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FISH DISTRIBUTION AND ABUNDANCE IN MEDITERRANEAN STREAMS: THE ROLE OF HABITAT QUALITY, SPATIAL CONTEXT, AND MOVEMENT PATTERNS

Daniel Filipe Carvalho Miranda Pires

Doutoramento em Biologia
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Preliminary notes

According to the Article 41 nr.º 1 of the Post-graduate Studies Regulation (Diário da República N.º 1506 de 2006, II série de 30 de Outubro de 2006), the current thesis includes results that were previously published in collaboration with other co-authors. The candidate states that he was responsible for planning the research activities, data collection and analyses, and writing the manuscripts.

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À Raquel

Ao Tomé

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RESUMO

Os fatores e processos que determinam os padrões de distribuição das populações e comunidades de peixes dulçaquícolas em rios são temas de investigação centrais em Ecologia Aquática. O esclarecimento destas questões é importante não só para o conhecimento fundamental do funcionamento dos ecossistemas lóticos, mas também sob o ponto de vista aplicado. O número de espécies de peixes dulçaquícolas que se encontram ameaçadas devido à crescente degradação dos habitats aquáticos por pressão de origem humana é elevado, e prevê-se que possa aumentar face aos cenários de alteração climática, que apontam para redução da precipitação e disponibilidade de água. Nesta perspetiva, compreender as relações entre as espécies e o ambiente aquático é uma base fundamental para o desenvolvimento de medidas de gestão e conservação dos sistemas lóticos e da sua ictiofauna.

Ao longo dos últimos anos a análise dos padrões de distribuição de peixes dulçaquícolas em rios sofreu uma mudança de paradigma, acompanhando o crescente reconhecimento da complexidade e do elevado dinamismo dos sistemas lóticos. Atualmente estes sistemas são percecionados como estruturas hierárquicas, organizadas por subunidades de habitat que refletem níveis sucessivos de organização espacial como microhabitats, macrohabitats, secção de rio, segmento de rio e bacia hidrográfica. Em cada nível da hierarquia, a estrutura e persistência do habitat são controladas por diferentes processos, que operam a escalas temporais e espaciais distintas. Simultaneamente, a atuação dos diferentes processos não é independente, mas interativa ao longo das diferentes escalas.

Devido a esta perceção da organização hierárquica do sistema lótico, a análise dos padrões de distribuição dos organismos evoluiu de uma perspetiva focada apenas nas características locais do habitat, para uma abordagem multiescala, integrando também características do habitat a escalas espaciais superiores às tradicionais. Em particular, existe um interesse crescente no papel da heterogeneidade espacial de habitat e das relações de conectividade entre os habitats, nos padrões contemporâneos de distribuição de populações e comunidades. Ao longo do seu ciclo de vida, os peixes podem utilizar habitats distintos, para satisfazer requisitos específicos de diferentes fases do ciclo de vida (e.g. habitats de desova, alimentação ou refúgio contra cheias ou secas), que se podem encontrar dispersos no sistema lótico. Como tal, a localização desses habitats e a forma como o movimento de indivíduos é facilitado entre os mesmos, são fatores que se julga poderem influenciar sensivelmente a estrutura e dinâmica das populações e comunidades locais. Nesta perspetiva, a capacidade de movimento, que pode ser específica de cada espécie, é considerada também um fator particularmente relevante, uma vez que, em

última análise, condiciona a ligação ecológica entre diferentes habitats e define a escala de exploração da heterogeneidade de habitat.

Apesar da relevância destas questões, em termos teóricos e aplicados, a sua abordagem em sistemas lóticos temporários é ainda muito incipiente. Neste tipo particular de sistemas lóticos, a redução ou cessação temporária do caudal provoca a interrupção da continuidade fluvial e conduz a alterações dramáticas na disponibilidade e configuração dos habitats aquáticos. Nestas condições, a existência de habitats de refúgios durante o período de seca é fundamental para a persistência das populações e comunidades que dependem exclusivamente de ambientes aquáticos. No entanto, o padrão de utilização de habitats de refúgio em rios temporários tem sido insuficientemente analisado, e desconhece-se em grande medida a forma como a configuração espacial dos refúgios e os padrões de movimento das espécies piscícolas podem influenciar os padrões de distribuição e estrutura das comunidades durante o período húmido.

A presente dissertação incide sobre esta problemática, analisando os padrões de distribuição e abundância de peixes em rios temporários de tipo mediterrânico. Estes rios são caracterizados pela ocorrência de cheias e secas com uma sazonalidade bem definida, mas de magnitude muito variável a nível interanual. Em geral, no final do período de seca estival verifica-se uma diminuição e fragmentação muito sensiveis do habitat aquático disponível, que se restringe fundamentalmente a pegos isolados ou troços permanentes. Atualmente, a ictiofauna nativa encontra-se muito ameaçada, com cerca de 70 % das espécies apresentando elevados estatutos de conservação, devido a degradação do habitat por pressão de origem humana. O esclarecimento dos fatores e processos envolvidos na estruturação das comunidades piscícolas nestes sistemas torna-se assim fundamental para sustentar ações de gestão e conservação destinadas a contrariar o declínio da ictiofauna nativa.

Especificamente, o trabalho desenvolvido nesta tese pretendeu avaliar o papel das características locais do habitat, do contexto espacial, e dos padrões de movimento na distribuição, abundância e estrutura das populações e comunidades piscícolas em rios mediterrânicos. Nesse sentido foram desenvolvidas três atividades de investigação distintas visando especificamente: i) avaliar o efeito do contexto espacial, da morfologia e das características físico químicas dos refúgios estivais na estrutura das comunidades piscícolas; ii) descrever os padrões de movimento dos peixes, quantificando as taxa e amplitude das deslocações a partir dos refúgios estivais, e entre diferentes habitats durante o período húmido, em condições de continuidade hidrológica, e iii) avaliar o efeito da qualidade do habitat, da conