records, distribution and dispersal pathways of *Sander lucioperca* in Iberian freshwaters. *Cybium*, **33**, 255-256.

Ricklefs, R.E. (1987) Community diversity: Relative roles of local and regional processes. *Science* , **235**, 167-171.

Ricklefs, R.E. & Schulter, D. (1993) Species diversity in ecological communities: historical and geographical perspectives *University of Chicago Press*, *Chicago, IL*.

Rivas-Martinez, S.P. (1987) Introducción: nociones sobre Fitosociología. Biogeografía y Bioclimatología. *La vegetación de España* (ed. by M. Peinado-Lorca and S. Rivas-Martinez), pp. 18-25. Universidad Alcalá de Henares, Madrid

Rogado, L., Alexandrino, P.J., Almeida, P.R., Alves, M.J., Bochechas, J., Cortes, R.V., Domingos, M.I., Filipe, A.F., Madeira, J. & Magalhães, M.F. (2005) Peixes dulciaquicolas e migradores. *Livro Vermelho dos Vertebrados de Portugal* (ed. by M.J. Cabral, J. Almeida, P.R. Almeida, T. Dellinger, N. Ferrand de Almeida, M.E. Oliveira, J.M. Palmeirim, A.I. Queirós, L. Rogado and M.Santos-Reis), Instituto da Conservação da Natureza, Lisboa.

Robalo, J.I., Almada, V.C., Levy, A. & Doadrio, I. (2006a) Re-examination and phylogeny of the genus Chondrostoma based on mitochondrial and nuclear data and the definition of 5 new genera, Molecular Phylogenetics and Evolution. *Molecular Phylogenetics and Evolution,* **42**, 362-372.

Robalo, J.I., Santos, C.S., Almada, V.C. & Doadrio, I. (2006b) Paleobiogeography of two Iberian endemic cyprinid fishes (*Chondrostoma arcasii - Chondrostoma macrolepidous*) inferred from mitocondrial DNA sequence data. *Journal of Heredity,* **97**, 143-149.

Saab, V. (1999) Importance of spatial scale to habitat use by breeding birds in riparian forests: a hierarchical analysis. *Ecological Applications,* **9**, 135-151.

Sala, O.E., Chapin III, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, M.T., Walker, B.H., Walker, M. & Wall, D.H. (2000) Global biodiversity scenarios for the year 2100. *Science,* **287**, 1770-1774.

Santos, J.M., Godinho, F.N., Ferreira, M.T. & Cortes, R.V. (2004) The organisation of fsh assemblages in the regulated Lima basin, Northern Portugal. *Limnologica*, **34**, 224-235.

Schlosser, I.J. (1991) Stream fish ecology: a landscape perspective. *BioScience*, **41**, 704-712.

Schlosser, I.J. (1995) Critical landscape attributes that influence fish population dynamics in headwater streams. *Hydrobiologia,* **303**, 71-81.

Segurado, P. & Araújo, M.B. (2004) An evaluation of methods for modelling species distributions. *Journal of Biogeography,* **31**, 1555- 1568.

Segurado, P., Araújo, M.B. & Kunin, W.E. (2006) Consequences of spatial autocorrelation for niche-based models. Journal of Applied Ecology 43, 433-444.

Selmi,S. & Boulinier,T. (2001) Ecological Biogeography of Southern Ocean Islands: The Importance of Considering Spatial Issues. *The American Naturalist,* **158**, 426-437.

Shreve, R.L. (1967) Infinite topologically random channel networks. *Journal of Geology,* **75**, 178-186.

Simpson, G.G. (1953) *Evolution and geography - an essay on historical biogeography with special reference to mammals* Condon Lectures, Oregon State System of Higher Education, Eugene, OR.

Smith, K.G. & Darwall, W.R.T. (2006) *The status and distribution of freshwater fish endemic to the Mediterranean Basin*. IUCN Freshwater Biodiversity Assessement Programme.

Smith, C.L. & Powell, C.R. (1971) The summer fish communities of Brier Creek, Marshall County, Oklahoma. *American Museum Novitates,* **2458**, 1-30.

Sparks, R.E. (1995) Need for ecosystem management of large rivers and their floodplains. *BioScience,* **45**, 168-182.

Strahler, A.N. (1964) Quantitative geomorphology of drainage basins and channel networks. *Handbook of applied hydrology* (ed. by V.T.Chow), pp. 4-39. McGraw-Hill, New York.

Sousa-Santos, C., Collares-Pereira, M.J. & Almada, V. (2007) Reading thehistoryof ahybridfishcomplex fromitsmolecular record. *Molecular Phylogenetics and Evolution*, **45**, 981–996.

Tonn, W.M., Magnuson, J.J., Rask, M. & Toivonen, J. (1990) Intercontinental comparison of small-lake fish assemblages: the balance between local and regional processes. *The American Naturalist,* **136**, 345-375.

Valone, T.J. & Hoffman, C.D. (2002) Effects of regional pool size on local diversity in small-scale annual plant communities. *Ecology Letters,* **5**, 477-480.

Vannote, R.L., Minshall, G.W., Cumming, K.W., Sedell, J.R. & Cushing, C.E. (1980) The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* **37**, 130-137.

Vié, J.-C., Hilton-Taylor, C. & Stuart, S. (2009) *Wildlife in a changing world - An analysis of the 2008 IUCN Red List of Threatened Species* IUCN, Gland, Switzerland.

Vörösmarty, C.J., Lévêque, C., Revenga, C., *et al*. (2005). Chapter 7: Fresh Water. In *Ecosystems and Human Well-being: Current State and Trends, Volume 1*. pp. 166-207*Findings of the Condition and Trends Working Group of the Millennium Ecosystem Assessment.* Edited by Hassan.R.; Scholes,R.; Ash,N. Island Press. Washington, DC. Available at http://www.millenniumassessment.org.

Wiens, J.A. (1989). Spatial scaling in ecology. *Functional Ecology* **3**, 385-397.

Wiens, J.A. (2002a) Predicting species occurrences: progress, problems, and prospects. *Predicting species occurrences: issues of accuracy and scale* (ed. by J.M.Scott, P.J.Heglund, and M.L.Morisson), p. 739-749. Island Press, Washington.

Wiens, J.A. (2002b) Riverine landscapes: taking landscape ecology into the water. *Freshwater Biology,* **47**, 501.

Wilkinson, C.D. & Edds, D.R. (2001) Spatial pattern and environmental correlates of a midwestern stream fish community: including spatial autocorrelation as a factor in community analyses. *The American Midland Naturalist,* **146**, 271-289.

Willis, K.J. & Whittaker, R.J. (2002) Ecology: Enhanced: Species Diversity--Scale Matters. *Science* **295**, 1245-1248.

Whittaker, R.J., Willis, K.J. & Field, R. (2001) Scale and species richness: towards a general, hierarchical theory of species diversity. *Journal of Biogeography,* **28**, 453-470.

Willig, M.R., Kaufman, D.M. & Stevens, R.D. (2003) Latitudinal Gradients of Biodiversity: pattern, process, scale, and synthesis. *Annual Review of Ecology and Systematics,* **34**, 273–309.

Chapter 2.

The freshwater fish databases

*“**Map**/noun [C] a diagram or collection of data showing the arrangement, distribution, or sequence of something. (....)”*

Oxford Dictionary of English. 2009

Filipe, A.F., Alves, M.J. & Collares-Pereira, M.J. (Submitted) How much data are we loosing? Challenges on building freshwater biodiversity databases to attain data quality. *Bioscience*.

Submitted as an Overview Article

How Much Data Are We Loosing? Challenges on Building Freshwater Biodiversity Databases to Attain Data Quality

Ana F. Filipe[1*], Maria J. Alves[1,2], and Maria J. Collares-Pereira[1]

[1]Universidade de Lisboa, Faculdade de Ciências, Centro de Biologia Ambiental, Campo Grande, 1749-016 Lisboa, Portugal; [2]Museu Nacional de História Natural, Rua da Escola Politécnica nº58, 1250-102 Lisboa, Portugal.

(Abstract)

Optimizing the biodiversity data collected and assuring data quality to researchers, managers and general public users is a challenging task that needs to be addressed, especially regarding data from freshwater biota, whose information is often scattered or incomplete. The new Inland Fish Database (InFISH.PT) designed to be a basal too for research and applied studies was used to evaluate the sampling completeness of a freshwater biodiversity database, and describe the likely uncertainties and voids on collecting and merging data from distinct sampling surveys. Also the new possibilities opened by building high quality regional databases are discussed.

Keywords: completeness, freshwater biodiversity, data quality, database, Natural History Museums

Short title: Completeness of freshwater biodiversity databases

(Introduction)

Knowing which species live in a place is widely recognized as fundamental for both research and conservation actions (Gaston 1996, Sutherland 2000). But even for regions with high investments on data collecting, databases remain mostly focused on terrestrial organisms, for which there is a long tradition of data collection at Natural History Museums (Lawton et al. 1994). However, a better knowledge on freshwater biodiversity has been frequently mentioned as a priority as well as the need for more investments on field surveys (Abell et al. 2008, Balian et al. 2008, 2009; Lévêque et al. 2008). But the question to begin with is how complete is the information already collected and how can we optimize both data sampling and storing, while assuring data quality. Here we use a new freshwater fish diversity database to identify the critical voids and uncertainties of data, and the possibilities opened by having high quality databases as tools for research on biodiversity and management.

Freshwater biota are subjected to multiple threats (see Dudgeon et al. 2009) and most are at higher risk than terrestrial ones (Allan and Flecker 1993). Obtaining sufficient and credible scientific information on freshwater biodiversity and making it available is the very beginning to successfully deal with sustainable management and conservation of the world's freshwater resources (Balian et al. 2008). Advances on fish biodiversity have been developed mostly from research on diversity gradients (e.g., Filipe et al. 2009), habitat changes (e.g., Wang et al. 2001), invasiveness (e.g., Gido and Brown 1999), and climate change predictions and impacts (e.g., Buisson and Grenoulliet 2009). Many of the shortcomings identified therein are pointed as the result of voids in the data sets used, which implies that complete and high quality biodiversity databases are required. Indeed, information on freshwater species distributions seems generally scattered and incomplete, when compared to other better known biota (but see Balian et al. 2008).

Biodiversity databases mostly specialized on specific taxa and/or regions are being computerized, offering the possibility of exploring ecological questions that might shed light on the mechanisms shaping diversity patterns, as well as sharing knowledge for conservation and management actions from regional to global extents. Regarding the freshwater fish databases available, the FishBase is a worldwide online relational database which is currently the main source of information on fishes (www.fishbase.org; Froese and Pauly 2008), being linked to other global database initiatives of storing information such as the Encyclopedia of Life (EoL, www.eol.org) and the Global Biodiversity Information Facility (GBIF, www.gbif.org/). Among such initiatives, there is a growing concern on providing indicators of data quality to accomplish the data reliability commitment for biodiversity information databases (Wilson 2003, Guralnick and Lane 2007). The reliability of information on these databases is commonly assured by referencing data sources and the sate of data checking and vetting - data validation - by collaborators or curators who were previously recognized as able to authenticate data (Lane 1996); also, the worldwide open access is considered to fuel the validation of the information among experts around the world (Bisby 2000).

Collecting field data is costly regarding time, expert knowledge and funding; compiling these data is also laborious, involving a huge effort on turning data consistent, complete, and effectively organized to be accessed by database users while assuring interoperability with other data sets on biodiversity data or even on other areas of knowledge (e.g., Beier and Degerman 2007). Never the less, it turns out biodiversity data are commonly treated more inaccurately than required, with drawbacks as incomplete sampling spatial coverage or non-exhaustive samplings at each site, which often require collecting additional field data to improve the set of useable data (Hortal et al. 2007). These difficulties are particularly evident for some ecosystems as fresh waters, where samplings can be complex due to the spatial and temporal environmental variability (e.g., Symes et al. 2009).

This paper aims to investigate how biodiversity data can be optimized, particularly for freshwater biota, using the new database on Inland Fish Species of Portugal – hereafter named InFISH.PT – by: i) identifying critical data voids and uncertainties, ii) proposing solutions to overcome such limitations, and iii) discussing the new possibilities opened when assuring data quality of biodiversity databases as key tools for research, conservation and management.

(Data, methods and results)

Setting up a regional biodiversity database

The regional freshwater fish database – InFISH.PT - containing data on distribution and life history traits of native species in the Portuguese freshwaters was built during the past five years to provide and manage information to be used on research and applied studies.

Portuguese native fish assemblages are dominated by the 25 primary species *sensu* Darlington (1957) most belonging to the Cyprinidae family, and occupying the North-west, Central-west, South-west and Mira Iberian biogeographical provinces defined by Filipe et al. (2009). Albeit relatively species-poor, species are typically Iberian endemics with distribution ranges typically constrained to few river basins, being the majority endangered according with the UICN species criteria (Rogado et al. 2005, Smith and Darwall 2005). Since the first known fish introduction (*Carassius auratus* at the year 1792), the number of successful non-native fish species has increased up to 15 at present mostly due to recreational fisheries interests (Ribeiro et al. 2009a). Portuguese landscapes are environmentally diverse, encompassing two major stream habitat types: the temperate and the Mediterranean-type (Gasith and Resh 1999, Pardo and Álvarez 2006), the latest is common southerly of Tagus River and at some north-eastern areas of low altitude. The stream network consists of 25 major river basins, five shared with Spain, and draining across the Iberian Peninsula into the Atlantic Ocean (figure 1).

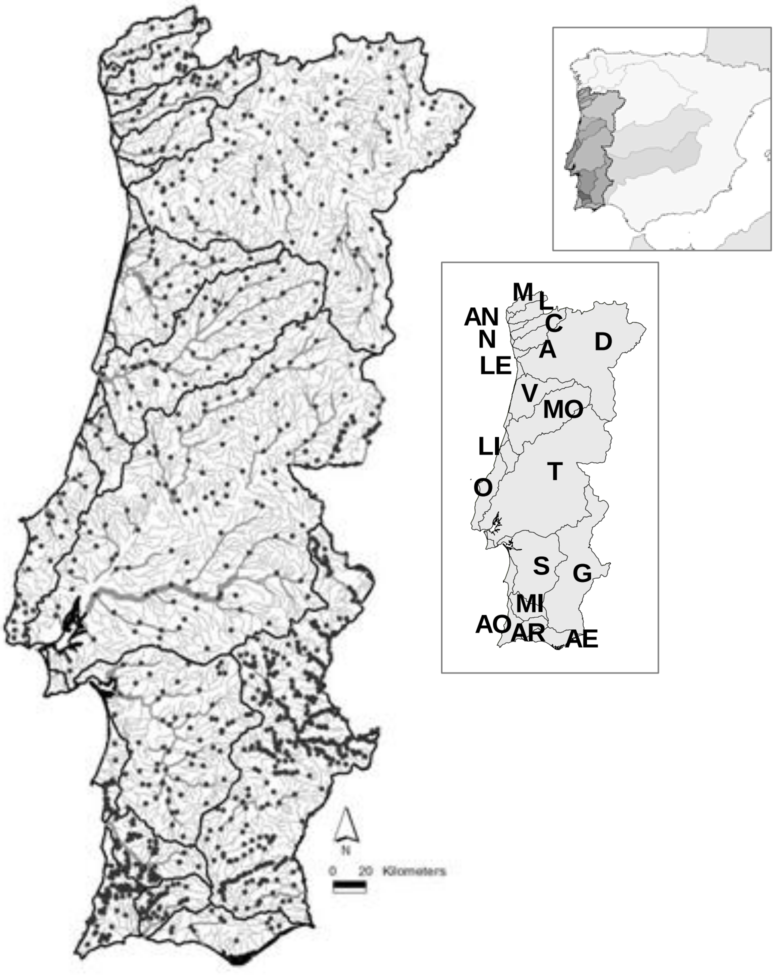


Figure 1. Location sampling records of the primary freshwater fishes. The data set was extracted from the InFISH.PT database and records obtained by electrofishing captures (2345 samplings at 1199 sites; see Table 1 for river basin codes). The area sampled is Portugal mainland, extending over 89,367 km^2, 15.3 % in the Iberian Peninsula and located at de most occidental part of Europe (42º to 37º N latitude and 6º10" to 9º20' W longitude).

The InFISH.PT database encompasses the sampling data records for all fish species occurring in mainland Portuguese watercourses, as well as information on species life-history traits. The database was built with user-friendly and widespread tools as Access (Microsoft Corp., US) for data storage in tables and queries building to cross data and extract information;

a live-link with a geographic information system (GIS) using ArcMap (ESRI Inc., US) was settled to allow mapping visualization and analysis. The spatial information encompasses around 40,650 km of watercourses total length, and 25 river basins with a surface area ranging from 69 to 11,590 km^2 (table 1). The data on sampling records started to be merged for the Portuguese Red Data Book (Rogado et al. 2005), and were completed with additional data of taxonomical descriptions, species life-history traits, and additional sampling records (see Filipe et al. 2009). Data sources included recent publications, gray literature, unpublished reports, and online national information (from DGRF at www.cartapiscicola.org, and ICN-B at www.icn.pt/sipnat). A special concern during data merging was evaluating the coherence of taxonomic identifications among data sources.

The InFISH.PT data records are mostly from surveys performed by electrofishing (near 85%), since this method is considered non-selective for a wide range of species, thus allowing valid comparisons among samplings and the likely of detecting the whole assemblage (Reynolds et al. 2003). For each sampling, it was recorded the number of individuals collected of each species, the sampling effort performed, and the site location (pathway and coordinates); this way data on species occurrence were recorded at the smallest scale grain available and able to be re-sampled – the sampling site. Herein the data sets used in analysis were restricted to primary fish species records sampled by electrofishing at stream habitats (see figure 1 for data visualization and table 2 for species list).

Table 1. Resume of the data set used per river basin from the complied 2345 samplings at 1199 sites and extracted from the InFISH.PT database. For each basin it is given the number of sites and sampling records, the basin surface area, the total watercourses length, the maximum stream order (sensu Strahler) and the densities (%) of samplings and sites per km2 of surface area and km of watercourse.

						Density sites (% per km^2)		Density samplings (% per km^2	
River Basin	Sites	Samplings	Basin (km^2)	Watercourse (km)	Order				
Ave (A)	9	10	1363	478	4	0.7	18.8	0.7	20.9
Eastern Algarve (AE)	13	14	1635	797	3	0.8	16.3	0.9	17.6
Âncora (AN)	2	3	124	29	2	1.6	69.3	2.4	103.9
Western Algarve (AO)	90	91	1338	789	4	6.7	114.0	6.8	115.3
Arade (AR)	43	43	124	702	4	34.7	61.2	34.7	61.2
Cávado C)	26	26	265	507	4	9.8	51.3	9.8	51.3
Douro (D)	112	128	18640	7854	7	0.6	14.3	0.7	16.3
Douro-Vouga (DV)*	1	2	233	130	3	0.4	7.7	0.9	15.4
Guadiana (G)	446	1488	11590	6232	6	3.8	71.6	12.8	238.8
Lima (L)	27	30	1185	470	4	2.3	57.4	2.5	63.8
Leça (LE)	2	2	69	83	2	2.9	24.1	2.9	24.1
Lis (LI)	8	8	897	482	5	0.9	16.6	0.9	16.6
Minho (M)	12	13	815	340	6	1.5	35.3	1.6	38.3
Mira (MI)	75	82	1580	939	5	4.7	79.9	5.2	87.3
Mondego-Lis (ML)*	1	1	138	37	2	0.7	26.7	0.7	26.7
Mondego (MO)	43	51	6640	3323	5	0.6	12.9	0.8	15.3
Neiva (N)	3	4	274	88	2	1.1	34.2	1.5	45.6

*The small coastal drainages located between large watersheds are designated with the name of both, with a hiphen between. The small coastal basins of Vouga-Mondego and Cavado-Ave were not included because they do not have sampling records.

Table 2. List of the 25 native species of Portugal (mainland) classified as primary fish following Darlington (1957). Data were extracted from the InFISH.PT database.

Species	Author
Cobitidae	
Cobitis calderoni	Bacescu, 1962
Cobitis paludica	(de Buen, 1930)
Cyprinidae	
Achondrostoma arcasii	(Steindachner, 1866)
Achondrostoma occidentale	(Robalo, Almada, Sousa-Santos, Moreira and Doadrio, 2005)
Achondrostoma oligolepis	(Robalo, Doadrio, Almada, and Kottelat 2005)
Anaecypris hispanica	(Steindachner, 1866)
Barbus bocagei	Steindachner, 1864
Barbus comizo	Steindachner, 1864
Barbus microcephalus	Almaça, 1967
Barbus steindachneri	Almaça, 1967
Barbus sclateri	Gunther, 1868
Iberochondrostoma almacai	(Coelho, Mesquita and Collares-Pereira 2005)
Iberochondrostoma lemmingii	(Steindachner, 1866)
Iberochondrostoma lusitanicum	(Collares-Pereira, 1980)
Iberochondrostoma olisiponensis	(Gante, Santos and Alves, 2007)
Iberochondrostoma oretanum	(Doadrio and Carmona, 2003)
Pseudochondrostoma duriense	(Coelho, 1985)
Pseudochondrostoma polylepis	(Steindachner, 1864)
Pseudochondrostoma willkommii	(Steindachner, 1866)
Squalius alburnoides complex	(Steindachner, 1866)
Squalius aradensis	(Coelho, Botutskaya, Rodrigues and Collares-Pereira, 1998)
Squalius carolitertii	(Doadrio, 1988)
Squalius pyrenaicus	(Günther, 1868)
Squalius torgalensis	(Coelho, Bogutskaya, Rodrigues and Collares-Pereira, 1998)
Tinca tinca	(Linnaeus, 1758)

Assessing completeness of data

Taxonomic information is considered the backbone of biodiversity databases, disregarding its extent and taxa (Bisby 2000). Overall, regarding the InFISH.PT taxonomical information, species identification of co-existing individuals with strong morphological similarities was found as being incongruent between the surveys performed at some areas. Also individuals with a hybrid origin from two distinct co-occurring species were rarely mentioned, despite several studies have already documented the existence of distinct hybrid zones (e.g., Pereira et al. 2009), which might have also lead to misidentifications.

On a first approach on evaluating the data quality, measures of sampling effort at each record can be accessed by quantifying the amount of sites and samplings for which this information was compiled (e.g., Hortal 2007). One feature that emerged from performing this analysis on the data set on primary species was the absence of measures of sampling effort at some records. Samplings for which effort measures have been registered summed 2,041 from the 2,345 total in the data set, which corresponds to 925 sites with registered effort measures for at least one sampling from the total of 1199 sites. When documented in an accessible way, the fishing sampling effort differed mostly between (1) sampling at a stream surface area considered adequate according with the site characteristics during a fixed time interval, and (2) sampling at a fixed area during a time range also according with the site characteristics. The void of information found might be resulting of the somehow incomplete documentation available.

Sampling completeness is a major concern on biodiversity data sets especially regarding fresh waters (Sánchez-Fernández et al. 2008). To evaluate the sampling completeness, measures of both sampling coverage (i.e. the completeness of samplings across the landscape) and sampling effort (i.e. the exhaustiveness of the samplings) were performed. In detail, sampling coverage measures were mapped 1) for each basin by calculating the density of the number of both sites and samplings by both the basin's total surface area and the total watercourses' length, and 2) for each 10*10km UTM grid cell, both the total number of sites and samplings recorded. Also, to evaluate the likely uncertainties of geographical gradients of species occurrence, the sampling coverage measures were correlated with both the species richness and the occurrence maps for each grid cell; these biodiversity maps were thereafter overlapped with the sampling coverage measures for visualization.

The data set on primary species records analyzed showed no sampling records for 370 from a total of 892 of 10*10km UTM grid cells, which corresponds to 41% of the whole territory. Most of these non-sampled grid cells are headwaters where, for the ones not fed by groundwater, surveys are restricted to the wet season, when stream's wetted habitats are found, thus with few sampling opportunities during the year. Because headwaters environments may function both as hatchery and/or nursery areas for some species, we included the non-sampled grid cells in calculations, in spite of the eventual risk of inflating sampling coverage incompleteness. The sampling coverage regarding densities of both sites and samplings was distinct among river basins and grid cells (figure 2 and table 1). The Arade river basin had the highest density of sites covered and the most revisited sites regarding the basin surface area, but when considering sampling densities of the total length of watercourses, the most well spatially covered was the Western Algarve and the one with the highest number of revisited sites was the Guadiana river basin. Another feature that emerged was the low density of sampling coverage at the two largest river basins, the Tagus and Douro. Also, the sampling coverage and re-sampling of sites differed among grid cells, and the Guadiana, Mira and Western Algarve basins had the highest number of multiple samplings (figure 3a), although the within-basins site coverage was not uniformly distributed (figure 3b). Moreover, the richness of primary fish species mapped was highly correlated with the number of samplings (Corr. Spearman = 0.351, *p*=.000), but not with the number of surveyed sites (Corr. Spearman = -0.055, *p*=.07) (figure 3c). When mapping the species distributions, data on some of most widespread species (e.g., *Barbus bocagei*) seemed particularly incomplete at some of the largest basins (Tagus and Douro).

(Discussion)

Data limitations of the InFISH.PT database

The database presented is an example of a compilation on biodiversity inventories of freshwater biota where a number of data limitations was identified, as well as ways to overcome them. Firstly, the observed taxonomic uncertainties and incoherencies between the data sources merged may reflect the taxonomic instability from recent years due to description of new species, enhanced by the difficulty of the persons involved at samplings to correctly assign species with high morphological similarities and to recognize hybrid swarms in some natural populations (e.g., Rogado et al. 2005, Pereira et al. 2009). The Natural History Museums can have an important role on surpass these uncertainties, prior and subsequent to field surveys, by achieving the taxonomic authentication through the curator's expertise and consulting the biological collections for the biota under analysis (Alberch 1993, Lane 1996). Indeed, museum collections are scientifically robust and thus one of the most valuable and comprehensive sources of biodiversity data, which are even more important because of the current lack of well trained taxonomists worldwide (see Skelton 2002, Valdecasas and Camacho 2003). This demise may be especially critical considering the current generalized advances on computing technology and online available tools for generating biodiversity databases.

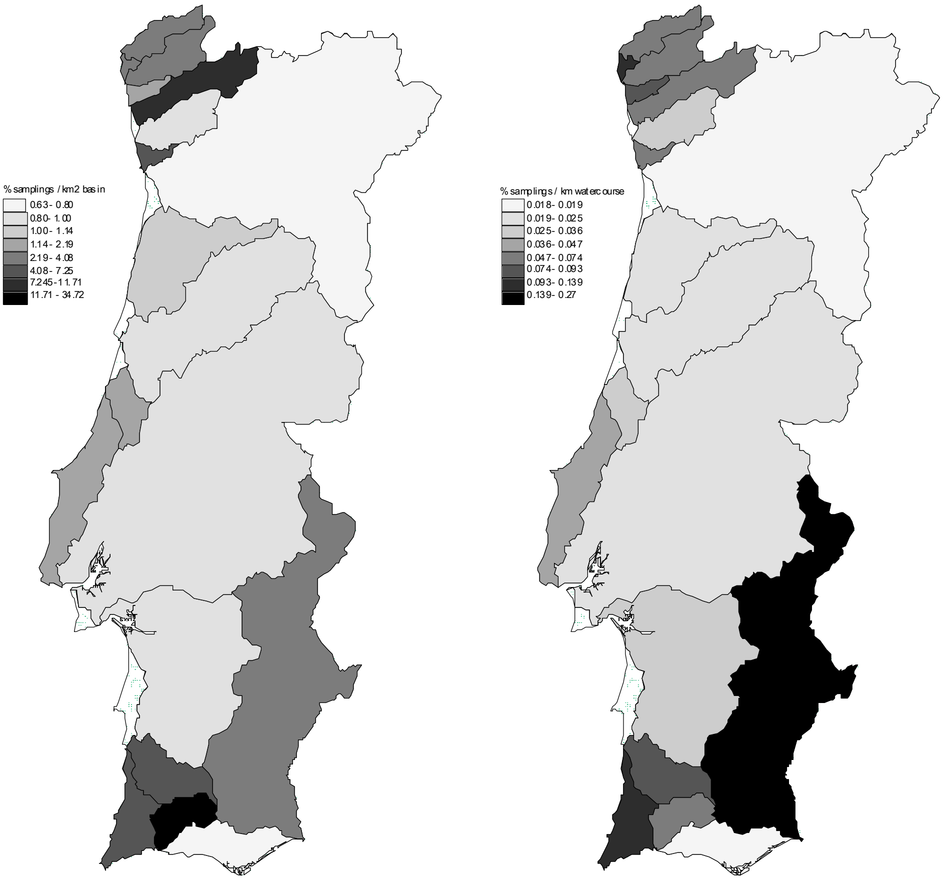


Figure 2. Sampling coverage (number of samplings and sites) per each river basin. The data used were extracted from the InFISH.PT database and correspond to the primary freshwater fishes detected in watercourses by electrofishing.

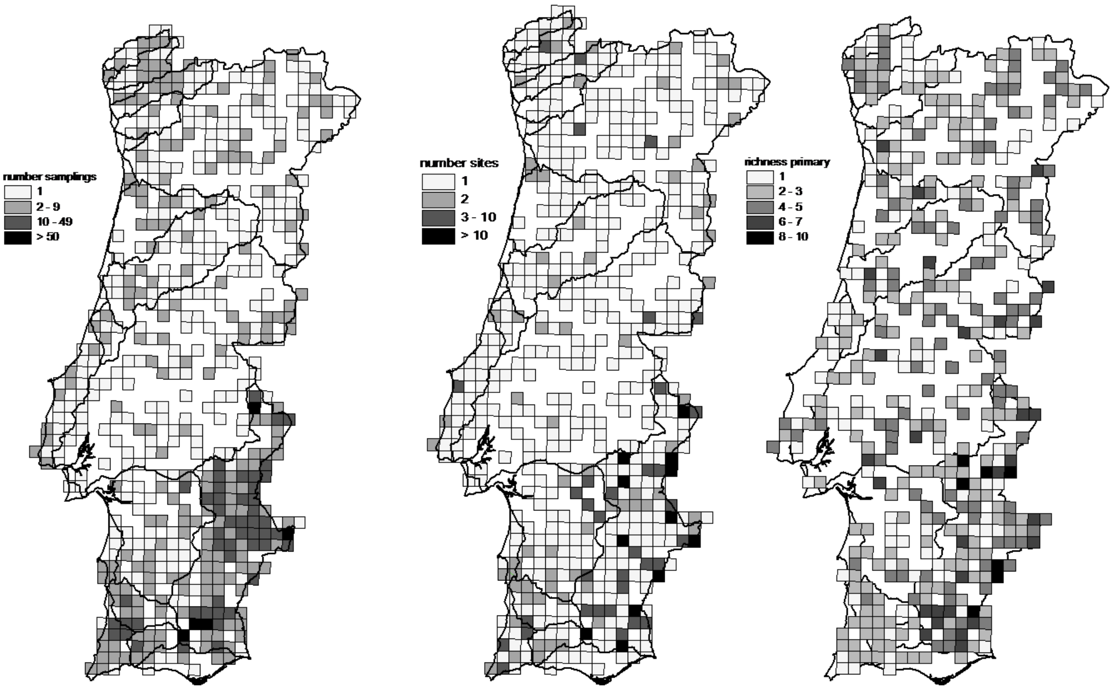


Figure 3. Database records for each 10*10km UTM grid cell on sampling coverage: number of samplings (a) and sites (b)), and on observed species richness (c). The data used were extracted from the InFISH.PT database and correspond to the primary freshwater fishes detected in watercourses by electrofishing.

Secondly, the scattered information found on measures of sampling effort was mostly due to the lack of such information in some inventories. Overcoming such void can be attained by registering all information during both sampling surveys and data digitalization on the sampling effort and sampling coverage measures, which are essential in order to standardize the data obtained and allow comparisons of records, and perform biodiversity estimates (e.g., Royle and Nichols 2003).

Thirdly, the analyses performed revealed some deficiencies in the data sampling coverage: some larger areas are particularly data-deficient on sampling coverage, such as the Douro and Tagus river basins. This information, when overlapped with species distributions indicates that occurrence data of some species are likely to be particularly incomplete. When overlapped with the observed species richness revealed higher richness for areas with more revisited sites but not for areas covered by higher number of sites, which raises the possibility that species detection was missed more often when visiting more sites in a stream than when performing re-samplings at a small number of sites. Therefore re-samplings are probably needed to obtain good sampling coverage, particularly during distinct seasonal periods in environmentally variable freshwater habitats (see Gasith and Resh 1999). But further field survey assessments of data sampling completeness are necessary since revisited sites can be biased to the ones where species richness is indeed higher. Assessments designed to obtain good variation coverage of particular species and areas which account for environmental and spatial variation might be particularly useful, as the rule-step procedure proposed by Hortal and Lobo (2005).

To achieve high quality biodiversity data

Overcoming the limitations identified in InFISH.PT database, which are likely shared by other freshwaters data sets, will certainly allow improvements on database reliability. As was illustrated here, if data are properly validated and quality attained, biodiversity databases can constitute trustworthy basal tools to examine ecological research hypotheses, and develop conservation-oriented actions (e.g., Usseglio-Polatera et al. 2000).

By focusing on freshwater faunas, we point to the necessity of building-up the current information on taxonomy and species distribution with special concerns for assuring data sampling completeness and taxonomic certification as a first step to enhance the knowledge of species distribution. Moreover this study turned obvious that database building needs concerted strategic measures among data providers, database developers and users, where certification entities as the Natural History Museums should have a crucial role for taxonomic authority certificates, and where constant maintenance is required (e.g., Sobero et al. 2000).

The GBIF initiative is becoming the main authority on biodiversity knowledge mostly because of its links with the Natural History Museums worldwide and the standardization and share of data procedures (Guralnick and Lane. 2007). It constitutes a more than welcomed initiative which seems efficient on minimizing the database problems identified across biota worldwide, which are interestingly similar to the ones identified here (Graham et al. 2004, Guralnick and Lane 2007). In spite the usefulness of global initiatives as GBIF, efforts on obtaining high quality regional data assessments remain as fundamental key-steps that cannot be neglected.

Regarding the InFISH.PT database

Considering data on fish species inhabiting Portuguese streams, it is well worth data collectors spend some extra-time on quantifying the amount of sampling effort carried out during each field journey. For example, the previously referred online Portuguese databases from DGRF, and ICN-B, they would gain on reliability if measures of data quality, namely completeness, were explicitly given, as generally recommended by other database developers (e.g., Hortal et al. 2007). Moreover, just recently the Portuguese Authority for Water Management (INAG) developed standard protocols for fish sampling methods in national waters (INAG 2008) for assuring data quality of samplings and proper implementation of the EU Water Framework Directive (WFD, www.euwfd.com). This was an important initiative since, as it was noted here, sampling methods standardization facilitates the possibility to efficiently gather comparable and reliable data and produce consistent outputs.

Regarding the records on the overall richness patterns, they must be treated with caution and consider the structure of the watercourses and their habitats. Particularly the watercourses with higher richness of primary fish were mostly recorded at main river stretches in Portuguese-Spanish border (Guadiana and Tagus river basins), which might suggest their relevance as propagule and refugee areas for this fauna. But, as part of international basins, these areas are also ‘open doors’ to invasive species dispersal between the two countries (Ribeiro et al. 2009b), which constitutes a growing problem needing to be addressed. Conversely, a low richness was recorded for headwaters across the landscape, which should be also treated with caution, as these habitats are particularly fragile and play a key role in what concerns the completion of some species life-cycles, especially in the Mediterranean-type streams (Gasith and Resh 1999, Collares-Pereira et al. 2002). Some small watercourses emerged as having high species richness, raising the possibility of being considered freshwater fish sanctuaries (see Magalhães et al. 2002, Filipe et al. 2004), but these are well sampled streams, and the possibility of finding other equally relevant areas should be also explored. Some of the uncertainties described here can be overcome by simply estimating species richness, which are more accurate than observed values (Cao et al. 2007).

The InFISH.PT database has demonstrated being a valuable fundamental research tool (e.g., Filipe et al. 2009), but currently the database needs expansion to take advantage of all its potential for other purposes as applied biodiversity matters. Solutions on how to strategically contextualize the database are being considered, which might involve stepwise procedures similar to the ones followed for other faunal databases where, in due course, the databases become accessible to all public (e.g., Statzner et al. 2007).

Brief overview on the opportunities to enhance knowledge and conservation of freshwater biodiversity

Knowledge and awareness on the freshwater fish fauna is generally scarce between policymakers and general public across the world, and is frequently focused on game-fish species which are mostly non-native for many regions. Currently opportunities are emerging for direct conservation actions at local and regional extents, namely for protecting critically endangered species, and for actively preventing the establishment of non-native species in protected areas; also the implementation of water impoundment schemes requires minimization and compensation programs, which should provide a good opportunity for some conservation and management actions (see Collares-Pereira et al. 2000, 2002). Additionally, governmental legislation and efforts are staring to be proactive. For example, for the European member-states, the WFD legislation compromises each member to achieve good ecological status in all waters by 2015 and to ensure that such status does not deteriorate. Implementing the WFD on freshwaters and adapting management to the likely impacts of the current climatic changes on such vulnerable ecosystems encompassing highly endangered faunas will constitute great challenges in the years to follow, while assuring the maintenance of the services provided. Particularly, Portugal faces great challenges by putting in the equation the construction of more ten water impoundment schemes planned to get 7,000 megawatts of energy power at year 2020 (http://pnbeph.inag.pt).

Not disregarding the database quality issues focused above, the effective conservation of freshwater biodiversity and water services go much beyond an inventory work for simplistic approaches of fish biodiversity spatial patterns (Symes et al. 2009, Thorp et al. 2009). On a global overview, some of the promising fields to invest are field experiments regarding species interactions based on native and non-native species removal or addition, lab experiments regarding physiological thresholds, empirical tests on the patterns found in nature to understand the biotic processes involved using current spatial genetic distances, and adaptive-management frameworks based on experimental programs.

Finally, protecting freshwater biota at the regional level and on a short term basis requires local and pragmatic approaches to raise consciousness at distinct sectors of the society and an effective protection of freshwater's biodiversity while assuring environmental services (Vörösmarty et al. 2005). Possible ways are the involvement of all stakeholders to bridge the gap with the natural world, which might need new proactive approaches where people take as valuable this patrimony and get truly involved on decision-making processes (Cullen et al. 1999, Fraser et al. 2009). Examples are (1) to give ecosystem services an economical value, and individuals can be then rewarded for helping to maintain services provided in good conditions (Wilson and Carpenter 1999), and (2) to perform regional decision-making with a bottom-up approach where a common vision of the region is built (Fraser et al. 2009). Natural History Museums have great challenges ahead, as the key institutions centralizing biological collections and knowledge on the natural world, while acting on outreach activities such as awareness, education, and conquering the appreciation of policymakers (Suarez and Tutsui 2004, Mares 2009).

Concluding remarks

Few studies were dedicated on attaining biodiversity data quality, namely regarding completeness of regional or particular taxa databases. This is somehow surprising since ongoing efforts on collecting data are likely to result in at least partially unreliable data sets. The InFISH.PT database offered the possibility of exemplifying the structure of a regional database, listing the uncertainties of the data merged, and ultimately building a framework to collect and store data efficiently. Special issues raised regarding freshwater biodiversity databases go to (1) attaining taxonomy data voids , (2) minimizing bias of sampling coverage for the nested freshwater environments (as among river basins, lakes, streams), and (3) revisiting sites accordingly with the common seasonality of freshwaters.

There is much to gain by improving data collecting and storing. While minimizing costs of biological surveys since funds are often small for both museums and conservation matters, new possibilities are opened for testing biological patterns, as well as for building efficient and user-friendly decision tools for conservation and management actions. Because specialized or regional databases, as those regarding freshwater biota, are the foundation of worldwide and highly accessible databases, investments on attaining high quality data will be soon rewarded.

Acknowledgements

This study is part of A.F.F. PhD project supported by the research grant SFRH/BD/10330/2002 and by the project grant PTDC/BIA-BDE/66519/2006 to M.J.A., both from the Portuguese Foundation for Science and Technology (FCT-Portugal, co-financed by FEDER). We greatly thank colleagues at FFISHGUL (ffishgul.fc.ul.pt/members.html) for sharing unpublished data and reports on species presence/absence, namely, L. da Costa, C. Cunha, H. Gante, M. Gromicho, M. F. Magalhães, N. Mesquita, A. Perdices, and F. Ribeiro, as well as the fish fauna authors and collaborators of the Portuguese Red Data Book (edited by ICN-B), especially the coordinator Leonor Rogado.

Authors contributions

A.F.F. conceived the idea, gathered the data and produced the database; M.J.A. and M.J.C. certified taxonomy and validated data on species occurrences; A.F.F. analyzed the data and led the writing with inputs from the remaining authors.

References cited

Abell R et al. 2008. Freshwater ecoregions of the World: A new map of biogeographic units for freshwater biodiversity conservation. BioScience 58: 403-414.

Alberch P. 1993. Museums, collections, and biodiversity inventories. Trends in Ecology and Evolution 8: 372-375.

Allan JD, Flecker AS. 1993. Biodiversity conservation in running waters. BioScience 43 32-43.

Balian EV, Segers H, Lévèque C, Martens K. 2008. The freshwater animal diversity assessment: an overview of the results. Hydrobiologia 595: 627-637.

Beier U, Degerman E. 2007. Processes of collating a European fisheries database to meet the objectives of the European Union Water Framework Directive. Fisheries Management and Ecology 14: 407-416.

Bisby FA 2000.The quiet revolution: biodiversity informatics and the internet. Science 289: 2309-2312.

Buisson L, Grenoulliet G. 2009. Contrasted impacts of climate change on stream fish assemblages along an environmental gradient. Diversity and Distributions 15: 613-626.

Cao Y, Hawkins CP, Larsen DP, Van Sickle J. 2007. Effects of sample standardization on mean species detectabilities and estimates of relative differences in species richness among assemblages. The American Naturalist 170: 381-395.

Collares-Pereira MJ, Cowx IG, Coelho MM (eds) 2002. Conservation of Freshwater Fishes: Options for the Future. Oxford (United Kingdom): Blackwell Science.

Collares-Pereira MJ, Cowx IG, Ribeiro F, Rodrigues JA, Rogado L. 2000. Threats imposed by water resource development schemes on the conservation of endangered fish species in the Guadiana River Basin in Portugal. Fisheries Management 7: 167-178.

Cullen PW, Norris RH, Resh VH, Reynoldson TB, Rosenberg DM, Barbour MT. 1999. Collaboration in scientific research: a critical need for freshwater ecology. Freshwater Biology 42: 131-142.

Darlington PJ. 1957. Zoogeography: the Geographical Distribution of Animals. New York: Wiley.

Dudgeon D, Arthington AH, Kawabata Z-I, Knowler DJ, Lévêque C, Naiman RJ, Prieur-Richard AH, Soto D, Stiassny MLJ, Sullivan C. 2009. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews 81: 163-182.

Filipe AF, Araújo MB, Doadrio I, Angermeier PL, Collares-Pereira MJ. 2009. Biogeography of Iberian freshwater fishes revisited: the roles of historical versus contemporary constraints. Journal of Biogeography 36: 2096-2110.

Filipe AF, Marques T, Seabra SG, Tiago P, Ribeiro F, Moreira da Costa L, Cowx IG, Collares-Pereira MJ. 2004. Selection of priority areas for fish conservation in the Guadiana River Basin, Iberian Peninsula. Conservation Biology 18: 189-200.

Fraser EDG, Dougill AJ, Mabeee WE, Reed M, McAlpine P. 2009a. Bottom up and top down: analysis of participatory processes for sustainability indicator identification as a pathway to community empowerment and sustainable environmental management. Journal of Environmental Management 78: 114-127.

Froese R, ,Pauly D. FishBase. 2008. World Wide Web electronic publication. (1 October 2009; www.fishbase.org).

Gasith A, Resh VH. 1999. Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. Annual Review of Ecology and Systematics 30: 51-81.

Gaston KJ.1996. Biodiversity: A Biology of Numbers and Difference. Portland (OR): Wiley, John and Sons.

Gido KB, Brown JH. 1999. Invasion of North American drainages by alien fish species. Freshwater Biology 42: 387-399.

Graham CH, Ferrier S, Huettman F, Moritz C, Peterson AT. 2004. New developments in museum-based informatics and applications in biodiversity analysis. Trends in Ecology and Evolution 19: 497-503.

Guralnick R, Hill AW, Lane M. 2007. Towards a collaborative, global infrastructure for biodiversity assessment.Ecology Letters 10: 663-672.

Hortal J, Lobo JM. 2005. An ED-based protocol for optimal sampling of biodiversity. Biodiversity and Conservation 14: 2913-2947.

Hortal J, Lobo JM, Jiménez-Valverde A. 2007. Limitations of biodiversity databases: case study on seed-plant diversity in Tenerife, Canary Islands. Conservation Biology 21: 853-863.

INAG. 2008. Manual para a Avaliação Biológica da Qualidade da Água em Sistemas Fluviais segundo a Directiva Quadro da Água - Protocolo de Amostragem e Análise para a Fauna Piscícola. Lisboa: Ministério do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional, Instituto da Água.

Lane M. 1996. Roles of natural history collections. Annals of the Missouri Botanical Garden 83: 536-545.

Lawton JH, Prendergast JR, Eversham JC.1994. The numbers and spatial distributions of species: analyses of British data. Pages 177-195 in Forey PI, Humphries CJ, Vane-Wright RI, eds. Systematic and Conservation Evaluation. Oxford (United Kingdom): Claredon Press.

Lévêque C, Oberdorff T, Paugy D, Stiassny MLJ, Tedesco PA. 2008. Global diversity of fish (Pisces) in freshwater. Hydrobiologia 595: 545-567.

Magalhães MF, Batalha DC, Collares-Pereira MJ. 2002. Gradients in stream fish assemblages across a Mediterranean landscape: contributions of environmental factors and spatial structure. Freshwater Biology 47: 1015-1031.

Mares MA. 2009. Natural science collections: America's irreplaceable resource. BioScience 59: 544-545.

Pardo I, Álvarez M. 2006. Comparison of resource and consumer dynamics in Atlantic and Mediterranean streams. Limnética 25: 271-286.

Pereira C, Neto A, Collares-Pereira MJ 2009. Cytogenetic survey of two Iberian Leuciscinae (Teleostei, Cyprinidae) genera hybridizing extensively in nature. I. Evidence of a similar and conserved chromosome pattern with some species-specific markers for hybrid genomes recognition. *Genetica* 137: 285-291.

Reynolds L, Herlihy AT, Kaufmann PR, Gregory SV, Hughes RM. 2003. Electrofishing effort requirements for assessing species richness and biotic integrity in western. North American Journal of Fisheries Management 23: 450-461.

Ribeiro F, Collares-Pereira MJ, Moyle PB. 2009a. Non-native fish in the fresh waters of Portugal, Azores and Madeira Islands: a growing threat to aquatic biodiversity. Fisheries Management and Ecology 16: 255-264.

Ribeiro F, Gante HF, Sousa G, Filipe AF, Alves MJ, Magalhães MF. 2009b. New records, distribution and dispersal pathways of *Sander lucioperca* in Iberian freshwaters. Cybium. in press.

Rogado L, Alexandrino PJ, Almeida PR, Alves MJ, Bochechas J, Cortes RV, Domingos MI, Filipe AF, Madeira J, Magalhães MF. 2005. Peixes dulciaquicolas e migradores. ini Cabral MJ, Almeida J, Almeida PR, Dellinger T, Ferrand de Almeida N, Oliveira ME, Palmeirim JM, Queirós AI, Rogado L, Santos-Reis M, eds. Livro Vermelho dos Vertebrados de Portugal. Lisboa: Instituto da Conservação da Natureza.

Royle A, Nichols JD. 2003. Estimating abundance from repeated presence-absence data or point counts. Ecology 84: 777-790.

Sánchez-Fernández D, Lobo JM, Abéellán P, Ribera I, Millán A. 2008. Bias in freshwater biodiversity sampling: the case of Iberian water beetles. Diversity and Distributions 14: 754-762.

Statzner B, Bonada N, Dolédec S. 2007. Conservation of taxonomic and biological trait diversity of European stream macroinvertebrate communities: a case for a collective public database. Biodiversity and Conservation 16: 3609-3602

Skelton P. 2002. An overview of the challenges of conserving freshwater fishes in South Africa. Pages 221-236 in Collares-Pereira MJ, Cowx IG, Coelho MM, eds. Conservation of Freshwater Fishes: Options for the Future. Oxford (United Kingdom): Blackwell Science.

Smith KG, Darwall WRT. 2005. The Status and Distribution of Freshwater Fish Endemic to the Mediterranean Basin. IUCN-SSC Gland and Cambridge: The World Conservation Union IUCN.

Sobero JM, Llorente JB, Oñate L. 2000. The use of specimen-label databases for conservation purposes: an example using Mexican Papilionid and Pierid butterflies. Biodiversity and Conservation 9: 1441-1446.

Suarez AV, Tutsui ND. 2004. The value of museum collections for research and society.BioScience 54: 66-74.

Sutherland JP. 2000. The Conservation Handbook: Research, Management and Policy. William J. Sutherland. Oxford (United Kingdom): Blackwell Science.

Symes KL, Armitage PD, Cannan CE. 2009. Application of the relational database approach to a nested study of biological and physical data from a lowland river.River Research and Applications 13: 185-198.

Thorp JH, Thoms MC, Delong MO. 2006. The riverine ecosystem synthesis: biocomplexity in river networks across space and time. River Research and Applications 22: 123-147.

Usseglio-Polatera P, Bournaud M, Richoux P, Tachet H.2000. Biomonitoring through biological traits of benthic macroinvertebrates: how to use species trait databases? Hydrobiologia 422-423: 152-162.

Valdecasas AG, Camacho A. 2003. Conservation to the rescue of taxonomy. Biodiversity and Conservation 12: 1113-1117.

Vörösmarty CJ, Lévêque C, Revenga C. 2005. Chapter 7: Freshwater. Millennium Ecosystem Assessment (1October 2009; *http://www.millenniumassessment.org*).

Wang L, Lyons J, Kanehl P, Bannerman R. 2001. Impacts of Urbanization on Stream Habitat and Fish Across Multiple Spatial Scales. Environmental Management 28: 255-266.

Wilson EO. 2003. The encyclopedia of life. Trends in Ecology and Evolution 18: 77-80.

Wilson MA, Carpenter SR. 1999. Economic valuation of freshwater ecosystem services in the United States: 1971-1997. Ecological Applications 9: 772-783.

Chapter 3.

Patterns of freshwater fish species in the Iberian Peninsula

"... A second great fact that strikes us in our general review is, that barriers of any kind, or obstacles to free migration, are related in a close and important manner to the differences between the productions of various regions."

Charles Darwin, 1859, *in* "On the Origin of Species by means of Natural Selection"

Filipe, AF, Araújo M.B., Doadrio, I., Angermeier, P.L. & Collares-Pereira, M.J. (2009) Biogeography of Iberian freshwater fishes revisited: the roles of historical versus contemporary constraints. *Journal of Biogeography,* **36**, 2096-2110

Published as Original Article

Journal of Biogeography (*J. Biogeogr.*) (2009) **36**, 2096–2110

ORIGINAL ARTICLE

Biogeography of Iberian freshwater fishes revisited: the roles of historical versus contemporary constraints

Ana F. Filipe[1]*, Miguel B. Araújo[2,3], Ignacio Doadrio[2], Paul L. Angermeier[4] and Maria J. Collares-Pereira[1]

[1]*Centro de Biologia Ambiental, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, Lisboa, Portugal,* [2]*Museo Nacional de Ciencias Naturales, CSIC, José Gutiérrez Abascal 2, Madrid, Spain,* [3]*Rui Nabeiro Biodiversity Chair, University of Évora, Largo dos Colegiais, Évora, Portugal and* [4]*US Geological Survey, Virginia Cooperative Fish and Wildlife Research Unit, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA*

*Correspondence: Ana F. Filipe, Centro de Biologia Ambiental, Departamento de Biologia Animal, Faculdade de Ciências de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal.
E-mail: affilipe@fc.ul.pt

ABSTRACT

Aim The question of how much of the shared geographical distribution of biota is due to environmental vs. historical constraints remains unanswered. The aim of this paper is to disentangle the contribution of historical vs. contemporary factors to the distribution of freshwater fish species. In addition, it illustrates how quantifying the contribution of each type of factor improves the classification of biogeographical provinces.

Location Iberian Peninsula, south-western Europe (*c.* 581,000 km^2).

Methods We used the most comprehensive data on native fish distributions for the Iberian Peninsula, compiled from Portuguese and Spanish sources on a 20-km grid-cell resolution. Overall, 58 species were analysed after being categorized into three groups according to their ability to disperse through saltwater: (1) species strictly intolerant of saltwater (primary species); (2) species partially tolerant of saltwater, making limited incursions into saltwaters (secondary species); and (3) saltwater-tolerant species that migrate back and forth from sea to freshwaters or have invaded freshwaters recently (peripheral species). Distance-based multivariate analyses were used to test the role of historical (basin formation) vs. contemporary environmental (climate) conditions in explaining current patterns of native fish assemblage composition. Cluster analyses were performed to explore species co-occurrence patterns and redefine biogeographical provinces based on the distributions of fishes.

Results River basin boundaries were better at segregating species composition for all species groups than contemporary climate variables. This historical signal was especially evident for primary and secondary freshwater fishes. Eleven biogeographical provinces were delineated. Basins flowing to the Atlantic Ocean north of the Tagus Basin and those flowing to the Mediterranean Sea north of the Mijares Basin were the most dissimilar group. Primary and secondary freshwater species had higher province fidelity than peripheral species.

Main conclusions The results support the hypothesis that historical factors exert greater constraints on native freshwater fish assemblages in the Iberian Peninsula than do current environmental factors. After examining patterns of assemblage variation across space, as evidenced by the biogeographical provinces, we discuss the likely dispersal and speciation events that underlie these patterns.

Keywords

Assemblage composition, basin boundaries, biogeography, climate, dispersal, environment, freshwater fish, history, Iberian Peninsula.

doi:10.1111/j.1365-2699.2009.02154.x

INTRODUCTION

A common assumption in ecological research is that organisms sharing the same geographical location also share the same current environmental constraints and at least some of the same evolutionary history. The question is how much of this shared geographical distribution is due to biotic responses to the current environmental conditions vs. historical changes in these conditions. Here, we use distributions of native Iberian freshwater fishes to examine the roles of historical and contemporary factors in determining patterns of fish assemblage composition in the Iberian Peninsula. To our knowledge, this is the first time that both types of factors – historical and contemporary – have been examined on an equal footing in Iberian biogeographical studies (but see Bonada *et al.*, 2005; Araújo *et al.*, 2008).

Barriers to dispersal often constrain distributions of species and strongly influence spatial patterns of assemblage composition. Barriers such as long-term basin boundaries are often considered more relevant to contemporary geographical patterns of freshwater fishes than are current environmental conditions (e.g. Hugueny & Lévêque, 1994; Yap, 2002; Smith & Bermingham, 2005). This view contrasts with that of assemblage organization in terrestrial and marine ecosystems, where current climate is often considered the main determinant of the geographical distributions of species (e.g. Chust *et al.*, 2006; Burgmer *et al.*, 2007).

The constrained dispersal of freshwater fishes among aquatic environments permits strong inferences about the roles of historical vs. contemporary factors, species dispersal and species responses to environmental changes. In particular, we would expect the influence of terrestrial barriers and hydrological connections to differ between species strictly restricted to freshwater and species that might disperse through marine environments, including those with current occasional or frequent incursions into saltwater. The Iberian Peninsula provides an excellent case study for comparing the contributions of historical vs. current environmental factors to spatial patterns of fish assemblages due to the long-term existence of basin divides, many endemic species and a well-known evolutionary history of fishes for the area.

Historical vs. contemporary factors

The contrasting roles of history and the modern environment in structuring biotic assemblages have spurred long debates (e.g. Davis & Scholtz, 2001; Bonada *et al.*, 2005; Svenning & Skov, 2005; Tedesco *et al.*, 2005). There is a growing consensus that both types of factor play important roles, but it is difficult to tease apart their respective contributions, in part because contemporary and historical factors tend to covary (Araújo *et al.*, 2008). Analysing patterns of species assemblages at macroecological scales might shed some light on this debate by revealing the extent to which current species distributions are at equilibrium with their environment and/or are constrained by historical factors. It is said that species distributions are at equilibrium with their current environment if they occur in all suitable areas and are absent from unsuitable ones (Hutchinson, 1957). One approach to assessing the degree of equilibrium of species with the present environment is to measure patterns of covariation between species assemblages and current environmental factors (see Araújo & Pearson, 2005, and references therein). It assumes that if species are at equilibrium with the environment, then covariation between assemblage composition and environment will be high. In contrast, if the covariation is weak it suggests non-equilibrium with the environment, possibly due to historical and/or ecological factors, such as limited dispersal ability.

Contemporary environmental barriers, such as climate, and historical dispersal barriers, such as drainage boundaries, are likely to have influenced Iberian freshwater fish assemblages in multiple ways. Barriers with low permeability to fish dispersal possibly induced vicariant differentiation between species, and permeability might have increased via new pathways created by environmental changes or through hydrological reconfigurations, such as river capture or river entry into estuaries. In particular, we would expect the influence of barriers to differ between species that are strictly restricted to freshwater and species that are tolerant of salt water if the latter use an additional dispersal pathway through the marine environment.

Indeed, the rise of physical barriers to fish dispersal accompanying the formation of river basins (in the Pliocene–Pleistocene, 2.5–1.8 Ma; Calvo *et al.*, 1993) is expected to rank highly among the key historical events affecting contemporary Iberian freshwater fish distributions. These barriers shaped 'biogeographical islands', with drainage reconfigurations occurring only sporadically (Calvo *et al.*, 1993). In addition, it is likely that previous speciation occurred following the formation of Iberian endorheic (closed) basins, which produced a system of inland lakes (in the Upper Miocene, 11–5 Ma; López-Martínez, 1989; Doadrio, 2002; Doadrio & Carmona, 2004; Robalo *et al.*, 2006). Subsequent sporadic events, such as the creation of new dispersal routes due to downstream confluences of adjacent river systems formed during drops in sea level (in the Pleistocene, *c.* 120 ka), might also be relevant (Machordom & Doadrio, 2001; Doadrio & Carmona, 2003; Sousa-Santos *et al.*, 2007).

Biogeographical provinces

Contemporary biogeographical provinces, defined by species assemblages, can be used to explore and understand patterns of similarity of species occurrences and the likely processes of dispersal, speciation and extinction among species and across landscapes. For example, if two provinces harbour distinct or endemic species one could hypothesize that the provinces' boundaries acted as strong barriers, which might have favoured allopatric speciation. If these provinces also harbour widespread species, one might hypothesize that the provinces' boundaries became more permeable after speciation. Patterns of freshwater fish distributions have been used to help delineate biogeographical provinces and to examine hypotheses regarding probable speciation events as well as post-speciation dispersal in North America (Hocutt & Wiley, 1986), Lower Mesoamerica (Smith & Bermingham, 2005), South America (Hubert & Renno, 2006), West Africa (Hugueny & Lévêque, 1994), Europe (Reyjol *et al.*, 2007), Asia (Yap, 2002) and across the world (Lévêque *et al.*, 2008). However, the delineation of such provinces has typically been based on species similarities among river basins or stream catchments without previous tests of the relevance of such units of analysis on current species assemblage patterns.

The importance of historical basin delineation as a main driver of fish assemblage composition in the Iberian Peninsula has been reported in several studies. Some authors found concordance between Iberian freshwater species assemblages and basin delineation (Arévalo, 1929; Hernando, 1990; Hernando & Soriguer, 1992; Vargas *et al.*, 1998; Mesquita *et al.*, 2007), whereas others found concordance between species distributions and current latitudinal patterns based on either Cyprinidae or all primary species (Lozano, 1952; Almaça, 1978; Doadrio, 1988). However, the role of current environmental conditions in shaping fish assemblage composition remains largely unexplored. Indeed, previous studies examining the role of contemporary environments have been limited to specific regions of the peninsula (e.g. Carmona *et al.*, 1999; Filipe *et al.*, 2002; Magalhães *et al.*, 2002).

Here, we examine relationships between assemblages of Iberian native freshwater fishes and sets of historical and contemporary environmental variables. In particular, we ask whether dispersal barriers among basins are a more accurate predictor of current patterns of native fish assemblage composition than contemporary environmental factors. Subsequently, we ask whether the identification of the most constraining factor might help us explore and comprehend occurrence patterns across space (Table 1). We first analyse the influence of the geographical structure of river basins vs. current climate on local native species composition. We do this by separating groups of species with distinct tolerance of salinity to assess the effects of marine and terrestrial barriers on fish distribution. Finally, in the light of our findings, we redefine biogeographical provinces and examine occurrence patterns and the permeability of dispersal barriers.

MATERIALS AND METHODS

Study area

The Iberian Peninsula is in south-western Europe and comprises approximately 581,000 km^2 (Fig. 1). Its climate is varied, including desert, Mediterranean, alpine and Atlantic areas (Rivas-Martínez, 1987). Current environmental conditions produce several types of watercourses, ranging from Mediterranean streams with harsh cycles of flooding and drying to more mesic streams with continuous flow. The geological and faunal evolutionary history of the Iberian Peninsula is complex. Iberian streams have been isolated from Central Europe since the rise of the Pyrenees Mountains during the Tertiary (80–20 Ma), and were only partially covered by ice during the Quaternary glaciations. This is reflected in the large number of species that exist there compared with northern Europe (both endemics and non-endemics), a pattern analogous to other European Mediterranean peninsulas (Griffiths, 2006; Smith & Darwall, 2006).

Data sources

We built the species database by merging information from 1990 to 2008 on freshwater fish distributions from Portuguese and Spanish sources. We excluded non-native species to allow a clearer interpretation of the influences of evolutionary history vs. current environment. Species occurrence records were compiled from three main sources: (1) electrofishing samples compiled for the Portuguese Red Data Book (Rogado *et al.*, 2005); (2) electrofishing samples designed to sample watercourses with limited previous collection efforts by the first author; and (3) data published in the Spanish Atlas and Red Data Book based on a 10-km grid cell (Doadrio, 2002). This grid was used as the unit of analysis for the database since it is the coarsest sampling grain among data sources and it ensures inclusion of at least one electrofishing sample per cell. Data aggregation for each grid cell was performed with the ArcMap 9.1 summarization tool (ESRI, 2001). The database contains 64 native fish species, based on Iberian specialists' taxonomic synthesis of phylogenetic studies summarized in the Portuguese and Spanish Red Data Books (Doadrio, 2002; Rogado *et al.*, 2005) and on subsequent species descriptions (Doadrio & Elvira, 2007; Doadrio *et al.*, 2007; Gante *et al.*,

Table 1 Mechanisms potentially driving contemporary patterns of fish assemblage composition in the Iberian Peninsula.

Forcing factors	Events	Processes	Predictions
Long-term river basin divides	River basin formation	Allopatric speciation due to impassable barriers	Areas of distinct basins harbour differentiated and endemic species
Current environmental variation	Current climate	Species distributions constrained by physiological limitations	Areas of distinct environments harbour differentiated and endemic species
Recent punctuated hydrological connections	For example, headwater captures, basin mouth connections	Post-speciation dispersal	Closely related species or the same species occur in neighbouring provinces linked by current or historical dispersal routes

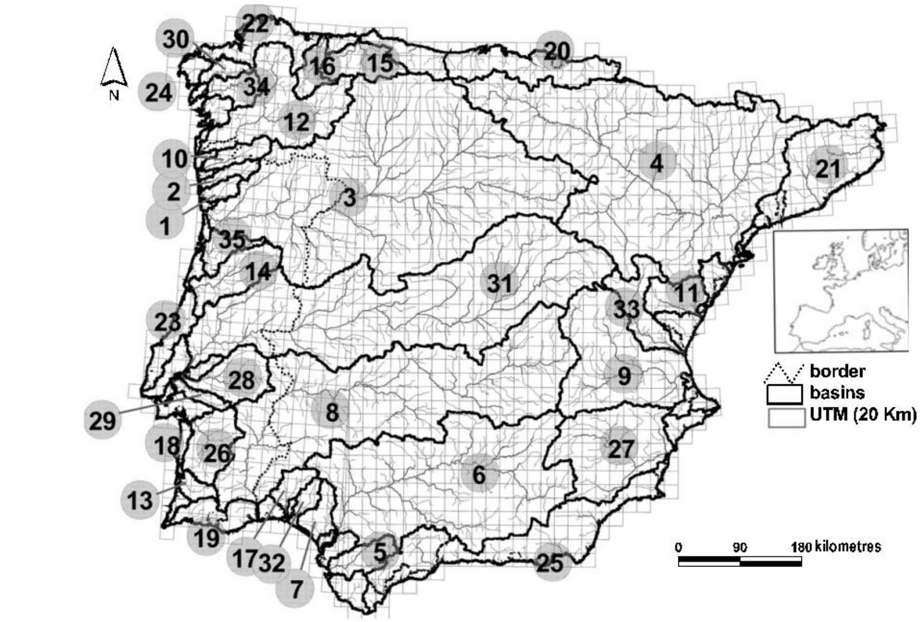


Figure 1 The 35 hydrological basins of the Iberian Peninsula, located in south-western Europe: 1, Ave; 2, Cávado; 3, Douro; 4, Ebro; 5, Guadalete; 6, Guadalquivir; 7, Guadiamar; 8, Guadiana; 9, Júcar; 10, Lima; 11, Mijares; 12, Minho; 13, Mira; 14, Mondego; 15, Narcea; 16, Navea; 17, Odiel; 18, Alentejo coastal basins; 19, Algarve coastal basins; 20, Cantabria and Basque coastal basins; 21, Catalunya and northern València coastal basins; 22, Galicia and Asturias coastal basins; 23, Extremadura coastal basins; 24, Northern coastal basins; 25, Southern València, Extremadura and Castilha coastal basins (designated from now on as Medit. basins); 26, Sado; 27, Segura; 28, Sorraia; 29, St. Estevão; 30, Tambre; 31, Tagus; 32, Tinto; 33, Túria; 34, Uila; 35, Vouga. Main watercourses, 20 × 20 km Universal Transverse Mercator (UTM) grid cells and borders of Portugal and Spain are also represented.

2007). We treated *Lampetra fluviatilis* (Linnaeus, 1758) and *Lampetra planeri* (Bloch, 1784) as a single entity due to the likelihood that they are different ecotypes of a single species (Rogado *et al.*, 2005; Espanhol *et al.*, 2007).

Species occurrence data were validated by the following procedure: (1) recent species records not previously documented in a river basin (i.e. the entire area drained by a river and its tributaries) were identified as possible translocations and excluded, and (2) questionable identifications of specimens, when available, were confirmed at the Natural History Museums of Lisbon and Madrid. Species for which information about their distributions was somehow limited were excluded from our analysis, while rare species were retained to minimize the chances of overlooking distinctive but sparsely represented communities. The 58 species used in our analyses are listed in Appendix S1 in Supporting Information, as are the reasons for excluding the six species previously listed. Since oceans can be a major barrier to fish dispersal, species were classified according to physiological saltwater tolerance (*sensu* Darlington, 1957). Forty species are strictly intolerant of saltwater (i.e. primary species), five can tolerate limited incursions into saltwaters (i.e. secondary) and 13 are saltwater tolerant (i.e. peripheral). The latter species migrate back and forth from the sea to freshwaters or have invaded freshwaters recently.

Data were resampled in 20-km grid cells to avoid computational limitations that would arise when using smaller grid cells. This procedure also reduced the effects of false species absences and of merging distinct (but not quantified) sampling efforts among collections. The final dataset comprised 1620 grid cells after excluding coastal cells containing less than 50% land (Fig. 1). For convenience, we assumed that observed species absences in grid cells were real. Across all 58 species, occurrences covered 1457 grid cells (i.e. 89.9%).

Climate variables and basin limits were built from digital cartography with free internet access. Climate data included 19 variables related to thermal and hydrological characteristics known to constrain freshwater fish distributions (Matthews, 1998). Climate variables were based on records from 1950 to 2000 at 1-km grid cell resolution (Hijmans *et al.*, 2005, 2007; see Appendix S2). Here, mean values were attributed to each grid cell in ArcMap 9.1 (ESRI, 2001). To be consistent with current topography, river basin boundaries (as defined previously) were generated based on a 90-m resolution digital terrain model (DTM of CGIAR-CSI, 2005) in ArcMap 9.1

(ESRI, 2001) using both the Catchment Polygon Processing and Drainage Point Processing tools (ESRI, 2005). The 290 Iberian river basins obtained when using a minimum basin area of 0.4 km^2 were considered as potentially suitable areas to support freshwater fishes, according to the terrain validation we performed for a subset of basins. Afterwards, the small adjacent basins were merged with the basic geographical tools. The 35 basins obtained were considered an appropriate match for the spatial resolution of the grid cells used (basin area range 1300–97,673 km^2; see Fig. 1).

Data analysis

Data analysis was designed to tease apart the relative contributions of historical (i.e. basin delineation) vs. contemporary environmental factors (i.e. climate variables). Because species with different capabilities of dispersing through saltwater may display different levels of association with river basins, analyses were conducted using four matrices of distinct species groups: (1) primary (40 species × 388 samples), (2) secondary (5 × 100), (3) peripheral (13 × 450), and (4) all species (58 × 1457). Since dispersal of organisms is contingent on proximity and the existence of pathways between locations, we expect nearby locations to have more similar species composition than distant ones (Whittaker, 1975; Wilkinson & Edds, 2001). Here we considered that the spatial structure of fish species composition – naturally clumped at this scale – might be a consequence of the ecological processes under investigation, which should not be removed. Using the multivariate nonparametric distance-based methods (Smith *et al.*, 1990; Clarke & Gorley, 2001) commonly used in biogeographical studies (e.g. Stickle & Hughes, 2000; Araújo *et al.*, 2004) allowed us to incorporate the spatial structure of the data and avoid the well-documented statistical problems that occur when using standard statistical tests on spatially autocorrelated data (see Legendre, 1993). The effect of the spatial structure of the data on the results obtained was tested by a second-order spatial autocorrelation analysis based on a geographical distances matrix (Legendre, 1993).

Analysis 1: To distinguish influences of historical vs. contemporary factors

To examine the relative influence of river basin and current climate on patterns of assemblage composition among native fish species, three analytical steps were performed. First, to assess which combination of climatic variables best explains spatial variation in fish assemblages, we applied a heuristic procedure termed BVSTEP (Clarke & Warwick, 1994) to select the 'best' subset of environmental variables that maximizes the correlation between each group of species and the environment. This step was performed instead of testing the Iberian climate zones defined by a cluster analysis because it enables us to minimize the possibility that the climatic variables used do not determine the occurrences of species for each species group considered. The procedure starts with the construction of one pairwise similarity matrix for species occurrences and another similarity matrix for climate variables. Regarding species occurrences, distances between grid cells were calculated with the Bray–Curtis index (Bray & Curtis, 1957) because it minimizes the effect of false absences by ignoring null values shared between assemblages. Regarding climate variables, to calculate climatic differences between grid cells we used Euclidean distances after standardizing variables to zero mean and unit variance. The BVSTEP routine starts by identifying the climate variable most strongly correlated with variation in species assemblages (Spearman's coefficient), then adding the best remaining variables via forward selection until the significance of the coefficient improves by less than 0.001 (Clarke & Warwick, 1994). In each BVSTEP analysis we performed five restarts, with initial species subsets chosen at random to assess the sensitivity of BVSTEP to the initial conditions. Each run used *c.* 50% of the variables.

Second, to allow statistical comparisons of climate and basin factors, we clustered climate variables previously identified by BVSTEP by forcing the production of 35 climate zones to match the number of river basins. The *k*-means non-hierarchical clustering method used performs an iterative relocation procedure for a given number of clusters, where the objects are moved to minimize within-cluster variability and maximize between-cluster variability until the most significant ANOVA results are obtained. To ensure the consistency and distinctiveness of the clusters obtained, the distance of each sample from the respective cluster centre, as well as the Euclidean distances between clusters, were checked. This method has been considered appropriate to classify environmental data despite the arbitrary divisions it imposes (see Fuller *et al.*, 2007, and references therein).

Finally, to test differences in assemblage composition between climate zones and basins, we used nonparametric analysis of similarity (ANOSIM) for each species matrix. This test operates directly on a pairwise distance matrix without the assumption of multivariate normality (Clarke, 1993). It compares ranked similarities of the matrix between and within groups selected a priori by calculating the global R statistic, and performs a randomization routine where the empirical distribution of the global R under a null model is generated to estimate significance (Clarke, 1993). Global R ranges between -1 and $+1$, where 0 indicates completely random grouping. Here, climate zones and basins were the groups previously defined and tested independently for significant differences in each species distance matrix. All ANOSIM tests involved 999 random group reassignments.

Analysis 2: To characterize biogeographical provinces

To delineate biogeographical provinces, occurrence data for the 58 species were aggregated by the factor that best explained variation in assemblage composition, i.e. the river basins or climate domains (see Analysis 1), and a 58 species × 35 units of analysis matrix was obtained. Then, a three-step procedure was adopted. First, a dissimilarity matrix between the selected

35 units of analysis was calculated using the Bray–Curtis measure. Hierarchical cluster analysis of the dissimilarity matrix was then performed with the unweighted pair-group method using arithmetic averages (UPGMA; Sneath & Sokal, 1973). Second, we mapped alternative solutions for province delineation by varying the dissimilarity cut-off in the clustering dendrogram. The adopted cut-off was the one that maximized dissimilarity while still preserving spatial contiguity of the clusters (i.e. selecting the maximum number of clusters with adjoining units of analysis). Third, we again used the ANOSIM global *R* statistic, now to test if between-province differences were significantly different from within-province differences (see Analysis 1 for procedure details).

To test how much of the similarity of the delineated provinces is due to spatial contiguity, we tested the second-order spatial autocorrelation by performing partial Mantel tests using three matrices: matrix 1 of geographical distance between the paired units of analysis, where '1' indicates adjoining areas and '2' indicates areas with no common boundary; matrix 2 of biogeographical membership, where '0' was assigned for units of analysis belonging to the same province and '1' otherwise; and matrix 3 of assemblage similarity based on the original 58 × 35 matrix. The partial Mantel test allowed us to control the effect of the spatial structure of the data by examining the hypothesis that fish similarities (matrix 3) and province membership (matrix 2) are strongly related even when the effect of geographical distance between units of analysis is removed (matrix 1) (Hugueny & Lévêque, 1994; Smith & Bermingham, 2005). A total of 10,000 randomized resampling iterations were generated to estimate significance.

Furthermore, to explore patterns of fish species composition and likely dispersal we quantified average faunal similarities among provinces and identified which fish species contributed the most to that similarity by performing a similarity-breakdown analysis (SIMPER) on the 58 × 35 species matrix. Also, based on the average of Bray–Curtis similarities, both among assemblages and among species within each province, we calculated: (1) the proportion of area occupied by a species within each province – province constancy, *C* (Clarke & Warwick, 1994); and (2) the ratio of a species' province constancy to its constancy across the entire Iberian Peninsula – province fidelity, *F* (Ibrahim *et al.*, 1972). Constancy varies between 0 and 1, reaching 1 if the species is detected among all units of analysis; a fidelity value < 1 indicates that a species is less widespread within the province than across the Iberian Peninsula overall, whereas a value of > 1 indicates that a species is more widespread in the considered province than across the peninsula overall (for a similar approach see Newall & Magnuson, 1999; Wells *et al.*, 2002).

For hierarchical cluster analyses, ANOSIM, BVSTEP and SIMPER routines we used PRIMER version 5.2.0 software (Clarke & Gorley, 2001); for non-hierarchical cluster analysis we used SPSS version 15.0 (SPSS Inc., 2006). Partial Mantel tests were performed using ZT version 1.0 software (Bonnet & Van de Peer, 2002).

RESULTS

Historical vs. contemporary factors

When examining fish distributions with regard to current climate, we found that variables reflecting extreme climatic seasonality correlated significantly with assemblage composition, especially for primary and secondary species (Table 2). For primary species, precipitation and temperature summer extremes, winter temperature and precipitation seasonality were strong significant correlates of species occurrence, while for secondary species, winter temperature and precipitation showed a stronger correlation. For peripheral species, annual and summer precipitations were the strongest correlates, and the climate zones obtained were less spatially clustered than the ones obtained for the remnant species groups (for maps of the climate zones see Appendix S3).

When analysing species composition with regard to current climate and basin delineation we found that both sets of variables were significantly associated with the four groups of species considered (Table 3). The degree of association with current climate and with basin divides was greater for primary species (do not disperse through saltwater) and for all species combined (i.e. greater ANOSIM global *R* statistics). Assemblages of secondary species (occasionally disperse through saltwater) were less discriminated by both climate and basin

Table 2 Environmental variables selected by BVSTEP (i.e. variables that maximize correlation with species assemblage variation at $P < 0.001$).

	Species groups			
Variables	Primary	Secondary	Peripheral	All
Mean temperature of wettest quarter	1	1		1
Mean temperature of driest quarter	2			2
Precipitation of driest month	3			3
Precipitation seasonality	4			4
Precipitation of driest quarter	5			5
Precipitation of warmest quarter	6		2	6
Mean temperature of coldest quarter		2		
Precipitation of wettest month		3		
Precipitation of coldest quarter		4		
Annual precipitation			1	
Correlation coefficient (r_s)	0.519	0.481	0.073	0.474

Cell numbers represent variable rankings (starting at the first variable selected). For species groups and variable details see Appendices S1 and S2.

Table 3 One-way ANOSIM global *R* statistics for species composition. Climatic zones and basin memberships were used (independently) as factors.

Species groups	Current climate	Basins
Primary	0.578**	0.802**
Secondary	0.306**	0.628**
Peripheral	0.096**	0.124*
All	0.559**	0.807**

For species details see Appendix S1.
Significant at: $^{*}P < 0.05$, $^{**}P < 0.001$.

divides. Both factors discriminated peripheral species assemblages (live mostly in saltwater) even less well. Comparing both factors tested, basin divides were consistently better than current climate at segregating species composition for all species groups.

For all species combined, both results on climate variables selected and on the degree of association with climate and basin divides closely resemble those for the primary species group, which has the highest number of species (Tables 2 and 3).

Biogeographical provinces of freshwater fishes

Given that river basin delineation was strongly associated with fish assemblages of all species groups, river basins were used as units of analysis to delineate biogeographical provinces. In the river basins' dendrogram, spatial contiguity of basins was lost for more than 11 clusters, corresponding to the cut-off value of 47% Bray–Curtis dissimilarity (Fig. 2). These 11 provinces differed significantly in species composition (ANOSIM global $R = 0.778$, $P < 0.001$). Atlantic basins north of the Tagus and Mediterranean basins north of the Mijares were the most dissimilar group of basins (named the North-west, Tambre, Navea-Narcea, North, and North-east provinces; Figs 2 & 3). The Mediterranean small coastal basins were the next most dissimilar (provinces of Túria-Mijares and Central-east; Figs 2 & 3). Three of the 11 provinces comprised solely a small coastal basin: the Tambre, Mira and North basins. Among these, the Mira Basin was the most dissimilar, being defined as a province when only four clusters were recognized.

Interbasin similarity in fish assemblages and province membership was significantly related even after removing the effect of geographical distance (partial Mantel test, $R = 0.411$, $P = 0.0001$). This pattern indicates that the delineation of biogeographical provinces is robust even when the second-order spatial autocorrelation is removed.

Regarding the provinces' ichthyofaunal characterization, average similarity among provinces for all species occurrences ranged between 94.12 in the Túria-Mijares Province and 63.84 in the Central-east Province (Table 4). The maximum contribution of a single species to those values was 33.33% in the Tinto-Odiel Province, which also had the fewest species contributing to the average similarity (three species contributed 80% of the average similarity); the North-east Province had the most contributing species (13 species contributed 80% of the average similarity). Overall, peripheral species contributed most to the average similarity within provinces and across the Iberian Peninsula. Accordingly, primary and secondary species occurred more frequently within a province than across the entire Iberian Peninsula (i.e. they had higher values of fidelity; Table 4). Species with the least province fidelity across the Iberian Peninsula, which occurred in almost all provinces,

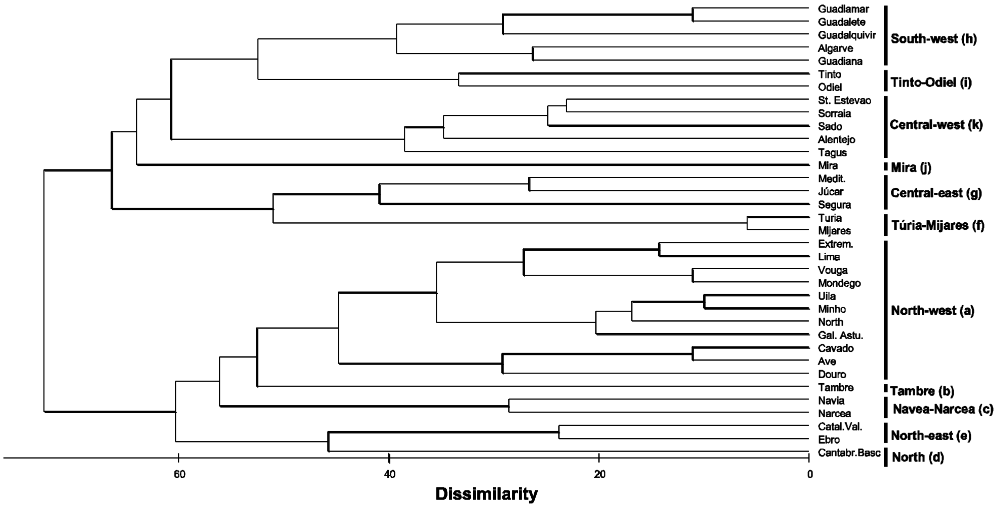


Figure 2 Dendrogram of the overall dissimilarity for the 58 species of freshwater fish among the 35 basins built by the unweighted pair-group method using arithmetic averages (UPGMA) based on the Bray–Curtis dissimilarity square matrix. The axis represents Bray–Curtis dissimilarity values; province membership of each basin is indicated by its name and code.

Figure 3 The 11 biogeographical provinces delimited by overall similarity of freshwater fish assemblages among basins and preserving geographical contiguity: a, North-west; b, Tambre; c, Navea-Narcea; d, North; e, North-east; f, Túria-Mijares; g, Central-east; h, South-west; i, Tinto-Odiel; j, Mira; k, Central-west.

were *Anguilla anguilla* (Linnaeus, 1758), *Cobitis paludica* (de Buen, 1930) and *Achondrostoma arcasii* (Steindachner, 1866). The most widespread species ($C > 0.6$) were *A. anguilla, C. paludica* and *Petromyzon marinus* Linnaeus, 1758. The 22 least frequently occurring species ($C < 0.1$) were primary, except the anadromous *Acipenser sturio* Linnaeus, 1758 and the secondary *Cottus gobio* Linnaeus, 1758. There were 18 species unique to a province (31.0% of all species considered). The South-west Province contains the greatest number (six) of these endemic species (i.e. 23.1% of Iberian species), suggesting that basin boundaries have especially constrained freshwater fish assemblages in this region.

DISCUSSION

River basin boundaries emerge as the main determinant of Iberian fish distributions when tested against current climate, especially for species restricted to freshwaters (primary). The results point to a non-equilibrium state of assemblages with contemporary environmental conditions, contingent on a species' ability to disperse through time via hydrological connections. The primacy of historical factors over the current environment also applies to European amphibians and reptiles (Araújo *et al.*, 2008), but was not observed for freshwater caddisflies of the south-eastern Iberian Peninsula, which might reflect the high dispersal capacity of caddisflies or the possible confounding effects of historical and environmental variables (Bonada *et al.*, 2005).

Also, by using data at a grid-cell scale, we clearly show that river basin boundaries are appropriate units of analysis in biogeographical studies of freshwater fishes, at least more than current climate. River basins have been adopted as units of analysis in previous studies, but without being properly tested with finer-resolution datasets against other alternatives (e.g. Hugueny, 1990).

The diminished role of river basin boundaries in determining distributions of peripheral species, thriving mainly in saltwater, relative to primary and secondary species, is probably due to the dispersal of fish along coastlines, which decreases the isolation of populations. Indeed a reduced genetic differentiation of peripheral species between basins has been recorded (Lucas *et al.*, 2001), which together with our results is evidence for the likely relevance of dispersion processes through saltwaters. On the other hand, our results show that distributions of secondary species seem more shaped by basin boundaries than those of peripheral species, which suggests that secondary species are less able to disperse through coastal seas, despite their tolerance of salinity. Additional support for this hypothesis comes from our results on the lower constancy of secondary species across the Iberian Peninsula. Similar patterns have been described in other regions of the world (Hugueny, 1990; Smith & Bermingham, 2005).

Quantifying the relevance of historical factors such as river basin boundaries provided new insights into the spatial patterns of Iberian freshwater fishes. Our analysis of similarity in species composition among basins and the delineation of biogeographical provinces provided useful information on likely speciation and dispersal mechanisms. In particular, basin boundaries seem to be responsible for the high rates of Iberian endemism observed for primary and secondary species, which is not surprising since basin boundaries can limit dispersal, even for some terrestrial animals (e.g. Lobo *et al.*, 2006). In provinces currently harbouring many endemic species, it is likely that multiple additional factors (such as past climate and/or local isolation processes) acted synergistically and allowed them to become centres of speciation (see the molecular studies of Zardoya & Doadrio, 1999; Mesquita *et al.*, 2007). For example, in the South-west Province, distinct speciation events probably occurred for *Aphanius baeticus* (Doadrio, Carmona &

Table 4 SIMPER analysis for each province.

North-west (a)					
S	26				
*S*unique	3				
N	11				
AvSim	64.53				
Species	*C*	*F*	AvSim	%	Cum%
Anguilla anguilla	1.00	1.06	8.30	12.86	12.86
Platichthys flesus	1.00	1.85	8.30	12.86	25.72
Squalius carolitertii	0.91	3.14	6.52	10.11	35.83
Pseudochondrostoma duriense	0.82	2.41	5.57	8.63	44.47
Petromyzon marinus	0.82	1.24	5.33	8.25	52.72
Cobitis paludica	0.73	0.95	4.22	6.54	59.25
Achondrostoma oligolepis	0.73	2.81	4.06	6.30	65.55
Barbus bocagei	0.73	2.15	4.06	6.30	71.85
Gasterosteus gymnurus	0.73	1.70	4.04	6.25	78.10
Achondrostoma arcasii	0.64	1.39	3.41	5.28	83.30
North-east (e)					
S	26				
*S*unique	2				
N	2				
AvSim	76.19				
Species	*C*	*F*	AvSim	%	Cum%
Phoxinus bigerrei	1.00	5.00	4.76	6.25	6.25
Squalius laietanus	1.00	16.67	4.76	6.25	12.50
Barbus graellsii	1.00	11.11	4.76	6.25	18.75
Barbus haasi	1.00	9.09	4.76	6.25	25.00
Salaria fluviatilis	1.00	5.88	4.76	6.25	31.25
Valencia hispanica	1.00	11.11	4.76	6.25	37.50
Cobitis paludica	1.00	1.30	4.76	6.25	43.75
Achondrostoma arcasii	1.00	2.17	4.76	6.25	50.00
Anguilla anguilla	1.00	1.06	4.76	6.25	56.25
Atherina boyeri	1.00	1.96	4.76	6.25	62.50
Alosa fallax	1.00	2.50	4.76	6.25	68.75
Gasterosteus gymnurus	1.00	2.33	4.76	6.25	75.00
Liza ramada	1.00	2.94	4.76	6.25	81.25
South-west (h)					
S	26				
*S*unique	6				
N	5				
AvSim	66.88				
Species	*C*	*F*	AvSim	%	Cum%
Pseudochondrostoma willkommii	1.00	5.00	7.36	11.00	11.00
Squalius pyrenaicus	1.00	1.96	7.36	11.00	22.00
Barbus sclateri	1.00	3.23	7.36	11.00	33.00
Iberochondrostoma lemmingii	1.00	3.85	7.36	11.00	44.00
Cobitis paludica	1.00	1.30	7.36	11.00	55.00
Anguilla anguilla	1.00	1.06	7.36	11.00	66.00
Petromyzon marinus	1.00	1.52	7.36	11.00	77.00
Aphanius baeticus	0.80	7.27	4.67	6.98	83.98

Table 4 Continued.

Central-east (g)					
S	19				
*S*unique	2				
N	3				
AvSim	63.84				
Species	*C*	*F*	AvSim	%	Cum%
Squalius pyrenaicus	1.00	1.96	8.28	12.97	12.97
Barbus guiraonis	1.00	5.00	8.28	12.97	25.95
Anguilla anguilla	1.00	1.06	8.28	12.97	38.92
Atherina boyeri	1.00	1.96	8.28	12.97	51.90
Syngnathus abaster	1.00	9.09	8.28	12.97	64.87
Aphanius iberus	1.00	5.88	8.28	12.97	77.85
Barbus sclateri	0.67	2.16	3.03	4.75	82.59
Túria-Mijares (f)					
S	9				
*S*unique	1				
N	2				
AvSim	94.12				
Species	*C*	*F*	AvSim	%	Cum%
Parachondrostoma turiense	1.00	16.67	11.76	12.50	12.50
Squalius pyrenaicus	1.00	1.96	11.76	12.50	25.00
Squalius valentinus	1.00	7.14	11.76	12.50	37.50
Barbus guiraonis	1.00	5.00	11.76	12.50	50.00
Barbus haasi	1.00	9.09	11.76	12.50	62.50
Cobitis paludica	1.00	1.30	11.76	12.50	75.00
Achondrostoma arcasii	1.00	2.17	11.76	12.50	87.50
Navea-Narcea (c)					
S	9				
*S*unique	0				
N	2				
AvSim	71.43				
Species	*C*	*F*	AvSim	%	Cum%
Phoxinus bigerrei	1.00	5.00	14.29	20.00	20.00
Pseudochondrostoma duriense	1.00	2.94	14.29	20.00	40.00
Alosa alosa	1.00	2.50	14.29	20.00	60.00
Anguilla anguilla	1.00	1.06	14.29	20.00	80.00
Tinto-Odiel (i)					
S	6				
*S*unique	0				
N	2				
Similarity	66.67				
Species	*C*	*F*	AvSim	%	Cum%
Squalius pyrenaicus	1.00	1.96	22.22	33.33	33.33
Barbus sclateri	1.00	3.23	22.22	33.33	66.67
Iberochondrostoma lemmingii	1.00	3.85	22.22	33.33	100.00

Table 4 Continued.

Central-west (k)

S	28				
*S*unique	1				
N	5				
Similarity	66.21				
Species	*C*	*F*	AvSim	%	Cum%
Pseudochondrostoma polylepis	1.00	5.00	7.01	10.59	10.59
Squalius alburnoides complex	1.00	2.70	7.01	10.59	21.18
Squalius pyrenaicus	1.00	1.96	7.01	10.59	31.76
Iberochondrostoma lusitanicum	1.00	7.14	7.01	10.59	42.35
Cobitis paludica	1.00	1.30	7.01	10.59	52.94
Anguilla anguilla	1.00	1.06	7.01	10.59	63.53
Barbus bocagei	0.80	2.35	4.17	6.30	69.83
Lampetra (*L. fluviatilis* and *L. planeri*)	0.80	4.00	4.17	6.30	76.12
Petromyzon marinus	0.80	1.21	3.93	5.93	82.06

For each province, species richness (*S*), number of species restricted to the province (*S*unique), number of basins analysed (*N*) and AvSim are presented. Three provinces were not subjected to SIMPER since they comprised only one basin. Species contributing up to 80% of the species assemblage similarity for each province are listed. Species constancy (*C*), fidelity (*F*), average similarity (AvSim) and its percentage (%) and cumulative percentage (Cum%) are also shown. For species details see Appendix S1.

Fernández-Delgado, 2002), *Anaecypris hispanica* (Steindachner, 1866), *Iberochondrostoma oretanum* (Doadrio & Carmona, 2003), *Squalius aradensis* (Coelho, Bogutskaya, Rodrigues & Collares-Pereira, 1998), and *Squalius palaciosi* (Doadrio, 1980). These species are thought to have evolved via allopatric speciation from widespread species not particularly constrained by dispersal barriers.

Basin boundaries seem to have exhibited punctuated permeability through: (1) intermittent connections of freshwaters due to hydrological reorganization associated with climate change and/or orogeny events (e.g. river capture); or (2) river-mouth freshwater pathways, most probably formed during the lowering of sea levels (e.g. during the Messinian salinity crisis). Recent phylogenetic studies in other regions mention the biogeographical importance of past rearrangements of drainage networks (Waters & Wallis, 2000; McGlashan & Hughes, 2001; Burridge *et al.*, 2007). For the Iberian Peninsula, genetic studies are shedding light on the role of punctuated connectivity among basins, such as river captures and other hydrological rearrangements (see Mesquita *et al.*, 2005; Sousa-Santos *et al.*, 2007), and downstream confluences of adjacent river systems due to sea-level fluctuations (see Mesquita *et al.*, 2007). Province distinctiveness may also reflect remnant influences of the historical formation of endorheic basin divides prior to current basin boundaries. For example, the Central-west Province was previously partially occupied by the Tagus-Sado Endorheic Basin (Calvo *et al.*, 1993), which may still account for some similarity among current species assemblages (see Carmona *et al.*, 2002). It is clear from these considerations that it will be instructive to assess the influence of other historical factors to better understand the biogeographical processes at finer spatial scales.

The 11 provinces defined here represent the first biogeographical approach for the Iberian Peninsula that considers both primary and non-primary fish species, and that incorporates recent taxonomic advances. These provinces reflect an evident distinctiveness of fish assemblages in some small coastal basins and strong latitudinal and Atlantic–Mediterranean gradients, both prominent in previous studies based on Cyprinidae or all primary fishes (Arévalo, 1929; Lozano, 1952; Almaça, 1978; Doadrio, 1988; Hernando, 1990; Hernando & Soriguer, 1992; Soriguer *et al.*, 2005) and those based on both primary fishes and amphibians (Vargas *et al.*, 1998). Our provinces are also similar to previous Iberian regionalizations based on non-fish fauna (e.g. García-Barros *et al.*, 2002), but are distinct from provinces delineated for pteridophyte flora (e.g. Márquez *et al.*, 2001). Given that biogeographical provinces are not necessarily similar for different groups of organisms (see Rabeni & Doisy, 2000; Abell *et al.*, 2008), we recommend developing regions independently for distinctive taxonomic groups rather than assuming that one group adequately represents biogeographical patterns for all biota (see Procheş, 2005; Rodrigues & Brooks, 2007).

Our results show that it is not necessary to invoke contemporary environmental variables to understand current patterns of assemblage variation of Iberian freshwater fish. However, this does not imply that climate is not important or that other factors, such as direct human interference, do not constrain species distributions at local or regional levels. The fact that isolation emerged as a major constraint on the composition of fish assemblages could be interpreted as a sign that global environmental changes might not be important in shaping the future distributions of species. This clearly would be a misleading interpretation. First, there is no evidence that climate does not affect local distributions of species. Our results show that different sets of species occur in different river basins, highlighting the relevance of terrestrial barriers to dispersal, but this does not disprove the hypothesis that climate constrains the distributions of species, and the probable great impacts of global environmental changes on such fauna limited to aquatic environments for dispersal and persistence. Indeed, the correlation of distinct climate variables with distinct species groups points to the necessity of exploring the ecological processes involved, namely during past climatic changes (see Tedesco *et al.*, 2005). A strong test of the hypothesis that climate constrains the distributions of species would involve laboratory or ecosystem experiments using translocations of species from one river basin to another, although this latter option might have potentially large impacts and be unsuitable on practical and ethical grounds. Second, environmental changes are not limited to the direct effects of climate on species distributions. In particular, the long history of human settlement in the Iberian Peninsula has altered landscapes, created anthropogenic barriers such as dams and reservoirs, caused translocations of fish between watersheds, and consequently led to local declines of native species and increased distribution and abundance of non-native species (see Clavero & García-Berthou, 2006).

The current global rate of homogenization of fish faunas (Olden, 2006) suggests that environmental effects will eventually overwhelm the effects of historical processes, and that biological interactions with non-native species may strongly influence distributions of native fishes (Hoeinghaus *et al.*, 2007). This scenario might be exacerbated by the massive impacts on fish distributions predicted by Portugal's plan to build 10 new hydroelectric dams (Paterson *et al.*, 2008). These are topics for future research (e.g. reanalysing our dataset under the assumption that all rare species go extinct) since a proper understanding of the role of these factors can be useful for predicting and mitigating the effects on biological assemblages. However, despite the future likelihood of pervasive anthropogenic change, our main conclusion remains intact; i.e. isolation of basins in space and time provides a parsimonious explanation for today's geographical patterns of variation in freshwater fish assemblages.

Here, we point out as a promising topic for future research the relative importance of possible dispersal pathways created by climate-driven sea-level fluctuations in the past as a model for understanding the dispersal consequences of sea-level fluctuations associated with current climate change. In addition, explicitly linking our approach to phylogeographical data might provide missing historical information that would help to understand current regional patterns of assemblage composition (Brown & Lomolino, 2000). As a way to strengthen hypothesis testing, additional efforts to quantify uncertainties should be made, such as the adequacy of typical fish sampling efforts (particularly concerning rare species; see Cao *et al.*, 2002) and the influence of using land-based grid cells for understanding patterns of freshwater biodiversity.

CONCLUSIONS

The long-term basin boundaries of the Iberian Peninsula (since the Pliocene–Pleistocene) represent major constraints on current fish assemblage composition. In other words, current climate does not need to be invoked to explain existing biogeographical patterns. As expected, peripheral freshwater species, which frequently inhabit saltwater, are less constrained by basin divides than primary or secondary species. Our analyses also revealed that permeability varies considerably among river basins, most likely due to intermittent basin connectivity in the past. Further investigation is needed to clarify the patterns of basin permeability. We also showed that basins are appropriate units of analysis for delimiting biogeographical provinces for Iberian fishes, since basins integrate the shared evolutionary history and current hydrological connectivity of freshwater assemblages.

ACKNOWLEDGEMENTS

We are grateful to all who helped with field collections and those who facilitated data gathering, particularly Leonor Rogado at the ICN (Portuguese Nature Conservation Institute). We are also grateful to Pedro Segurado for GIS help. A.F.F, P.L.A. and M.J.C.-P. were supported by the Portuguese Foundation for Science and Technology (SFRH/BD/10330/2002 to A.F.F.). M.B.A. was supported by the IBERIA GAP project (CGL2006-10196, Spanish Ministry of Education and Science). The Synthesis Project (http://www.synthesys.info/) partially supported this research (under the European Community Research Infrastructure Action of the FP6 'Structuring the European Research Area' Programme). The Virginia Cooperative Fish and Wildlife Research Unit is jointly sponsored by the US Geological Survey, Virginia Polytechnic Institute and State University, Virginia Department of Game and Inland Fisheries, and Wildlife Management Institute. The Portuguese Forest Resources Service (DGRF) provided the necessary fishing permits.

REFERENCES

Abell, R., Thieme, M.L., Revenga, C., Bryer, M.T., Kottelat, M., Botutskaya, N.G., Coad, B., Mandrak, N.E., Contreras Balderas, S., Bussing, W., Stiassny, M.L.J., Skelton, P.H., Allen, G.R., Unmack, P.J., Naseka, A.M., Ng, R., Sindorf, N., Robertson, J., Armijo, E., Higgins, J.V., Heibel, T.J., Wikramanayake, E.D., Olson, D., López, H.L., Reis, R.E., Lundberg, J.G., Sabaj Pérez, M.H. & Petry, P. (2008) Freshwater ecoregions of the world: a new map of biogeographic units for freshwater biodiversity conservation. *BioScience*, **58,** 403–414.

Almaça, C. (1978) Répartition géographique des Cyprinidae ibériques et secteurs ichthyogéographiques de la Péninsule Ibérique. *Vestník Ceskoslovenské Spolecnosti Zoologické*, **XLII,** 241–248.

Araújo, M.B. & Pearson, R.G. (2005) Equilibrium of species' distributions with climate. *Ecography*, **28,** 693–695.

Araújo, M.B., Densham, P.J. & Williams, P.H. (2004) Representing species in reserves from patterns of assemblage diversity. *Journal of Biogeography*, **31,** 1037–1050.

Araújo, M.B., Nogués Bravo, D., Diniz-Filho, J.A.F., Haywood, A.M., Valdes, P.J. & Rahbek, C. (2008) Quaternary climate changes explain diversity among reptiles and amphibians. *Ecography*, **31**, 8–15.

Arévalo, C. (1929) *La vida en las aguas dulces*. Labor, Barcelona.

Bonada, N., Zamora-Munoz, C., Rieradevall, M. & Prat, N. (2005) Ecological and historical filters constraining spatial caddisfly distribution in Mediterranean rivers. *Freshwater Biology*, **50**, 781–797.

Bonnet, E. & Van de Peer, Y. (2002) Zt: a software tool for simple and partial Mantel tests. *Journal of Statistical Software*, **7**, 1–12.

Bray, J.R. & Curtis, J.T. (1957) An ordination of the upland forest communities of Southern Wisconsin. *Ecological Monographs*, **27**, 325–349.

Brown, J.H. & Lomolino, M.V. (2000) Concluding remarks: historical perspective and the future of island biogeography theory. *Global Ecology and Biogeography*, **9**, 87–92.

Burgmer, H., Hillebrand, H. & Pfenninger, M. (2007) Effects of climate-driven temperature changes on the diversity of freshwater macroinvertebrates. *Oecologia*, **151**, 93–103.

Burridge, C.P., Craw, D. & Waters, J.M. (2007) An empirical test of freshwater vicariance via river capture. *Molecular Ecology*, **16**, 1883–1895.

Calvo, J.P., Daams, R., Morales, J., López-Martínez, N., Augustí, J., Anadon, P., Armenteros, I., Cabrera, L., Civis, J., Corrochano, A., Díaz-Molina, M., Elizaga, E., Hoyos, M., Martín-Suárez, E., Martínez, J., Moissenet, E., Muñoz, A., Pérez-García, A., Pérez-González, A., Portero, J., Robles, F., Santistebas, C., Torres, T., Van Der Meulen, A.J., Vera, J.A. & Mein, P. (1993) Up-to-date Spanish continental Neogene synthesis and paleoclimatic interpretation. *Revista de la Sociedad Geológica de España*, **6**, 29–40.

Cao, Y., Larsen, D.P., Hughes, R.M., Angermeier, P.L. & Patton, T.M. (2002) Sampling effort affects multivariate comparisons of stream assemblages. *Journal of the North American Benthological Society*, **21**, 701–714.

Carmona, J.A., Doadrio, I., Márquez, A.L., Real, R., Hugueny, B. & Vargas, J.M. (1999) Distribution patterns of indigenous freshwater fishes in the Tagus River basin, Spain. *Environmental Biology of Fishes*, **54**, 371–387.

Carmona, J.A., Olivero, J., Doadrio, I., Real, R., Márquez, A.L. & Vargas, J.M. (2002) Testing for inter-drainage connections on the basis of the distribution pattern of endemic freshwater. *Archives of Hydrobiology*, **150**, 101–116.

CGIAR-CSI (2005) *Digital elevation data (DEM) for the entire globe of Shuttle Radar Topographic Mission (SRTM) 90m vs. 2*. Consultative Group for International Agricultural Research, Consortium for Spatial Information. Available at: http://srtm.csi.cgiar.org/ (last accessed 12 January 2008).

Chust, G., Pérez-Haase, A., Chave, J. & Pretus, J.Ll. (2006) Floristic patterns and plant traits of Mediterranean communities in fragmented habitats. *Journal of Biogeography*, **33**, 1235–1245.

Clarke, K.R. (1993) Non parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, **18**, 117–143.

Clarke, K.R. & Gorley, R.N. (2001) *PRIMER (vs 5.2.0): user manual/tutorial*. PRIMER-E, Plymouth.

Clarke, K.R. & Warwick, R.M. (1994) *Change in marine communities: an approach to statistical analysis and interpretation*. Natural Environment Research Council, Plymouth.

Clavero, M. & García-Berthou, E. (2006) Homogenization dynamics and introduction routes of invasive freshwater fish in the Iberian Peninsula. *Ecological Applications*, **16**, 2313–2324.

Darlington, P.J. (1957) *Zoogeography: the geographical distribution of animals*. Wiley, New York.

Davis, A.L.V. & Scholtz, C.H. (2001) Historical vs. ecological factors influencing global patterns of scarabaeine dung beetle diversity. *Diversity and Distributions*, **7**, 161–174.

Doadrio, I. (1988) Delimitation of areas in the Iberian Peninsula on the basis of freshwater fishes. *Bonner Zoologische Beitrage*, **39**, 113–138.

Doadrio, I. (ed.) (2002) *Atlas y Libro Rojo de los Peces Continentales de España*. Dirección General de Conservación de la Naturaleza. Museo Nacional de Ciencias Naturales, Madrid.

Doadrio, I. & Carmona, J.A. (2003) Testing freshwater Lago Mare dispersal theory on the phylogeny relationships of Iberian cyprinid genera *Chondrostoma* and *Squalius* (Cypriniformes, Cyprinidae). *Graellsia*, **59**, 457–473.

Doadrio, I. & Carmona, J.A. (2004) Phylogenetic relationships and biogeography of the genus *Chondrostoma* inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, **33**, 802–815.

Doadrio, I. & Elvira, B. (2007) A new species of the genus *Achondrostoma* Robalo, Almada, Levy & Doadrio, 2007 (Actynopterigii, Cyprinidae) from western Spain. *Graellsia*, **63**, 295–304.

Doadrio, I., Perea, S. & Alonso, F. (2007) A new species of the genus *Squalius* Bonaparte, 1837 (Actinopterygii, Cyprinidae) from the Tagus River Basin (Central Spain). *Graellsia*, **63**, 89–100.

Espanhol, R., Almeida, P.R. & Alves, M.J. (2007) Evolutionary history of lamprey paired species *Lampetra fluviatilis* (L.) and *Lampetra planeri* (Bloch) as inferred from mitochondrial DNA variation. *Molecular Ecology*, **16**, 1909–1924.

ESRI (2001) *ArcMap version 9.1*. Environmental Systems Research Institute, Redlands, CA.

ESRI (2005) *Arc Hydro distribution package version 1.1*. Environmental Systems Research Institute, Redlands, CA. Available at: http://www.crwr.utexas.edu/ (last accessed 6 July 2008).

Filipe, A.F., Cowx, I.G. & Collares-Pereira, M.J. (2002) Spatial modelling of freshwater fish in semi-arid river systems: a tool for conservation. *River Research and Applications*, **18**, 123–136.

Fuller, R.M., Devereux, B.J., Gillings, S., Hill, R.A. & Amable, G.S. (2007) Bird distributions relative to remotely sensed habitats in Great Britain: towards a framework for national modeling. *Journal of Environmental Management*, **84**, 586–605.

Gante, H.F., Santos, C.D. & Alves, M.J. (2007) A new species of *Chondrostoma* Agassiz, 1832 (Cypriniformes: Cyprinidae) with sexual dimorphism from the lower Rio Tejo Basin, Portugal. *Zootaxa*, **1616**, 23–35.

García-Barros, E., Gurrea, P., Luciáñez, M.J., Cano, J.M., Munguira, M.L., Moreno, J.C., Sainz, H., Sanz, M.J. & Simon, C. (2002) Parsimony analysis of endemicity and its application to animal and plant geographical distributions in the Ibero-Balearic region (western Mediterranean). *Journal of Biogeography*, **29**, 109–124.

Griffiths, D. (2006) Pattern and process in the ecological biogeography of European freshwater fish. *Journal of Animal Ecology*, **75**, 734–751.

Hernando, J.A. (1990) Ictiofauna del río Guadalquivir: características y perspectivas. *Avances en el conocimiento y gestion del medio ambiente de Córdoba* (ed. by A. López and J.M. Recio), pp. 35–62. Liberia, Andaluzia.

Hernando, J.A. & Soriguer, M.C. (1992) Biogeography of the freshwater fish of the Iberian Peninsula. *Limnética*, **8**, 243–253.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A.M. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978.

Hijmans, R.J., Cameron, S.E. & Parra, J.L. (2007) *WorldClim version 1.4*. Museum of Vertebrate Zoology, University of California, Berkeley. Available at: http://www.worldclim.org/ (last accessed 6 July 2008).

Hocutt, C.H. & Wiley, E.O. (eds) (1986) *The zoogeography of North American freshwater fishes*. John Wiley and Sons, New York.

Hoeinghaus, D.J., Winemiller, K.O. & Birnbaum, J.S. (2007) Local and regional determinants of stream fish assemblage structure: inferences based on taxonomic vs. functional groups. *Journal of Biogeography*, **34**, 324–338.

Hubert, N. & Renno, J.-F. (2006) Historical biogeography of South American freshwater fishes. *Journal of Biogeography*, **33**, 1414–1436.

Hugueny, B. (1990) Geographic range of west African freshwater fishes: role of biological characteristics and stochastic processes. *Acta Oecologica*, **11**, 351–375.

Hugueny, B. & Lévêque, C. (1994) Freshwater fish zoogeography in West Africa: faunal similarities between river basins. *Environmental Biology of Fishes*, **39**, 365–380.

Hutchinson, G.E. (1957) Concluding remarks. *Cold Spring Harbor Symposia on Quantitative Biology*, **22**, 415–427.

Ibrahim, K.M., West, N.E. & Goodwin, D.L. (1972) Phytosociological characteristics of perennial atriplex-dominated vegetation of southeastern Utah. *Plant Ecology*, **24**, 13–22.

Legendre, P. (1993) Spatial autocorrelation: trouble or new paradigm? *Ecology*, **74**, 1659–1673.

Lévêque, C., Oberdorff, T., Paugy, D., Stiassny, M.L.J. & Tedesco, P.A. (2008) Global diversity of fish (Pisces) in freshwater. *Hydrobiologia*, **595**, 545–567.

Lobo, J.M., Verdú, J.R. & Numa, C. (2006) Environmental and geographical factors affecting the Iberian distribution of flightless *Jekelius* species (Coleoptera: Geotrupidae). *Diversity and Distributions*, **12**, 179–188.

López-Martínez, N. (1989) Tendencias en plaeobiogeografía: el futuro de la biogeografía del pasado. *Paleontología* (ed. by E. Aguirre), pp. 271–299. CSIC, Madrid.

Lozano, L. (1952) *Los peces fluviales de España*. Ministerio Agricultura, Madrid.

Lucas, M.C., Baras, E., Thom, T.J., Duncan, A. & Slavik, O. (2001) *Migration of freshwater fishes*. Blackwell Science, Oxford.

Machordom, A. & Doadrio, I. (2001) Evidence of a Cenozoic Betic–Kabilian connection cased on freshwater fish phylogeography (*Luciobarbus*, Cyprinidae). *Molecular Phylogenetics and Evolution*, **18**, 252–263.

Magalhães, M.F., Batalha, D.C. & Collares-Pereira, M.J. (2002) Gradients in stream fish assemblages across a Mediterranean landscape: contributions of environmental factors and spatial structure. *Freshwater Biology*, **47**, 1015–1031.

Márquez, A.L., Real, R. & Vargas, J.M. (2001) Methods for comparison of biotic regionalizations: the case of pteridophytes in the Iberian Peninsula. *Ecography*, **1**, 659–670.

Matthews, W.J. (1998) *Patterns in freshwater fish ecology*. Chapman and Hall, New York.

McGlashan, D.J. & Hughes, J.M. (2001) Genetic evidence for historical continuity between populations of the Australian freshwater fish *Craterocephalus stercusmuscarum* (Atherinidae) east and west of the Great Dividing Range. *Journal of Fish Biology*, **59**, 55–67.

Mesquita, N., Hanfling, B., Carvalho, G.R. & Coelho, M.M. (2005) Phylogeography of the cyprinid *Squalius aradensis* and implications for conservation of the endemic freshwater fauna of southern Portugal. *Molecular Ecology*, **14**, 1939–1954.

Mesquita, N., Cunha, C., Carvalho, G.R. & Coelho, M.M. (2007) Comparative phylogeography of endemic cyprinids in the south-west Iberian Peninsula: evidence for a new ichthyogeographic area. *Journal of Fish Biology*, **71**, 45–75.

Newall, P.R. & Magnuson, J.J. (1999) The importance of ecoregion versus drainage area on fish distributions in the St. Croix River and its Wisconsin tributaries. *Environmental Biology of Fishes*, **55**, 245–254.

Olden, J.D. (2006) Biotic homogenization: a new research agenda for conservation biogeography. *Journal of Biogeography*, **33**, 2037–2039.

Paterson, J.S., Araújo, M.B., Berry, P.M., Piper, J.M. & Rounsevell, M.D.A. (2008) Mitigation, adaptation and the threat to biodiversity. *Conservation Biology*, **22**, 1352–1355.

Procheş, Ş. (2005) The world's biogeographical regions: cluster analyses based on bat distributions. *Journal of Biogeography*, **32**, 607–614.

Rabeni, C.F. & Doisy, K.F. (2000) Correspondence of stream benthic invertebrate assemblages to regional classification schemes in Missouri. *Journal of the North American Benthological Society*, **19**, 419–428.

Reyjol, Y., Hugueny, B., Pont, D., Bianco, P.G., Beier, U., Caiola, N., Casals, F., Cowx, I.G., Economou, A., Ferreira, M.T., Haidvogl, G., Noble, R., Sostoa, A., Vigneron, T. & Virbickas, T. (2007) Patterns in species richness and endemism of European freshwater fish. *Global Ecology and Biogeography*, **16**, 65–75.

Rivas-Martínez, F. (1987) Introduction: nociones sobre fitosociología, biogeografía y bioclimatología. *La vegetation de España* (ed. by M. Peinado and S. Rivas-Martínez), pp. 18–25. Universidad de Alcalá de Henares, Madrid.

Robalo, J.I., Santos, C.S., Almada, V.C. & Doadrio, I. (2006) Paleobiogeography of two Iberian endemic cyprinid fishes (*Chondrostoma arcasii – Chondrostoma macrolepidotus*) inferred from mitochondrial DNA sequence data. *Journal of Heredity*, **97**, 143–149.

Rodrigues, A.S.L. & Brooks, T.M. (2007) Shortcuts for biodiversity conservation planning: the effectiveness of surrogates. *Annual Review of Ecology, Evolution, and Systematics*, **38**, 713–737.

Rogado, L., Alexandrino, P.J., Almeida, P.R., Alves, M.J., Bochechas, J., Cortes, R.V., Domingos, M.I., Filipe, A.F., Madeira, J. & Magalhães, M.F. (2005) Peixes dulciaquícolas e migradores. *Livro Vermelho dos Vertebrados de Portugal* (ed. by M.J. Cabral, J. Almeida, P.R. Almeida, T. Dellinger, N. Ferrand de Almeida, M.E. Oliveira, J.M. Palmeirim, A.I. Queirós, L. Rogado and M. Santos-Reis), pp. 63–114. Instituto da Conservação da Natureza, Lisbon.

Smith, S.A. & Bermingham, E. (2005) The biogeography of lower Mesoamerican freshwater fishes. *Journal of Biogeography*, **32**, 1835–1854.

Smith, K.G. & Darwall, W.R.T. (2006) *The status and distribution of freshwater fish endemic to the Mediterranean Basin.* IUCN, Gland, Switzerland and Cambridge. Available at: http://data.iucn.org/dbtw-wpd/edocs/2006-005.pdf.

Smith, E.P., Pontasch, K.W. & Carns, J., Jr (1990) Community similarity and the analysis of multispecies environmental data: a unified statistical approach. *Water Research*, **24**, 507–514.

Sneath, P.H.A. & Sokal, R.R. (1973) *Numerical taxonomy*. W. H. Freeman and Company, San Francisco.

Soriguer, M.C., Vallespín, C., Gomez-Cama, C. & Hernando, J.A. (2005) Age, diet, growth and reproduction of a population of *Cobitis paludica* (de Buen, 1930) in the Palancar Stream (southwest of Europe, Spain) (Pisces: Cobitidae). *Hydrobiologia*, **436**, 51–58.

Sousa-Santos, C., Collares-Pereira, M.J. & Almada, V. (2007) Reading the history of a hybrid fish complex from its molecular record. *Molecular Phylogenetics and Evolution*, **45**, 981–996.

SPSS Inc. (2006) *SPSS 15.0 for Windows*. SPSS Inc., Chicago.

Stickle, J.V. & Hughes, R.M. (2000) Classification strengths of ecoregions, catchments and geographic clusters for aquatic vertebrates in Oregon. *Journal of the North American Benthological Society*, **19**, 370–384.

Svenning, J.C. & Skov, F. (2005) The relative roles of environment and history as controls of tree species composition and richness in Europe. *Journal of Biogeography*, **32**, 1019–1033.

Tedesco, P.A., Oberdorff, T., Lasso, C.A., Zapata, M. & Hugueny, B. (2005) Evidence of history in explaining diversity patterns in tropical riverine fish. *Journal of Biogeography*, **32**, 1899–1907.

Vargas, J.M., Real, R. & Guerrero, J.C. (1998) Biogeographical regions of the Iberian Peninsula based on freshwater fish and amphibian distributions. *Ecography*, **21**, 371–382.

Waters, J.M. & Wallis, G.P. (2000) Across the southern Alps by river capture? Freshwater fish phylogeography in South Island, New Zealand. *Molecular Ecology*, **9**, 1577–1582.

Wells, F., Metzeling, L. & Newall, P. (2002) Macroinvertebrate regionalization for use in the management of aquatic ecosystems in Victoria, Australia. *Environmental Monitoring and Assessment*, **74**, 271–294.

Whittaker, R.H. (1975) *Communities and ecosystems*. Macmillan Publishing, New York.

Wilkinson, C.D. & Edds, D.R. (2001) Spatial pattern and environmental correlates of a Midwestern stream fish community: including spatial autocorrelation as a factor in community analyses. *The American Midland Naturalist*, **146**, 271–289.

Yap, S.Y. (2002) On the distributional patterns of Southeast–East Asian freshwater fish and their history. *Journal of Biogeography*, **29**, 1187–1199.

Zardoya, R. & Doadrio, I. (1999) Molecular evidence on the evolutionary and biogeographical patterns of European Cyprinids. *Journal of Molecular Evolution*, **49**, 227–237.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Freshwater fish species analysed for the Iberian Peninsula and classified according to Darlington (1957).
Appendix S2 Contemporary climate variables examined as potential correlates of fish distributions.
Appendix S3 Contemporary climate zones (of 35 predefined clusters) that maximize the correlation with occurrences of the four species groups.

BIOSKETCH

This study is part of **Ana Filipa Filipe**'s PhD project, focused on understanding the patterns and processes of freshwater fish distribution and diversity in the Iberian Peninsula (http://ffishgul.fc.ul.pt). Her main research interests encompass macroecology, in particular freshwater biodiversity and its relation with the environment, past events and anthropogenic stressors.

Author contributions: A.F.F., M.B.A., P.L.A. and M.J.C-P. conceived the ideas; A.F.F. and I.D. gathered the data; A.F.F. produced the database; A.F.F. and M.B.A. analysed the data; A.F.F. led the writing with inputs from the remaining authors.

Editor: Robert McDowall

Supporting Information available online

Appendix S1 – Freshwater fish species analysed for the Iberian Peninsula and classified according to Darlington (1957): P – primary; S – secondary; Ph – peripheral (A - anadromous; C – catadromous; M – amphidromous). Note that the following species, despite occurring in the Iberian Peninsula, were not considered: (1) *Barbus steindachneri* because of current data incongruence. Despite being recorded by Portuguese researchers in the international Tagus and Guadiana river basins, it has not been recorded in Spain. Further, all its recorded localities (in 20 x 20-km grid cells) overlap with at least one other species with high morphological resemblance (*B. sclateri* and *B. comizo*); (2) *Tinca tinca* because the distribution had been strongly modified by human activities; (3) *Salmo trutta* because both the populations structure and range size had been altered via widespread stocking from central and northern Europe; and (4) *Mugil cephalus*, *Liza aurata*, and *Dicentrarchus labrax* due to deficient sampling efforts. Regarding all species, known translocated populations were excluded during data analyses (indicated below by superscripts).

Species	Author	Darlington Classification
Acipenseridae		
Acipenser sturio	Linnaeus, 1758	Ph, A
Anguillidae		
Anguilla anguilla	(Linnaeus, 1758)	Ph,C
Atherinidae		
Atherina boyeri	Risso, 1810	Ph, M
Balitoridae		
Barbatula quignardi 1	(Băcescu-Mester, 1967)	P
Blenniidae		
Salaria fluviatilis	(Asso, 1801)	S
Clupeidae		
Alosa alosa	(Linnaeus, 1758)	Ph, A
Alosa fallax	(Lacepède, 1803)	Ph, A
Cobitidae		
Cobitis calderoni	Bacescu, 1962	P
Cobitis paludica	(de Buen, 1930)	P
Cobitis vettonica	Doadrio & Perdices, 1997	P
Cottidae		
Cottus gobio	Linnaeus, 1758	S
Cyprinidae		
Achondrostoma arcasii	(Steindachner, 1866)	P
Anaecypris hispanica	(Steindachner, 1866)	P
Achondrostoma occidentale	(Robalo, Almada, Sousa-Santos, Moreira & Doadrio, 2005)	P
Achondrostoma oligolepis	(Robalo, Doadrio, Almada, & Kottelat 2005)	P
Achondrostoma salamantinum	Doadrio & Elvira 2007	P
Barbus bocagei	Steindachner, 1864	P
Barbus comizo	Steindachner, 1864	P
Barbus graellsii	Steindachner, 1866	P
Barbus guiraonis	Steindachner, 1866	P
Barbus haasi	Mertens, 1925	P
Barbus meridionalis	Risso, 1827	P
Barbus microcephalus	Almaça, 1967	P
Barbus sclateri	Gunther, 1868	P
Gobio lozanoi 2	Doadrio & Madeira 2004	P

Species	Author	Darlington Classification
Iberochondrostoma almacai	(Coelho, Mesquita & Collares-Pereira 2005)	P
Iberochondrostoma lemmingii	(Steindachner, 1866)	P
Iberochondrostoma lusitanicum	(Collares-Pereira, 1980)	P
Iberochondrostoma olisiponensis	(Gante, Santos & Alves, 2007)	P
Iberochondrostoma oretanum	(Doadrio & Carmona, 2003)	P
Parachondrostoma arrigonis	(Steindachner, 1866)	P
Parachondrostoma miegii	(Steindachner, 1866)	P
Parachondrostoma turiense	(Elvira, 1987)	P
Phoxinus bigerrei	Kottelat, 2007	P
Pseudochondrostoma duriense	Coelho, 1985	P
Pseudochondrostoma polylepis 3	(Steindachner, 1864)	P
Pseudochondrostoma willkommii	(Steindachner, 1866)	P
Squalius alburnoides complex	(Steindachner, 1866)	P
Squalius aradensis	(Coelho, Botutskaya, Rodrigues & Collares-Pereira, 1998)	P
Squalius carolitertii	(Doadrio, 1988)	P
Squalius castellanus	Doadrio, Perea & Alonso, 2007	P
Squalius laietanus	Doadrio, Kottelat & de Sostoa, 2007	P
Squalius malacitanus	Doadrio & Carmona, 2006	P
Squalius palaciosi	(Doadrio, 1980)	P
Squalius pyrenaicus 4	(Gunter, 1868)	P
Squalius torgalensis	(Coelho, Bogutskaya, Rodrigues & Collares-Pereira, 1998)	P
Squalius valentinus	Doadrio & Carmona, 2006	P
Cyprinodontidae		
Aphanius baeticus	Doadrio, Carmona & Fernández-Delgado, 2002	S
Aphanius iberus	(Valenciennes, 1846)	S
Gasterosteidae		
Gasterosteus gymnurus	Cuvier, 1829	Ph, M
Mugilidae		
Chelon labrosus	(Risso, 1827)	Ph, C
Liza ramada	(Risso, 1810)	Ph, C
Petromyzontidae		
Lampetra fluviatilis	(Linnaeus, 1758)	Ph, A.
and *Lampetra planeri* *	(Bloch, 1784)	
Petromyzon marinus	Linnaeus, 1758	Ph, A.
Pleuronectidae		
Platichthys flesus	(Linnaeus, 1758)	Ph,C
Salmonidae		
Salmo salar	Linnaeus, 1758	Ph, A.
Valenciidae		
Valencia hispanica	(Valenciennes, 1846)	S
Syngnathidae		
Syngnathus abaster	Risso, 1827	Ph, M

1 Translocated to Douro basin (Leon, Orbigo River; Doadrio, 2002); 2 Translocated to basins south of Douro and Ebro basins (Doadrio & Madeira, 2004); 3 Translocated to Júcar and Segura basins (Doadrio, 2002); 4 Translocated to downstream freshwaters of Ebro Basin (I. Doadrio pers. comm.).

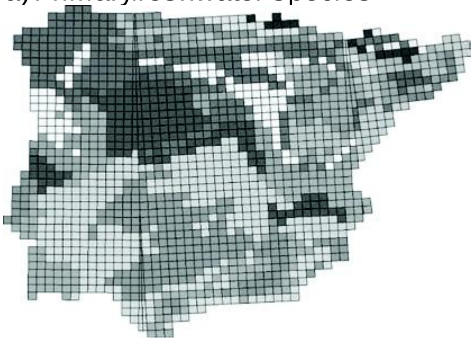
Appendix S2 - Contemporary climate variables examined as potential correlates of fish distributions.

Variables	Units
Annual mean temperature	degrees-C x 10
Mean diurnal range (MDR)1	degrees-C x 10
Isothermality (ISO)2	unitless
Temperature seasonality (TSE)3	degrees-C x 10
Maximum temperature of warmest month (MAT)	degrees-C x 10
Minimum temperature of coldest month (MIT)	degrees-C x 10
Temperature annual range (TAR) 4	degrees-C x 10
Mean temperature of wettest quarter	degrees-C x 10
Mean temperature of driest quarter	degrees-C x 10
Mean temperature of warmest quarter	degrees-C x 10
Mean temperature of coldest quarter	degrees-C x 10
Annual precipitation	mm
Precipitation of wettest month	mm
Precipitation of driest month	mm
Precipitation seasonality (PSE)	unitless
Precipitation of wettest quarter	mm
Precipitation of driest quarter	mm
Precipitation of warmest quarter	mm
Precipitation of coldest quarter	mm

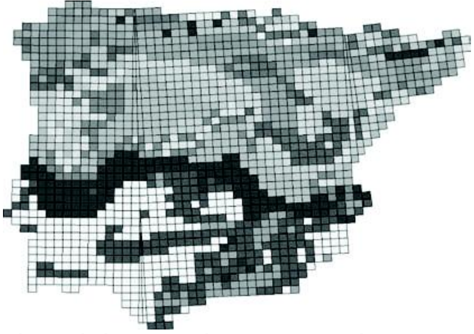
MDR=mean of monthly (max temp - min temp); 2 ISO=[(MDR)/TAR] x 100; 3 TSE=standard deviation x 100; 4 TAR=MAT - MIT; PSE=coefficient of variation.

Appendix S3 – Contemporary climate zones (of 35 predefined clusters) that maximize the correlation with occurrences of the four species groups.

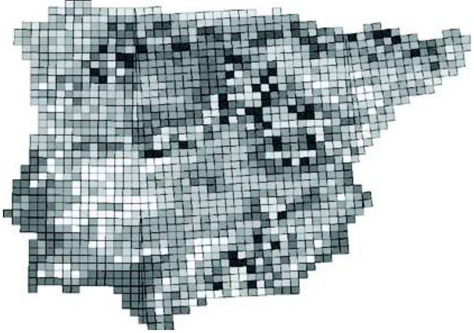
a) Primary freshwater species



b) Secondary freshwater species



c) Peripheral freshwater species



d) All freshwater species

Chapter 4.

Local richness of fish species in the south-western Iberian basins

"... for we should never forget that to range widely implies not only the power of crossing barriers, but the more important powers of being victorious in distant lands in the struggle for life with foreign associates."

Charles Darwin, 1859, *in* "On the Origin of Species by means of Natural Selection"

Filipe, A.F., Magalhães, M.F. & Collares-Pereira, M.J. (Submitted). Native and invasive fish species richness in Mediterranean streams: the role of multiple landscape influences. *Diversity and Distributions.*

Submitted as Biodiversity Research

Native and invasive fish species richness in Mediterranean streams: the role of multiple landscape influences

Ana F. Filipe[1*], M. Filomena Magalhães[1], Maria João Collares-Pereira[1]

[1]Universidade de Lisboa, Faculdade de Ciências, Centro de Biologia Ambiental, Campo Grande, Lisboa, Portugal

Abstract

Aim

To examine the role of multiple landscape factors on the species richness patterns of native and invasive freshwater fish.

Location

Mediterranean streams in the south-western Iberian Peninsula (c. 87,000 km^2).

Methods

We used a compiled dataset of fish occurrences from 436 stream sampling sites. We quantified the incremental explanatory power of multiple landscape factors in native, invasive and overall local species richness using regression analysis procedures. First, we related variation in local species richness across basins to regional species richness (here corresponding to the basin species pool), area, and factors of climate and topography. Second, we related within-basin variation in local species richness to site’s climate and topographic characteristics, and spatial structure derived using PCNM coordinates, after testing for species richness autocorrelation along the stream network; predicted local richness were finally mapped for each species group.

Results

Patterns of local species richness across basins showed no relation to basin area, but were strongly associated with regional species richness for overall, native and invasive species; at this scale, annual rainfall, showed a significant incremental contribution to variation in invasive species richness only. Within-basins, environmental factors were associated with local richness for all species groups, though their contributions to the total explained variation were inferior to those of spatial structure; rainfall seasonality and stream slope were the most consistent environmental correlates for all three species groups, while the influence of spatial structure was most prevalent for native species.

Main Conclusions

Landscape factors operating among and within basins seem to play a relevant role in shaping local species richness of both native and invasive species, through the regional species pools’ ‘filtering’, and may be contingent on basin-specific contexts. Nevertheless, local factors such as habitat characteristics and biotic interactions, and human-induced disturbances on habitat and introduction of invasive species may also be at play. Multiscale approaches incorporating a multitude of factors are strongly encouraged to facilitate a deeper understanding of the biodiversity patterns of Mediterranean streams, and to promote more effective conservation and management strategies that inhibit current biotic homogenization.

Keywords: Environmental factors, fish assemblages, invasive species, Mediterranean streams, multiscale, regional species richness, spatial structure.

Running title: Fishes richness in Mediterranean streams

Introduction

Understanding the processes that shape spatial variation in species diversity remains a challenging issue in community ecology (Brown, 1995; Lawton, 2000). Some emphasis has been given to the view that multiple processes interact to influence patterns of species diversity at different scales (Gaston & Blackburn, 2000). Specifically, the richness of local assemblages may depend on contemporary factors such as landscape context, habitat characteristics, and local biotic interactions, which act as environmental hierarchical 'filters' over the potential colonizers of a region (i.e., on the regional species pool; Poff, 1997); however, the extent to which these influences may limit species diversity and generate differences in local assembly organization remains poorly understood for many biota and ecosystems.

The unprecedented current risk of faunal homogenization due to the spread of invasive species worldwide prompts a quest for better understanding and distinguishing factors shaping diversity gradients in native and invasive species (Olden & Rooney, 2006). In particular, it is important to evaluate the role of species pools and contemporary landscape 'filters' on shaping local native and invasive species richness, and the extent to which other processes may be acting, as for instance, human influences on invasive species from the introduction to the establishment steps (Olden & Poff, 2003; Wilson *et al*., 2009).

Fish inhabiting Mediterranean river basins in the Iberian Peninsula seems an ideal study case for analysing the interplays between multiple processes in shaping local species richness of native and invasive species, because i) basins are well defined and isolated from each other since the Pliocene-Pleistocene (2.5-1.8 Ma; Calvo *et al*., 1993), ii) stream environments are highly variable over a broad range of spatial and temporal scales (Gasith & Resh, 1999), and their heterogeneity seems to strongly influence assembly organization of fish (Magalhães *et al*., 2002; Filipe *et al*., 2004; Mesquita *et al*., 2006), iii) the region is one of the world hotspots of biodiversity for fish, holding several endemic and steadily declining species with very restricted distributions (Smith & Darwall, 2005), and iv) this fauna faces important conservation threats, as the increasing spread and establishment of invasive species may seriously impact population and assemblage dynamics, and the structure and function of the ecosystem (e.g. Cowx & Collares-Pereira, 2000; Ribeiro *et al.*, 2009). Nevertheless, multiscale assessments on the influence of a multitude of factors shaping species diversity patterns in Mediterranean streams remain poorly understood (but see Bonada *et al*., 2008).

Analysing the shape and strength of the relationships between local species richness and the regional species pool has been traditionally used to draw inferences on whether assemblages are saturated or not, with linearity indicating that species in the region constitute the primary limiting factor to local richness, and curvilinearity indicating saturation and prevalence of biotic interactions (Cornell, 1985; Ricklefs, 1987; Gering & Crist, 2007). The utility of such approach to infer whether assemblages are prone to invasion has been questioned (e.g. Hillebrand & Blenckner, 2002), though it remains an interesting approach for examining the contribution of regional influences to local species richness and ultimately for unveiling the key biotic processes involved throughout ecological history (Srivastava, 1999; He *et al*., 2005). In this context, and for faunas constrained by stream basin’s boundaries, basins usually match regions (e.g., Angermeier & Winston, 1998; Irtz *et al*., 2004).

Moreover, quantifying the environmental influences from climate and topography on local species richness might be useful to explain the variation in local diversity gradients across large scale extents (e.g. Rahbek & Graves, 2001; Whittaker *et al*., 2001), and probably reflects species responses to contemporary prevailing conditions, by mechanisms as physiological tolerance and niche coexistence (Hutchinson, 1959; Chesson, 2000). In Mediterranean streams, climate and topography have been often related to fish assemblage structure as they characterize aridity and stream size and location (Filipe *et al*., 2004; Magalhães *et al*., 2002).

Finally, the region’s area has been advanced as an essential factor to include in analysis of species diversity patterns (e.g. Kadmon & Allouche, 2007), as it can be a surrogate of other factors favouring the co-occurrence of large number of species (Rosenzweig, 1995; Storch & Gaston, 2004). Particularly, species-area relationships may be related to habitat size and immigration and extinction dynamics (MacArthur & Wilson, 1967), and habitat heterogeneity and niche diversification (Williamson, 1988), each to varying degrees (for stream fish see Angermeier & Shlosser, 1989).

Here we examine the influence of landscape factors (i.e. regional species pool, basin area, and environmental climate and topography factors) on the geographic patterns of diversity of native and invasive fish inhabiting the Mediterranean river basins in south-western Iberian Peninsula. We adopted a regression approach to quantify the added contribution of each set of factors to variation in local species richness. Spatial structure was also considered in the analysis to account for eventual trends in local species richness resulting from unmeasured and spatially structured abiotic and biotic factors (Borcard & Legendre, 1994). Specific goals of our work are to: i) quantify multiple factors associated with variation in local species richness, ii) examine how these associations vary with geographical extent and between native and invasive species, and iii) describe and map spatial patterns in species diversity. Finally, we consider some management options to promote native diversity and inhibit biotic homogenization in Mediterranean streams.

Methods

Study area

The study was conducted in the south-western region of Iberian Peninsula at the 23 independent river basins including the Lower Guadiana (thereafter Guadiana), Sorraia, Sado, Mira, Stº. Estevão, Arade, and other small coastal basins (Fig. 1; Table 1). The orography of the region is dominated by the mountain chains of São Mamede (1,025 m high) and Monchique (902 m), and the elevations of Ossa (653 m) and Caldeirão (580 m). The climate is typically Mediterranean, with 80% of the annual rain occurring between October–March and virtually none falling during the hot months of July-September (Miranda et al., 2002). The mean annual temperature varies between 12.5 and 15.0 ºC near the coast, rising above 17.5 ºC farther inland (APA 1984). Generally, the mean annual rainfall decreases southward, and towards inland; the wetter areas correspond to the mountaintops of Monchique (1,400 mm) and S. Mamede (1,000 mm), whereas minimal rain (< 400 mm) fall in the Guadiana basin. Stream flow regimes are highly dependent on rainfall, with large floods occurring in winter, and droughts prevailing in summer, and being the most severe near the coast and farther inland, respectively. Streams encompass two major infrastructures for water irrigation: the Santa Clara dam in the Mira basin operating since 1968, and the Alqueva dam in the Guadiana flooded in 2002.

Table 1 – Estimated regional species richness (Chao2) and completeness of inventories for overall (RSR-t), native (RSR-n), and invasive (RSR-i) fish species in river basins in south-western Iberian Peninsula. The area of each river basin is also presented.

Basins	Area (km^2)	RSR-t		RSR-n		RSR-i	
		Chao2	Compl	Chao2	Compl	Chao2	Compl
Alcantarilha	207	4.5	66.7	2.5	80.0	2.5	40.0
Aljezur	180	3.0	100.0	3.0	100.0	0.0	-
Alportel	230	5.5	90.9	5.5	90.9	0.0	-
Arade	860	9.0	100.0	6.0	100.0	3.0	100.0
Beliche	118	5.7	88.2	4.2	94.8	1.0	100.0
Carrapateira	96	4.0	100.0	3.0	100.0	1.0	100.0
Coina	97	4.8	84.2	3.3	92.3	1.0	100.0
Espinche	79	3.0	100.0	2.0	100.0	1.0	100.0
Foupana and Odeleite	771	9.0	100.0	8.0	100.0	1.0	100.0
Foz Arade	77	4.0	100.0	4.0	100.0	0.0	-
Foz Mira Norte	42	3.0	100.0	3.0	100.0	0.0	-
Guadiana	11,600	19.0	100.0	13.0	100.0	6.0	100.0
Junqueira	39	5.0	100.0	5.0	100.0	0.0	-
Marateca	425	3.3	90.1	2.7	75.2	1.0	100.0
Mira	1,480	9.0	100.0	6.0	100.0	3.0	100.0
Muge	697	9.0	88.9	5.2	96.9	3.0	100.0
Odiaxere	158	5.7	88.2	3.0	100.0	2.7	75.2
Quarteira	409	6.8	88.2	5.0	100.0	1.0	100.0
Sado	6,229	16.1	93.1	10.9	82.3	6.0	100.0
Sao Martinho	252	11.5	78.3	10.5	66.9	2.0	100.0
Seixe	257	5.0	100.0	4.0	100.0	1.0	100.0
Sorraia	6,322	15.2	98.8	9.0	100.0	6.5	92.9
Sto Estevao	1,385	14.1	92.2	7.0	100.0	6.0	100.0

Species dataset

Data on local occurrences of fish species were extracted from the database containing sites sampled between 1997 and 2008 described by Filipe *et al*. (2009). In detail, the dataset used was screened to encompass sites with single electrofishing samples (with a single pass in wadeable stream reaches, 300-600 V and 4-6 A, with pulsed direct current), in wetted areas ranging between 300 to 600 m^2, in fourth to seventh order stream reaches (*sensu* Strahler, 1964), and between October and June; sites are at least 500 m apart, and have minimal human impact. This way the dataset used contained information of species occurrences obtained with similar sampling efforts from stream habitats located at middle courses. The invasive species considered for analysis were the ones introduced prior to 1990 and currently established (Ribeiro *et al.*, 2009), thus assuring an effective basin species pool for proper testing (Srivastava, 1999). Overall, the dataset included 436 sites at 23 independent basins, with 15,369 fish records of 27 species (see Appendix 1 for species list); the number of sites per basin was roughly proportional to basin area, ranging from two to 143 (Table 1).

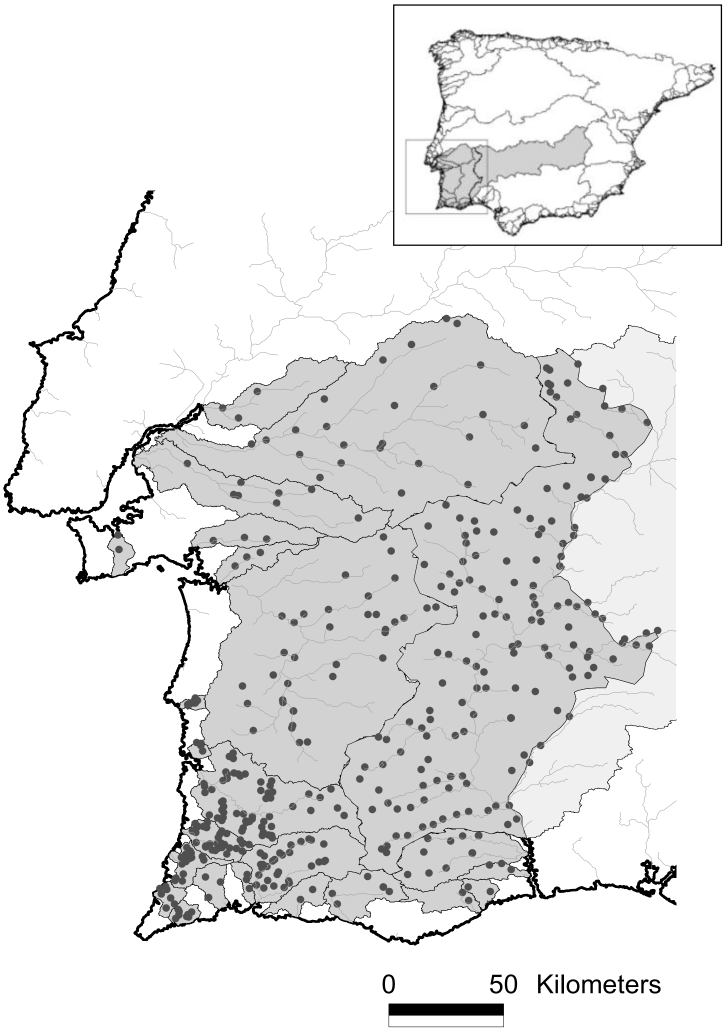


Figure 1 – The river basins considered for analysis and the 436 sites sampled for fish across the stream networks. The region comprises nearly 87,000 km2 and is located in south-western Iberian Peninsula (Europe).

Explanatory variables

The regional species richness pool was quantified for native, invasive, and all species. As for previous studies on stream biota (e.g., Angermeier & Winston, 1998; Irz *et al*., 2004), the regional species pool corresponds to the number of species in a river basin because it constitutes an independent regions *sensu* Srivastava (1999) due to their spatially nested nature (Frissell *et al*., 1986) and similar evolutionary histories among species (Filipe *et al*., 2009). For each basin, we computed the Chao2 estimator of absolute species richness (Chao, 1987; Walther & Morand, 1998) to reduce the effects of undetected rare species, resulting from variation in the number of sites and individuals sampled across basins, and interdependency of regional and local species richness datasets (Table 1). Chao2 is an asymptotical incidence-based richness estimator that has been highly recommended for datasets with few sampling records (Maiphae *et al.,* 2005), and poor species pools (Herzog *et al*., 2002). We calculated Chao2 estimates using the EstimateS vs. 8.0 (Colwell, 2005) and performing 50 randomized accumulation curves to avoid arbitrary effects (Colwell & Coddington, 1994). The completeness of each basin inventory was assessed by the ratio between the total number of species recorded and the Chao 2 estimate (Sobero *et al*., 2000).

Thirteen environmental variables were compiled for each site, using digital cartography with free internet access (Table 2). The selected climate and topographic variables reflect the extent of relative dryness of each site, and stream size and location, and have been associated with assemblage organization in Mediterranean fish (Filipe *et al.,* 2002, 2004; Magalhães *et al.,* 2002; Mesquita *et al*., 2006). Rainfall and temperature variables were described from 50-year data series (1950 to 2000), and interpolated at 1-km grid resolution (Hijmans *et al*., 2005, 2007). Topographical variables were derived from a Digital Terrain Model (DTM), with a 90-m grid cell resolution (CGIAR-CSI, 2005), using the ArcMap 9.1 Spatial Analysis and ArcHydro extensions (ESRI 2001, 2005). Specifically, we have: i) eliminated DEM's spurious sinks, ii) calculated flow accumulation, as the drainage area upstream of each cell, iii) defined the flow accumulation threshold that eliminates ephemeral stretches holding no fish (i.e. 150 cells, ~1.2 km^2), according with previous field surveys and satellite images (images with 1 m pixel grain of the Portuguese Forestry Authority (DGRF); see Maidment & Djokic, 2000), and iv) delineated drainage network surfaces using the flow accumulation threshold. All stream segments between confluences were vectorised and tagged with values of each environmental variable. We also tagged each stream segment with values of stream link, by assigning each segment with no tributaries as first order and summing the order of all upstream segments (Shreve, 1967), and stream slope, by using the percent variation in altitude between segment edges.

The area of each river basin was derived by summing the wetted area of all stream segments, from interpolations using the satellite images. Finally, the within-basin spatial structure was addressed from pairwise site distances along the stream network, using the PathMatrix Arcview 3.2 extension (Ray, 2005).

Table 2 – The environmental variables used to characterize sampling sites in river basins in south-western Iberian Peninsula. Variables in bold were retained for building multiple OLS linear regression models, after collinearity screening.

Variable	Units
Climate	
Annual Temperature (AMT)	degC*10
Temperature Seasonality (TES)	Coefficient of variation of monthly temperatures
Maximum Temperature of the Warmest Month (MAT)	degC*10
Minimum Temperature of the Coldest Month (MIT)	degC*10
Annual Rainfall (APR)	mm
Rainfall Seasonality (PRS)	Coefficient of Variation of monthly rainfall
Rainfall in the Wettest Month (PWM)	mm
Rainfall in the Driest Month (PDM)	mm
Topography	
Stream Link (STL)	
Distance Downstream (DDW)	m
Flow Accumulation measures (FAC)	Measured by drainage area upstream, km^2
Stream Elevation (SEL)	m
Stream Slope (SSL)	%

Data analysis

Analysis focused on quantifying variation in local richness for native, invasive and all species, independently, at two spatial scales: across river basins (N=23), and within selected basins (Guadiana and Mira, number of sites ≥60). Variation in local species richness was related to explanatory variables by ordinary least square (OLS) linear regressions, using a step-by-step procedure to quantify the increments of the variance explained by each set of explanatory variables. Here the entry of each explanatory variable or sets of variables was defined *a priori* (Quinn & Keogh 2002), in accordance with its inherent interdependence, and hierarchical scales (Schneider 2001).

Variation in local species richness across basins was related to basin area, regional species richness (i.e., total richness of basins), and environmental factors. Here local species richness per basin was derived as the average number of species recorded among sites, and the averages of climate and topographic variables for each basin were used. To take potential linear and curvilinear relationships between local and regional species richness into account, we evaluated both linear and quadratic terms of basin species richness.

Variation in local species richness within-basins was related to environmental and spatial variables. Prior to analysis, spatial autocorrelation of species richness in sites along the stream network was assessed using Moran' I correlograms (Legendre & Legendre 1998) by dividing the range of pairwise distances into 15 classes, with similar number of pairs, to assure that tests of significance have the same power across all distance classes (Legendre & Legendre 1998). The significance of Moran´s I coefficients was evaluated from Monte Carlo permutation tests using SAM vs. 2.0 software (Rangel *et al*. 2006). Spatial structure was then quantified using the Principal Coordinates of Neighbour Matrices Analysis approach (PCNM; Borcard & Legendre, 2002; Dray *et al*., 2006). The PCNM accounts for the spatial structure across a wide range of scales by producing a set of orthogonal spatial variables, based on linear distances, which are adequate for stream networks (e.g. Urban *et al*., 2006). Here PCNMs were built with SpacemakeR Package for R 2.3.1 (http://biomserv.univ-lyon1.fr/~dray/software.php;

(The R Team 2006), using the maximum pairwise stream distance within each basin as thresholds (Urban *et al*. 2006).

To improve the association between explanatory variables and each dependent variable, the former were transformed and tested in univariate regressions; transformed variables were then used in further analyses if resulted in regressions with higher adjusted coefficient of determination. Moreover, to reduce collinearity problems in OLS model building, Pearson's correlations (R) were computed between each pair of explanatory variables, dropping for further analysis the variable from each pair with $|R| > 0.60$ with the least association with the dependent variable in univariate analysis.

To produce parsimonious subsets of explanatory variables, selection of variables was carried within the sets of environmental and spatial variables based on the two-step forward selection procedure of Blanchet *et al*. (2008), thus preventing the potential shortcomings associated with classical stepwise selection methods: a preliminary fitting of a full model relating the dependent variable to all explanatory variables in the set was followed by a forward selection based on the alpha significance level only if the full model was statistically significant. The global fit of the OLS linear regression models was evaluated using the adjusted coefficient of multiple determination (adj-R^2). The Variance Inflation Factor (VIF) was used for assessing the multicolinearity of model coefficients (Quinn & Keogh, 2002). To illustrate the regression results for each basin, the fitted values of species richness for each site were mapped using the ArcGIS tool.

Results

Across basins richness patterns

The average local species richness per river basin ranged between 1.9 and 6.7 for all species, 1.4 and 6.2 for native species, and 0.9 and 2.7 for invasive species. Local species richness was not related to basin area whether species were considered altogether or whether analysis was restricted to native or to invasive species (Table 3). Conversely, local species richness was positively, linearly associated with the regional species richness for the three species groups (Fig. 2). The incremental contributions of regional species richness to explained variation in local richness was 0.593 and 0.639 for native and invasive species, respectively, being 0.605 for all species. Quadratic terms of regional species richness were not significant in any case, indicating that no asymptotes were reached in local richness for overall, native and invasive species. Moreover, similar trends were observed even if basin area was excluded from the analyses. Environmental variables showed a significant contribution to variation in local richness only for invasive species, with basins with higher-annual rainfall (APR) tending to hold higher numbers of invasive species. Taken together, explanatory variables accounted for large proportions of variation in local richness for all species groups (>0.600).

Table 3 – Summary of results of OLS regressions for variation in local richness (LSR) of all, native and invasive fish species across river basins in south-western Iberian Peninsula. In all cases, entry of explanatory variables in the models followed a predefined three-step sequence, whereby basin area (AREA) was entered first, regional species richness (RSR) second, and environmental variables third. Directions of association (+ or -), cumulative amounts of explained variation (Adj-R^2), increments of explained variation (AdjR^2-inc), Standard errors (Std. Error), and *P*-levels are given for each variable. The quadric term of regional species richness was not significant in any case.

Step	Variable	Adj. R^2	Std. Error	Adj.R^{2-}inc	*P*
All species					
1st	AREA (+)	0.022	1.160		0.235
2nd	RSR-t (+)	0.627	0.716	0.605	<0.0001
3rd	-				
Native species					
1st	AREA (+)	0.047	1.201		0.934
2nd	RSR-n (+)	0.686	0.658	0.639	<0.0001
3rd	-				
Invasive species					
1st	AREA (+)	0.123	0.611		0.084
2nd	RSR-i (+)	0.716	0.348	0.593	<0.0001
3rd	APR (+)	0.794	0.296	0.078	<0.0001

Within basins richness patterns

Local richness per site in the Guadiana ranged between 1 and 12 for overall species, 0 and 9 for native species, and 0 and 4 for invasive species. Local species richness per site in the Mira ranged between 1 to 6 species, 1 to 5 for native and 0 to 3 for invasive species. The highest positive autocorrelations in species richness occurred in the first distance classes for overall and native species in both basins, and invasive species in the Mira basin, decreasing for subsequent lags, and fluctuating up and down thereafter (Fig. 3).

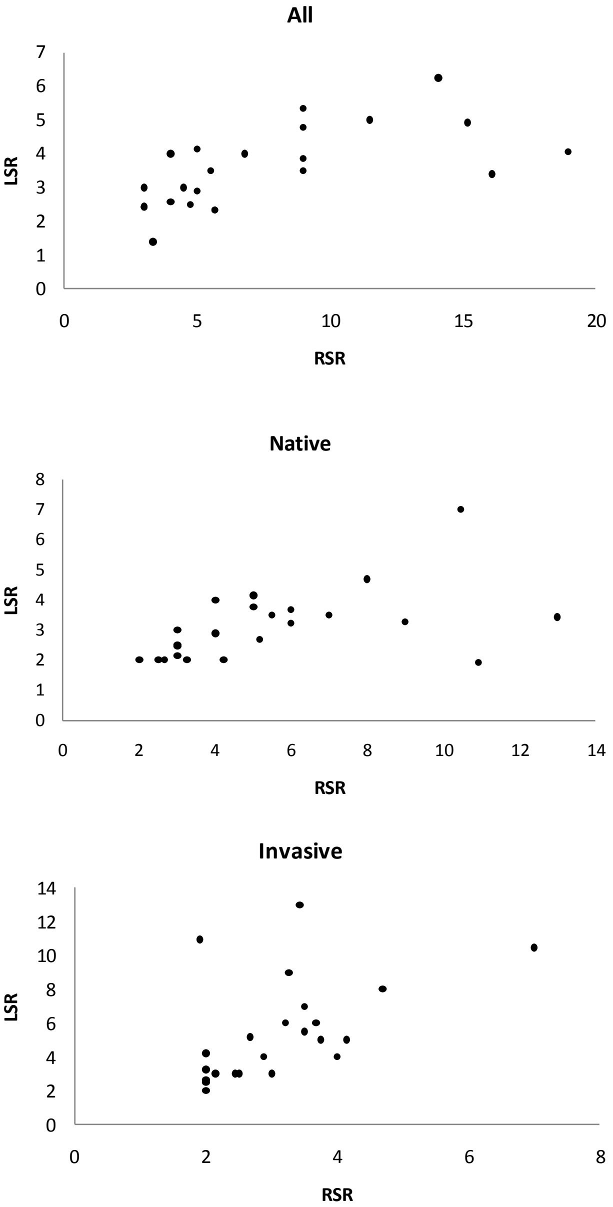


Figure 2 – Local species richness (average values of LSR) versus regional species richness (RSR; Chao2 estimates) for all, native and invasive fish species in south-western Iberian Peninsula.

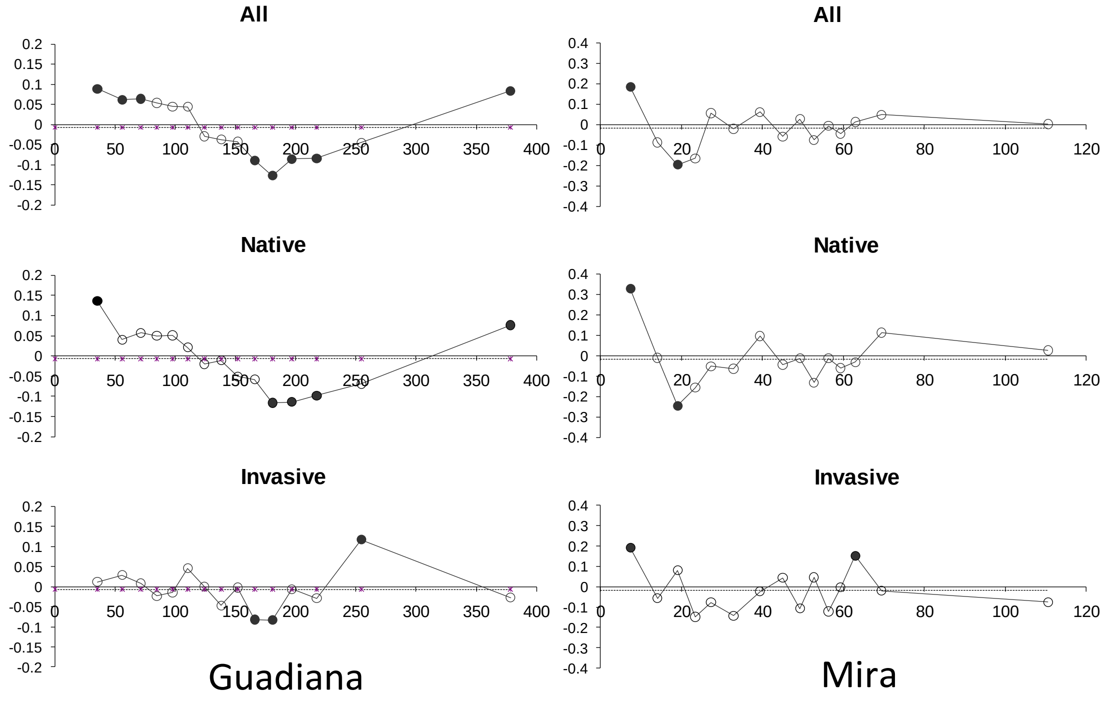


Figure 3 - Moran's I correlograms for local richness of all, native and invasive fish species in the Guadiana and Mira basins, using pairwise site distance along stream networks (km). Dark symbols indicate Moran'I is statistically significant (*P*<0.05). Positive significant values indicate positive autocorrelation, and thus that nearer sites hold more similar species richness than sites farther apart. Significant negative values have the opposite meaning.

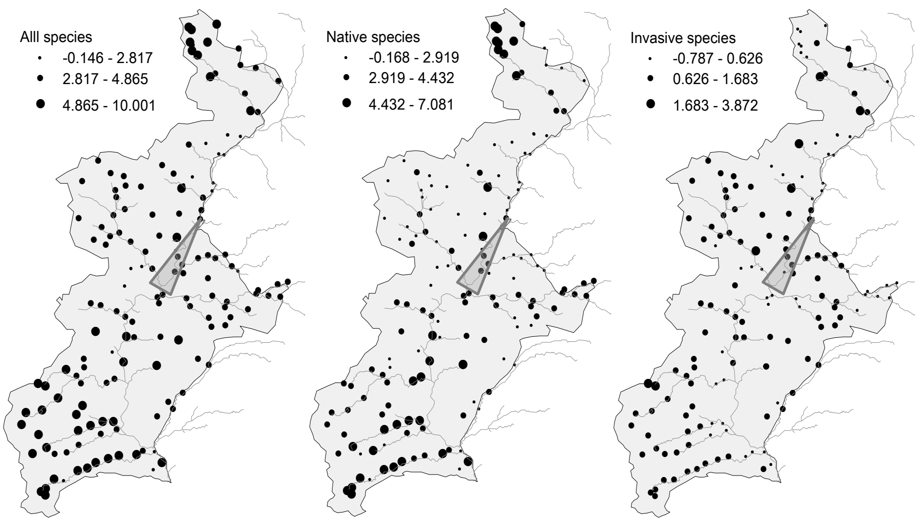
Environmental variables at each site showed significant associations with local richness of overall, native, and invasive species in both the Guadiana and the Mira basins (Table 4). Nevertheless, the proportion of variation in local richness accounted for by this set of variables was generally higher in the Mira basin, and for native species. In the Guadiana basin, the local richness of the three species groups increased with rainfall seasonality, with annual rainfall also showing positive associations with local richness of overall and native species. Stream slope was also significantly related to local richness for both native and invasive species, though the former increased and the later decreased in steeper sites (Table 4). Unlike the results of Guadiana basin, in the Mira basin climate variables were not significant correlates of local species richness. Local richness of overall species increased with stream link and distance downstream, whereas that of invasive species associated with the former factor only and that of native species tended to decrease in stepper sites. Spatial variables showed significant association with local species richness in both basins, for the three species groups, and accounted for superior amounts of explained variation than environmental variables for native and invasive species in the Guadiana basin and native species in the Mira basin.

Taken together, the sets of environmental and spatial variables explained a high proportion of variation in local richness of native species (>0.500) only in the Mira basin for all and native species groups (Table 4). The amounts of unexplained variation were always higher for invasive species. The maps of the fitted local richness showed no spatial discontinuities in the Guadiana basin, though there was a tendency for the northern and southern tributaries of the Guadiana holding the highest numbers of both native and invasive species (Fig. 4a). Conversely, in the Mira basin there were was a clear break in species richness distribution, with tributaries downstream the Santa Clara dam holding more native species and less invasive species than those upstream (Fig. 4b).

Table 4. Summary of results of OLS regressions for variation in local richness (LSR) of overall, native and invasive fish species among sites in the Guadina and Mira basins. In all cases, environmental variables were entered first and spatial variables second in the regression models. Directions of association of variables selected using forward procedures (+ or -), cumulative amounts of explained variation (Adj-R^2), increments of explained variation (AdjR^2-inc), Standard errors (Std. Error), and *P*-levels are given for each set of variables. See Table 2 for environmental variable codes. Prefix V designates spatial variables built in PCNM analyses; the prefix ln indicates that variables were ln-transformed prior to analysis.

Step	Variable	Adj.R^2	Std. error	Adj. R^2 inc	*P*
Guadiana					
All species					
1st	PRS (+), APR (+)	0.071	2.332		0.002
2nd	V_{52}(-),V_{24}(+),V_{53}(-),V_{4}(-),V_{54}(+),V_{67}(+),V_{29}(-),V_{9}(+),V_{35}(+),V_{61}(-)	0.330	1.980	0.259	<0.0001
Native species					
1st	SSL(+), APR(+), PRS(+)	0.140	1.847		<0.0001
2nd	V_{4}(-),V_{24}(+),V_{54}(+),V_{52}(-),V_{29}(-),V_{9}(+),V_{67}(+),V_{61}(-),V_{53}(-),V_{56}(+),V_{5}(+)	0.376	1.573	0.300	<0.0001
Invasive species					
1st	SSL(-),lnPRS(+)	0.052	0.997		0.009
2nd	V_{35}(+),V_{52}(-),V_{24}(+),V_{53}(-),V_{11}(+),V_{51}(-)	0.236	0.895	0.183	<0.0001
Mira					
All species					
1st	lnSTL(+),lnDDW(+)	0.340	0.864		<0.0001
2nd	V_{4}(-),V_{28}(-)	0.479	0.768	0.139	<0.0001
Native species					
1st	SSL(-)	0.153	0.956		0.004
2nd	V_{6}(-),V_{5}(+),V_{28}(-),V_{12}(+), V_{23}(+),V_{16}(-),V_{20}(-),V_{11}(-)	0.716	0.554	0.563	<0.0001
Invasive species					
1st	lnSTL	0.097	0.569		0.010
2nd	V_{3}(+)	0.172	0.544	0.075	0.002

a) Guadiana Basin



b) Mira Basin

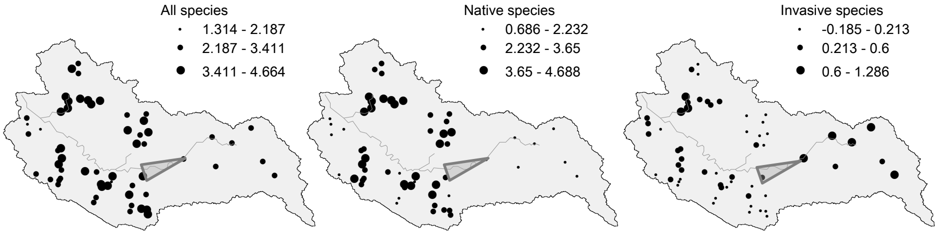


Figure 4 – Maps of the fitted local richness for all, native, and invasive fish species in the Guadiana and Mira basins. The Santa Clara Dam in the Mira Basin and the Alqueva Dam in the Guadiana Basin are represented by a triangle.

Discussion

The present study added support to the view that local species richness is a function of multiple factors operating at different spatial and temporal scales (e.g. Rahbek & Graves, 2001; Moreno-Rueda & Pizarro, 2009), as previously evidenced for other freshwater faunas (e.g. for fish, Griffiths, 1997; Angermeier & Winston, 1998; Irz *et al*., 2004; Hoeinghaus *et al*., 2007; e.g., for Mediterranean stream macroinvertebrates, Bonada *et al*., 2008). The south-western Iberian freshwater fish showed a considerable variation in diversity patterns between native and invasive species, both across and within Mediterranean river basins, suggesting distinct filtering processes are operating on each regional pool of species.

Across basins, the regional pool of species emerged as a strong correlate of local richness for the three species groups, pointing to the importance of large-scale, spatial and temporal processes, in shaping local diversity gradients (see Ricklefs, 1987). Moreover, the relationship between regional and local species richness was linear disregarding the species group considered, which is somehow similar to what has been found elsewhere (Hugueny & Paugy, 1995; Oberdorff *et al,.* 1998), and when only native species are considered (Angermeier & Winston 1998). Although it is difficult to disentangle the primary mechanisms underlining the associations found herein, they probably differ between the native and invasive species. In the case of native species the perceived relationship might reflect mechanisms acting across historical time, such as niche adaptation, large-scale dispersal and immigration-extinction events (see Filipe *et al*., 2009). This is certainly not the case for invasive species, for which the perceived relationship most likely reflect the recent processes of species establishment (Olden & Poff, 2004). Furthermore, the linearity found for both native and invasive groups suggests that all species might be at non-equilibrium with the local environments (Whittaker *et al*., 2001), with immigration-emigration and dispersal processes probably playing a major role throughout the ecological history of native species and more recently also of invasive ones, as previously suggested for Mediterranean streams (Magalhães *et al.* 2002).

Moreover, the lack of association between local species richness and basin area is in accordance with other studies on stream faunas (e.g. Angermeier & Winston, 1998; Heino *et al*., 2003). This may indicate that increased heterogeneity and availability of habitats at larger basins is not driving local species richness thus supporting the non-equilibrium idea mentioned above or, alternatively, that habitat availability and heterogeneity were not adequately represented by basin area. Whatever their ultimate causes, the results obtained strengthened the importance of testing within-basin species richness patterns against landscape heterogeneity as herein, but it is advisable to pursue a deeper evaluation of the influence of region size to understand properly its role (as Belkessam, *et al.,* 1997; Matthews & Robinson, 1998).

The tendency for basins with higher annual rainfall holding higher richness of invasive species, whereas no climatic or topographic factors were associated with local richness of native species, is consistent with previous evidences pointing to the importance of climate as a primary environmental filter for Mediterranean fish fauna (Filipe 2002; Magalhães *et al*. 2002, 2007). It is likely that regional pools of native species are constituted by the ones evolutionarily adapted to cope with the prevailing patterns of seasonal droughts and floods of variable intensity prevailing in Mediterranean streams. This is not the case for invasive species, which evolved under distinct environmental contexts, thus probably lacking the life-history attributes to cope with such harsh rainfall events (e.g. Bernardo *et al.,* 2003). This does not mean that contemporary climate is not important in shaping native species richness, but rather that climate may function as a filter at distinct scales for each species group.

Within basins, environmental factors at each site were important correlates of local richness for both native and invasive species. The significant topographic variables identified in this study, namely stream link and stream slope, were often implicated in longitudinal variation in fish assemblages (e.g., Corbacho & Sánchez, 2001; Filipe *et al.*, 2002, Magalhães *et al*. 2002), though perceived associations may depend on the basins examined. Likewise, climate variables were associated with local richness in the Guadiana basin only, and for native species mostly, which is not surprising given this basin encompass the broader range of climatic conditions and the largest diversity of species. Spatial structure also accounted for a significant component of variability in local species richness of native and invasive species. This may be a consequence of unmeasured, spatially structured environmental and/or geographic factors, and it may reflect colonization and dispersal processes throughout historical and current times, or even both. For example, the smaller species richness upstream of the Santa Clara Dam in the Mira basin suggests the loss of stream connectivity associated with dam building might have disrupted dispersal and immigration/extinction processes, likely provoking the extirpation of some native species upstream; additionally, biotic interactions might have been at play, given that local richness of invasive species tended to be higher upstream of the dam. A similar species richness discontinuity was not observed in Guadiana basin, since sampling at this basin was mostly performed before closing the Alqueva Dam, and also it was likely too early to detect any effects on fish richness gradients.

Considerable amounts of variation in local richness at the basin scale remained unexplained. This seems unlikely to be due to effects of sampling completeness since the fish assemblages studied tend to show low spatial turnover and similar proportional occurrences of both common and rare species (Filipe et al., 2002; Magalhães et al., 2002). Even if some biases occurred for some rare species, they probably were consistent across sites within basins, and are therefore unlikely to weaken the prevailing patterns observed. The unexplained variation was most severe regarding invasive species, for which future studies explicitly examining the influence of human colonization pressure, dispersal factors, and change of local natural flows and habitat conditions might be instructive (Wilson et al. 2009**).** Also, testing factors operating at smaller spatial and temporal scales may be important to advance the understanding of diversity patterns of both native and invasive species. Few studies presented clear evidences that local factors, as biotic interactions, do influence assemblage structure of fish, especially in Mediterranean streams. The absence of clear asymptotic relations of local species richness which regional pool of species in this study also argues against the importance of such factors, but their influence cannot be disregarded (see Smith et al., 2004). Assessing the added contribution of small-scale factors to assemblage organization is thus a worthy subject for future studies, which will be particularly insightful on untangling the mechanisms involved if coupled with field experiments designed to isolate the potential contributions of other competing factors, such as stochasticity trigged by human disturbances (see Corbacho & Sánchez, 2001).

Contributions to stream management options

Our results highlight that conservation of native species diversity and control of biotic homogenization in Mediterranean streams would gain effectiveness if strategies are framed in a basin-specific context. In particular we suggest that (i) preventing new introductions and the increase of regional pools of invasive species may be the most effective tool to prevent biotic homogenization in Mediterranean streams, and (ii) the wettest basins and streams are likely key areas for colonization, establishment and dispersal of non-native species, and may be most critical for conservation and management efforts.

The European Water Framework Directive (WFD) mandates every state-member to apply measures for accessing stream's ecological status at the basin scale, but, surprisingly, the monitoring schemes are most focused on local-scale assemblage assessments, largely neglecting larger-scale climatic and topographical contexts and stream spatial structure. This may be a major obstacle on attaining species and ecosystems conservation, as changes in stream network connectivity seriously affect diversity gradients by altering processes, as dispersal (Veech *et al*. 2002), even if the local conditions are maintained. Additionally, ecological consequences due to current climatic changes, as the expected shifts in the distribution ranges of native and invasive species and disruption of colonization and extinction rates in local assemblages (see Buisson & Grenoulliet, 2009) cannot be effectively evaluated only from local assessments. The adoption of a multiscale perspective (as evidenced by Hoeinghaus *et al*., 2007) in management actions within the WFD will facilitate the understanding of natural and human-induced processes shaping species diversity patterns and assembly organization, and will result in more effective water management and conservation practices.

Conclusions

We conclude that the number of fish species coexisting in Mediterranean streams is influenced by multiple factors operating over distinct scales, as early envisioned by Tonn *et al*. (1990) and Poff (1997). The influence of regional species pool was obvious for both native and invasive species but distinct environmental and spatial filters are at play on shaping local richness within basins for each species group. Nevertheless, the amount of variance unexplained leaves room to hypothesise factors acting locally and human-induced influences might have a relevant role in shaping current fish diversity patterns. Meanwhile conservation management strategies must be framed on a multiscale perspective to assure effectiveness.

Acknowledgements

This study is part of the doctoral project of Ana Filipa Filipe, funded by the Portuguese Science Foundation (FCT) through SFRH/BD/10330/2002 grant. We are grateful to the numerous biologists who contributed to data gathering, particularly to the members of the Freshwater Fish Group of the University of Lisbon (http://ffishgul.fc.ul.pt/members.html) and the persons involved in the Portuguese Red Data Book of Vertebrates published by the Institute for Nature

Conservation and Biodiversity (ICNB). Special thanks are due to Pedro Peres-Neto for assistance with the PCNM method. The authors are truly grateful to Paul Angermeier for insightful comments on an early version of the manuscript. The Portuguese Forestry Authority (DGRF) provided the necessary fishing permits and the visualization of stream aerial images.

Authors contributions

AFF gathered the data and produced the database. AFF and MFM performed data analyses. All authors contributed with species data records, conceived the ideas, and wrote the manuscript, which was lead by AFF.

Biosketches

Ana Filipa Filipe is a PhD student at the Sciences Faculty of Lisbon University, Centre of Environmental Biology. Her main research interests are focused on the processes controlling species diversity. Her current research project is focused on macroecology of freshwater fishes at Iberian Peninsula. Also she has interest on public awareness of nature conservation.

Maria Filomena Magalhães is an assistant professor at the Faculty of Sciences of the University of Lisbon and member of the Centre of Environmental Biology. Her main research interests are community ecology and conservation biology of stream fish.

Maria João Collares-Pereira is a professor at the Sciences Faculty of Lisbon University, and member of the Centre of Environmental Biology. Her research interests include life-history strategies and speciation pathways of Iberian fish.

Supporting information

Appendix S1 – List of all (native and invasive) fish species in river basins in south-western Iberian Peninsula considered for analysis.

Fish species list for river basins in south-western Iberian Peninsula, based on records obtained between 1997 and 2008. PF – primary freshwater species; SF – secondary freshwater species; CAT – catadromous species; AMP – amphidromous species; N – native species, I – invasive species.

Family	Species	Author	Origin	Life cycle
Anguillidae				
	Anguilla anguilla	(Linnaeus, 1758)	N	SF, CAT
Atherinidae				
	Atherina boyeri	Risso, 1810	N	SF, AMP
Blenniidae				
	Salaria fluviatilis	(Vinyoles 1986)	N	PF
Cobitidae				
	Cobitis paludica	(de Buen, 1930)	N	PF
Cyprinidae				
	Anaecypris hispanica	(Steindachner, 1866)	N	PF
	Barbus bocagei	Steindachner, 1864	N	PF
	Barbus comizo	Steindachner, 1864	N	PF
	Barbus microcephalus	Almaça, 1967	N	PF
	Barbus sclateri	Gunther, 1868	N	PF
	Barbus steindachneri	Almaça, 1967	N	PF
	Carassius auratus	(Linnaeus, 1758)	I	PF
	Cyprinus carpio	Linnaeus, 1758	I	PF
	Gobio lozanoi	(Doadrio & Madeira 2004)	I	PF
	Iberochondrostoma almacai	(Coelho, Mesquita & Collares-Pereira 2005)	N	PF
	Iberochondrostoma lemmingii	(Steindachner, 1866)	N	PF
	Iberochondrostoma lusitanicum	(Collares-Pereira, 1980)	N	PF
	Pseudochondrostoma polylepis	(Steindachner, 1864)	N	PF
	Pseudochondrostoma willkommii	(Steindachner, 1866)	N	PF
	Squalius alburnoides	(Steindachner, 1866)	N	PF
	Squalius aradensis	(Coelho, Botutskaya,	N	PF

Family	Species	Author	Origin	Life cycle
		Rodrigues, & Collares-Pereira 1998)		
	Squalius pyrenaicus	(Gunter, 1868)	N	PF
	Squalius torgalensis	(Coelho, Bogutskaya, Rodrigues & Collares-Pereira, 1998)	N	PF
	Tinca tinca	(Linnaeus, 1758)	N	PF
Centrarchidae				
	Lepomis gibbosus	(Linnaeus,1758)	I	PF
	Micropterus salmoides	(Lacepède, 1802)	I	PF
Cichlidae				
	Australoheros facetum	(Jenyns, 1842)	I	PF
Poecillidae				
	Gambusia holbrooki	(Girard, 1859)	I	PF

Note: The species *Alosa alosa*, *Alosa fallax*, *Salmo trutta*, and *Gasterosteus aculeatus* were not considered for analysis due to incomplete sampling.

References

Angermeier P.L. & Schlosser I.J. (1989) Species-area relationships for stream fishes. *Ecology*, **70**, 1450-1462.

Angermeier,P.L. & Winston,M.R. (1998) Local vs. Regional Influences on Local Diversity in Stream Fish Communities of Virginia. *Ecology***, 79**, 911-927.

APA (1984) *Atlas do Ambiente Digital*. Agencia Portuguesa do Ambiente. Dataset from 1931 to 2003. Accessed 2009. http://www.iambiente.pt/atlas/est/index.jsp.

Belkessam, D., Oberdorff, T., & Hugueny, B. (1997) Unsatured fish assemblages in rivers of north-western France: potential consequences for species introductions. *Bulletin Français de la Pêche et de la Pisciculture,* **344/345**, 193-204.

Bernardo, J.M., Ilhéu, M., Matono, P., & Costa, A.M. (2003) Interannual variation of the fish assemblage structure in a mediterranean river: implications of streamflow on the dominance of native or exotic species. *River Research and Applications,* **19**, 521-532.

Blanchet, F.G., Legendre, P., & Borcard, D. (2008) Forward selection of explanatory variables. *Ecology,* **89**, 2623-2632.

Bonada, N., Rieradevall, M., Dallas, H., Davis, J., Day, J., & Figueroa, R. (2008) Multi-scale assessment of macroinvertebrate richness and composition in Mediterranean-climate rivers. *Freshwater Biology,* **53**, 772-788.

Borcard, D. & Legendre, P. (1994) Environmental control and spatial structure in ecological communities: an example using oribatid mices (Acari, Oribatei). *Environmental and Ecological Statistics*, **1**, 37-53.

Borcard, D., Legendre, P. (2002) All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. *Ecological Modelling*, **153**, 51-68.

Buisson, L. & Grenoulliet, G. (2009) Contrasted impacts of climate change on stream fish assemblages along an environmental gradient. *Diversity and Distributions*, **15**, 613-626.

Calvo, J.P., Daams, R., Morales, J., López-Martínez, N., Augustí, J., Anadon, P., Armenteros, I., Cabrera, L., Civis, J., Corrochano, A., Díaz-Molina, M., Elizaga, E., Hoyos, M., Martín-Suárez, E., Martínez, J., Moissenet, E., Muñoz, A., Pérez-García, A., Pírez-González, A., Portero, J., Robles, F., Santistebas, C., Torres, T., Van Der Meulen, A.J., Vera, J.A., & Mein, P. (1993) Up-to-date Spanish continental Neogene Synthesis and paleoclimatic interpretation. *Revista de la Sociedad Geológica de España,* **6**, 29-39.

Chao, A. (1987) Estimating the population size of capture-recapture data with unequal catchability. *Biometrics,* **43**, 783-791.

Chesson, P. (2000) Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics,* **31**, 343-366.

Colwell, R. K. *EstimateS: statistical estimation of species richness and shared species from samples. (Version 8.0)*. 2005. User's guide and application published at: http://purl.oclc.org/estimates, Department of Ecology & Evolutionary Biology, University of Connecticut.

Colwell, R.K., Coddington, J.A. (1994) Estimating terrestrial biodiversity through extrapolation. *Philosophical Transactions of the Royal Society of London B*, **345**, 101-118.

Corbacho, C., Sánchez, J.M. (2001) Patterns of species richness and introduced species in native freshwater fish faunas of a Mediterranean-type basin: The Guadiana River (Southwest Iberain Peninsula). *Regulated Rivers: Research & Management*, **17**, 699-707.

Cornell, H.V. (1985) Local and regional richness of cynipid gall wasps on California oaks. *Ecology*, **66**, 1247-1260.

Cornell, H.V. & Lawton, J.H. (1992) Species interactions, local and regional processes, and limits to the richness of ecological communities: a theoretical perspective. *Journal of Animal Ecology,* **61**, 1-12.

Cowx, I.G., Collares-Pereira, M.J. (2000) Conservation of endangered fish species in the face of water resource development schemes in the Guadiana River, Portugal: harmony of the incompatible. *Management and Ecology of River Fisheries* (ed. by M.J. Collares-Pereira, I.G. Cowx and M.M. Coelho). pp. 428-438. Fishing News Books, Blackwell Science, Oxford.

Davis, A.L.V., Scholtz, C.H., & Deschodt, C. (2008) Multi-scale determinants of dung beetle assemblage structure across abiotic gradients of the Kalahari-Nama Karoo ecotone, South Africa. *Journal of Biogeography,* **35**, 1465-1480.

Diniz-Filho, J.A.F., Bini, L.M., Hawkins, B.A. (2003) Spatial autocorrelation and red herrings in geographical ecology. *Global Ecology and Biogeography*, **12**, 53-64.

Dray, S., Legendre, P., Peres-Neto, P.R. (2006) Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). *Ecological Modelling*, **196**, 483-493.

ESRI (2001) ArcMap version 9.1. Environmental Systems Research Institute, Redlands, CA.

ESRI (2005) Arc Hydro distribution package version 1.1. Environmental Systems Research Institute, Redlands, CA. Available at: http://www.crwr.utexas.edu/ (last accessed 6 July 2008).

Filipe, A.F., Araújo, M.B., Doadrio, I., Angermeier, P.L., & Collares-Pereira, M.J. (2009) Biogeography of Iberian freshwater fishes revisited: the roles of historical versus contemporary constraints. *Journal of Biogeography,* **36**, 2096-2110.

Filipe, A.F., Cowx, I.G., & Collares-Pereira, M.J. (2002) Spatial modelling of freshwater fish in semi-arid river systems: a tool for conservation. *River Research and Applications,* **18**, 123-136.

Filipe, A.F., Marques, T., Seabra, S.G., Tiago, P., Ribeiro, F., Moreira da Costa, L., Cowx, I.G., & Collares-Pereira, M.J. (2004) Selection of priority areas for fish conservation in the Guadiana River Basin, Iberian Peninsula. *Conservation Biology,* **18**, 189-200.

Frissell, C.A., Liss, W.J., Warren, C.F., & Hurley, M.D. (1986) A hierarchical framework for stream habitat classification - viewing streams in a watershed context. *Environmental Management,* **10,** 199-214.

Gasith, A. & Resh, V.H. (1999) Streams in Mediterranean Climate Regions: abiotic influences and biotic responses to predictable seasonal events. *Annual Review of Ecology and Systematics,* **30**, 51-81.

Gaston, K.J. & Blackburn, T.M. (2000) *Pattern and process in macroecology.* Blackwell Science, Oxford.

Gering, J.C. & Crist, T.O. (2007) The alpha–beta–regional relationship: providing new insights into local–regional patterns of species richness and scale dependence of diversity components. *Ecology Letters*, **5**, 437-452.

GIAR-CSI (2005) *Digital elevation data (DEM) for the entire globe of Shuttle Radar Topographic Mission (SRTM) 90m vs. 2*. Consultative Group for International Agricultural Research, Consortium for Spatial Information. Available at: http://srtm.csi.cgiar.org/ (last accessed 12 January 2008).

Griffiths, D. (1997) Local and regional species richness in North American lacustrine fish. *Journal of Animal Ecology,* **66**, 49-56.

Griffiths, D. (1999) On investigating local-regional species richness relationships. *Journal of Animal Ecology,* **68**, 1051-1055.

He, F., Gaston, K.J., Connor, E.F., & Srivastava,D.S. (2005) The local-regional relationship: immigration, extinction and scale. *Ecology,* **86**, 360-365.

Heino, J., Muotka, T., & Paavola, R. (2003) Determinants of macroinvertebrate diversity in headwater streams: regional and local influences. *Journal of Animal Ecology,* **72**, 425-434.

Hijmans, R.J., Cameron, S.E. & Parra, J.L. (2007) WorldClim version 1.4. Museum of Vertebrate Zoology, University of California, Berkeley. Available at: http://www.worldclim.org/ (last accessed 6 July 2008).

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A.M. (2005) Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology, **25**, 1965–1978.

Hillebrand, H. & Blenckner, T. (2002) Regional and local impact on species diversity – from pattern to processes. *Oecologia,* **132**, 479-491.

Hoeinghaus D.J., Winemiller K.O., & Birnbaum S. (2006) Local and regional determinants of stream fish assemblage structure: inferences based on taxonomic vs. functional groups. Journal of Biogeography, **34**, 324-338.

Hugueny, B. & Paugy, D. (1995) Unsaturated Fish Communities in African Rivers. *The American Naturalist,* **146**, 162-169.

Hutchinson, G.E. (1959) Homage to Santa Rosalia, or why are so many kinds of animals? *The American Naturalist,* **XCIII**, 145-159.

Irz, P., Argillier, C., Oberdorff, T. (2004) Native and introduced fish species richness in French lakes: local and regional influences. *Global Ecology and Biogeography*, **13**, 335-344.

Kadmon, R. & Allouche, O. (2007) Integrating the Effects of Area, Isolation, and Habitat Heterogeneity on Species Diversity: A Unification of Island Biogeography and Niche Theory. *The American Naturalist,* **170**, 443-454.

Lawton, J.H. (2000) *Community ecology in a changing world*. Ecology Institute, Oldendorf, Luhe, Germany.

Legendre, P. & Legendre, L. (1998) *Numerical Ecology*, 2nd edn. Elsevier, Amsterdam.

MacArthur, R.H., Wilson, E.O. (1967) *The theory of island biogeography*, Princeton University Press, Princeton, N.J.

Magalhães, M.F., Batalha, D.C., & Collares-Pereira, M.J. (2002) Gradients in stream fish assemblages across a Mediterranean landscape: contributions of environmental factors and spatial structure. *Freshwater Biology,* **47**, 1015-1031.

Magalhães, M.F., Beja, P., Schlosser, I.J., Collares-Pereira, M.J. (2007) Effects of multi-year droughts on fish assemblages of seasonally drying Mediterranean streams. *Freshwater Biology*, **52**, 1494-1510.

Maidment, D., Djokic, D. (2000) *Hydrologic and Hydraulic Modelling Support with Geographic Information Systems*. ESRI Press, USA.

Maiphae, S., Pholpunthin, P., Dument, H.J. (2005) Species richness of the Cladocera (Branchiopoda: Anomopoda and Ctenopoda) in southern Thailand, and its complementarity with neighbouring regions. *Hydrobiologia*, **537**, 147-156.

Matthews, W.J. & Robinson, H.W. (1998) Influence of drainage connectivity, drainage area and regional species richness on fishes of the interior highlands of Arkansas. *American Midland Naturalist,* **139**, 1-19.

Mesquita, N., Coelho, M.M., & Magalhães, F. (2006) Spatial Variation in Fish Assemblages across small Mediterranean Drainages: Effects of Habitat and Landscape Context. *Environmental Biology of Fishes,* **77**, 105-120.

Miranda, P., Coelho, F.S., Tomé, A.R., Valente, M.A. (2002) 20th century Portuguese climate and climate scenarios. *Climate change in Portugal: scenarios, impacts and adaptation measures - SIAM project*, pp. 23-84, Gradiva, Lisboa, Portugal.

Moreno-Rueda, G. & Pizarro, M. (2009) Relative influence of habitat heterogeneity, climate, human disturbance, and spatial structure on vertebrate species richness in Spain. *Ecological Research,* **24**, 335.

Oberdorff, T., Hugueny, B., Compin, A., & Belkessam, D. (1998) Non-interactive fish communities in the coastal streams of North-western France. *Journal of Animal Ecology,* **67**, 472-484.

Olden, J.D. & Poff, N.L. (2003) Towards a mechanistic understanding and prediction of biotic homogenization. *American Naturalist,* **162**, 442-460.

Olden, J.D. & Poff, N.L. (2004) Clarifying biotic homogenization. *Trends in Ecology & Evolution,* **19**, 283-284.

Olden, J.D. & Rooney, T.P. (2006) On defining and quantifying biotic homogenization. *Global Ecology & Biogeography,* **15**, 113-120.

Poff, N.L. (1997) Landscape filters and species traits: towards mechanistic understanding and prediction in stream ecology. *Journal of the North American Benthological Society,* **16**, 391-409.

Quinn, G.P. & Keogh, J.S. (2002) *Experimental design and data analysis for biologists*. Cambridge University Press, Cambridge.

Rahbek, C. & Graves, G.R. (2001) Multiscale assessment of patterns of avian species richness. *Proceedings of the National Academy of Sciences USA,* **98**, 4534-4539.

Rangel, T.F.V.B., Diniz-Filho, J.A.F., & Bini, L.M. (2006) Towards an integrated computational tool for spatial analysis in macroecology and biogeography. *Global Ecology & Biogeography*, **15**, 321-327.

Ray, N. (2005) Pathmatrix: a geographical information system tool to compute effective distances among samples. *Molecular Ecology Notes*, **5**, 177-180.

Ribeiro, F., Collares-Pereira, M.J., & Moyle, P.B. (2009) Non-native fish in the fresh waters of Portugal, Azores and Madeira Islands: a growing threat to aquatic biodiversity. *Fisheries Management & Ecology*, **16**, 255-264.

Ricklefs, R.E. (1987) Community diversity: Relative roles of local and regional processes. *Science,* **235**, 167-171.

Rosenzweig, M.L. (1995) *Species diversity in space and time*. University Press, Cambridge.

Schneider, D.C. (2001) The rise of the concept of scale in ecology. *BioScience*, **51**, 553.

Shreve, R.L. (1967) Infinite topologically random channel networks. *Journal of Geology*, **75**, 178-186.

Shurin, J.B., Havel, J.E., Leibold, M.A., & Pinel-Alloul, B. (2000) Local and regional zooplankton species richness: a scale independent test for saturation. *Ecology,* **81**, 3062-3073.

Smith, S.A., Bell, G., & Bermingham, E. (2004) Cross-Cordillera exchange mediated by the Panama Canal increased the species richness of local freshwater fish assemblages. *Proceedings of the Royal Society of London B*, **271**, 1889-1896.

Smith, K.G. & Darwall, W.R.T. (2006) *The status and distribution of freshwater fish endemic to the Mediterranean Basin*. IUCN, Gland, Switzerland and Cambridge. Available at: http://data.iucn.org/dbtw-wpd/edocs/2006-005.pdf.

Sobero, J.M., Llorente, J.B., Oñate, L. (2000) The use of specimen-label databases for conservation purposes: an example using Mexican Papilionid and Pierid butterflies. *Biodiversity and Conservation*, **9**, 1441-1446.

SPSS Inc. (2005) *SPSS 15.0 for Windows*. SPSS Inc., Chicago.

Srivastava, D.S. (1999) Using local-regional richness plots to test for species saturation: pitfalls and potentials. *Journal of Animal Ecology*, **68**, 1-16.

Storch, D. & Gaston, K.J. (2004) Untangling ecological complexity on different scales of space and time. *Basic and Applied Ecology,* **5**, 389-400.

Strahler, A.N. (1964) Quantitative geomorphology of drainage basins and channel networks, Chow,V.T. (ed.), *Handbook of Applied Hydrology*, p. 4-39. McGraw-Hill, New York.

The R Team. 2006. R: A language and Environment. Vienna, Austria, R Foundation for Statistical Computing.

Tonn, W.M., Magnuson, J.J., Rask, M., & Toivonen, J. (1990) Intercontinental comparison of small-lake fish assemblages: the balance between local and regional processes. *The American Naturalist,* **136**, 345-375.

Urban, M.C., Skelly, D.K., Burchsted, D., Price, W., Lowry, S. (2006) Stream communities across a rural–urban landscape gradient. *Diversity and Distributions*, **12**, 337-350.

Veech, J.A., Summerville, K.S., Crist, T.O., & Gering, J.C. (2002) The additive partitioning of species diversity: recent revival of an old idea. *Oikos,* **99**, 3-9.

Walther, B.A. & Morand, S. (1998) Comparative performance of species richness estimation methods. *Parasitology,* **116**, 395-405.

Whittaker, R.J., Willis, K.J., & Field, R. (2001) Scale and species richness: towards a general, hierarchical theory of species diversity. *Journal of Biogeography,* **28**, 453-470.

Williamson, M.H. (1988) Relationship of species number to area, distance and other variables. *Analytical Biogeography* (ed. by A.A. Myersand and P.S. Guiller, pp. 91-115. Chapman and Hall, London.

Wilson, J.R.U., Dormontt, E.E., Prentis, P.J., Lowe, A.J., & Richrdson, D.M. (2009) Something in the way you move: dispersal pathways affect invasion success. *Trends in Ecology & Evolution*, **24**, 136-144.

Chapter 5.

Examining spatial patterns of freshwater fish

"Consider two extreme cases. In the first, the habitat is highly fragmented, the isolation has a long history, and dispersal among the patches is very infrequent. This scenario favours low diversity but a high degree of endemism in each patch. This extreme is approached by freshwater fishes. At the other extreme, the environment is much more continuous ..."

James H. Brown, 1995, in "Macroecology" pp 171

Filipe, A.F., Angermeier, P.L. & Collares-Pereira, M.J. (Submitted). Spatial gradients of species diversity in freshwater fishes: testing theory with empirical evidence. *Global Ecology and Biogeography*.

Submitted as a Research Review

Spatial gradients of species diversity in freshwater fishes: testing theory with empirical evidence

Ana F. Filipe[1*], Paul L. Angermeier[2], and Maria J. Collares-Pereira[1]

[1]Universidade de Lisboa, Faculdade de Ciências, Centro de Biologia Ambiental, Campo Grande, Lisboa, Portugal, [2]U.S. Geological Survey, Virginia Cooperative Fish and Wildlife Research Unit, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA.

Abstract

Aim

To integrate knowledge regarding spatial gradients in freshwater fish species richness and develop an innovative approach for future empirical tests; ultimately, we aim to advance the overall knowledge of species diversity gradients.

Location

Freshwater systems across the world.

Methods

Literature review aiming for a improve knowledge of biodiversity gradients, freshwater fish species in particular. Reviewing current knowledge of spatial gradients of freshwater fish species richness, identifying shortcomings in the empirical tests performed, and linking those shortcomings with a set of useful alternative tests constitutes the backbone of our approach.

Results

Studies of freshwater fishes added or subtracted support for the hypotheses proposed to explain species richness gradients found in nature. The three main hypotheses were examined, and the current environment-based hypothesis was both the most tested and the best supported. The role of historical factors might emerge from exploring patterns of freshwater fishes, particularly from testing groups of species with dissimilar dispersal constraints. When performing such empirical tests, researchers must be especially cautious regarding the following: a) uncertainty in species' spatial data, b) inaccuracy when merging data from distinct regions, species attributes and histories, c) concerns related to scale, d) the spatial structure of freshwater habitats, and e) the design of hypothesis testing. Studies discerning among species attributes and incorporating evolutionary approaches are currently producing interesting results.

Main Conclusions

Our synthesis included an integration of the empirical tests explaining factors regulating broad-scale spatial gradients of freshwater fish species richness and may provide new knowledge into the processes that shape biodiversity gradients in general. Freshwater fishes seemed appropriate for testing the role of historical factors while accounting for freshwater spatial structure. Studies focusing on species interactions, including field experiments, will advance on examining of the biotic mechanisms involved.

Keywords: Area, diversity gradient, environment, freshwater fish, history, species richness

Running title: Freshwater fish diversity gradients

Introduction

Recent advances in the analyses of geographic biodiversity gradients are resolving questions that have intrigued ecological and evolutionary biologists for decades. However, available evidence invoked to support competing hypotheses is typically biased toward terrestrial biota, which narrows the scientific debate more than would be desirable (Hawkins *et al*., 2003a; Menge, 2009). In regard to freshwater biota, namely fishes, this bias is mostly due to the lack of integration and synthesis of knowledge concerning biodiversity patterns found in nature. In the present study, we propose to address this issue by synthesising current knowledge of freshwater fish species richness gradients from the literature, then exploring how this knowledge contributes to the general understanding of biodiversity patterns.

The main advances in freshwater ecological research, as for terrestrial and marine ecosystems, derive from shifts in the scale of analyses: traditionally studies were focused on trends in local populations and habitat quality, but the 1990s brought a surge of new scientific papers which began to recognise that freshwater fish population persistence may depend on an array of processes operating at multiple spatial and temporal scales (e.g. Schlosser, 1991, 1995). These developments, along with a general increase in studies of other biota, resulted mostly from an enhanced availability of large-scale data on geographic information systems (GIS), computer processing improvements, and new analytical methods applicable to species distributions (Johnson & Gage, 1997). Apart from new developments in conservation and management research, such progress allowed testing ecological and evolutionary hypotheses of larger scales. When coupled with molecular markers, ecological data examined ecological and evolutionary processes acting at different levels of the biological hierarchy (e.g. Manel *et al*., 2003; Diniz-Filho *et al*., 2008).

Nevertheless, considerable room for a synthesis of knowledge concerning freshwater biodiversity patterns remains because new studies have been scattered across areas of interest, taxa, and study scales (Bailan *et al*., 2008a), which has limited the entry of freshwater knowledge into scientific debates. Freshwater fishes are well suited to test alternative hypotheses related to species diversity gradients, to infer the relative importance of the mechanisms involved, and ultimately to synthesise current knowledge of species diversity gradients (Tedesco *et al.*, 2005; Filipe *et al*., 2009); key strengths of analysing freshwater fishes include species 1) are well known worldwide, 2) vary widely in dispersal ability and life-histories, 3) segregate across major habitat types (e.g. lotic *vs.* lentic), 4) dispersal is strongly constrained by basin boundaries, and 5) dispersal pathways can often be inferred from geologic and phylogenetic histories.

Several hypotheses have been proposed to explain broad patterns of species diversity (Rohde, 1992; Gaston & Blackburn, 2000; Willig*, et al*., 2003; Hillebrand 2004), with latitudinal gradients of species richness - more species are generally found closer to the tropics - receiving the most attention. For decades, this species richness gradient has been documented for terrestrial, marine biota, and also for fresh waters (Barbour & Brown, 1974; Oberdorff *et al*., 1995). In freshwater environments, the relationship of species richness with latitude has been referred to as weaker and less steep than in other environments and dissimilar among continents and habitat types; this suggests that the mechanisms involved are somehow dissimilar to those acting in terrestrial and marine environments and biota (Hillebrand, 2004). The interest in examining this worldwide gradient lies in the fact that the mechanisms invoked to explain broad-scale gradients are also instructive in clarifying the mechanisms operating at smaller spatial scales (e.g. Gaston & Blackburn, 2000; Stevens, 2006); the reverse is also likely (e.g. Hawkins & Porter, 2003). For example, the ability to disperse seems to determine the species richness patterns observed for European freshwater fishes (Hof *et al*., 2008), which may partially explain the patterns observed both worldwide and in other regions.

Here we review the empirical studies performed on broad-scale freshwater fish species richness patterns. In particular, we (1) synthesise evidence from broad-scale freshwater fish studies that do or do not support the main hypotheses proposed to explain geographic species diversity gradients and their biotic mechanisms, (2) identify key shortcomings of the data and methods used, as well as possible research pathways to overcome them, and (3) ultimately offer contribitions into a better knowledge of the ecological and evolutionary processes that regulate freshwater fish diversity and species diversity in general. Finally, we aim to catalyse new research so that a more mature and cohesive knowledge of species diversity patterns emerge.

Background on species diversity gradients

Although hypotheses to explain broad-scale species diversity patterns have long been proposed, recent empirical tests on a range of taxa and spatial grains and extents are generating new ideas and re-kindling old debates. More than thirty specific hypotheses have been proposed to explain patterns of species diversity, and many of these hypotheses are closely related (Kerr, 2001). Here we focus on those supported by empirical evidence; we have grouped them according to the mechanisms involved and factors tested, as have Guégan *et al*. (1998) and others, into current environment-based, history-based, and area-based hypotheses (Table 1).

The first group of hypotheses asserts that contemporary environmental conditions drive existing patterns of species diversity (Brown, 1981; Huston, 1994) by operating directly on the physiology of organisms and/or indirectly on ecosystem productivity (Rohde, 1992; Whittaker *et al*., 2001; Hawkins *et al*., 2003b). This hypothesis is framed on niche characteristics, where functional distinctions among species, species interactions, and environmental preferences combine to determine the equilibrium species diversity in an assemblage (Hutchinson, 1959). A number of versions have been developed according to the factor tested (Rohde, 1992). The second general hypothesis asserts that historical factors prevail in shaping species diversity patterns, despite the persistent need for species to cope with environments (Rohde, 1992; Gaston & Blackburn, 2000). This hypothesis is consistent with current recognition of the role of the stochastic rates of speciation, extinction, and dispersal in assemblages throughout evolutionary time (Rohde, 1992; Currie *et al*., 2004; Wiens & Graham, 2007). The third general hypothesis is focused on extinction-colonisation rates and dispersal mechanisms occurring within an area with spatial characteristics (Rosenzweig, 1995). Area and its spatial structure drive species richness gradients via either (1) species-area relationships, where larger areas encompass more species due to favourable colonisation and extinction rates and/or higher habitat diversity (MacArthur, 1963) or (2) 'mid-domain effects', where geometric constraints on species ranges largely determine the observed species diversity patterns, as the observed peaks in the middle of bounded areas (Colwell & Lees, 2000).

Table 1 Main hypotheses and driving factors proposed to explain complex broad-scale geographic patterns in species richness. Most-cited references are included.

Hypothesis	Driving factors	References
Current environment-based hypothesis		
1) Climate	Current climate	Brown, 1981; Mittelbach *et al.*, 2001; Rohde, 1992
2) Energy	Energy availability. The greater the available energy, the more individual organisms, hence species, can be supported	(Wright, 1983; Wright *et al.*, 1993
3) Water-energy	Water regime and energy regime simultaneously. The breakpoint is related to species' physiological tolerances.	Hawkins *et al.*, 2003a, b, 2004
4) Environment stability	Environment stability. A constant environment allows specialization on narrower niches and a boost in speciation	Rohde, 1992;Turner, 1992
5) 'Metabolic effect'	Temperature dependence of metabolic enzyme kinetics. Annual temperature has effects on metabolic enzyme kinetics, and thus on mechanisms related to generation time and mutation rates related to individual-level rates of genetic divergence	Allen *et al.*, 2002, 2006
History-based hypothesis		
1) Speciation rate	Climate. The opportunity to evolutionary diversification arises from higher evolutionary rates and biotic interactions at warmer climates	Rohde, 1992; Currie *et al.*, 2004
2) 'Niche conservatism' and time for speciation	Species from ancient niches have difficulty in colonizing new areas.	Wiens & Donoghue, 2004
Area-based hypothesis		
1) Species-area	Larger areas can support more species	MacArthur & Wilson, 1963; Gaston & Blackburn, 2000; Ricleffs, 2004
2) 'Mid-domain effect'	Geometric constraints on species ranges	Colwell & Lees, 2000

The influence of the main driving factors on species richness gradients has been increasingly tested, though mostly in applications of the environment-based hypothesis over a range of taxa. Empirical tests of the environment-based hypothesis based on new data and analytical tools are revealing some inconsistencies with predictions: studies suggest environmental explanations of species diversity patterns (1) may indirectly focus on the mechanisms regulating current species diversity (Currie *et al.*, 2004) thus failing to demonstrate particular associations (as pointed out by Ricklefs (2004), (2) often do not properly test alternative factors on equal footing (Hawkins *et al.*, 2003b; but see Currie, 1991), and (3) apply mostly to species with large range sizes (Lennon *et al.*, 2004. The metabolic activity version of the hypothesis is not supported by some empirical data (e.g. Hawkins *et al.*, 2007a) and is not a valid prediction for non-ectothermic organisms at the least (Allen *et al.*, 2006).

Meanwhile, the historical hypothesis has been renewed by recent analyses providing evidence for the role of the ‘niche conservatism’ concept, i.e., the tendency of species to retain ancestral niches, and the time needed for speciation (Wiens & Donoghue, 2004; see also Wiens, 2004 and Wiens & Graham, 2007). Such ideas were rapidly tested at a range of spatial scales (e.g. Mittelbach *et al.*, 2007), and coupled with evolutionary approaches (e.g. Harrison & Grace, 2007; Hawkins *et al.*, 2007b; Roy & Goldberg, 2007; Wiens, 2007; Hawkins *et al.*, 2006). These studies represent great advances in hypothesis testing, since historical factors have traditionally been diminished because 1) of the difficulty of quantifying past patterns and events (Ricklefs, 2004) from scarce and/or spatially discontinuous data allowing merely *post* *hoc* inferences (e.g. Latham & Ricklefs, 1993), 2) when explicitly tested, they often exhibit weak signals, which might be the result of species tracking environmental conditions and thus shifting geographically over time (Currie, 1991; Kerr & Currie, 1999; Roy & Goldberg, 2007), and 3) of the greater availability and reliability of contemporary environmental data, which has lead to more robust predictions.

Proper assessments of the quantification of the influence of area are still awaiting more careful testing and interpretation. For example, the conventional approach of linearising species-area relations via log-transformation can obscure scale-dependent factors and consequently outcomes regarding species diversity patterns (Lomolino, 2000; Lomolino & Weiser, 2001). One analytical option is to first control for area, which might hide the influence of unaccounted factors co-varying with area, while revealing the influence of other direct drivers of biodiversity patterns (Whittaker *et al*., 2001). Also, empirical tests of the 'mid-domain effect' are able to predict species-richness patterns as the peaks in the middle of regions and the likely influence of spatial characteristics such as landscape barriers (e.g. Jetz & Rahbec, 2001), but outcomes in distinct scale extents are less clear because model assumptions restrict testing to spatially delimited taxa and regions (Whittaker *et al*., 2001).

In summary, reconciling the influences of history, current environment, and spatial structure on species diversity seems to be progressing (e.g. Storch *et al*., 2006); meanwhile, the environment-based hypothesis stands as being able to providing capable models to explain current global biodiversity patterns (Whittaker & Field, 2000).

Freshwater fish diversity patterns

In this section, we searched for scientific papers in the World Web of Science database to quantify the amount of studies on species diversity patterns conducted over the last 50 years on freshwater biota in general and fishes in particular. We compared this amount with the total amount of studies not restricted to any taxa. We then identified studies using larger scale extent datasets of freshwater fish that might shed light onto the three hypotheses previously presented. For each study, we identified the type of evidence, dispersal capability of the species studied (*sensu* Darlington, 1957), the factors most strongly correlated with species richness (hereafter called 'driving factor', similar to the 'primacy variable' of Hawkins (2003a)), habitats occupied, species dataset used, and scale of analysis (grain and extent) (Table 2).

Scientific literature on freshwater biota, particularly fishes, followed the growth of the number of studies for all biota with a lag delay of approximately two years, starting around year 1990 (Figure 1). The contemporary-environment hypothesis has been widely cited as determining freshwater fish diversity patterns among species groups with distinct dispersal capability, stream and lake ecosystems, and spatial scale extents from regional to global, often using basins or lakes as scale grains (Table 2). Environmental factors (and consequently versions of the hypothesis) have been tested worldwide, but we found it difficult to distinguish between versions of climate, species-energy, or water-energy balance. Here key driving factors identified were mean annual discharge, surface area of the drainage (Oberdorff *et al*., 1995), and net primary productivity (Guégan *et al*., 1998); climatic factors have also been highlighted, mostly across continents and regions (Oberdorff *et al*., 1997; Rathert *et al*., 1999). The 'metabolic-effect' version was also tested and recognised as significant (Allen *et al.*, 2002). In these studies, historical factors were also tested (except Guégan *et al.*, 1998), which suggests that it is not necessary to invoke history to understand patterns of freshwater fish diversity.

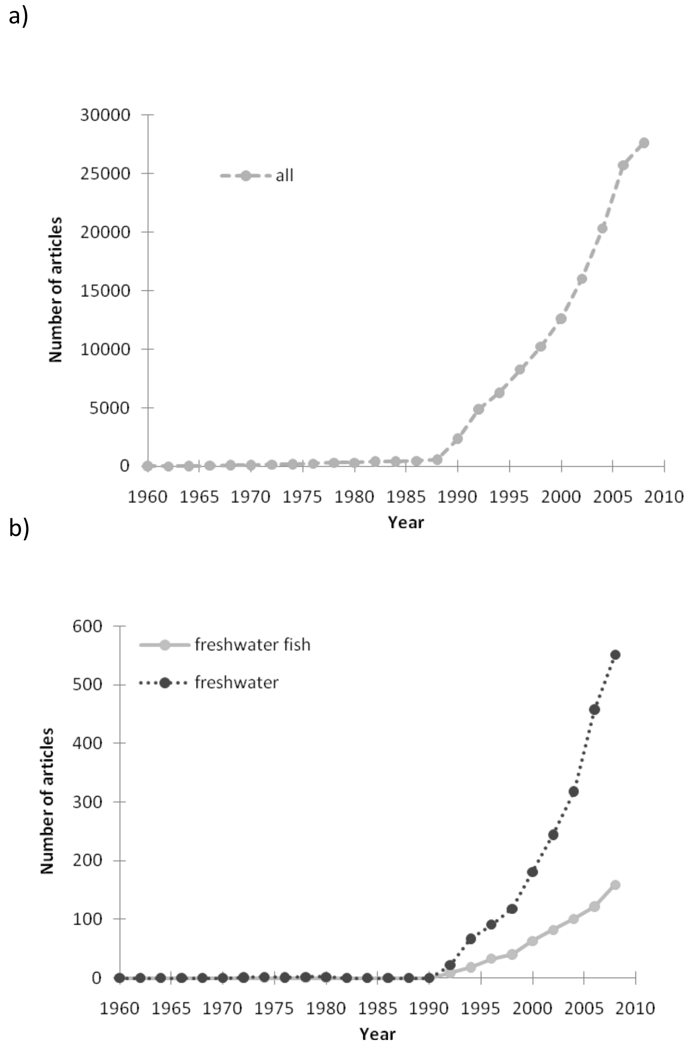


Figure 1 Number of scientific papers published each year in journals included in the Web of Science (http://scientific.thomson.com) from 1960 to 2009 that contained within their keyword lists both (a) either 'diversity' or 'biodiversity' (studies on any biota) and (b) containing 'freshwater', or both 'fish' and 'freshwater' (studies filtered to freshwater biota, or freshwater fish).

Table 2 Evidence regarding geographic patterns of freshwater fish species diversity categorised by the three general hypotheses; versions were merged if evidence did not clearly distinguish them (Table 1). For each table entry, we indicate evidence type (empirical test vs. argumentation-based), species groups (according with dispersal pathways; n effect', LGM – refuges during the last glacial (Darlington 1957)), occurrence datasets (species lists vs. sampling sites), driving factors (or 'primacy variable'; Hawkins *et al.*, 2003), type of freshwater ecosystem, and both scale grains and extent. Potentially, species called primary are solely dependent on orographic events that produce changes in the stream network, secondary species can also occasionally disperse through saltwater, and peripheral species move through saltwater frequently during their lifecycles. Species lists refer to data from atlases and species maps, whereas sampling sites refer to data collected directly from field surveys. Streams refer to surface lotic waters, lakes to lentic surface waterbodies. MAT – Mean annual temperature, MAP – mean annual precipitation, PET – potencial evapotranspiration, AET – actual evapotranspiration, FR Annual flow regime, MAD – mean annual discharge at river mouth, NPP – net primary productivity, SDA – surface of drainage area, SLA – surface lake area, MDE – 'mid-domain effect', LGM – refuges during the last glacial maximum (Pliocene).

Reference	Evidence type	species	Occurrence dataset	Driving factors	Ecosystem	Scale Extent	Scale Grain
Current environment-based hypothesis							
1-3) Climate, energy, water-energy							
Oberdorff *et al.*, 1995	Yes	All native	Species lists	SDA, MAD (not LGM area)	Streams	World	292 basins
Oberdorff *et al.*, 1997	Yes	All	Species lists	SDA(~MAD), NPP and climate (less LGM connectivity and LGM area)	Streams	Western Europe, North America	132 rivers
Guégan *et al.*, 1998	Yes	All primary	Species lists	NPP, FR (not SDA)	Streams	World	183 basins
Kerr & Currie, 1999	Yes	All primary	Species lists	PET (not cladistic phylogenies)	Streams	North America (north of Mexico)	11 square grid of Currie & Paquin (1987)
Zhao *et al.*, 2006	Yes	All native	Species lists	Altitude, PET, AET, MAT, SLA	lakes	China	109 lakes
Béguer *et al.*, 2007	Yes	All peripheral	Species lists	Interaction of MAT, SDA, FR*slope (=stream power), NPP	Streams	Western Europe	41 basins
Sax, 2001	Yes	All exotic and established	Species lists	Current environment (arguing against historical factors)	All	North and South America, Europe, Asia, Africa, oceanic islands	Species distribution ranges
4) Environment stability							
Rathert *et al.*, 1999	Yes	All native	Species lists	climate extremes (not density of watercourses)	Streams	Oregon State, USA	375 hexagons grid
5) 'Metabolic-effect'							
Allen *et al.*, 2002	Yes	All native	(Oberdorff *et al.*, 1995) species lists	MAT and population densities fit the 3 predict ions	Streams	World	292 basins

Table 2 Continued.

Reference	Evidence type	species	Occurrence dataset	Driving factors	Ecosystem	Scale Extent	Scale Grain
History-based hypothesis							
Mayden, 1988	Yes	All	Species lists	Pre-Pleostocenic drainage connections	Streams	Northeastern America	
Tedesco *et al.*, 2005	Yes	All primary	Species list	LGM refuges connectivity, SDA (not NPP)	Tropical forest streams	Tropical South and Central America, West Africa	141 basins
Griffiths, 2006	Yes	All native	Species lists	habitat availability, LGM connectivity	Streams	Europe	25 regions
Hof *et al.,* 2008	No	All primary (discerned by habitat type)	Species lists	SLA, maximum altitude (not climate). Infers relevance ecological traits, history of regions and lineages	Groundwaters, streams, lakes	Europe	25 regions
Li *et al.*, 2009	Yes (elevational richness gradient)	Genus *Schizothorax*	Sampling sites	MAT and time of colonization (not, MAP, area, MDE, diversification rates)	Streams	Tibetan Plateau and adjacent regions	Sites
Area-based hypothesis							
1) Species-area							
Barbour & Brown, 1974	Yes	All	Species lists	SLA , suggests time of colonization	lakes	World	70 lakes
Oberdorff *et al.*, 1995	Yes	All native	Species lists	SDA, MAD (not LGM area)	Streams	World	292 basins
Oberdorff *et al.*, 1997	Yes	All	Species lists	SDA(~MAD), NPP and climate (less LGM connectivity and LGM area)	Streams	Western Europe, North America	132 rivers
Zhao *et al.*, 2006	Yes	All native	Species lists	Altitude, PET, AET, MAT, SLA	lakes	China	109 lakes
Béguer *et al.*, 2007	Yes	All peripheral	Species lists	Interaction of MAT, SDA, FR*slope (=stream power), NPP	Streams	Western Europe	41 basins
2)' Mid-domain effect'							
Hof *et al.*, 2008	No	All primary	Species lists (grouped by habitat type)	Suggests MDE at Central Europe	Groundwaters, streams, lakes	Europe	25 regions
Li *et al.*, 2009	Yes	All primary	Species lists	MDE for elevation gradient	Streams	Tibetan Plateau	Interpolations from site data

History-based factors, despite their supposedly reduced role in large-scale extents, have been considered relevant since the release of early studies on the evolutionary mechanisms of speciation in freshwater fish (Matthews, 1998; Table 2). Early researchers related past events, phylogenetic data, and current species distributions using spatial data (e.g. Mayden, 1988), or drew *post hoc* historical inferences on current species diversity - for example, the influence of Pleistocenic Glaciations on one or a few species at regional scales (e.g. Hurwood & Hughes, 1998). More recent broad-scale empirical tests revealed evidence for the strong influence of historical factors on current richness patterns across distinct groups of species, freshwater environments, and regions and continents (Table 2). For example, the 'niche conservatism' concept has been illustrated with cases where freshwater fish species are absent from habitats very similar to those actually occupied (Wiens, 2004) and supported by other empirical tests (e.g. McNyset, 2009). The relevance of historical climatic events was underlined recently in an empirical test of West African and Latin American fish species richness (Tedesco *et al*., 2005). Moreover, some studies have supported the importance of time lag in speciation on shaping species diversity patterns found (e.g. Barbour & Brown, 1974); however, to our knowledge, no empirical testing was performed on large broad-scale datasets. Oberdorff (1999) found that glaciation events are still 'printed' at endemic species distributions whereas widely distributed fish species were more related to contemporary environmental factors, and thus concluded a clearer role for historical signals in the distribution of endemic species. Because most endemic species hotspots occur in arid or semi-arid regions (Oberdorff, 1999), the influence of historical factors may be more marked in these regions (see Filipe *et al*., 2009).

Finally, considerable evidence points to the importance of area in shaping freshwater fish diversity patterns from groundwaters to streams and lakes and from continents to worldwide. Such studies most often take basins and lakes as grains of analyses. The influence of drainage basin area or lake surface area has received some attention in studies at large spatial extents (e.g. Oberdorff *et al*., 1997; Barbour & Brown, 1974; Table 2). Within Europe, only lentic species showed a significant species–area relationship, with a richness peak in lakes in central Europe (Hof *et al*., 2008). Within a given region, stream area measures are commonly positively correlated with species diversity along a watercourse's longitudinal gradient, as habitat volume, diversity, and stability increases in larger watercourses (e.g. Filipe *et al*., 2004). Also, mid-course peaks in species richness have been reported (e.g. Ward, 1998), as postulated by Vannote *et al*. (1980) at the River Continuum Concept: the result of sequential shifts in species composition (species additions and replacements) is probably reflecting longitudinal shifts in available resources.

All hypotheses are now gaining empirical support through new tests on broad empirical datasets. Particularly, the current environment characteristics as the main driver of current diversity for freshwater fishes has been reinforced by two studies: 1) a test on invasive species worldwide, which obviously lacks a coherent historical explanation (Sax, 2001); and 2) phylogenetic data for three families of North American freshwater fishes for which species richness appeared to be consistently related to current climate patterns, with little reason to postulate evolutionary explanations (Kerr & Currie, 1999). Conversely, other recent empirical tests do not indicate that the environment-based hypothesis prevails uniformly; rather, factors such as life–history traits, species resilience and dispersal ability, habitat preferences, and biotic interactions are likely to have strong effects on biodiversity patterns. For example, knowledge of historical glaciations, isolations and dispersal routes largely explains current diversity patterns of European fishes with distinctive life–history traits and mobility (Griffiths, 2006); also, species inhabiting distinctive habitats (e.g. lotic, groundwater, and lentic habitats) exhibited distinctive relationships between species richness and latitude (though lentic habitats, the less stable habitats over geologic time, exhibited a weaker relationship) (Hof *et al.*, 2008).

Shortcomings in testing freshwater fish diversity patterns

We now identify the major weaknesses of current knowledge and possible solutions for building a research framework and improving future empirical tests. Figure 2 systematises the features to consider and outlines the following key points.

Data uncertainties of freshwater fish diversity

Databases of freshwater fish species distributions, despite great advances in the amount of data available, are significantly limited in data quality and completeness. Dataset assessments to overcome this problem are often nonexistent or incomplete for this fauna, which ultimately results in inaccurate outcomes. The records of species presences/abundances rarely include accurate measures of sampling bias, species detectabilities, or sampling effort (Filipe, unpublished); many records of absences are not reliable, especially for rare species in highly seasonal environments (Cao *et al*., 1998), as is the case of freshwaters in arid and semi-arid regions. There is a need to account for and quantify species-specific detectabilities and sampling efforts across habitats and spatial grains using techniques such as species accumulation plots during field surveys (Cao *et al*., 2001; Gotelli & Colwell, 2001; Smith & Jones, 2005). Accurate estimates of species diversity measures and presence are essential for proper comparability and integration when compiling inventory data and, ultimately, for conducting valid empirical tests (Cao *et al*., 2006). Additionally, a concerted effort is needed to computerise and centralise the scattered datasets on species occurrence into a worldwide database and to obtain better geographic coverage (see Bailan *et al*., 2008a). For lesser-studied regions, there is a need to increase the completeness and accuracy of freshwater fish inventories (Figure 2). Freshwater fishes are supposedly more speciose and endemic in the Neotropical Region (e.g. Amazonia), but it is likely that fish diversity is underestimated in regions as Central Africa and Southeastern Asia since, overall, the Holarctic Region has been most studied (Bailan et al., 2008b; Lévêque et al., 2008).

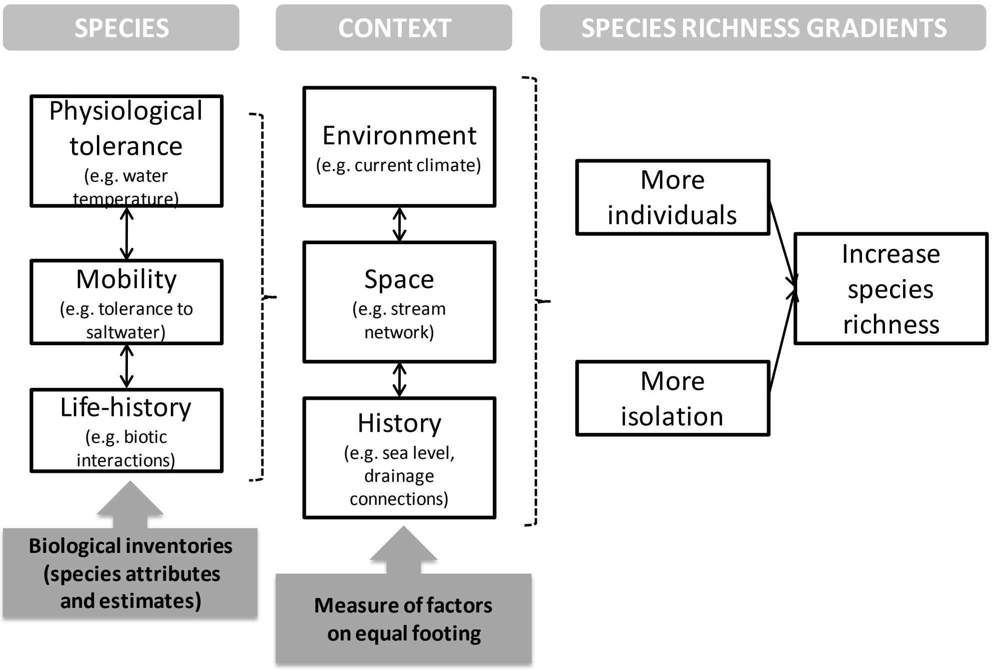


Figure 2 Overview of the components involved in empirical tests regarding freshwater fish diversity gradients. Species respond differently to distinct components of the current environment, history, and area attributes depending on their distinct physiological characteristics, life-histories, and ability to disperse. Arrows indicate associations between species attributes, driving factors of species richness, and mechanisms invoked on trigging species richness based on the current literature. Shaded boxes below indicate ways to improve empirical tests.

Incomplete knowledge of biological attribute, and history of species and regions

There is a lack of knowledge regarding fish species' physiological thresholds, habitat needs, dispersal abilities, probability of colonisation success, life-history traits, and assemblage relationships; other freshwater fauna are even less well documented (Bailan *et al.*, 2008b). As an example, even the species classifications of primary, secondary and peripheral in accordance with overall potential dispersal pathways *sensu* Darlington (1957) are not entirely known. This information is necessary to match species with historical events, habitats, and dispersal routes and thus refine empirical tests (see Hof *et al*., 2008, and Pearson & Boyero, 2009 for freshwater taxa in general). Additionally, explicit data on species lineages as clade-based phylogenies, if available, can be used to relate past speciation events to current species diversity and to test the influence of historical factors (.g. Kerr & Currie, 1999). Also, data on the history of regions as past orographic changes, glaciations, stream captures and sea-level changes are usually scattered; when spatially incorporated, they could be tested simultaneously with current environmental data, allowing for more rigorous empirical tests (Tedesco *et al*., 2005). Together with current environmental data, these data could lead to a better knowledge of fish species richness patterns. A supplementary effort is needed to summarise such germane information, as previously emphasised (Angermeier, 1995).

Concerns regarding scale

Several studies strongly suggest that factors influencing diversity of freshwater fishes, as for other biota, are scale-dependent and ultimately, the scale of analysis determines the outcome of empirical tests (Angermeier, 1998; Rathert *et al*., 1999; Tedesco *et al*., 2005). This highlights the need for an explicit accounting of scale in analytical designs, as generally recommended (Whittaker *et al*., 2001; Willis & Whittaker, 2002). Freshwater fish diversity patterns have been tested at a multitude of spatial grains and extents, which ultimately impedes the comparison of findings across studies. Namely, the spatial extents of analyses varies from regional to worldwide; the spatial grains of analyses used are also variable in area, for example, when considering sub-basins or basins or when examining fixed area sizes composed of grids or hexagons (e.g. Rathert *et al*., 1999). The wide variation in analysis scale grain makes the use of biodiversity checklists from areas varying in size especially challenging (Keil & Hawkins, 2009).

Unaccounted role of dispersal constraints and structure of freshwaters

Despite the potential interest of investigating the effects of dispersal on freshwater fish diversity on a global scale, to our knowledge no empirical tests addressed this issue (but see Hof *et al*., 2008). In fact, the spatial structure of freshwaters, which strongly constrain fish dispersal, have been difficult to describe quantitatively so that their influences on species diversity patterns can be tested. However, recent advances in GIS techniques and spatial statistics are enabling researchers to evaluate the influences of drainage networks on fish diversity; this is currently shedding light on how such dispersal constraints shape species richness patterns (Olden *et al*., 2001; Ganio *et al*., 2005; Ray, 2005; Imbert *et al*., 2008). These analyses require special attention to the effects of spatial autocorrelation, a property that renders conventional statistical analyses more difficult to apply (Dormann *et al*., 2007).

Weaknesses of empirical testing

A serious issue regarding empirical testing is that some predictions are inconclusive at distinguishing between hypotheses - or even misleading - because the factors associated with different hypotheses are often correlated or are based on indirect measures regarding the variables of interest (as noted for other biota; Willig *et al*., 2003). Also, the difficulty of rigorously and simultaneously testing the relative importance of a multitude of factors seems to be delaying progress in knowing how well each general hypothesis accounts for the observed spatial patterns of freshwater fish diversity. Thus, proper empirical tests should include efforts (1) to disentangle potential driving factors of species diversity patterns through careful analysis of correlated factors and data on the likely biotic mechanisms involved, and (2) to examine those potential driving factors on an *a priori* effective, equal power (variation and scale).

New knowledge and emerging research topics

What do we know thus far regarding spatial patterns of freshwater fish diversity? And what do we need to know? In this paper, we have 1) summarised current knowledge of the geographic patterns associated with interspecific distinct motilities and habitats occupied, and spatial scales (extents and grains), 2) clarified possible links among themes in freshwater ecology and evolution, and 3) demonstrated that environment, history, and area-based factors might be complementary in controlling freshwater fish diversity. Although, more rigorous tests on the multitude of hypotheses are needed to clarify the main driving factors and mechanisms involved. Specifically, our synthesis of the empirical literature on the broad-scale spatial gradients of freshwater fish species richness allowed suggestions for improving empirical tests and opened new possibilities for clarifying the processes regulating spatial patterns species diversity.

We propose fishes as appropriate taxa to determine the influence of historical factors on current species richness patterns because these organisms are strongly constrained and are better known than other freshwater fauna. We expect historical events to be detectable in freshwater fish assemblages which might be useful in explaining the mechanisms of species diversity and evolution concurrent with species' tracking with environmental conditions. Using freshwater fishes to test general hypotheses about species diversity not only helps build biodiversity knowledge; it also sheds light on feasible strategies for the conservation and management of freshwater biota. Species relying mostly on freshwater habitats (as invertebrates, fish, and amphibians) tend to be highly threatened (MA Millennium Assessment, 2005). The current loss of biodiversity has been referred to as related with an increase in faunal homogenisation and a consequent decrease of ecological integrity and ecosystem services (Vorosmarty *et al*., 2008). Ongoing climate change is likely to exacerbate these trends (Poff *et al*., 2001; Burgmer *et al*., 2007; Buisson & Grenoulliet, 2008). A clearer picture of the processes and patterns driving biodiversity on a multitude of spatial and temporal scales is critical to preventing likely losses of freshwater fish diversity. In summary, this synthesis underlines the role of spatial constraints on freshwater fish diversity through history, with implications for the ability of fishes to adapt to current threats and tracking the available favourable environments and habitats. These are issues that are fundamental on how to effectively manage and conserve this fauna.

While scrutinising the outcomes and implications of our study and the inputs for biodiversity knowledge in general, it is important to note the caveats of the synthesis performed. First, the distinct scales—both extent and grain—among the studies analysed allow some comparisons, but the multitude of scales used implies that they are in some sense tenuous, thus weakening knowledge integration (see Lyons, 1999). Second, the relevance of factors at larger scales tends to average out at smaller scales, which might explain why climatic variables are often the strongest correlates of species richness at large grains and extents (Field, 2009). Third, the synthesis performed was based on correlational studies, excluding field experiments (e.g. Smith *et al*., 2004), which might turn out to be inconclusive for deciphering the relative importance of the factors proposed to explain some of the global patterns of species diversity and the mechanisms underlying them. Starting from these considerations, we outline key-research topics germane to our knowledge of fish diversity patterns (topics contextualised at Figure 2).

Spatial constraints and dispersal

Testing the role of spatial structure of freshwaters, namely the constraints to dispersal, seems a promising research pathway, especially for exploring historical and area- based hypotheses. Indeed, fishes constitute examples of assemblages in a likely non-equilibrium with present environments because the colonisation of new areas depends on rare geological events such as changes in physical connections between river basins (Beisner *et al*., 2006; Filipe *et al*., 2009). This is in contrast to less spatially constrained biota, such as large mammals (Brown, 1995). It might therefore be interesting to compare fishes with other fauna with restricted pathways of dispersal (e.g. highly mobile terrestrial species whose dispersal is restricted by unsuitable habitats such as urban areas). In fact, freshwater fish species vary in their dispersal pathways mostly according to their biological attributes and are thus appropriate for multiple simultaneous comparisons: some are solely dependent on orographic events that produce changes in the stream network, others can also occasionally disperse through saltwater, and still others move through saltwater frequently during their lifecycles (called primary, secondary, and peripheral species, respectively, *sensu* Darlington, 1957). Additionally the dispersal pathways and current patterns of invasive species richness are likely to be driven by additional factors related to human-mediated dispersal (Leprieur *et al*., 2009).

Testing 'mid-domain effects' on freshwater fish diversity is also promising but poorly developed to date (but see Li *et al*., 2009). These tests are especially interesting because they rely on null models and may be able to explain species richness peaks in the middle of study regions and environmental gradients in face of strong dispersal constraints. If so, 'mid-domain effects' tests may help reconcile the roles of historical constraints and current environmental conditions in shaping biodiversity patterns (e.g. Gaston, 2003; Storch *et al*., 2006).

Some emerging ideas

Genetic divergence, when coupled with geographic and environmental distances, allows inferences about the relative importance of evolutionary history, dispersal, and current environment on freshwater fish diversity gradients (e.g. Bermingham & Martin, 1998; Reeves & Bermingham, 2006). Thus the development of clade-based phylogenies to characterise faunal age and evolutionary history will increase current knowledge of biota across scales and allow more rigorous testing of hypotheses related to history. In particular, taxonomic distinctness, a diversity measure of phylogenetic relatedness of species, was used as a complementary diversity measure of species richness, first in assessments on the extent to which species can withstand changes/disturbances (Rogers, 1999) and more recently in tests regarding spatial variation in species diversity on broader scales (Brennan *et al.*, 2005; Krasnov *et al.*, 2005; Peat *et al.*, 2009). There is evidence that this measure can reflect phylogenetic structure - thus the evolutionary and ecological history of taxa - more than effects of anthropogenic pressures (Abéellán, 2006) and has the advantage of being independent of sampling effort and thus quite suited to datasets based on species lists (Warwick & Clarke, 1998). This measure has not yet been empirically tested on freshwater fishes' broad-scale spatial gradients as a complementary measure of species richness (but see Bhat & Magurran, 2006); this seems a worthwhile investigation, as Heino *et al*. (2005) envisioned. While species biological attributes habitat preferences have been studied for decades, new evidence of the role of biotic interactions - neutral, positive, or negative - on current patterns of species diversity is now emerging from empirical datasets (e.g. Araújo & Luoto, 2007) and simulation (e.g. Travis *et al*., 2005) experiments. To date, the influence of biotic interactions on freshwater fish diversity has been mostly tested on local or regional scale extents (e.g. Oberdorff *et al.*, 1998) where these factors were generally considered unimportant. But there is no evidence that biotic interactions do not play a role on broader scales, which might be especially pertinent in the Neotropics (MacArthur, 1972). Biotic interactions data are rather complex to parameterise; they present both a challenge and one more reason to progress to a multi-scale approach, from local to broad scales, as recommended for achieving a better knowledge of what drives species coexistence (Whittaker *et al*., 2001). Examining species interactions together with biological attributes and habitat preferences which species tend to retain, will allow us to investigate the long-term evolutionary biotic mechanisms.

Conversely, the possibility of performing field experiments opened by landscape interventions, such as dams, or the implementation of hydrological canals, has given some accurate answers on how communities react and is offering unique, valuable opportunities for research. For example, Smith *et al*. (2004) found fish communities permeable to invasions and mostly dependent on regional effects, which was argued as consistent with neutral theory models in which ecological differences between species are not determinant (Bell, 2001; Hubbell, 2001); surprisingly, this is the opposite of the studies above that evidenced the role of such differences and thus the relevance of ecological niches.

At last, we turn to Iberian Peninsula freshwater fishes as an example of how many research questions remain at least at some regions. Encompassing a highly endemic and threatened freshwater fish fauna, where communities appear mostly driven by historical events (Filipe *et al*., 2009, and references therein), this Iberian fauna has been the stage of strong advances in our knowledge of biogeography, evolution, and ecology. But researchers still seek many answers: Why do old lineages still occur despite likely extinction? What is the role of species' ecological niches in the face of broad-scale landscape events across evolutionary time?

Conclusions

Based on current literature, our synthesis allowed for the integration of knowledge on freshwater fish diversity gradients, namely of empirical tests of species richness patterns found in nature. Identifying the shortcomings involved in these previous studies allowed us to sketch approaches for future empirical tests aiming to unveil the processes that shape freshwater fish diversity gradients.

Knowledge of diversity patterns of terrestrial taxa has guided much of the research attempting to understand the diversity patterns of freshwater biota (Hawkins *et al.*, 2003a). Here we propose that the opposite could be considered: proper tests of species diversity patterns in fresh waters could advance the knowledge of the patterns of species diversity observed in terrestrial environments. The effort regarding knowledge integration from the body of literature constituted a step forward in this direction.

Acknowledgments

The ideas presented here were developed during the doctoral degree research of the first author and financed by the Portuguese Foundation for Science and Technology (FCT; SFRH/BD/10330/ 2002). Support was also provided by the Centre of Environmental Biology, Portugal (CBA), and by Virginia Polytechnic Institute and State University, USA (VPI&SU); any use of trade or product names does not imply endorsement by the US government. We are grateful to Luís Borda d'Água for helpful comments on an earlier version of this manuscript.

Biosketches

Ana Filipa Filipe is a PhD student at the Sciences Faculty of Lisbon University, Centre of Environmental Biology. Her main research interests are focused on the processes controlling species diversity. Her current research project is focused on macroecology of freshwater fishes at Iberian Peninsula. Also she has interest on public awareness of nature conservation.

Paul Angermeier is a research scientist with the U.S. Geologic Survey and a professor at Virginia Polytechnic Institute and State University, USA, where the Virginia Cooperative Fish and Wildlife Research Unit is jointly sponsored by the US Geological Survey, Virginia Polytechnic Institute and State University, Virginia Department of Game and Inland Fisheries, and Wildlife Management Institute. His research interests include the ecology and conservation of fishes and the sustainable use of aquatic ecosystems.

Maria João Collares-Pereira is a professor at the Sciences Faculty of Lisbon University, and member of the Centre of Environmental Biology. Her research interests include life-history strategies and speciation pathways of Iberian fish.

References

Abéellán, P., Bilton, D.T., Milán, A., Sánchez-Fernández, D. & Ramsay, P.M. (2006) Can taxonomic distinctness assess anthropogenic impacts in inland waters? A case study from a Mediterranean river basin. *Freshwater Biology*, **51**, 1744-1756.

Allen, A.P., Brown, J.P. & Gillooly, J.F. (2002) Global biodiversity, biochemical kinetics, and the energetic-equivalence rule. *Science*, **297**, 1548.

Allen, A.P., Gillooly, J.F., Savage, V.M. & Brown, J.H. (2006) Kinetic effects of temperature on rates of genetic divergence and speciation. *Proceedings of the National Academy of Sciences of USA*, **103,** 9130-9135.

Angermeier, P.L. (1995) Ecological Attributes of Extinction-Prone Species: Loss of Freshwater Fishes of Virginia. *Conservation Biology,* **9**, 143-158.

Angermeier, P.L. & Winston, M.R. (1998) Local vs Regional Influences on Local Diversity in Stream Fish Communities of Virginia. *Ecology*, **79**, 911-927.

Araújo, M.B. & Luoto, M. (2007) The importance of biotic interactions for modelling species distributions under climate change. *Global Ecology & Biogeography*, **16**, 743-743.

Bailan, E.V., Seguers, H., Lévêque, C. & Martens, K. (2008a) An introduction to the Freshwater Animal Diversity Assessment (FADA) project. *Hydrobiologia*, **595**, 3-8.

Bailan, E.V., Seguers, H. & Lévêque, C. (2008b) The Freshwater Animal Diversity Assessment: an overview of the results. *Hydrobiologia*, **595**, 627-637.

Barbour, C.D.& Brown, J.H. (1974) Fish species diversity on lakes. The *American Naturalist*, **108**, 473-489.

Béguer, M., Beaulaton, L. & Rochard, E. (2007) Distribution and richness of diadromous fish assemblages in Western Europe: large-scale explanatory factors. *Ecology of Freshwater Fish,* **16**,221-237.

Beisner, B.E., Peres-Neto, P.R., Lindstrom, E.S., Barnett, A. & Longhi, M.L. (2006) The role of environmental and spatial processes in structuring lake communities from bacteria to fish. *Ecology,* **87**, 2985-2891.

Bell, G. (2001) Neutral Macroecology. *Science,* **293**, 2413-2418.

Bermingham, E. & Martin, A.P. (1998) Comparative mtDNA phylogeography of neotropical freshwater fishes: testing shared history to infer the evolutionary landscape of lower Central America. *Molecular Ecology,* **7**, 499-517.

Bhat, A. & Magurran, E. (2006) Taxonomic distinctness in a linear system: a test using a tropical freshwater fish assemblage. *Ecography,* **29**, 104-110.

Brennan, K.E.C., Majer, J.D. & Moir, M.L. (2005) Refining sampling protocols for inventorying invertebrate biodiversity: influence of drift-fence length and pitfall trap diameter on spiders. *Journal of Aracnology,* **33**, 681-702.

Brown, J.M. (1981) Two Decades of Homage to Santa Rosalia: Toward a General Theory of Diversity. *American Zoologist*, **21**, 877-888.

Brown, J.H. (1995) *Macroecology.* The University of Chicago Press, Chicago.

Bhat, A. & Magurran, E. (2006) Taxonomic distinctness in a linear system: a test using a tropical freshwater fish assemblage. *Ecography,* **29**, 104-110.

Buisson, L. & Grenouillet, G. (2008) Climate change hastens the turnover of stream fish assemblages. *Global Change Biology,* **14**, 2232-2248.

Burgmer, H., Hillebrand, H. & Pfenninger, M. (2007) Effects of climate-driven temperature changes on the diversity of freshwater macroinvertebrates. *Oecologia,* **151**, 93-103.

Cao, Y., Hawkins, C.P. & Storey, A.W. (2006) A method for measuring the comparability of different sampling methods used in biological surveys: implications for data integration and synthesis. *Freshwater Biology*, **50**, 1105-1115.

Cao, Y., Larsen, D.P. & Hughes, R.M. (2001) Evaluating sampling sufficiency in fish assemblage surveys: a similarity-based approach. *Canadian Journal of Fisheries and Aquatic Sciences*, **58**, 1782-1793.

Cao, Y., Williams, D. & Williams, N. (1998) How important are rare species in aquatic community ecology and bioassessment? *Limnology and Oceanography*, **43**, 1403-1409.

Colwell, R.K. & Lees, D.C. (2000) The mid-domain effect: geometric constraints on the geography of species richness. *Trends in Ecology & Evolution*, **15**, 70-76.

Currie, D.J. (1991) Energy and Large-Scale Patterns of Animal- and Plant-Species Richness. *American Naturalist*, **137**, 27.

Currie, D.J., Mittelbach, G.G., Cornell, H.V., Field, R., Guégan, J., Hawkins, B.A., Kaufman, D.M., Kerr, J.T., Oberdorff, T., O'Brien, E.M. & Turner, J.R.G. (2004) Predictions and tests of climate-based hypotheses of broad-scale variation in taxonomic richness. *Ecology Letters*, **7**, 1121-1134.

Currie, D.J. & Paquin, V. (1987) Large-scale biogeographical patterns of species richness in trees. *Nature*, **329**, 326-327.

Darlington, P.J. (1957) *Zogeography: the geographical distribution of animals.* Wiley, New York.

Diniz-Filho, J.A.F., Telles, M.P.C., Bonatto, S.L., Eizirik, E., O de Freitas, T.R., De Marco, P., Santos, F.R., Sole-Cava, A. & Soares, T.N. (2008) Mapping the evolutionary twilight zone: molecular markers, populations and geography. *Journal of Biogeography,* **35**, 753-763.

Dormann, C.F., McPherson, J.M., Araújo, M.B., Bivand, R., Bolliger, J., Carl, G., Davies, R.G., Hirzel, A., Jetz, W., Kissling, W.D., Kuhn, I., Ohlemüller, R., Peres-Neto, P.R., Reineking, B., Schroder, B., Schurr, F.M., Wilson, R. (2007) Methods to account for spatial autocorrelation in the analysis of species distributional data: a review. *Ecography*, **30**, 609-628.

Field, R., Hawkins, B.A., Cornell, H.V., Currie, D.J., Diniz-Filho, J.A.F., Guégan, J., Kaufman, D.M., Kerr, J.T., Mittelbach, G.G., Oberdorff, T., O'Brien, E.M. & Turner,J.R.G. (2009) Spatial species-richness gradients across scales: a meta-analysis. *Journal of Biogeography* **36**, 132-147.

Filipe, A.F., Araújo, M.B., Doadrio, I., Angermeier, P.L. & Collares-Pereira, M.J. (2009a) Biogeography of Iberian freshwater fishes revisited: the roles of historical versus contemporary constraints. *Journal of Biogeography*. **36**, 2096-2110.

Filipe, A.F., Marques, T., Seabra, S.G., Tiago, P., Ribeiro, F., Moreira da Costa, L., Cowx, I.G. & Collares-Pereira, M.J. (2004) Selection of priority areas for fish conservation in the Guadiana River Basin, Iberian Peninsula. *Conservation Biology*, **18**, 189-200.

Ganio, L.M., Torgersen, C.E. & Gresswell, R.E. (2005) A geostatistical approach for describing spatial pattern in stream networks. *Frontiers in Ecology and the Environment*, **3**, 138-144.

Gaston, K.J. (2003) *The structure and dynamics of geographic ranges*, Oxford University Press, Oxford.

Gaston, K.J. & Blackburn, T.M. (2000) *Pattern and process in macroecology*, Blackwell Science, Oxford.

Gehrke, P.C. & Harris, J.H. (2000) Large-scale patterns in species richness and composition of temperate riverine fish communities, south-eastern Australia. *Marine and Freshwater Research*, **51**, 165-182.

Gotelli, N.J. & Colwell, R.K. (2001) Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters*, **4**, 379-391.

Griffiths, D. (2006) Pattern and process in the ecological biogeography of European freshwater fish. *Journal of Animal Ecology*, **75**, 734-751.

Guégan, J., Lek, S. & Oberdorff, T. (1998) Energy availability and habitat heterogeneity predict global riverine fish diversity. *Nature*, **391**, 382-384.

Harrison, S.P. & Grace, J.B. (2007) Biogeographic affinity helps explain productivity-richness relationships at regional and local scales. *The American Naturalist*, **170**, S5-S15.

Hawkins, B.A. (2004) Are we making progress toward understanding the global diversity gradient? *University of California Post-prints*, **2309**, 1-4.

Hawkins, B.A., Diniz-Filho, J.A.F., Bini, L.M., Araújo, M.B., Field, R., Hortal, J., Kerr, J.T., Rahbek, C., Rodríguez, M.A. & Sanders, N.J. (2007a) Metabolic theory and diversity gradients: where do we go from here? *Ecology*, **88**, 1898-1902.

Hawkins, B.A., Diniz-Filho, J.A.F., Jaramillo, C.A. & Soeller, S.A. (2006) Post-Eocene climate change, niche conservatism, and the latitudinal diversity gradient of New World birds. *Journal of Biogeography*, **33**, 770-780.

Hawkins, B.A., Diniz-Filho, J.A.F., Jaramillo, C.A. & Soeller, S.A. (2007b) Climate, niche conservatism, and the global bird diversity gradient. *The American Naturalist*, **170**, S16-S27.

Hawkins, B.A., Field, R., Cornell, H.V., Currie, D.J., Guégan, J., Kaufman, D.M., Kerr, J.T., Mittelbach, G.G., Oberdorff, T., O'Brien, E.M., Porter, E.E. & Turner, J.R.G. (2003a) Energy, water, and broad-scale geographic patterns of species richness. *Ecology*, **84**, 3105-3117.

Hawkins, B.A., Porter, E.E. & Diniz-Filho, J.A.F. (2003b) Productivity and history as predictors of the latitudinal diversity gradient of terrestrial birds. *Ecology*, **84**, 1608-1623.

Heino, J., Janne, S., Lappalainen, J. & Virtanen, J. (2005) The relationship between species richness and taxonomic distinctness in freshwater organisms. *Limnology and Oceanography,* **50**, 978-986.

Hillebrand, H. (2004) On the generality of the latitudinal diversity gradient. *The American Naturalist*, **163**, 192-211.

Hof, C., Brandle, M. & Brandl, R. (2008) Latitudinal variation of diversity in European freshwater animals is not concordant across habitat types. *Global Ecology and Biogeography*, **14**, 539-546.

Hubbell, S.P. (2001) Neutral theory in community ecology and the hypothesis of functional equivalence. *Functional Ecology,* **19**, 166-172.

Hurwood, D.A. & Hughes, J.M. (1998) Phylogeography of the freshwater fish, *Mogurnda adspersa*, in streams of northeastern Queensland, Australia: evidence for altered drainage patterns. *Molecular Ecology*, **7**, 1507-1517.

Huston, M.A. (1994) *Biological diversity: the coexistence of species in changing landscapes*. Cambridge University Press, Cambridge, UK.

Hutchinson, G.E. (1959) Homage to Santa Rosalia, or why are so many kinds of animals? *The American Naturalist* **XCIII**, 145-159.

Imbert, H., de Lavergne, S., Gayou, F., Rigaud, C. & Lambert, P. (2008) Evaluation of relative distance as new descriptor of yellow European eel spatial distribution. *Ecology of Freshwater Fish*, **17**, 520-527.

Jetz, W. & Rahbek, C. (2001) Geometric constraints explain much of the species richness pattern in African birds. *Proceedings of the National Academy of Sciences of USA***, 98**, 5661-5666.

Johnson, L.B. & Gage, S.H. (1997) Landscape approaches to the analysis of aquatic ecosystems. *Freshwater Biology*, **37**, 113-132.

Keil, P. & Hawkins, B.A. (2009) Grids versus regional species lists: are broad-scale patterns of species richness robust to the violation of constant grain size? *Biodiversity and Conservation*, **18**, 3127-3137.

Kerr, J.T. (2001) Global biodiversity patterns: from description to understanding. *Trends in Ecology & Evolution*, **16**, 424-425.

Kerr, J.T. & Currie, D.J. (1999) The relative importance of evolutionary and environmental controls on broad-scale patterns of species richness in North America. *Ecoscience*, **6**, 329-337.

Krasnov, B.R., Shenbrot, G.I., Moulliot, D., Khokhlova, I.S. & Poulin, R. (2005) Spatial variation in species diversity and composition of flea assemblages in small mammalian hosts: geographical distance or faunal similarity? *Journal of Biogeography,* **32**, 633-644.

Latham, R.E. & Ricklefs, R.E. (1993) Global patterns of tree species richness in moist forests: Energy-diversity theory does not account for variation in species richness. *Oikos*, **67**, 325-333.

Lennon, J.J., Koleff, P., Greenwood, J.J.D. & Gaston, K.J. (2004) Contribution of rarity and commonness to patterns of species richness. *Ecology Letters*, **7**, 81-87.

Leprieur, F., Olden, J.D., Lek, S. & Brosse, S. (2009) Contrasting patterns and mechanisms of spatial turnover for native and exotic freshwater fish in Europe. *Journal of Biogeography*, **36**, 1899-1912.

Lévêque, C., Oberdorff, T., Paugy, D., Stiassny, M.L.J. & Tedesco, P.A. (2008) Global diversity of fish (Pisces) in freshwater. *Hydrobiologia,* **595**, 545-567.

Li, J.L., He, Q., Hua, X., Zhou, J., Xu, H., Chen, J. & Fu, C. (2009) Climate and history explain the species richness peak at mid-elevation for *Schizothorax* fishes (Cypriniformes: Cyprinidae) distributed in the Tibetan Plateau and its adjacent regions. *Global Ecology & Biogeography,* **18**, 264-272.

Lomolino, M.V. (2000) A species-based theory of insular biogeography. *Global Ecology & Biogeography,* **9**, 39-58.

Lomolino, M.V. & Weiser, M.D. (2001) Towards a more general species-area relationship biodiversity on all islands, great and small. *Journal of Biogeography*, **28**, 431-445.

Lyons, S.K. & Willig, M.R. (1999) A hemispheric assessment of scale dependence in latitudinal gradients of species richness. *Ecology*, **80**, 2483–2491.

MA, Millennium Assessment (2005) Chapter 7 Fresh water. *Millenium Ecossystem Assessment: Current State & Trends Assessment.* Millenium Assessment. (Accessed at www.millenium assessment.org).

MacArthur, R.H. & Wilson, E.O. (1963) An equilibrium theory of island biogeography. *Evolution*, **17**, 373-387.

MacArthur, R.H. (1972) *Geographical ecology: patterns in the distribution of species.* Harper & Row, New York.

Manel, S., Schwartz, M.K., Luikart, G, & Taberlet, P. (2003) Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology & Evolution,* **18**, 189-197.

Matthews, W.J. (1998) *Patterns in freshwater fish ecology*. Chapman & Hall, New York.

Mayden, R.L. (1988) Vicariance Biogeography, Parsimony, and Evolution in North American Freshwater Fishes. *Systematic Zoology*, **37**, 329-355.

Menge, B.A. (2009) Do terrestrial ecologists ignore aquatic literature? *Frontiers in Ecology and Environment,* **7**, 182-183.

McNyset, K.M. (2009) Ecological niche conservatism in North American freshwater fishes. *Biological Journal of the Linnean Society*, **96**, 282-295.

Mittelbach, G.G., Schemske, D.W., Cornell, H.V., llen, A.P., Brown, J.M., Bush, M.B., Harrison, S.P., Hurlbert, A.H., Knowlton, N., Lessios, H.A., McCain, C.M., McDade, L.A., McPeek, M.A., Near, T.J., Price, T.D., Ricklefs, R.E., Roy, K., Sax, D.F., Schluter, D., Sobel, J.M. & Turelli, M. (2007) Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. *Ecology Letters*, **10**, 315-331.

Mittelbach, G.G., Steiner, C.F., Scheiner, S.M., Gross, K.L., Reynolds, H.L., Waide, R.B. & Willig, M.R. (2001) What is the observed relationship between species richness and productivity? *Ecology*, **82**, 2381-2396.

Oberdorff, T., Guégan, J. & Hugueny, B. (1995) Global scale patterns of fish species richness in rivers. *Ecography*, **18**, 345-352.

Oberdorff, T., Hugueny, B., & Guégan, J. (1997) Is there an influence of historical events on contemporary fish species richness in rivers? Comparisons between Western Europe and North America. *Journal of Biogeography*, **24**, 461-467.

Oberdorff,T., Hugueny,B., Compin,A., & Belkessam,D. (1998) Non-interactive fish communities in the coastal streams of North-western France. *Journal of Animal Ecology* **67**, 472-484.

Olden, J.D., Jackson, D.A. & Peres-Neto, P.R. (2001) Spatial isolation and fish communities in drainage lakes. *Oecologia*, **127**, 572-585.

Pearson, R.G. & Boyero, L. (2009) Gradients in regional diversity of freshwater taxa. *Journal of the North American Benthological Society*, **28**, 504-514.

Peat, H.J., Clarke, A. & Convey, P. (2009) Diversity and biogeography of the Antarctic flora. *Journal of Biogeography,* **34**, 132-146.

Poff, N.L., Angermeier, P.L., Cooper, S.D., Lake, P.S., Fausch, K.D., Winemiller, K.O., Mertes, L.A.K., Oswood, M.W., Reynolds, J.H. & Rahel, F.J. (2001) Fish Diversity in Streams and Rivers. *Global Biodiversity in Changing Environmment. Scenarios for the 21st Century* (ed. by F.S.Chapin III, O.E.Sala, and E.Huber-Sannwald), pp. 315-349. Springer-Verlag, NewYork.

Rathert, D., White, D., Sifneos, J.C. & Hughes, R.M. (1999) Environmental correlates of species richness for native freshwater fish in Oregon, U.S.A. *Journal of Biogeography*, **26**, 257-273.

Ray, N. (2005) Pathmatrix: a geographical information system tool to compute effective distances among samples. *Molecular Ecology Notes*, **5**, 177-180.

Reeves, R.G. & Bermingham, E. (2006) Colonization, population expansion, and lineage turnover: phylogeography of Mesoamerican characiform fish. *Biological Journal of the Linnean Society,* **88**, 235-255.

Ricklefs, R.E. (2004) A comprehensive framework for global patterns in biodiversity. *Ecology Letters*, **7**, 1-15.

Rogers, S.I., Clarke, K.R. & Reynolds, J.D. (1999) The taxonomic distinctness of coastal bottom-dwelling fish communities of the North-East Atlantic. *Journal of Animal Ecology* **68**, 769-782.

Rohde, K. (1992) Latitudinal gradients in species diversity: the search for the primary cause. *Oikos*, **65**, 514-527.

Rosenzweig, M.L. (1995) *Species diversity in space and time*. Cambridge University Press, Cambridge.

Roy, K. & Goldberg, E.E. (2007) Origination, Extinction, and Dispersal: Integrative Models for understanding present day diversity gradients. *The American Naturalist*, **170**, S71-S85.

Sax, D.F. (2001) Latitudinal gradients and geographic ranges of exotic species: implications of biogeography. *Journal of Biogeography*, **28**, 139-150.

Schlosser, I.J. (1991) Stream fish ecology: a landscape perspective. *BioScience*, **41**, 704-712.

Schlosser, I.J. (1995) Critical landscape attributes that influence fish population dynamics in headwater streams. *Hydrobiologia*, **303**, 71-81.

Smith, S.A., Bell, G., & Bermingham, E. (2004) Cross-Cordillera exchange mediated by the Panama Canal increased the species richness of local freshwater fish assemblages. *Proceedings of the Royal Society B,* **271**, 1889-1896.

Smith, K.L. & Jones, M.L. (2005) Watershed-level sampling effort requirements for determining riverine fish species composition. *Canadian Journal of Fisheries and Aquatic Sciences*, **62**, 1580-1587.

Stevens, R.D. (2006) Historical processes enhance patterns of diversity along latitudinal gradients. *Proceedings of the Royal Society B,* **273**, 2283-2289.

Storch, D., Davies, R.G., Zajicek, S., Orme, C.D.L., Olson, V.A., Thomas, G.H., Ding, T.-S., Rasmussen, P.C., Bennett, P.M., Blackburn, T.M., Owens, P.F. & Gaston, K.J. (2006) Energy, range dynamics and global species richness patterns: reconciling mid-domain effects and environmental determinants of avian diversity. *Ecology Letters*, **9**, 1308-1320.

Tedesco, P.A., Oberdorff, T., Lasso, C.A., Zapata, M. & Hugueny, B. (2005) Evidence of history in explaining diversity patterns in tropical riverine fish. *Journal of Biogeography*, **32**, 1899-1907

Turner, J.R.G. (1992) Stochastic processes in populations: the horse behind the cat? In: *Genes in Ecology*, 29-33, Blackwell, Oxford.

Travis, J.M.J., Brooker, R.W. & Dytham, C. (2005) The interplay of positive and negative species interactions across an environmental gradient: insights from an individual-based simulation model. *Biology Letters*, **1**, 5-8.

Vannote, R.L., Minshall, G.W., Cumming, K.W., Sedell, J.R. & Cushing, C.E. (1980) The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences*, **37**, 130-137.

Ward, J.V. (1998) Riverine landscapes: biodiversity patterns, disturbances regimes, and aquatic conservation. *Biological Conservation*, **83**, 269-278.

Warwick, R.M. & Clarke, K.R. (1998) Taxonomic distinctness and environmental assessment. *Journal of Applied Ecology,* **35**, 532.

Whittaker, R.J. & Field, R. (2000) Tree species richness modeling: an approach of global applicability? *Oikos*, **89**, 399-402.

Whittaker, R.J., Willis, K.J. & Field, R. (2001) Scale and species richness: towards a general, hierarchical theory of species diversity. *Journal of Biogeography*, **28**, 453-470.

Wiens, J.J. (2004) Speciation and ecology revisited: phylogenetic niche conservatism and the origin of species. *Evolution*, **58**, 193-197.

Wiens, J.J. (2007) Global patterns of diversification and species richness in amphibians. *The American Naturalist*, **170**, S86-S106.

Wiens, J.J. & Donoghue,M.J. (2004) Historical biogeography, ecology and species richness. *Trends in Ecology & Evolution*, **19**, 639-644.

Wiens, J.J. & Graham,C.H. (2007) Niche conservatism: integrating evolution, ecology, and conservation biology. *Annual Review of Ecology and Systematics*, **36**, 519-539.

Willig, M.R., Kaufman, D.M. & Stevens, R.D. (2003) Latitudinal Gradients of Biodiversity: pattern, process, scale, and synthesis. *Annual Review of Ecology and Systematics,* **34**, 273-309.

Willis, K.J. & Whittaker, R.J. (2002) Ecology: Enhanced: Species Diversity-Scale Matters. *Science*, **295**, 1245-1248.

Wright, D.H. (1983) Species-energy theory: an extension of species-area theory. *Oikos*, **41**, 96-506.

Wright, D.H., Currie, D.J. & Maurer, B.A. (1993) Energy supply and patterns of species *richness on local and regional scales. Species diversity in ecological communities* (ed. by R. E. Ricklefs and D. Schluter), pp. 66-72. University of Chicago Press, Chicago, Illinois, USA.

Zhao, S., Fang, J., Peng, C., Tang, Z. & Piao, S. (2006) Patterns of fish species richness in China's lakes. *Global Ecology and Biogeography*, **15**, 386-394.

Chapter 6.

General discussion

If sometimes I say flowers smile
And if sometimes I say streams sing,
It is not because I think there are smiles in flowers
or songs in streams..
It is because this way
I might make faked humans feel the truly existence of flowers and streams

"Se às vezes digo que as flores sorriem
E se eu disser que os rios cantam,
Não é porque eu julque que há sorrisos nas flores
E cantos no correr dos rios...
É porque assim
Faço mais sentir aos homens falsos
A existência verdadeiramente real das flores e dos rios."

Fernando Pessoa como Alberto Caeiro *in* O Guardador de Rebanhos ", CXXXI, 1914

6.

General Discussion

This chapter constitutes an integrated discussion of the main findings of the study. It is presented a brief summary of the outcomes, some matters focused throughout the dissertation, the main key strengths and weaknesses, and the implications of the knowledge gained.

6.1 Summary of the outcomes

The results of this dissertation improved the understanding of Iberian freshwater fish spatial patterns, by identifying the main constraints and the ecological processes shaping the spatial patterns of current assemblages across the Iberian Peninsula landscapes (IP; Chapter 3), and of local species richness across the south-western area (SW; Chapter 4). We have shown that a multitude of large-scale factors constrain current fish assemblages, from historical dispersal barriers to current environments. With the databases built, we improved the data available of freshwater fish species occurrences in the IP, and particularly in the SW streams (Chapters 2, 3, 4). Moreover, future research studies were suggested regarding the biodiversity spatial patterns of freshwater fish after a revision of the current scientific literature (Chapter 5).

It is expected the outcomes will constitute a knowledge background for theoretical aspects of ecology, but also for dealing with human-induced changes in fresh waters, and detect biodiversity modifications over time, as studies over large-scale for Iberian freshwater biota are just now beginning. The study answered to the questions previously formulated within fundamental and applied contexts, as summarized below:

Questions (I)

It was identified some inaccuracy and incompleteness in the data merged on freshwater fish species distributions in mainland Portugal by examining the primary species dataset (1990-2008; InFISH.PT database). The lack of data quality identified was due to taxonomic uncertainties, scattered information of sampling effort measures, and unbalanced coverage of records. Procedures adopted which improved data quality include: 1) filtering samplings according with sampling efforts, 2) checking taxonomy according with the current species identification, and 3) performing additional directed samplings. These procedures were applied in InFISH.IP database when possible (see also Chapter 3), and are likely shared by other regional freshwater biota databases. Investments on the data quality of the freshwater biodiversity databases can 1) optimize the data collected, 2) share knowledge between researchers, managers, and educators, and ultimately 3) increase awareness on the current freshwater ecosystems and biodiversity problems (Chapter 2).

Questions (II)

The native fish assemblage composition varied across the Iberian Peninsula, and the long-term river basins boundaries formed since the Pliocene–Pleistocene (2.5-1.8 Ma) seem to play an important role, more than current climate, which did not need to be invoked to explain existing biogeographical patterns. The relevance of basin boundaries was distinct among species with distinct dispersal abilities: peripheral species, which frequently inhabit salt waters showed to be less constrained by basin boundaries than secondary and primary species. Peripheral species might be using additional pathways through the coastline to disperse. Based on the river basins delineation, eleven biogeographical provinces were proposed. These new provinces improved information available on dispersal processes of fish species. Permeability among provinces was most likely due to past events of punctual stream captures (Chapter 3).

Questions (III)

The fish species richness varied across the Mediterranean-type south-western streams and is likely to be influenced by multiple factors operating at distinct scales. The influence of basin species pools was obvious on the average of local richness among basins, for both native and invasive species. The amount of annual rainfall was relevant for non-native species. Within basins, distinct environmental and spatial filters were at play on shaping local richness for native and invasive species, but, overall, spatial filters incremented the variance explained more than topographic and climate factors (Chapter 4).

Questions (IV)

Previous studies regarding empirical tests performed on freshwater fish species at global, continental, or regional scales covered the main hypotheses regarding species richness pattern, here the environment-based, the history-based and the area-based hypotheses. The environmental-based hypothesis was the most tested and supported. While the history-based hypothesis has been the less supported, according with our analysis, that may be due to limitations of testing historical factors on equal footing with the contemporary ones (Chapter 5).

6.2 Spatial patterns of freshwater fish species

The drivers of current assemblages

As referred, the present study clearly showed that local assemblages of Iberian freshwater fish are driven by a multitude of large-scale factors. This is a somehow expected finding since it has been observed in previous studies in other world regions, disregarding the type of stream (e.g. Schlosser, 1991; Angermeier & Winston, 1997; Rahert *et al*., 1999; see also Allan & Johnson, 1997; see also Matthews, 1998), and in smaller extents studies in the IP (e.g. Aparício *et al*., 2000; Filipe *et al*., 2002, 2004; Ferreira *et al*., 2008). Therefore, our results add evidence to the view which recognizes that factors operating at broad spatial and temporal scales act synergistically and have a strong influence on structuring current biodiversity patterns of freshwater assemblages.

Historical factors appear as being more relevant than current environmental factors on constraining the studied assemblages (Chapter 2 and 3). This was a surprisingly result, since other studies focused on freshwater fish or other freshwater, marine and terrestrial faunas supported the view that past events have limited impact on current species assemblages because species have tracked environmental changes through range shifts (e.g. Gray, 2001; Svenning & Skov, 2005; see also Chapter 5). Nevertheless, the relevance of historical factors on the spatial patterns of freshwater fish species was also supported in other works (e.g. Tedesco et al., 2005; for more references see Table 2 in Chapter 5; see also Araújo *et al*., 2008 for amphibians and reptiles). In Bonada *et al*. (2005), for example, it was tested historical and ecological variables on caddisfly species distribution in some Iberian Mediterranean river basins and, despite the importance of historical factors, ecological variables were better at explaining distributions of those stream macroinvertebrates; the authors attributed these results to the time length of historical events, the high dispersion and colonization capacity of many caddisfly species, and the strong environmental gradient in the area, which probably allowed species to track contemporary environments. With this dissertation evidences were added to the view, less supported, that historical factors may be the main constrain of current assemblages for strongly spatial restricted groups as freshwater fish (Chapter 5). The assemblages studied seem to be in a non-equilibrium state with contemporary environmental conditions, and contingent on species' ability to disperse through time via hydrological connections (Chapter 3).

In fact, the importance of historical factors emerged from our studies, in the IP and SW areas. In the IP, the composition of assemblages was strongly associated with river basin boundaries, more than with current climate (Chapter 3). Iberian river basin boundaries constitute barriers to fish which influence dispersal and colonization processes, and drive fish assemblage's composition. These barriers have been emphasized as relevant for stream fish in other regions (Smith *et al*., 2004; Leprieur *et al.,* 2009). But, to our knowledge, this is the first study that compares historical – here river basin boundaries – and current environments – here climate – of stream fish on an equal footing. In the SW streams, the local species richness across basins was more correlated with the pool of species at each basin than with current environmental variables of climate and topography, and both last variables did not improve considerably the variability already explained by the pool of species (see text below and Chapter 4).

However, the results do not disprove that current environments do constrain the distribution of freshwater fish species, in spite of the emphasis on the historical dimension of biodiversity on the current debate of historical *versus* contemporary influences. Evidences were added that current environments do influence the Iberian fish assemblages, which is similar to results of previous studies in IP at smaller extents (e.g. Filipe *et al*., 2002, 2004; Ferreira *et al*., 2008; see also Chapter 1 for references in other regions), and findings do not exclude the likely imprinting caused by direct human interference on habitats, or more local factors, as species interactions (Wiley *et al*., 1997; see Araújo & Luoto, 2007 for recent evidences on the importance of species interactions).

Actually, across the Peninsula, fish assemblage composition was strongly correlated with current climate (although less than with basins boundaries). Across the SW river basins, topographical and climate variables were not significant, except the annual rainfall for established invasive species. Within basins, local environmental characteristics of climate and topography revealed as important (Chapter 4). Moreover, spatial factors reflecting the hydrological network within the SW river basins explained the majority of fish richness local variability, probably because spatial contagious processes are acting at this scale. These ecological processes are more likely to be associated with current stream environments than historical factors, because fish species remained mostly free to move throughout the river network across time (for similar results see Grenouillet *et al*., 2004; see also Chapter 3). Spatial stream variables are likely to surrogate variables as the hydrological regime, habitat availability, and habitat stability (for similar results see Urban *et al*., 2006).

In summary, the results gave additional data for the local *versus* regional influences debate, highlighting the variability explained by large-scale factors. For the historical *versus* contemporary debate, it supported the prevalence of historical factors on explaining current variability of assemblages, which has been the less supported view so far. However, the literature review evidenced that if more stringent hypothesis tests are developed considering all factors on equal footing (current and past environments, and spatial characteristics), the likely importance of historical factors might emerge (Chapter 5). Examining patterns of stream fish species helped to clarify the imprint of past heritage and current conditions - a central theme for researchers - for strongly spatially confined faunas as the ones restricted to freshwater environments.

Species with distinct dispersal ability and biogeographical origin

Our findings evidenced that freshwater fish species with distinct dispersal ability or originated in foreign biogeographical regions have distinct spatial patterns and are likely to be constrained by distinct factors, and helped to understand the processes that underline the patterns observed. Results support the view that species with distinct attributes can be constrained by distinct factors (e.g. Griffiths, 2006; but see Smith *et al.,* 2004).

Iberian freshwater fish species with different dispersal abilities use distinct dispersal pathways (Chapters 3 and 5). Particularly, peripheral species are likely to use marine dispersal pathways, while primary, and surprisingly, secondary species dispersal along coastlines is probably infrequent. Secondary species were expected to use more often this dispersal pathway than primary species due to their higher tolerance to salinity, but our results do not support that hypothesis. Smith and Bermingham (2005) found similar evidences for secondary species in Mesoamerican freshwater fish, which suggests that secondary species experience similar constraints on dispersal as the primary species.

Therefore, the distinct ability to disperse among stream fish species is likely to determine spatial broad-scale patterns. For example, Griffiths (2006) found that, as the result of the limited dispersal ability of fish species confined to fresh waters, the glaciated northern European streams are species-poor, mostly inhabited by larger peripheral species. In southern areas (as in the Balcans, and in Iberian and Italian Peninsulas, especially at mountainous areas), fish faunas are richer in endemic species, and colonization and isolation processes are likely to be important. Generally, peripheral species - which live in salted and non-salted waters - showed less geographical variation in species richness across Europe. Similar patterns were found in North American streams (see McAllister *et al*., 1986; Moyle & Herbold, 1987). Moreover, the relevance of peripheral fish dispersal past events throughout salt waters has been evidenced (e.g. McDowall, 1999).

When considering alien biogeographical origin of species in the SW study, richness of invasive species was associated with distinct landscape factors of those regarding native species (Chapter 4). In fact, species with a foreign biogeographical place of origin – called invasive or non-native - do not seem to be so well adapted to basins with low annual rainfall as native species, where streams are less permanent and can dry-up or be reduced (e.g. Gasith & Resh, 1999; Magalhães *et al*., 2002). Current native assemblages are mostly limited by basins species pool, which add support to the idea that the establishment and/or persistence of native fish species in Mediterranean-type environments is mostly related with species evolutionary history (see Magalhães *et al*., 2002). Additional evidences underline the role of past evolutionary events in other Mediterranean-type streams (e.g. Bonada *et al.*, 2008). In the current investigation, locally assemblages with established invasive species are correlated with spatial attributes of the stream network, which indicates the importance of colonization and dispersal processes for all assemblage members. Additional evidences on the relevance of such processes in these Mediterranean-type fauna can be found at Pires *et al*. (2009).

Again, the hierarchical faunal filters

Regarding the hierarchical faunal filtering framework previously presented (Chapter 1), the investigation performed allowed to identify the main factors and ecological processes implicated in shaping Iberian freshwater fish assemblages.

As pointed previously, evidences pointed that historical factors (as long term river basins barriers) constitute the main drivers of current local assemblages of stream fish at large extents (here at the IP), while current environment acts as a relevant filter in lower hierarchical levels: within river basins level, current environment (as climate) showed being an important constrain (here at some SW basins) to local species richness. Also, stream network characteristics and, of less importance, stream topography, appear as pertinent within river basins, likely most related with current environments. At intermediate hierarchical levels, among some basins (as SW areas), climate revealed being a main constraint for invasive species most likely because they originally evolved in foreign places and could not persist in some areas. Figure 6.1 illustrates the hierarchical levels operating and, although processes at stream reach level were not directly examined, the level is illustrated as it was considered throughout the manuscript.

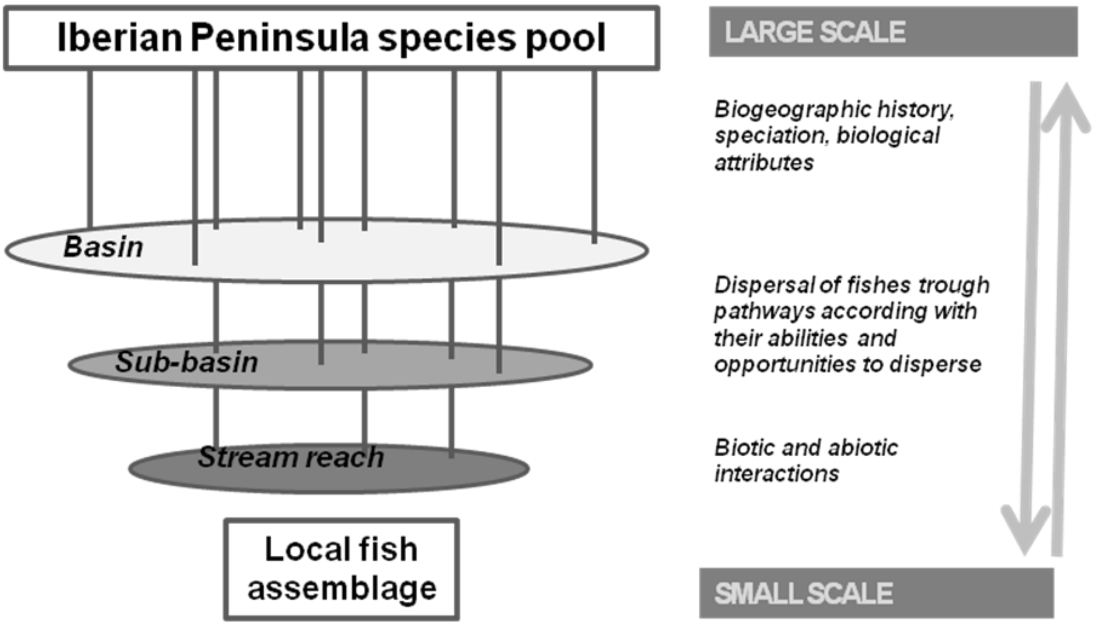


Figure 6.1 The hierarchical constraints of freshwater fish of the Iberian Peninsula from broader to finer temporal and spatial scales. Arrows indicate the interconnection of both scales and filters. Local assemblages are the result of a range of filters related with the landscape history of the Peninsula, the evolution of species and their attributes, the

opportunities to disperse and colonize through time and across landscapes, and biotic/abiotic interactions between species occupying their ecological niches (adapted from Smith & Powell, 1971).

6.3 Study key–strengths and weaknesses

This investigation constituted the first study on Iberian stream fish using recent taxonomical advances, large occurrences datasets, and new macroecological and biogeographical approaches regarding data organization and analyses. Particularly, coupling biodiversity databases with Geographic Information Systems (GIS) and various modern statistical techniques allowed achieving the aims proposed, advance knowledge on the IP freshwater fish, and added support to some ecological views in debate (see Allan & Johnson, 1997; Johnson & Gage, 1997). The main key-strengths are identified below:

- Spatial data on IP freshwater fish species obtained were compiled and are now available at InFISH.PT and InFISH.IP.
- Knowledge was added regarding the main constraints acting in some faunal filter hierarchical levels:
    - Regarding IP freshwater fish assemblages, historical constraints were evidenced; spatial patterns of assemblage composition were the basal information to delineate the biogeographical provinces. Dispersal and colonization are main ecological processes operating. Results do not disregard the relevance of current environment constrains.
    - Regarding SW freshwater fish assemblages, local fish species richness was related with a multitude of landscape factors. The species pool available and the stream network spatial structure were important hierarchical faunal filters. Results do not disregard the relevance of current environment constrains.
- More stringent empirical tests are needed to obtain a sound basal knowledge regarding freshwater fish broad-scale patterns.

In spite there were no evidences pointing drawbacks that might have influenced the results obtained, some aspects can be improved as:

- Explicit measures regarding data completeness and bias in the databases are important to attain. Such limitations were indentified and minimized before analyses, but this likely weakness could be better managed with data quality investments during data collecting and gathering (Chapter 2).
- Some factors tested are somewhat intercorrelated. Disentangling current environment and spatial factors is being the focus of major concerns and debates (see Currie 2007; Chapter 3).
- Some relevant factors might not have been addressed. It is the case of:
    - Other historical factors, as the influence of endorreic basins and sea level fluctuations at the IP (Chapter 3).
    - Both colonization pressure of invasive species (see Lockwood *et al*., 2009; Wilson *et al.,* 2009) and local influences (as habitat characteristics) at the SW streams (e.g. Angermeier & Winston, 1998; Irz *et al*., 2004; Chapter 4).
    - Human impacts on stream habitats, besides fish invasions (Chapters 3 and 4).

Accounting such factors on future studies might potentiate some of the findings achieved here. For example, the role of biotic interactions, which have not been considered relevant for this fauna on previous studies (e.g. Oberdorff, *et al*., 1998; Peres-Neto, 2004) might be clarified, if properly examined (see Araújo & Luoto, 2007).

6.4 Study implications

The present investigation is particularly directed to researchers dedicated to the ecological matters addressed, especially those interested in understanding Iberian and Mediterranean-type systems. Other potential readers are educators focused in public awareness of the natural values, designers of biodiversity databases, and Natural History Museum staff. Concerning the implications of the dissertation findings to managers, they are detailed below, since they might constitute a useful tool for underpinning peninsular and regional conservation planning efforts for Iberian stream faunas, particularly fish species. But beforehand, a picture of the ongoing conservation and management concerns is presented.

Current environmental concerns

We now face challenges regarding the maintenance of the natural values and the services provided in a changing world, which imply to attain immediate conservation and management actions but also to forecast future scenarios (Sala *et al*., 2000; Rahel *et al.,* 2008). Indeed, biotic homogenization, climate change, and habitat destruction are serious concerns for freshwater fish, with consequences predicted to be devastating when acting synergistically, and even more for the increasing demands regarding waters services (see Revenga *et al*., 2000; Daufresne & Boët, 2007; Rahel & Olden, 2008; Buisson & Grenouillet, 2009). South-western Europe is experiencing a decrease and alteration of stream flows, due to the increasing implementation of water reservoirs and other water uses. This is expected to be potentiated by climate changes by provoking an increase in the severity of draughts, especially in streams with a Mediterranean-type regime, with consequences already pointed regarding freshwater fish (e.g. Magalhães *et al.,* 2007). The current rates of biotic homogenization, which might be even more potentiated with the environmental changes described above, are expected to have serious consequences beyond biodiversity loss in the functional characteristics of ecosystems, with immediate and future impacts on ecological and evolutionary processes (Olden *et al*., 2004; Clavero & García-Berthou, 2006).

Considerable efforts are in agenda of European Union (EU) countries to implement the WFD by attaining the ‘good ecological state’ before 2015 at the basin level (EC, 2009; EEB, 2009 a,b). The European Environmental Bureau (EEB) has been stressing the opportunity to restore Europe’s rivers by 2015 based on public consultations approaches and former cooperation on building River Basin Management Plans, which set out the specific objectives and actions necessary to restore the healthy ecology of inland waters, as well as the financial and regulatory instruments required to do so (for a case study see Videira *et al*., 2009).

Regarding Iberian Peninsula, although recent advances have been made for the management and conservation of stream ecosystems and their biota, especially on increasing (although insufficient) awareness of inland water problems (e.g. see information online at www.icnb.pt, www.lpn.pt, www.mma.es), insufficient effective effort has been done to attain main threats to protect stream faunas in both Iberian countries (e.g. Collares-Pereira *et al*., 1999). Particularly, advances were made on designing areas to protect fresh waters (Filipe *et al*., 2004 in Appendix II), but effective efforts to protect watercourses and maintain hydrological connectivity has been almost absent, resulting in the implementation of barriers to fish dispersal, as dams, even at protected areas (the same as in other regions, see Pringle, 2001). Additionally, some non-native species are used for recreational fisheries activities, and the economical value implicated triggers the human-induced colonization pressure, and it is superimposed without proper balancing impacts on the remnant freshwater biota (Marta *et al*., 2001; see also Clavero & García-Berthou, 2006; Ribeiro *et al*., 2009). There are also difficulties in integrating decision-making at the basin scale, in spite pointed as necessary in the WFD, and a larger collaborative effort among existing river basin authorities is needed (Allan *et al*., 1997; Collares-Pereira & Cowx, 2004; Naiman, 2009). For the Portuguese and Spanish shared basins, the basin-scale management is even more difficult to attain (Sadoff *et al*., 2008).

Attaining effective conservation and management

As a general consequence of the work undertaken, it arises that the traditional local approaches are not enough to accomplish challenges on both basic and applied research, and on conservation and management of riverine fauna. Multiscale and integrative approaches considering influences from fine to broader spatial and temporal scales constitute useful frameworks for understanding, evaluating and predicting biodiversity patterns, and the likely adaptations to current environmental changes. Indeed, multiscale and integrative broad-scale frameworks have been recognized at a range of areas of society related with freshwater natural resources, from research to jurisdiction and management (e.g. Allan *et al.*, 1997; Naiman, 2009).

Essentially, this dissertation highlighted the historical dimension of the patterns found in nature regarding freshwater fish species diversity, which depend on processes such as dispersal and colonization of organisms. This denotes that limitations to dispersal are an important factor to consider regarding conservation and management of freshwater faunas and ecosystems from across the Peninsula to within river basins. Such outcomes need to be crossed with the ongoing conservation and management strategies of both Portugal and Spain. Below, some aspects are addressed.

Specifically, the eleven biogeographical provinces delineated for the IP based on freshwater fish species obtained can be a valuable tool on attaining the WDF aims by facilitating resource inventories, monitoring, and coordination of for large-scale conservation and management strategies (Fig. 3, Chapter 3). Knowing where species are and which can occur in certain areas is essential to the establishment of effective conservation strategies (see Abell *et al.*, 2008, Zogaris *et al.*, 2009 and references therein). The Iberian provinces proposed contemplate recent taxonomy, and complete and accurate occurrence data, and were based on exact basin limits using the similarity of assemblage species composition information (see Appendix I for previous studies; Almaça, 1978; Doadrio 1988; Vargas *et al.*, 1998; Mesquita *et* *al.*, 2007). Moreover, information provided includes similarity of fish assemblages between and within provinces (Chapter 3).

Knowing that freshwater fish biodiversity in the IP is strongly related with both dispersal and colonization of organisms, and that long-term barriers to dispersal, such as natural river basin boundaries, and within basin stream network contiguity are determinant brings some implications to conservation and management strategies.

Firstly, it implies that management and conservation of freshwaters must be attained at the basin and across basins scales. This consideration has been referred in other studies, which recognized that river basins are functional units with well-defined barriers that must be attained in investigation and management plans (Filipe *et al.*, 2004; see also Moyle & Yoshiyama, 1994; Puth & Wilson, 2001). Although Iberian protected areas cover only parts of river basins and sub-basins, additional protection measures at those extents are required to maintain aquatic natural values (Moyle & Yoshiyama, 1994; Angermeier & Winston, 1999; Collares-Pereira & Cowx, 2004).

Secondly, it dictates that managers and conservationists cannot disregard the effects of stream network rearrangements due to human impacts. Namely, the plan to build ten new hydroelectric dams in Portuguese waters (www.inag.pt) should be reevaluated, as evidences of the likely negative consequences are summing (e.g. Paterson et al., 2008). Moreover, dams are not favourable additional impacts to the expected climate change and faunal homogenization, since they reduce the habitats available and constitute dispersal barriers for native species, whereas they benefit the spread and establishment of invasive fauna by increasing their habitats, and routes of dispersal. Moreover, the likely alterations due to climate changes, as the sea-level and stream flow expected shifts (Sutherland *et al.*, 2006; see also Unmack, 2001) can disrupt current fish dispersal pathways. Such connectivity changes in fresh waters might also alter biotic homogenization rates (Rahel, 2007). From the results achieved, it seems that predicting the freshwater fish distributions future shifts can be rather complex since the role of historical and contemporary constraints might evolve. If current human induced environmental trends continue, the primacy of historical effects on fish distributions may not persist. Current rates of biotic homogenization suggest that environmental effects will eventually overwhelm effects of historical processes which take place much slower (see Sax *et al.*, 2007).

Identifying the basins more susceptible to fish invasion, as the SW basins in the present study with higher annual rainfall, can be used as a tool for predicting and preventing local invasions. But, as the pool of species dictates most of the species established locally, the most effective measure is being precautionary and prevent any fish species introduction at the basin scale, by acting on human induced colonization pressure, as was also defended in other studies (see e.g. Ribeiro, 2008).

Not disregarding the items previously addressed, it must be underlined that having high quality biodiversity databases is of great value, on acting and planning in face of current freshwater biodiversity environmental challenges. To be achieved, urgent and concerted efforts are needed on designing strategies for storage and manage data. Technicians, researchers and Natural History Museum curators are recognized as the key-actors to improve data quality, but financial and human investments are required.

Also, it must be mentioned that public awareness concerning freshwater biodiversity and stream ecosystems must be in the top list of priorities to attain. Education of all, including policymakers, about how humans affect the natural world and how biodiversity loss diminishes life quality must be catalyzed by informed educators, researchers and technicians (Angermeier, 2007). Increasing literacy of the relations among aquatic biodiversity, stream ecosystems and services provided is indispensable to promote the sustainable use of natural resources.

In a synthesis, protection of freshwater biodiversity constitutes a difficult task since it is influenced by the upstream drainage network, the surrounding land, the riparian zone, for which prerequisites are hardly met (Dudgeon, 2006). Immediate actions are needed where opportunities exist to set aside intact inland waters, while proceeding with data gathering and research, and concealing human uses with biodiversity values. Adopting a continued and adaptive management strategy and an investigation-action approach seems to be the right option.

6.5 References

Abell, R., Thieme, M.L., Revenga, C., Bryer, M.T., Kottelat, M., Botutskaya, N.G., Coad, B., Mandrak, N.E., Contreras Balderas, S., Bussing, W., Stiassny, M.L.J., Skelton, P.H., Allen, G.R., Unmack, P.J., Nascka, A.M., Ng, R.; Sindorf, N., Robertson, J., Armijo, E., Higgins, J.V., Heibel, T.J., Wikramanayake, E.D., Olson, D., López, H.L., Reis, R.E., Lundberg, J.G., Sabaj Pérez, M.H. & Petry, P. (2008) Freshwater Ecoregions of the World: A New Map of Biogeographic Units for Freshwater Biodiversity Conservation. *BioScience,* **58**, 403-414.Almaça, C. 1976. Zoogeografia e especiação dos ciprinídeos da Península Ibérica. *Natura Nova Série*, **4**, 1-28.

Almaça, C. 1978. Répartition géographique des Cyprinidae ibériques et secteurs ichthyogéographiques de la Péninsule Ibérique. *Vestnik Ceskoslovenské Spolecnosti Zoologické*, **XLII**, 241-248.

Allan, J.D., Erickson, D.L. & Fay, J. (1997) The influence of catchment land use on stream integrity across multiple spatial scales. *Freshwater Biology,* **37**, 149-161.

Allan, J.D. & Johnson, L.B. 1997. Catchment-scale analysis of aquatic ecosystems. *Freshwater Biology*, **37**, 107-111.

Angermeier, P.L. (2007) The Role of Fish Biologists in Helping Society Build Ecological Sustainability. *Fisheries*, **32**, 9-20.

Angermeier, P.L. & Winston, M.R. 1997. Assessing conservation value of stream communities: a comparison approach based on centres of density and species richness. *Freshwater Biology*, **37**, 699-710.

Angermeier, P.L. & Winston, M.R. 1998. Local vs. regional influences on local diversity in stream fish communities of Virginia. *Ecology*, **79**, 911-927.

Angermeier, P.L. & Winston, M.R. (1999) Characterizing fish community diversity across Virginia landscapes: prerequisite for conservation. *Ecological Applications*, **9**, 335-349.

Aparicio, E., Vargas, M.J., Olmo, J.M. & Sostoa, A. (2000) Decline of native freshwater fishes in a Mediterranean watershed an the Iberian Peninsula: a quantitative assessment. *Environmental Biology of Fishes,* **59**, 11-19.

Araújo, M.B. & Luoto, M. (2007) The importance of biotic interactions for modelling species distributions under climate change. *Global Ecology & Biogeography*, **16**, 743-743.

Araújo, M.B., Nogués Bravo, D., Diniz-Filho, J.A.F., Haywood, A.M., Valdes, P.J. & Rahbek, C. (2008) Quaternary climate changes explain diversity among reptiles and amphibians. *Ecography*, **31**, 8–15

Bonada, N., Zamora-Munoz, C., Rieradevall, M. & Prat, N. (2005) Ecological and historical filters constraining spatial caddisfly distribution in Mediterranean rivers. *Freshwater Biology,* **50**, 781-797.

Bonada, N., Rieradevall, M., Dallas, H., Davis, J., Day, J. & Figueroa, R. (2008) Multi-scale assessment of macroinvertebrate richness and composition in Mediterranean-climate rivers. *Freshwater Biology*, **53**, 772-788.

Buisson, L. & Grenouillet, G. (2009) Contrasted impacts of climate change on stream fish assemblages along an environmental gradient. *Diversity and Distributions,* **15**, 613-626.

Clavero, M. & García-Berthou, E. (2006) Homogenization dynamics and introduction routes of invasive freshwater fish in the Iberian Peninsula. *Ecological Applications,* **16**, 2313-2324.

Collares-Pereira, M.J. & Cowx, I.G. (2004) The role of catchment scale environmental management in freshwater fish conservation. *Fisheries Management,* **11**, 303-312.

Collares-Pereira, M.J., Cowx, I.G., Rodrigues, J.A., Rogado, L. & Costa, L.M. (1999) The status of *Anaecypris hispanica* in Portugal: problems of conserving a highly endangered Iberian species. *Biological Conservation,* **88**, 201-212.

Currie, D.J. (2007) Disentangling the roles of environment and space in ecology. *Journal of Biogeography* **34**, 2009-2011.

Daufresne, M. & Boët, P. (2007) Climate change impacts on structure and diversity of fish communities in rivers. *Global Change Biology,* **13**, 2467-2478.

Doadrio, I. 1988. Delimitation of areas in the Iberian Peninsula on the basis of freshwater fishes. *Bonner Zoologische Beitraege*, **39**, 113-138.

Doadrio, I. (2002) *Atlas y Libro Rojo de los Peces Continentales de España*. Dirección General de Conservación de la Naturaleza. Museo Nacional de Ciencias Naturales, Madrid.

Dudgeon, D. (2006) Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biological Reviews,* **81, 163-182**.

EC (European Commission) (2009) Introduction to the new EU Water Framework Directive. EC. Available at http://ec.europa.eu/environment/water/ (last accessed 7 December 2009).

EEB (European Environmental Bureau) (2009a) *Europe's water at the crossroads*. EEB of European Union. Available at http://www.eeb.org (last accessed 7 December 2009).

EEB (European Environmental Bureau) (2009b) *European Environmental Bureau* Activities. EEB of European Union. Available at http://www.eeb.org (last accessed 7 December 2009).

Ferreira,M.T., Sousa,L., Santos,J.M., Reino,L., Oliveira,J.M., Almeida,P.R., & Cortes,R.V. (2008) Regional and local environmental correlates of native Iberian fish fauna. *Ecology Freshwater Fish*, **16**, 504-514.

Filipe, A.F., Cowx, I.G. & Collares-Pereira, M.J. (2002) Spatial modelling of freshwater fish in semi-arid river systems: a tool for conservation. *River Research and Applications,* **18**, 123-136.

Filipe, A.F., Marques, T., Seabra, S.G., Tiago, P., Ribeiro, F., Moreira da Costa, L., Cowx, I.G. & Collares-Pereira, M.J. (2004) Selection of priority areas for fish conservation in the Guadiana River Basin, Iberian Peninsula. *Conservation Biology,* **18**, 189-200.

Gasith, A. & Resh, V.H. (1999) Streams in Mediterranean Climate Regions: abiotic influences and biotic responses to predictable seasonal events. *Annual Review of Ecology and Systematics*, **30**, 51-81.

Gray, J.S. (2006) Marine diversity: the paradigms in patterns of species richness examined. *Scientia Marina*, **65**, 41-56.

Grenouillet, G., Pont, D. & Hérissé, C. (2004) Within-basin fish assemblage structure: the relative influence of habitat versus stream spatial position on local species richness. *Canadian Journal of Fisheries and Aquatic Sciences*, **61**, 93-102.

Griffiths, D. (2006) Pattern and process in the ecological biogeography of European freshwater fish. *Journal of Animal Ecology*, **75**, 734-751.

Irz, P., Argillier, C., Oberdorff, T. (2004) Native and introduced fish species richness in French lakes: local and regional influences. *Global Ecology and Biogeography*, **13**, 335-344.

Johnson, L.B. & Gage, S.H. (1997) Landscape approaches to the analysis of aquatic ecosystems. *Freshwater Biology*, **37**, 113-132.

Leprieur, F., Olden, J.D., Lek, S. & Brosse, S. (2009) Contrasting patterns and mechanisms of spatial turnover for native and exotic freshwater fish in Europe. *Journal of Biogeography,* **36**, 1899-1912.

Lockwood, J.L., Cassey, P., & Blackburn, T.M. (2009) The more you introduce the more you get: the role of colonization pressure and propagule pressure in invasion ecology. *Diversity and Distributions*, **15**, 904-910.

Magalhães, M.F., Beja, P., Canas, C. & Collares-Pereira, M.J. (2002) Functional heterogeneity of dry-season fish refugia across a Mediterranean catchment: the role of habitat and predation. *Freshwater Biology,* **47**, 1919-1934.

Magalhães, M.F., Beja, P., Schlosser, I.J. & Collares-Pereira, M.J. (2007) Effects of multi-year droughts on fish assemblages of seasonally drying Mediterranean streams. *Freshwater Biology,* **52**, 1494-1510.

Marta, P., Bochechas, J. & Collares-Pereira, M.J. (2001) Importance of recreational fisheries in the Guadiana River Basin in Portugal. *Fisheries,* **8**, 345.

Matthews, W.J. (1998) *Patterns in freshwater fish ecology*. Chapman & Hall, New York.

McAllister, D.E., Platania, S.P., Schueler, F.W., Baldwin, M.E. & Lee, D.S. (1986) Ichthyofaunal patterns on a geographic grid. *The Zoogeography of North American Freshwater Fishes* (ed. by C.H. Hocutt and E.O. Wiley), pp. 17–51. John Wiley & Sons, New York.

McDowall, R.M. 1999. Diadromy and genetic diversity in Neartic and Palearic fishes. *Molecular Ecology*, **8**, 527-528.

Mesquita, N., Cunha, C., Carvalho, G.R. & Coelho, M.M. (2007) Comparative phylogeography of endemic cyprinids in the south-west Iberian Peninsula: evidence for a new ichthyogeographic area. *Journal of Fish Biology,* **71**, 45-75.

Moyle, P.B. & Herbold, B. (1987) Life-history patterns and community structure in stream fishes of western North America: comparisons with eastern North America and Europe. *Community and Evolutionary Ecology of North American Stream Fishes* (ed. by W.J. Matthews and D.C. Heins), pp. 25–32. University of Oklahoma Press, Norman, OK.

Moyle, P.B. & Yoshiyama, R.M. (1994) Protection of Aquatic Biodiversity in California: A Five-tiered Approach. *Fisheries,* **19**, 6-18.

Naiman, R.J. (2009) New perspectives for watershed management: balancing long-term sustainability with cumulative environment change (ed. by R.J. Naiman). *Watershed management: balancing sustainability and environmental change*, pp. 3-11. Springer-Verlag, New York.

Oberdorff,T., Hugueny,B., Compin,A., & Belkessam,D. (1998) Non-interactive fish communities in the coastal streams of North-western France. Journal of Animal Ecology, **67**, 472-484.

Olden, J.D., Poff, N.L., Douglas, M.R., Douglas, M.E. & Fausch, K.D. (2004) Ecological and evolutionary consequences of biotic homogenization. *Trends in Ecology & Evolution,* **19**, 18-24.

Paterson, J.S., Araújo, M.B., Berry, P.M., Piper, J.M. & Rounsevell, M.D.A. (2008) Mitigation, adaptation and the threat to biodiversity. Conservation Biology, 22, 1352–1355.

Peres-Neto, P.R. (2004) Patterns in the co-occurrence of fish species in streams: the role of site suitability, morphology and phylogeny versus species interactions. *Oecologia*, **140**, 352-360.

Pires, D.F., Pires, A.M., Collares-Pereira, M.J., & Magalhães, M.F. (2010) Variation in fish assemblages across dry-season pools in a Mediterranean stream: effects of pool morphology, physicochemical factors and spatial context. *Ecology Freshwater Fish*, doi: 10.1111/j.1600-0633.2009.00391.x.

Poff, N.L. (1997) Landscape filters and species traits: towards mechanistic understanding and prediction in stream ecology. *Journal of North American Benthological Society,* **16**, 391-409.

Pringle, C.M. (2001) Hydrologic connectivity and the management of biological reserves: a global perspective. *Ecological Applications,* **11**, 981-998.

Puth, L.M. & Wilson, K.A. (2001) Boundaries and corridors as a continuum of ecological flow control: lessons from rivers and streams. *Conservation Biology,* **15**, 21-30.

Rahel, F.J. (2007) Biogeographic barriers, connectivity and homogenization of freshwater faunas: it's a small world after all. *Freshwater Biology,* **52**, 696-710.

Rahel, F.J., Bierwagen, B. & Taniguchi, Y. (2008) Managing Aquatic Species of Conservation Concern in the Face of Climate Change and Invasive Species. *Conservation Biology,* **22**, 551-561.

Rahel, F.J. & Olden, J.D. (2008) Assessing the Effects of Climate Change on Aquatic Invasive Species. *Conservation Biology,* **22**, 521-533.

Rahert, D.; White, D.; Difneos, J.C. & Hughes, R. M. 1999. Environmental correlates of species richness for native freshwater fish in Oregon. *Journal of Biogeography*, **26**, 257-273.

Revenga, C., Brunner, J., Henninger, N., Kassem,K. & Payne,R. (2000) *Pilot Analysis of Global Ecosystems: Freshwater Systems.* World Resources Institute, Washington D.C.

Ribeiro, F. (2008) *Patterns and processes of fish invasions in Iberian rivers: the lower Guadiana drainage ad a case study*. PhD Thesis, University of Lisboa, Lisboa, Portugal.

Ribeiro, F., Gante, H.F., Sousa, G., Filipe, A.F., Alves, M.J., Magalhães, M.F. (2009) New records, distribution and dispersal pathways of *Sander lucioperca* in Iberian freshwaters. *Cybium*, **33**, 255-256.

Sadoff, C., Greiber, T., Smith, M. & Bergkamp, G. (2008) *Share – Managing Water Across Boundaries*. IUCN, Gland, Switzerland.

Sala, O.E., Chapin III, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans,R ., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, M.T., Walker, B.H., Walker, M. & Wall, D.H. (2000) Global biodiversity scenarios for the year 2100. *Science,* **287**, 1770-1774.

Sax, D.F., Schluter, D., Sobel, J.M. & Turelli, M. (2007) Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. *Ecology Letters*, **10**, 315-331.

Schlosser, I.J. 1991. Stream fish ecology: a landscape perspective. *BioScience*, **41**, 704-712.

Smith, S.A., Bell, G. & Bermingham, E. (2004) Cross-Cordillera exchange mediated by the Panama Canal increased the species richness of local freshwater fish assemblages. *Proceedings of the Royal Society B,* **271**, 1889-1896.

Smith, S.A. & Bermingham, E. (2005) The biogeography of lower Mesoamerican freshwater fishes. *Journal of Biogeography,* **32**, 1835-1854.

Smith, C.L. & Powell, C.R. (1971) The summer fish communities of Brier Creek, Marshall County, Oklahoma. *American Museum Novitates*, **2458**, 1-30.

Sutherland, W.J., Amstrong-Brown, S., Armsworth, P.R., Tom, B., Brickland, J., Campbell, C.D., Chamberlain, D.E., Cooke, A.I., Dulvi, N.K., Dusic, N.R., Fitton, M., Freckleton, R.P., Godfray, H.C.J., Grout, N., Harvey, H.J., Hedley, C., Hopkins, J.J., Kift, N.B., Kirby, J., Kunin, W.E., Macdonald, D.W., Marker, B., Naura, M., Neale, A.R., Oliver, T., Osborn, D. & Pullin, A.S. (2006) The identification of 100 ecological questions of high policy relevance in the UK. *Journal of Applied Ecology,* **43**, 617-627.

Svenning, J.C. & Skov, F. (2005) The relative roles of environment and history as controls of tree species composition and richness in Europe. *Journal of Biogeography*, **32**, 1019–1033.

Tedesco, P.A., Oberdorff, T., Lasso, C.A., Zapata, M. & Hugueny, B. (2005) Evidence of history in explaining diversity patterns in tropical riverine fish. *Journal of Biogeography,* **32**, 1899-1907.

Tonn, W.M., Magnuson, J.J., Rask, M. & Toivonen, J. (1990) Intercontinental comparison of small-lake fish assemblages: the balance between local and regional processes. *The American Naturalist,* **136**, 345-375.

Unmack, P.J. (2001) Biogeography of Australian freshwater fishes. *Journal of Biogeography,* **28**, 1053-1089.

Urban, M.C., Skelly, D.K., Burchsted, D., Price, W., & Lowry, S. (2006) Stream communities across a rural–urban landscape gradient. *Diversity and Distributions,* **12**, 337-350.

Vargas, J.M., Real, R. & Guerrero, J.C. (1998) Biogeographical regions of the Iberian peninsula based on freshwater fish and amphibian distributions. *Ecography*, **21**, 371-382.

Videira, N., Antunes, P. & Santos, R. (2009) Scoping river basin management issues with participatory modelling: The Baixo Guadiana experience. *Ecological Economics,* **68**, 965-978.

Wiley, M.J.; Kohler, S.L. & Seelbach, P.W. (1997) Reconciling landscape and local views of aquatic communities: lessons from Michigan trout streams. *Freshwater Biology*, **37**, 133-148.

Wilson, J.R.U., Dormontt, E.E., Prentis, P.J., Lowe, A.J. & Richrdson, D.M. (2009) Something in the way you move: dispersal pathways affect invasion success. *Trends in Ecology & Evolution,* **24**, 136-144.

Zogaris, S., Economou, A., & Dimopoulos, P. (2009) Ecoregions in the Southern Balkans: Should Their Boundaries Be Revised? *Environmental Management*, **42**, 682-697.

Chapter 7.

Concluding remarks

"You might be a big fish
In a little pond
Doesn't mean you've won..."

Coldplay band music, 2008, *in* lyric from "Lost "song

7.

Concluding Remarks

The main aim of this dissertation was to examine the spatial patterns of species composition and richness of freshwater fish inhabiting Iberian Peninsula. By using occurrence data at the Iberian Peninsula and South-western area scales, the research conducted allowed to identify the main driving factors and ecological processes, answering the questions initially raised. They can be summarized as follows:

(A) The hydrological constraints formed since the Pliocene–Pleistocene constituted the most obvious factor related to composition of native fish assemblages across the Iberian Peninsula, and contemporary climate did not need to be invoked to explain existing biogeographical patterns (Chapter 3). In the south-western part of the Iberian Peninsula the pool of species of each river basin seems to be driving the local species richness, for both native and invasive species; the distances throughout hydrological network accounted for a major amount of variability in species richness, while topography and climate landscape characteristics played a significant but minor role (Chapter 4);

(B) The current patterns of freshwater fish species, across the Iberian Peninsula or in the south-western area were strongly related with ecological contagious processes, such as dispersal and colonization. This denotes that dispersal limitation is an important factor to consider across all spatial and temporal scales, and has strong implications on theoretical and applied research but also regarding conservation and management of freshwater faunas and ecosystems, which need joint efforts of all Iberian regions to obtain better results. It is essential to cross these outcomes with conservation and management strategies and actions for Iberian stream faunas, and fish species in particular.

At last, Iberian freshwater fish assemblages emerged as being in non-equilibrium state with contemporary environmental conditions, and contingent on species' ability to disperse through time via hydrological connections. Both past landscape events and evolutionary history of species are much imprinted in the current assemblages. This knowledge is indeed fundamental to the understanding of why we can find a species in a certain place, and goes far beyond considering only local environment characteristics, as frequently done in freshwater biodiversity assessments which are mainly based solely on local scales.

7.1 Future directions

While asking specific questions, new questions were raised. The analyses conducted generated several ideas and hypotheses, and some can be pointed as topics for future research, as:

a Linking explicitly phylogenetic data might provide the missing historical information. For example, regarding the study cases conducted here, the within genus genetic variability and reconstitution of past hydrological events might help to understand current regional patterns of Iberian freshwater fish assemblage composition;

b Examining the relative importance of possible faunal dispersal pathways created by climate-driven sea-level fluctuations in the past might help to better understand the dispersal consequences of sea-level fluctuations associated with current climate change, which seems likely relevant for species constrained to fresh waters;

c Adding factors of human induced colonisation actions of non-native species seems a promising approach on examining the key-properties of dispersal pathways, as colonization pressure, genetic diversity, potential of colonization of coevolved species, and establishment mechanisms. This will not only help to understand invasiveness patterns and mechanisms, but also will enlighten ecological processes in general;

d Examining local mechanisms, as species interactions, species life-histories and biological attributes, and crossing with information on dispersal boundaries and altered habitats will add relevant information how local processes that underline spatial broad-scale patterns act.

Finally, especially relevant in future studies is to design efficient strategies of field survey assessments that attain knowledge gaps of biodiversity databases, including recommendation protocols on data collecting and gathering to all collectors. Specifically, main recommendations are to examine the adequacy of fish sampling, the influence of merging datasets of distinct sizes, and the influence of using specific data grains, as land-based grid-cells.

As a general conclusion of the present dissertation, it arises that the traditional local approaches are not enough to accomplish challenges on both basic and applied research, and on conservation and management of riverine fauna. Multiscale and integrative approaches considering influences from fine to broader spatial and temporal scales are essential frameworks for understanding, evaluating and predicting biodiversity patterns, and the likely adaptations to current environmental changes.

Thus our study may be considered as a knowledge basis to the definition of conservation strategies in an Iberian context and should help promoting public awareness concerning stream ecosystems natural values and threats. Not disregarding this important issue, freshwater conservation must be based on sound theory and high quality data. This dissertation raised several new questions when trying to get accurate answers to the ones initially proposed, but as referred biodiversity studies at large scales in Iberian freshwaters are just beginning. At last, we hope to have contributed to evidence that there is still a long way to go, by pointing out some of the current challenges and by opening new research avenues as regards the topics under analysis.

Appendix 1.

Biogeographical provinces delineated in previous studies

Appendix I.

Among the previous biogeographical provinces of fish species delineated for the Iberaian Peninsula, those most referenced are presented below (Figures A1, A2).

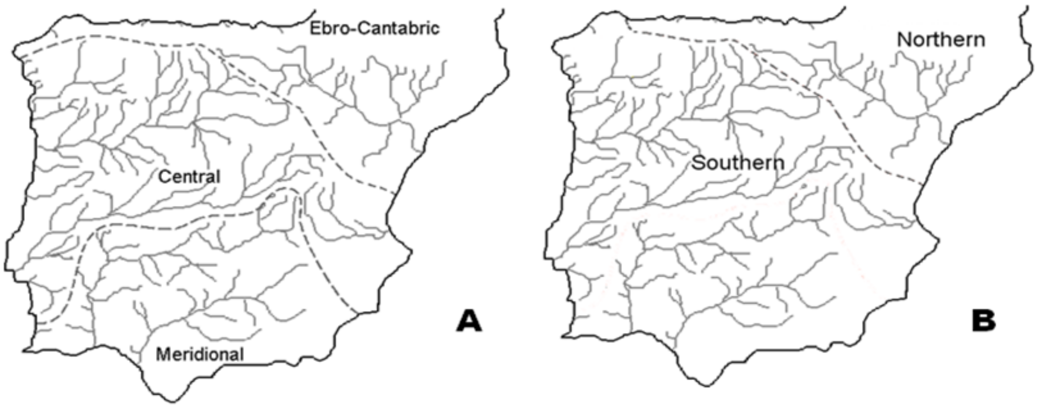


Figure A 1 Zoogeographical sectors considered by Almaça, (1978) and recognized by Vargas (1998) (A), and by Doadrio (1988) (B) (figure adapted from the referenced works). (A) The Ebro-Cantabric or Cantabric (Ebro catchment and Cantabric region), the Central or Atlantic (Douro and Tejo catchments, including also Túria, Jucar e Mijares catchments), and the Meridional or Mediterranean (Guadiana and Guadalquivir catchments and the south region of Spain). (B) The northern Iberian region, constituted by the basins of Ebro and Cantabric region, and the southern Iberian region that includes the other basins in the Peninsula.

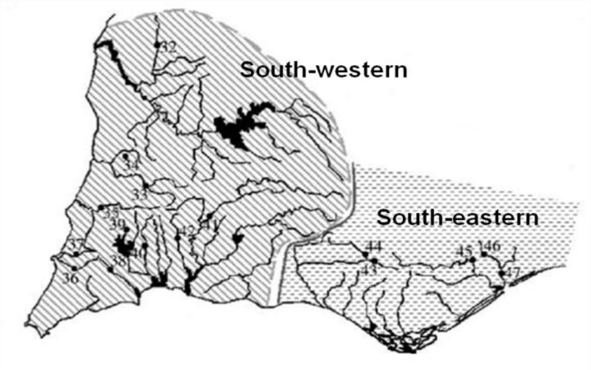


Figure A 2 A detail of the zoogeographical sector considered by Mesquita *el al*. (2007) which discerned areas in Meridional sector of Almaça (1978) by analyzing studying the evolutionary history of some Iberian and north Arican fish taxa,. (figure adapted from the referenced works).

References

Almaça, C. (1978) Répartition géographique des cyprinidae ibériques et secteurs ichthyogéographiques de la Péninsule Ibérique. *Vestník Ceskolovenské Spolecnosti Zoologické,* **XLII**, 241-248.

Doadrio,I. (1988) Delimitation of areas in the Iberian Peninsula on the basis of freshwater fishes. *Bonner Zoologische Beitraege,* **39**, 113-138.

Mesquita, N., Cunha, C., Carvalho, G.R. & Coelho, M.M. (2007) Comparative phylogeography of endemic cyprinids in the south-west Iberian Peninsula: evidence for a new ichthyogeographic area. *Journal of Fish Biology*, **71**, 45–75.

Vargas, J.M., Real, R. & Guerrero, J.C. (1998) Biogeographical regions of the Iberian Peninsula based on freshwater fish and amphibian distributions. *Ecography,* **21**, 371-382.

Appendix II.

Additional study: selection of priority areas for fish conservation

Appendix II.

Filipe, A.F., Marques, T., Seabra, S.G., Tiago, P., Ribeiro, F., Moreira da Costa, L., Cowx,I.G., & Collares-Pereira,M.J. (2004) Selection of priority areas for fish conservation in the Guadiana River Basin, Iberian Peninsula. *Conservation Biology*, 18**,** 189-200.

Selection of Priority Areas for Fish Conservation in Guadiana River Basin, Iberian Peninsula

A. F. FILIPE,* T. A. MARQUES,* S. SEABRA,* P. TIAGO,* F. RIBEIRO,* L. MOREIRA DA COSTA,*
I. G. COWX,† AND M. J. COLLARES-PEREIRA*‡

*Centro de Biologia Ambiental and Departamento de Biologia Animal, Faculdade de Ciências, 1749-016 Lisboa, Portugal
†Hull International Fisheries Institute, University of Hull, HU6 7RX, United Kingdom

Abstract: *We developed a species-by-species approach for selecting protected areas for conservation of native freshwater fishes in semiarid regions, with catchment as the fundamental landscape unit for conservation efforts. Input data were composed of occurrences of freshwater fishes and landscape variables, and general quantification of occurrence, abundance, and endemicity of each fish species, to derive an index of conservation value for each species. Probability of occurrence of each species was performed with logistic-regression analysis based on the landscape variables and extrapolated to the entire study area with a geographic information system. We estimated the conservation value of a stream reach by summing the predicted probability of occurrence of each species multiplied by its corresponding conservation value. To define and select reserves, we used a threshold that maximizes conservation value of the stream reaches but identifies the minimum number of reaches for protection. The approach was applied to native freshwater fishes in the Guadiana River basin (southern Iberian Peninsula), which are threatened by the construction of two major dams. We used the data from 1 sampling year (1999) to produce the models, which were validated based on data collected in 2000 and 2001. We used variables of climate (3), geomorphology (4), hydrology (7), and human influence (6) to build the predictive models, which revealed that native species occur over a wide range of riverine habitats, with stream order and location in the basin the most frequently selected variables. The conservation value of species varied considerably, with* Anaecypris hispanica *the highest-ranked species. The catchments selected for reserves were the mainstream of the Guadiana River (upstream and downstream of the Alqueva and Pedrogão reservoirs) and the Degebe, Ardila, and Enxoé catchments. Our approach is a pragmatic way to address the urgent need to protect Guadiana native fish species in light of the ongoing anthropogenic degradation of aquatic environments.*

Key Words: Alqueva Reservoir, conservation areas, conservation index, Cyprinidae, distribution, freshwater reserves, Iberian Peninsula, landscape variables, modeling

Selección de Áreas Prioritarias para la Conservación de Peces en la cuenca del Río Guadiana, Península Ibérica

Resumen: *Desarrollamos un método especie-específico para seleccionar áreas protegidas para la conservación de peces nativos de agua dulce en regiones semiáridas, con la subcuenca como unidad paisajística fundamental para los esfuerzos de conservación. Los datos de entrada fueron las ocurrencias de peces de agua dulce y las variables de paisaje, una cuantificación general de la ocurrencia, abundancia y endemicidad de cada especie piscícola para obtener un índice del valor de conservación para cada especie. La probabilidad de ocurrencia de cada especie se obtuvo con análisis de regresión logística basado en las variables del paisaje y extrapolado a toda el área de estudio con un sistema de información geográfica. Estimamos el valor de conservación de un tramo sumando la probabilidad de ocurrencia de cada especie multiplicado por su valor de conservación correspondiente. Para definir y seleccionar reservas, utilizamos un umbral que maximiza el valor de conservación de los tramos de río pero identifica el número mínimo de tramos a proteger. El método*

---

‡Address correspondence to M. J. Collares-Pereira, email mcolares@fc.ul.pt
Paper submitted December 17, 2001; revised manuscript accepted August 21, 2003.

Conservation Biology, Pages 189–200
Volume 18, No. 1, February 2004

fue aplicado a peces nativos de agua dulce en la cuenca del Río Guadiana (sur de la Península Ibérica), que están amenazados por la construcción de dos grandes embalses. Utilizamos los datos de un año (1999) de muestreo para producir modelos, que fueron validados con base en datos colectados en 2000 y 2001. Utilizamos las variables de clima (3), geomorfología (4), hidrología (7) e influencia humana (6) para construir los modelos predictivos, que revelaron que las especies nativas se distribuyen en un amplio espectro de hábitats ribereños, con el orden de río y su localización en la cuenca como las variables seleccionadas más frecuentemente. Los valores de conservación de las especies variaron considerablemente, con Anaecypris hispanica *como la especie de mayor jerarquía. Las subcuencas seleccionadas como reservas fueron el cauce principal del Río Guadiana (aguas arriba y aguas abajo de los embalses de Alqueva y del Pedrogão) y las subcuencas Degbe, Ardila y Enxoé. Nuestro método es una manera pragmática de atender la urgente necesidad de proteger las especies de peces nativas debido a la continua degradación antropogénica de ambientes acuáticos.*

Palabras Clave: áreas de conservación, Cyprinidae, Embalse del Alqueva, índice de conservación, modelo de distribución, Península Ibérica, Reservas de agua dulce, variables del paisaje

Introduction

Freshwater biodiversity is being lost at an alarming rate, even more rapidly than terrestrial biodiversity and especially in semiarid and arid regions (Moyle & Yoshiyama 1994; Cowx & Collares-Pereira 2002), where demands for water resources are growing. Efforts to conserve freshwater biodiversity are often lacking or ineffective, in part because protected areas are usually created for terrestrial biota (e.g., Williams et al. 1996; Kerr 1997; Lombard et al. 1997) with little consideration of the needs of aquatic species (Crivelli 2002; Saunders et al. 2002).

The design of reserve networks must ensure the appropriate representation and persistence of biodiversity (Araújo & Williams 2000; Margules & Pressey 2000). Saunders et al. (2002) conclude that adequate selection of freshwater reserves and adequate conservation efforts can maintain freshwater biodiversity but, where designed to conserve freshwater biota, reserves often fail because organizational processes (Angermeier & Winston 1999) and threats to freshwater biodiversity are not necessarily eliminated in the reserve selection. In fact, all life stages and threats related to land use, altered hydrology, and introductions of non-native species occur over scales much larger that those typically encompassed by terrestrial reserves and generally can only be addressed at the catchment scale, the fundamental landscape unit for conservation efforts (Naiman et al. 1993; Moyle & Yoshiyama 1994; Puth & Wilson 2001; Crivelli 2002; Saunders et al. 2002).

Therefore, one of the key constraints on progress in the conservation of freshwater fishes is proper selection of reserves that can support conservation objectives. This is a fundamental issue in the European Union, where countries are required to designate "special areas for conservation" under the Habitats Directive 92/43/EEC, the so-called Natura 2000 network. Despite many fish species being threatened in Europe (World Conservation Union 2000), few sites have been proposed or designated for conservation. Some procedures have been proposed to rank freshwater areas based to a certain extent on expert opinion (e.g., Rabe & Savage 1979; Maitland 1985; Minns 1987), but these fail to use objective or quantitative criteria for decision support or independent validation. Winston and Angermeier (1995) focused on density as an indicator of source populations and low extinction probability and therefore high population persistence. This procedure invokes source and sink dynamics—which are crucial when fresh waters are involved because local extinction is common, especially in semiarid regions—but it does not have a landscape-scale perspective. The application of gap analysis that defines complementary areas within a landscape (Rowe 1997; see aquatic GAP Analysis, http://www.gap.uidaho.edu/Projects/Aquatic/default.htm) has been proposed as an additional step in the prioritization of freshwater conservation areas for addressing quantification and nonarbitrary criteria, but the metapopulation aspects referred to above were not included.

Here we present a methodology for selecting important areas for the conservation of native freshwater fish on a regional scale based on quantifiable criteria integrating the concept of metapopulations.

We provide a protocol for reserve selection in which the main conservation goal is to protect species of limited distribution and abundance, through protecting catchments that may support the source populations of valued species. The procedure is based on a weighted ranking of species and reaches and on probability models of species occurrence.

The specific objectives of this work are to (1) present detailed steps of a new method to select freshwater reserves, (2) apply the method to a real case study, (3) point out possible sensitivity of the method in the face of data limitations, and (4) give insights into the management of the proposed freshwater reserves and conservation of the freshwater fish species. To achieve these objectives, we present an overview of the approach and illustrate its application with the case study of native freshwater fishes in the Guadiana River basin, a semiarid region with growing pressure on water resources.

Methods

Study Area

The Guadiana River basin, located in the southwestern Iberian Peninsula, originates in Spain at an elevation of 1700 m and flows south to the Atlantic Ocean through Portugal. The basin area is 66,960 km^2 and has a typical Mediterranean hydrological regime of highly intra- and interannual discharge variation, with severe droughts and floods (Gasith & Resh 1999; Cowx & Collares-Pereira 2000). During summer, most streams are reduced to a series of isolated pools.

In the last 20 years, water-development schemes have been constructed in this basin to supply water for agriculture and human consumption. The most important is Alqueva Reservoir, in the Portuguese middle reaches of the Guadiana River, which was under construction during the study period but is now being filled (dam closed in February 2002). Alqueva and Pedrogão, another reservoir still under construction further downstream, will flood a combined area of 262 km^2.

The study area is in the middle region of the Guadiana River basin and encompasses 5900 km^2 (Fig. 1). Elevation ranges from 0 to 650 m, and riparian vegetation is sparse and dominated by the genus *Fraxinus* L., *Nerium oleander* L., and *Securinega tinctoria* (L.) Rothm. The soil is schist-derived and highly impermeable. Groundwater is scarce. The watercourses studied include 140 km of the Guadiana River and 11 tributaries, 10 of which will be inundated in their downstream reaches when the two reservoirs are full. The region supports a diverse freshwater fish fauna comprising 11 primary native species: the 4 barbel species (*Barbus comizo*, *Barbus sclateri*, *Barbus steindachneri*, *Barbus microcephalus*), the 2 nase species (*Chondrostoma willkommii*, *Chondrostoma lemmingii*), *Anaecypris hispanica*, and *Salaria fluviatilis* are all classified as threatened (Serviço Nacional de Parques [SNPRCN] 1991). Only the *Squalius alburnoides* complex, *Squalius pyrenaicus*, and *Cobitis paludica* are not threatened (SNPRCN 1991). These species are endemic to the Iberian Peninsula, except *S. fluviatilis*, a circum-Mediterranean endemic. There are also 9 exotic fish species (*Cyprinus carpio*, *Carassius auratus*, *Tinca tinca*, *Lepomis gibbosus*, *Micropterus salmoides*, *Herichthys facetum*, *Gambusia holbrooki*, *Esox lucius*, and *Ameiurus melas*). The distribution patterns of the native species are highly variable, both through time within a stream (Pires et al. 1999) and through space within a catchment (Filipe et al. 2002). Some studies suggest that the endemic cyprinids move upstream to reproduce in spring and downstream

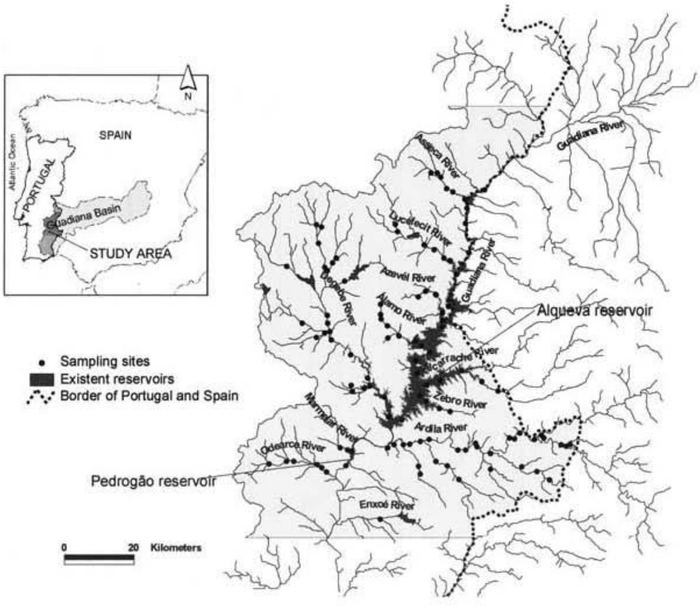


Figure 1. Sampling sites in the middle region of the Guadiana River basin, Portugal, including the designation of the rivers or tributaries (the same as the correspondent catchments) and of the existent reservoirs.

to avoid the harsh drought conditions during summer (Collares-Pereira et al. 2000*a*; Ribeiro et al. 2000). The range and abundance of many native fishes has declined (e.g., Collares-Pereira et al. 2000*a*, 2000*b*); by contrast, alien species are flourishing, mainly because they are adapted to the lentic conditions typically found in the numerous impoundments (Godinho & Portugal e Castro 1999; Collares-Pereira et al. 2000*b*; Corbacho & Sánchez 2001).

Fish Data

During 1999 we selected 110 sites extended across the 11 catchments and in the main river, maximizing the river distances within each catchment (Fig. 1). Where possible, we collected several samples from a site in different seasons to improve the completeness of species lists (27 sites sampled 2–6 times, for a total of 207 surveys). Ninety-six new sites, in addition to the 27 referred to above, were sampled in 2000 and 85 in 2001. Sites were 30–50 m long and representative of the habitat types available in the reach. We sampled sites with electrofishing (pulsed direct current, 300/600V, 4–6A, without block nets) for 15 minutes to estimate the presence or absence and abundance of each fish species. Site length was proportional to river width. Capture-efficiency estimates are not available for the area, but previous studies indicate that this sampling effort was sufficient to capture most species present, except in large river sites (Collares-Pereira et al. 2000*b*). All fish were returned alive to the river after we identified individuals to species. However, cyprinids <2 cm in total length (TL) and juveniles of the genus *Barbus* (TL < 10 cm) were excluded from further analysis because they could not be reliably identified to species. On some occasions we could not distinguish two of the four barbel species (*B. steindachneri* and *B. sclateri*) as adults (TL > 10 cm), so we used the term *B. steindachneri/sclateri* for these species.

Landscape Variables

Because occurrence data can be incomplete and biased toward accessible sites, in the first step of our protocol we applied a predictive model approach based on species occurrence, with landscape variables as a surrogate of complete data to supply information on species ranges (Borjóquez-Tapia et al. 1995). This approach also may clarify whether environmental changes will affect the probability that a target species will inhabit a specified location (Fleishman et al. 2002).

We acquired data on the landscape variables from across the region (vectorial digital maps with resolutions of 1:100,000 to 1:1,000,000) from Comissão Nacional do Ambiente (CNA 1983) and Empresa de Desenvolvimento e Infra-Estruturas de Alqueva (EDIA 1995–1999). Of the 20 landscape variables used for species modeling, 3 were climatic, 4 were geomorphological, 7 were hydrological, and 6 were related to human influence; all were treated in a geographic information system (GIS), ArcView 3.2 for Windows (Environmental Systems Research Institute), and converted into several classes (Appendix 1). The size and number of classes depended on the data available, resulting in a somewhat arbitrary class structure. We calculated some of the landscape measures in a GIS by using the original landscape layers (designated "derived from" in Appendix 1). All layers were clipped with a template map for the river hydrological network (resolution 1:1,000,000) (CNA 1983), resulting in 20 linear landscape layers in which each line represents a stream reach (i.e., a continuous array of pool-riffle configurations) bounded by the size and shape of the polygons of original landscape layers (resolution 1:1,000,000).

Modeling Analysis: Prediction of Species Occurrence

We constructed a logistic-regression model for each species to identify the landscape variables that are the best predictors of species occurrences, allowing further extrapolation for unsampled areas (see also Hosmer & Lemeshow 1989; Brito et al. 1999; Filipe et al. 2002).

We combined the 20 linear landscape layers of the landscape variables into a single layer in a GIS and overlaid it with point data on species presence or absence, resulting in an output matrix that included information for all sampling sites on species presence or absence and the nearest value of each landscape variable. For pairs of variables with a correlation coefficient Kendall-Tau *b* (Norusis 1995) of >0.75, we excluded the variable with the less clear biological interpretation (Mladenoff et al. 1995). Next, we built a data matrix for each species. Because predictive success is sensitive to the proportion of presences (Hosmer & Lemeshow 1989), for species that did not have 40–60% of sites with species present, we randomly selected rows (i.e., sites) to ensure a similar number of presences and absences (i.e., a subset of 40–60% of presences) (Brito et al. 1999).

For building each model, we chose the best combination of predictive variables with step-wise selection (Hosmer & Lemeshow 1989; Wald's test with probability of entry = 0.15 and probability of removal = 0.20). Categorical variables were codified as dummy, and interactions between variables were included in the variables selection. We assessed the goodness of fit of each model—how well the model described the data used on its construction—with a G test (statistics based on the likelihood ratio) and a classification table that includes correct classification of presences, absences, and total correct classification (TCC) (Tabachnick & Fidell 1996). To validate each model—how well the model describes new data—we used the year 2000 data set (96 new sampling sites, localized on the same study area) and compared the TCC of these observed occurrences against the ones predicted by the model generated with the 1999 data set. We also constructed logistic-regression models with the data from 1999–2001 (summing 291 sampling sites) to compare TCC rates with those obtained with models built only with 1999 data. For calculation of correct classifications, we transformed probability of occurrence into a binary variable of presence or absence of the species, with presence assigned at =0.5 (i.e., cut-off point of 0.5). We used SPSS 7.0 for Windows (Norusis 1995) for statistical analyses.

We extrapolated species probability of occurrences for each reach across the entire study area based on the models above. Because the landscape variables were defined at different resolutions, each layer of species probability of occurrence had the coarsest resolution of variables included in the model.

Conservation Value of Species and Reaches

To select priority areas for conservation of watercourses in the region according to the predetermined conservation goals, it is necessary to maximize biodiversity representation based on the available species data. Therefore, for the second step we used a method that ranks stream reaches according to probability of occurrence of a species, taking into account whether the species is endemic to a particular area and whether it is rare or abundant.

For the quantification of the relative conservation value of each species (VS), we used data on frequency of occurrence, total number of captured individuals of each species, and evaluation of the degree of endemism, assuming that the aggregation of these three criteria quantifies the species' relative importance for conservation better than occurrence alone. Therefore, the conservation value for species k (VS_k) is

$$\mathrm{VS}_k = \frac{\left(a\dfrac{1/O_k}{\sum_{i=1}^{S} 1/O_i} + b\dfrac{1/\ln T_k}{\sum_{i=1}^{S} 1/\ln T_i} + c\dfrac{1/E_k}{\sum_{i=1}^{S} 1/E_i}\right)}{3} \times 100,$$

where S is the number of species considered, O_k is the total number of sampling sites where species k occurred in all samples, T_k is the total number of captured individuals of species k in all samples, E_k is the endemic value of species k according to its distribution range (the species with lowest value has the most restricted distribution), and a, b, and c are weighting factors that may vary according to the importance placed on conserving distribution (a), abundance (b), or endemicity (c).

We constructed this index so that the sum of the conservation values of all species considered would be 100, which was true as long as $a + b + c = 3$. The scores for species endemism were 1, Guadiana endemic; 2, Meridional Sector of Iberian Peninsula endemic (sensu Almaça 1978); 3, Iberian endemic; 4, circum-Mediterranean endemic. Because most species encountered in the case study are endemic to Iberia or the Meridional Sector, the weighting for endemicity (c) in the index was attributed a lower weight ($c = 0.6$) than species distribution ($a = 1.2$) and species abundance ($b = 1.2$). We assigned greater weight to the latter two variables because our conservation goal was to select priority watercourses at a regional level. This weighting needs to be adjusted to fit the conservation goals and scenario to which the approach is being applied. For example, in the extreme case where the entire range of each species is included in the study area, the endemism value (E_k) and number of occurrences (O_k) are necessarily redundant, and E_k should not be considered ($c = 0$).

The conservation value of each area j ($j = 1, \ldots, N$) (VA_j) across the study region was calculated as the sum of the products of the probability of occurrence of each species at each area and the corresponding species conservation value (VS_k):

$$\mathrm{VA}_j = \sum_{k=1}^{S} (P_{kj} \times \mathrm{VS}_k),$$

where P_{kj} is the probability of occurrence of species k in area j and VS_k is the conservation value of species k. The conservation value of area j varies between 0 and 100.

In the case study we present, the area j is better described as reach j, so we refer to VAj as the conservation value of each reach j. We constructed the layer of the reach conservation value in GIS based on the layers of probability of occurrence of each native freshwater species.

Priority Areas for Conservation

At the third step of our framework, to define the threshold of VA, the value above which the reaches are selected for reserves, we plotted a cumulative-frequency curve of reaches with increasing VA values, excluding those to be flooded by the reservoirs. The threshold VA optimizes the relation of VA and the watercourses to be protected (i.e., the highest VA with the minimum number of selected reaches). This value is identified by the stabilizing point of the curve referred to above. Thereafter, the catchments layer was overlaid with those selected reaches, allowing the identification of the catchments with the majority of reaches with VA above the threshold because the catchment scale is the fundamental landscape unit for conservation of freshwater fish. All watercourses that link the selected catchments must be considered to act as corridors and ensure continuation of the regional ecological processes necessary for maintaining freshwater fish diversity.

Table 1. **Goodness-of-fit measures (*G* test results and percentage of correct classification of the predictions vs. observations) and explanatory variables of the occurrence models (including the estimated coefficients β and the wald test results) for native freshwater species (except *Anaecypris hispanica*) in the middle region of the Guadiana River basin.**

	Overall model[a]				*Explanatory variables*		
Species	G (p)	*TCC*	*PCC*	*ACC*	*variable*[b]	β^c	*Wald (p)*
Barbus steindachneri/sclateri	8.404 (0.015)	81.2	82.4	79.4	TRI	0.334	1.145 (0.285)
					ORD	0.707	14.583 (0.000)
Barbus comizo	14.826 (0.001)	80.0	85.7	76.2	EVA ∗ ORD	0.484	7.201 (0.007)
					TEM	2.141	6.039 (0.014)
Salaria fluviatilis	24.957 (0.000)	78.3	70.8	78.3	ORD	0.952	14.350 (0.000)
					DDO	−0.508	4.553 (0.033)
Chondrostoma lemmingii	29.566 (0.000)	76.7	75.0	77.8	ORD	−1.220	10.756 (0.001)
					DGU	0.539	5.923 (0.015)
					WAT	1.079	4.638 (0.031)
Chondrostoma willkommii	16.963 (0.001)	73.9	61.5	82.1	ORD	0.622	6.172 (0.013)
					DGU	0.383	5.034 (0.025)
					POL	−0.328	3.308 (0.069)
Squalius pyrenaicus	23.539 (0.000)	72.2	55.6	83.3	DGU	0.501	14.663 (0.000)
					ROC	−0.497	6.166 (0.013)
Barbus microcephalus	27.868 (0.000)	72.0	70.8	73.1	ORD	0.799	15.625 (0.000)
					Dam		
					Dam (1)	1.257	0.032 (0.858)
					Dam (2)	3.187	0.205 (0.650)
					POL	−0.257	3.144 (0.076)
Squalius alburnoides complex	10.385 (0.006)	70.9	93.9	36.4	POL	−0.240	3.279 (0.702)
					SOL	−1.137	5.635 (0.018)
Cobitis paludica	13.921 (0.001)	63.0	48.3	85.0	ROC	−0.756	9.825 (0.002)
					SOL	−0.249	0.409 (0.522)

[a] *Abbreviations: TCC, percentage of total correct classification; PCC, percentage of presence correct classification; ACC, percentage of absence correct classification.*

[b] *Variables are defined in Appendix 1.*

[c] *β-estimated coefficients.*

Results

Modeling Analysis: Prediction of Species Occurrence

The predictive models for species explained most occurrences (Table 1; all *G* tests were significant, $p < 0.05$) except for the rarest species, *A. hispanica*, for which the *G* test was not significant and most of the occurrence predictions were wrong. For the other rare species, prediction accuracies were high, although for *C. willkommii* there were considerable errors of omission (species detected but not predicted). Also, *S. pyrenaicus* and the abundant *C. paludica* had more omission than commission errors (species not detected but predicted). Only the most abundant, the *S. alburnoides* complex, had higher commission errors. The model for *C. paludica* had the lowest total correct predictions (TCC = 63%), whereas the *B. steindachneri/sclateri* model had the highest (81%). The models constructed with data from 1999, when validated with the data from 2000, yielded lower, but similar TCC for each species (average change per species of TCC between the two data sets: 8.02%). Furthermore, modeling based on 1 year of data seems acceptable for the presented case study because models constructed with 3 years of data improved predictive ability little (3.09% of average change per species of TCC).

Stream order and location in the basin played an important role in the occurrence of species (Table 1), as illustrated by maps of the predicted distribution (Appendix 2). Human influence also constrained species distributions, in particular for *B. microcephalus*. *C. willkommii*, *B. comizo*, *B. microcephalus*, *B. steindachneri/sclateri* group, and *S. fluviatilis* all occurred in large streams, and the last species occurrences were close to the Alqueva Dam area. The first species occurred in reaches distant from the main river and away from sources of pollution. Among the barbels there were several differences in occurrence that suggest different habitat requirements: *B. comizo* occurred in regions with high air temperatures, *B. microcephalus* in areas upstream of dams with no pollution source, and *B. steindachneri/sclateri* group mainly in the northern watercourses of the study area. *C. lemmingii* occurred at sites distant from the main river with small stream orders and abundant groundwater. Some species distributions were related to geomorphological variables, namely those of *S. pyrenaicus*, *S. alburnoides* complex, and *C. paludica*. The latter two species occurred in areas with acid soils, and *C. paludica* was associated with sedimentary rocks. *S. pyrenaicus* occurred in watercourses distant from the main river, and the *S. alburnoides* complex did not occur in highly polluted waters.

Conservation Value of Species and Reaches

The conservation value of species varied considerably (Table 2). *A. hispanica* had the highest conservation

Table 2. Conservation value for each of the native freshwater fish species (VS) in the middle region of the Guadiana River basin and the data used for calculation.

Species	No. of sampling sites with occurrence	No. of captured individuals	Endemic value*	VS
Anaecypris hispanica	7	58	1	22.68
Barbus comizo	14	37	3	14.33
Chondrostoma lemmingii	24	554	2	9.38
Salaria fluviatilis	24	274	4	8.94
Chondrostoma willkommii	26	352	1	11.15
Barbus microcephalus	48	1032	1	8.89
Squalius pyrenaicus	38	576	3	7.32
Cobitis paludica	60	874	3	6.19
Barbus steindachneri/sclateri	66	2553	2	6.18
Squalius alburnoides complex	79	8581	3	4.95

**Endemism value of the species according to its distribution range: 1, Guadiana endemic; 2, Meridional Sector of Iberian Peninsula endemic (sensu Almaça 1978); 3, Iberian endemic; 4, Circum-Mediterranean endemic.*

value because it is a rare species endemic to the Guadiana River basin. *B. comizo* had a lower endemism value but was ranked second in conservation value because of its rarity and low total abundance. *S. alburnoides* complex had the lowest VS value because it was the most abundant and widespread taxon.

The Guadiana River reaches, downstream reaches of the biggest tributaries (Degebe, Alcarrache, and Ardila), and some smaller southern tributaries (Enxoé, Marmelar) had the highest conservation values (Fig. 2a). Because it was impossible to model the species *A. hispanica*, the sites where this species occurred were overlaid on the VA map to ensure that the areas where it occurred were included (for a similar approach see Borjóquez-Tapia et al. 1995). All occurrences were in watercourses that already had a high conservation value, except for the Álamo catchment. This catchment was highly polluted in the downstream reaches, but there was a small area in the headwaters where *A. hispanica* occurred.

Priority Areas for Conservation

The optimum VA for selecting reaches as reserves for conservation was >53, which would designate 15% of the study area (Fig. 3). To include the corridors between reserves, however, we used a cut-off point of VA > 45, which designated 40% of the reaches for protection. After overlaying the reaches equivalent to VA > 45 with the catchment polygons, the selected reserves were the main stream of the Guadiana River (upstream and downstream of the Alqueva and Pedrogão reservoirs) and the Degebe, Ardila, and Enxoé catchments (Fig. 2b).

Discussion

Selecting Priority Areas for Conservation

Our method is a practical way of ranking watercourses for conservation based on probability of occurrence of

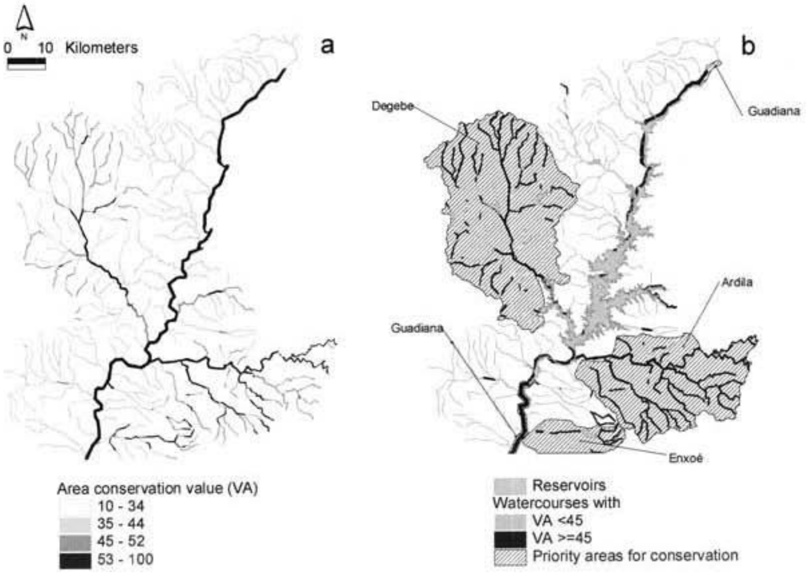


Figure 2. (a) Conservation value of each reach presented in four classes (minimum 10 and maximum 100) and (b) priority catchments for conservation within the middle region of the Guadiana River basin, Portugal.

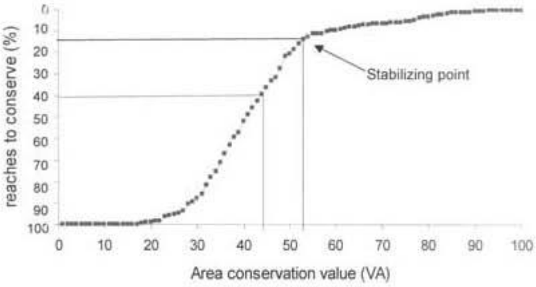


Figure 3. Cumulative frequency curve of reaches conservation value (VA) of the middle region of the Guadiana River basin, excluding reaches that will be flooded by the reservoirs. The stabilizing point of the curve defines the optimum VA; the lower VA of 45 is the suggested value to ensure the existence of ecological corridors between reserves to be selected.

species and criteria for rarity, abundance, and endemic value. In the application presented, the group of selected catchments represented all native fish species and was required to contribute to species persistence. Despite criticism (e.g., Smith & Theberge 1987; Williams & Araújo 2002), the use of aggregating criteria assessments was considered necessary because we assumed that an area's conservation value must take into account the relative importance of highly vulnerable species with localized distributions rather than merely species richness. The method also did not require intensive field surveys of fish species abundance, which is often a limitation in describing freshwater fish communities.

The cumulative frequency curve of reaches with increasing VA values can define the threshold of VA values and therefore the percentage of area that optimizes the criteria used. Furthermore, it is possible to present different reserve scenarios to land-use managers according to conservation resources. For our case study, we suggested a VA value lower than optimal to ensure the inclusion of corridors between protected areas.

Areas for conservation were recognized based on the modeling of species occurrence in a GIS environment. When GIS is used with modeling techniques for prediction occurrences, complex relationships can be elucidated and predicted, improving one's ability to examine correlations and advance hypotheses over larger and more heterogeneous regions than was previously possible (Johnson & Gage 1997). The GIS technology is also a practical way to produce visual images of landscape patterns, thus enabling development of spatially explicit management plans. Furthermore, the use of predictive models of species occurrence is advantageous because data on species distribution are often limited, and predictive models can overcome the need for expensive and extensive biological surveys (Prendergast et al. 1999). Also, logistic modeling helps managers understand the implications for species occurrence of altering the natural conditions by considering the influence of each variable included in the models.

Expert opinion is needed for cases with rare and unmodeled species at the final stage of the evaluation to verify whether the selected areas include the occurrence of those species. In our case study, for example, the Álamo catchment was not selected despite the presence of an isolated population of *A. hispanica*. This species had the highest conservation value but was too rare to achieve a model fitted to data. Thus, planners and conservation managers should consider protecting and rehabilitating the Álamo catchment. The decision must, however, weigh both the need to ensure the species protection and the social and economic costs of rehabilitating a highly degraded river.

Our data have limitations that may constrain their interpretation. First, sampling coverage may be a problem, mainly for rare and low-abundance species with a low probability of detection and for juveniles, because most of the sites were sampled only once and sampling effort may have been insufficient. Therefore, to assure that the models constructed have minimum bias relative to missed rare species and juveniles occurrences, we suggest that future researchers (1) quantify the probability of capture of the sampling protocol with data from intensively sampled sites and (2) test the sensitivity of the models to detecting uncertainty. This way detectability can be incorporated into the models (Stauffer et al. 2002) and survey protocols can be developed to assure the likelihood of detection, especially by defining the number of repeated visits needed for each sampling unit. Furthermore, the use of additional sampling methods and improving species identifications in the field should ensure detection of all individuals present and consequently the inclusion of all habitats needed by fish to complete their life cycle in the selected reserves (Schlosser 1991; Paller 1994).

The second limitation of our data is that the regression analysis assumes that variables are independent, but spatial autocorrelation among sites can increase the probability of committing a Type I error (Legendre 1993). Irrespective of sampling strategy, quantification of spatial autocorrelation is advisable in future analyses (Magalhães et al. 2002).

Third, model accuracy seemed to be affected by ecological attributes of species such as the seasonal migration of fishes. This might explain some incorrect classifications in our models: for *C. willkommii* the errors of omission were probably due to their aggregating behavior during migration. Furthermore, many species are confined to permanent refuge pools during the dry summer period, which can also lead to misclassifications. Omission errors with *S. pyrenaicus* and *C. paludica* suggest a weak habitat association with the landscape surrogates used. Only the most abundant species, the *S. alburnoides* complex, had higher commission errors, probably because it occupied a wide range of riverine habitats in the region and sufficient absence data was lacking (absences = 36.4%) (Table 1).

Fourth, other factors, such as failure to incorporate critical habitat variables with the proper scale, can be responsible for incorrect classifications. In addition, insufficient spatial resolution in the mapped environmental variables may be increased by arbitrarily choosing the classes used in landscape variables (number and cut-off point). Testing the freshwater landscape variables used to detect patterns and a sensitivity analysis of the models to the class structure and scale used should be conducted in the future (Hill & Binford 2002; Pearce et al. 2002).

In short, predictions from our species-by-species models may contain some level of error due to data limitations that need quantification, such as detection uncertainty, spatial autocorrelation and ecological characteristics of species, and other unmeasured factors. Despite these weaknesses, the validation with a distinct data set from the same landscape increased our confidence in the models and in the reliability of the conservation value of species and of areas. Also, the comparison with the 3-year data set for the same study area suggests that 1 year of sampling under nonextreme hydrological conditions can provide presence and absence data for modeling, and indicates that the assigned models can predict species occurrences across distinct sites (at the same study area) and years. However, the data collected cannot address long-term shifts in population dynamics and species ranges, so larger spatial and temporal data need to be considered. Our approach is a general procedure that can be used with diverse taxonomic groups and ecoregions at different grains and extents to determine areas of high conservation value. As in our case study, evaluation of the nature and magnitude of the prediction error of the models is imperative for identifying particular weaknesses of individual species models and their applicability to reserve selection.

For selection of areas to protect rare species, iterative methods are advisable to ensure representativeness, because it is not necessary to do an a posteriori overlap analysis as we performed here for the rare species *A. hispanica*. Therefore, the species modeling and reserve-selection methods we present, when applied to regions with several rare species or a high number of species (a frequent situation in large study areas), may be inadvisable. Also, some researchers (e.g., Pressey & Nicholls 1989) have compared the efficiency of scoring versus iterative approaches to achieve maximum species richness in a minimum number of reserves and indicate that the former are less efficient, at least with presence and absence data. Despite this argument, we consider the scoring approach appropriate for identifying a reserve network where the presumably persistent populations of uncommon, rare, or endemic species are represented. But the assessment of probability of extinction of freshwater species in the selection of conservation areas through iterative approaches should also be developed, as in terrestrial landscapes (Araújo & Williams 2000). Such an application is independent of expert opinion and avoids the use of weighting on the conservation-value-of-species index and thus increases the reliability of future decision-making. Although the method raises questions about the patterns and processes of freshwater environments, investigations are still needed into how to explicitly quantify metapopulation dynamics, connectivity, and species persistence, and how to measure freshwater landscape factors and detect freshwater patterns.

Management Considerations

Once reserve areas have been selected, they must be integrated within a basin-management approach to harmonize development opportunities and exploitation of aquatic resources (Meffe 2002). There is also a need for ecologists, conservationists, social scientists, and stakeholders to negotiate use rights (Cullen et al. 1999). In multinational water bodies, such as the Guadiana River basin, international collaboration is needed, and all social, economic, and political constraints should be considered. Additionally, the establishment of discrete reserves is not enough to protect freshwater fishes (Angermeier 2000; Meffe 2002). Interventions upstream or downstream must be considered in the management of reserves because these activities could have implications for the species for which the reserve is designated (Cowx & Collares-Pereira 2002). In particular, the construction of a dam outside of the reserve network has implications for the recolonization of each reserve area because it may disrupt migration pathways. Similarly, the introduction of alien species elsewhere in the watershed may have long-term implications if the introduced species is able to disperse into the reserves. In our case study, the Alqueva and Pedrogão reservoirs will create unsuitable habitats for native fishes by affecting their movement and enhancing the populations of exotic species. In addition, the lack of facilities for fish passage around Alqueva has permanently isolated the populations upstream and downstream of the dam.

Freshwater fishes throughout the world are now in peril because conservation efforts targeting them have been weak and little has been done to protect the habitats of endangered species. The approach we propose offers a defensible methodology to identify areas to protect vulnerable populations of high conservation value. It represents an additional and valuable problem-solving tool for conservation managers in protecting freshwater fish diversity for future generations.

Acknowledgments

This study was performed under the Multipurpose Alqueva Program, project PMo2.1, Monitoring Program of the Natural Patrimony of Alqueva and Pedrogão Areas, funded by "Empresa de Desenvolvimento e Infra-Estruturas de Alqueva SA" and the Specific Program for the Integrated Development of the Alqueva Area of the European Funds for Regional Development (PEDIZA/FEDER). We thank all collaborators who helped with the fieldwork and the reviewers who greatly improved the manuscript.

Literature Cited

Allan, D. J., D. L. Erickson, and J. Fay. 1997. The influence of catchment land use on stream integrity across multiple spatial scales. Freshwater Biology **37**:149–161.

Almaça, C. 1978. Répartition géographique des Cyprinidae ibériques et secteurs ichthyogéographiques de la Péninsule Ibérique. Vestnik Ceskoslovenské Spolecnosti Zoologické **XLII**:241–248.

Angermeier, P. L. 2000. The natural imperative for biological conservation. Conservation Biology **14**:373–381.

Angermeier, P. L., and M. R. Winston. 1999. Characterizing fish community diversity across Virginia landscapes: prerequisite for conservation. Ecological Applications **9**:335–349.

Araújo, M. B, and P. H. Williams. 2000. Selecting areas for species persistence using occurrence data. Biological Conservation **96**:331–345.

Beaumont, P. 1975. Hydrology. In Whitton B. A., editor. River ecology. Blackwell Science, Oxford, United Kingdom.

Borjóquez-Tapia, L. A., I. Azuara, and E. Ezcurra. 1995. Identifying conservation priorities in Mexico through geographic information systems and modelling. Ecological Applications **5**:215–231.

Brito, J. C., E. G. Crespo, and O. S. Paulo. 1999. Modelling wildlife distributions: logistic multiple regression vs overlap analysis. Ecography **22**:251–260.

Brunke, M., and T. Gonser. 1997. The ecological significance of exchange processes between rivers and groundwater. Freshwater Biology **37**:1–33.

Collares-Pereira, M. J., I. G. Cowx, F. Ribeiro, J. A. Rodrigues, and L. Rogado. 2000*a*. Threats imposed by water resource development schemes on the conservation of endangered fish species in the Guadiana River Basin in Portugal. Fisheries Management and Ecology **7**:167–178.

Collares-Pereira, M. J., A. F. Filipe, F. Ribeiro, P. Tiago, R. Silva, S. Seabra, T. Marques, and I. G. Cowx. 2000*b*. Programa de Monitorização para o Património Natural: PMo2.1—Monitorização de peixes dulciaquícolas da área de regolfo de Alqueva e Pedrogão. Relatório de Progresso. Centro de Biologia Ambiental, Faculdade de Ciências da Universidade de Lisboa, Lisboa.

Comissão Nacional do Ambiente (CNA). 1983. Atlas digital do ambiente. Versão 3.0 Internet. Direcção Geral do Ambiente, Ministério do Ambiente e dos Recursos Naturais, Lisboa.

Corbacho, C., and J. M. Sánchez. 2001. Patterns of species richness and introduced species in native freshwater fish faunas of a Mediterranean-type Basin: the Guadiana River (Southwest Iberian Peninsula). Regulated Rivers: Research & Management **17**:699–707.

Cowx, I. G., and M. J. Collares-Pereira. 2000. Conservation of endangered fish species in the face of water resource development schemes in the Guadiana River, Portugal: harmony of the incompatible. Pages 428–438 in I. G. Cowx, editor. Management and ecology of river fisheries. Fishing News Books, Blackwell Science, Oxford, United Kingdom.

Cowx, I. G., and M. J. Collares-Pereira. 2002. Freshwater fish conservation: options for the future. Pages 443–452 in M. J. Collares-Pereira, I. G. Cowx, and M. M. Coelho, editors. Conservation of freshwater fishes: options for the future. Fishing News Books, Blackwell Science, Oxford, United Kingdom.

Crivelli, A. 2002. The role of protected areas in freshwater fish conservation. Pages 373–388 in M. J. Collares-Pereira, I. G. Cowx, and M. M. Coelho, editors. Conservation of freshwater fishes: options for the future. Fishing News Books, Blackwell Science, Oxford, United Kingdom.

Cullen, P. W., R. N. Norris, V. H. Resh, T. B. Reynoldson, D. M. Rosemberg, and M. T. Barbour. 1999. Collaboration in scientific research: a critical need for freshwater ecology. Freshwater Biology **42**:131–142.

Empresa de Desenvolvimento e Infra-Estruturas de Alqueva (EDIA). 1995–1999. Dados cartográficos digitais do núcleo de informação geográfica da Empresa de Desenvolvimento e Infra-estruturas de Alqueva e Pedrogão. EDIA, Lisboa, Portugal.

Filipe, A. F., I. G. Cowx, and M. J. Collares-Pereira. 2002. Spatial modeling of freshwater fishes in semi-arid river systems: a tool for conservation. River Research and Applications **18**:123–136.

Fleishman, E., D. D. Murphy, and P. Sjogren-Gulve. 2002. Modeling species richness and habitat suitability for taxa of conservation interest. Pages 506–517 in J. M. Scott, P. J. Heglund, M. L. Morrison, et al., editors. Predicting species occurrences: issues of accuracy and scale. Island Press, Washington, D.C.

Gasith, A., and V. H. Resh. 1999. Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. Annual Review of Ecology and Systematics **30**:51–81.

Godinho, F. N., and M. I. Portugal e Castro. 1999. Utilização piscícola de pequenas albufeiras do sul—estrutura da ictiocenose da Albufeira da Tapada Pequena (Mértola, Mina de São Domingos): diagnose e gestão potencial. Silva Lusitana **special issue**:93–115.

Hill, K. E., and M. W. Binford. 2002. The role of category definition in habitat models: practical and logical limitations of using Boolean, indexed, probabilistic, and fuzzy categories. Pages 97–106 in J. M. Scott, P. J. Heglund, M. L. Morrison, et al., editors. Predicting species occurrences: issues of accuracy and scale. Island Press, Washington, D.C.

Hosmer, D. W. Jr., and S. Lemeshow. 1989. Applied logistic regression. Wiley, New York.

Johnson, L. B., and S. H. Gage. 1997. Landscape approaches to the analysis of aquatic ecosystems. Freshwater Biology **37**:113–132.

Kerr, J. T. 1997. Species richness, endemism, and the choice of areas for conservation. Conservation Biology **11**:1094–1100.

Legendre, P. 1993. Spatial autocorrelation: trouble or new paradigm? Ecology **74**:1659–1673.

Lombard, A. T., R. M. Cowling, R. L. Pressey, and P. J. Mustart. 1997. Reserve selection in a species-rich and fragmented landscape on the Agulhas Plain, South Africa. Conservation Biology **11**:1101–1116.

Magalhães, M. F., D. C. Batalha, and M. J. Collares-Pereira. 2002. Gradients in stream fish assemblages across a Mediterranean landscape: contributions of environmental factors and spatial structure. Freshwater Biology **47**:1015–1031.

Maitland, P. S. 1985. Criteria for the selection of important sites for freshwater fish in the British Isles. Biological Conservation **31**:335–353.

Margules, C. R., and R. L. Pressey. 2000. Systematic conservation planning. Nature **405**:243–253.

Matthews, W. J. 1998. Patterns in freshwater fish ecology. Chapman & Hall, New York.

Meffe, G. K. 2002. Connecting science to management and policy in freshwater fish conservation. Pages 186–197 in M. J. Collares-Pereira, I. G. Cowx, and M. M. Coelho, editors. Conservation of freshwater fishes: options for the future. Fishing News Books, Blackwell Science, Oxford, United Kingdom.

Minns, C. K. 1987. A method of ranking species and sites for conservation using presence-absence data and its application to native freshwater fish in New Zealand. New Zealand Journal of Zoology **14**:43–49.

Mladenoff, D. J., T. A. Sickley, R. G. Haight, and A. P. Nydeven. 1995. A regional landscape analysis and prediction of favorable grey wolf habitat in the Northern Great Lakes Region. Conservation Biology **9**:279-294.

Moyle, P. B., and R. M. Yoshiyama. 1994. Protection of aquatic biodiversity in California: a five-tiered approach. Fisheries **19**(2):6-18.

Naiman, R. J., H. Déchamps, and M. Pollock. 1993. The role of riparian corridors in maintaining regional biodiversity. Ecological Applications **3**:209-212.

Norris, R. H., and M. C. Thoms. 1999. What is river health? Freshwater Biology **41**:197-209.

Norusis, M. J. 1995. SPSS for Windows: SPSS PC base system users guide. Advanced and professional statistics. Version 7.0. SPSS, Chicago.

Oberdorff, T., J. Guégan and B. Huguenty. 1995. Global scale patterns of fish species richness in rivers. Ecography **18**:345-352.

Osborne, L. L., and M. J. Wiley. 1992. Influence of tributary spatial position on the structure of warmwater fish communities. Canadian Journal of Fisheries and Aquatic Sciences **49**:671-681.

Paller, M. H. 1994. Relationships between fish assemblage structure and stream order in the Carolina Coastal Plain Streams. Transactions of the American Fisheries Society **123**:150-161.

Pires, A. M., I. G. Cowx, and M. M. Coelho. 1999. Seasonal changes in fish community structure of intermittent streams in the middle reaches of the Guadiana basin, Portugal. Journal of Fish Biology **54**:235-249.

Poff, N. L., and J. D. Allan. 1995. Functional organization of stream fish assemblages in relation to hydrological variability. Ecology **76**:606-27.

Prendergast, J. R., R. M. Quinn, and J. H. Lawton. 1999. The gaps between theory and practice in selecting nature reserves. Conservation Biology **13**:484-492.

Pressey, R. L., and A. O. Nicholls. 1989. Efficiency in conservation evaluation: scoring versus iterative approaches. Biological Conservation **50**:199-218.

Pusey, B. J., M. J. Kennard, and H. Arthington. 2000. Discharge variability and the development of predictive models relating stream fish assemblage structure to habitat in northeastern Australia. Ecology of Freshwater Fish **9**:30-50.

Puth, L. M., and K. A. Wilson. 2001. Boundaries and corridors as a continuum of ecological flow control: lessons from rivers and streams. Conservation Biology **15**:21-30.

Rabe, F. W., and N. L. Savage. 1979. A methodology for the selection of aquatic natural areas. Biological Conservation **15**:291-300.

Rahert, D., D. White, J. C. Difneos, and R. M. Hughes. 1999. Environmental correlates of species richness for native freshwater fish in Oregon. Journal of Biogeography **26**:257-273.

Ribeiro, F., I. G. Cowx, and M. J. Collares-Pereira. 2000. Life history traits of the endangered Iberian cyprinid *Anaecypris hispanica* and their implications for conservation. Archiv für Hydrobiologie **149**:569-86.

Rowe, J. M. 1997. Watershed and stream-based inventory and assessment of the fish biodiversity of the Monongahela and Potomac River basins, West Virginia. Final report. West Virginia gap analysis project: aquatic biodiversity analysis. West Virginia University, Morgantown, West Virginia.

Saunders, D. L., J. J. Meeuwig, and C. J. Vincent. 2002. Freshwater protected areas: strategies for conservation. Conservation Biology **16**:30-41.

Schlosser, I. J. 1991. Stream fish ecology: a landscape perspective. BioScience **41**:704-712.

Serviço Nacional de Parques, Reservas e Conservação da Natureza (SNPRCN). 1991. Peixes dulciaquícolas e migradores. Livro Vermelho dos Vertebrados de Portugal. Secretaria de Estado dos Recursos Naturais, Lisboa.

Smith, P. G., and J. B. Theberge. 1987. Evaluating natural areas using multiple criteria: theory and practice. Environmental Management **11**: 447-460.

Stauffer, H. B., C. J. Ralph, and S. L. Miller. 2002. Incorporating detectability into power and sample size selection for presence/absence surveys with application to marbled murrelets. Pages 357-365 in J. M. Scott, P. J. Heglund et al., editors. Predicting species occurrences: issues of accuracy and scale. Island Press, Washington, D.C.

Tabachnick, B. G., and L. S. Fidell. 1996. Using multivariate statistics. 3rd edition. Harper Collins College Publishers, New York.

Williams, P., D. Gibbons, C. Margules, A. Rebelo, C. Humphries, and R. Pressey. 1996. A comparison of richness hotspots, rarity hotspots, and complementary areas for conserving diversity of British birds. Conservation Biology **10**:155-174.

Williams, P. H., and M. B. Araújo. 2002. Apples, oranges and probabilities: integrating multiple factors into biodiversity conservation with consistency. Environmental Modeling and Assessment **7**:139-151.

Winston, M. R., and P. L. Angermeier. 1995. Assessing conservation value using centers of population density. Conservation Biology **9**:1518-1527.

World Conservation Union (IUCN). 2000. IUCN red list of threatened animals. IUCN, Gland, Switzerland.

Appendix 1. Digital variables considered in developing the model of occurrences for native species in the middle watercourses of the Guadiana River basin, with examples of their use in the literature.

Variable	*Description**	*Class (class interval)*	*References*
Climatic			
TEM	average annual air temperature (° C)[a]	1 (<16); 2 (16-17.5); 3 (>17.5)	Paller 1994; Oberdorff et al. 1995
PRE	average annual precipitation (mm)[a]	1 (400-500); 2 (500-600); 3 (600-700)	Paller 1994; Rahert et al. 1999
EVA	average annual evapotranspiration (mm)[a]	1 (<400); 2 (400-450); 3 (<450)	
Geomorphologic			
SOL	soil pH[a]	1 (acid); 2 (neutral); 3 (alkaline)	
SOI	soil type[b]	1 (cambisoils); 2 (litosoils); 3 (luvisoils); 4 (vertisoils)	
ROC	rock type (lithology)[b]	1 (clastic); 2 (sedimentary rock); 3 (intrusive igneous rock); 4 (metamorphic rock)	Norris & Thoms 1999
ELE	elevation (m)[c]	0 (0); 1 (100); 2 (200); 3 (300)	Norris & Thoms 1999; Pusey et al. 2000
Hydrologic			
DGU	distance to the main river (km)[d]	0 (0); 1 (1-9); 2 (10-19); 3 (20-29); 4 (30-39); 5 (40-49); 6 (50-59); 7 (60-69)	Osborne & Wiley 1992
DMO	distance between site and Guadiana mouth (km)[d]	1 (108-139); 2 (140-159); 3 (160-179); 4 (180-199); 5 (200-219); 6 (220-239); 7 (240-259)	Pusey et al. 2000
TRI	distance between tributary mouth and Guadiana mouth (km)[d]	1 (106-139); 2 (140-169); 3 (170-199); 4 (≥200)	Osborne & Wiley 1992
BAS	catchment[c]	1 (Álamo); 2 (Alcarrache); 3 (Ardila); 4 (Asseca); 5 (Azevél); 6 (Degebe); 7 (Enxoé); 8 (Guadiana); 9 (Lucefécit); 10 (Marmelar); 11 (Odearce); 12 (Zebro)	Matthews 1998

continued

Appendix 1. (continued)

Variable	*Description**	*Class (class interval)*	*References*
ORD	stream order by Strahler—see Beaumont (1975)[d]	1 (1); 2 (2); 3 (3); 4 (4); 5 (5); 6 (> 6)	Paller 1994; Pusey et al. 2000
FLO	average annual run off (mm)[a]	1 (50–100); 2 (100–150); 3 (150–200)	Poff & Allan 1995
WAT	underground water resources ($m^3/[day.km^2]$)[a]	1 (50); 2 (250); 3 (300)	Brunke & Gonser 1997
Human influence			
LAN	land use[b]	1 (arable farming); 2 (grazing and/or holm and cork oaks); 3 (submediterranean policulture)	Schlosser 1991; Allan et al. 1997
DAM	dam influence (only dams with capacity >0.9hm³) upstream and downstream at a distance within 30 km[d]	1 (absence of dam influence); 2 (presence of dam influence upstream of the site); 3 (presence of dam influence downstream of the site)	
DDO	distance to Alqueva dam of downstream sites (km)[d]	0 (the site is not downstream); 1 (0–19); 2 (20–39); 3 (40–59); 4 (60–79)	
DUP	distance to Alqueva dam of upstream sites (km)[d]	0 (the site is not upstream); 1 (0–19); 2 (20–39); 3 (40–59); 4 (60–79)	
POL	amount of source pollution upstream of the site[d]	0 (0); 1 (1–5); 2 (6–10); 3 (11–15); 4 (16–20); 5 (21–50); 6 (>50)	
PDE	population density in the municipality (number of inhabitants km^{-2} in 1998)[a]	1 (1000–3999); 2 (4000–6999); 3 (7000–9999); 4 (10000–12999); 5 (13000–19999); 6 (≥20000)	Rahert et al. 1999

* *Variable resolutions:* [a] *Comissão Nacional do Ambiente (CNA) (1983), 1:100 000;* [b] *Empresa de Desenvolvimento e Infra-Estruturas de Alqueva (EDIA) (1995–1999), several scales more detailed than CNA scale;* [c] *derived from EDIA (1995–1999);* [d] *derived from CNA (1983).*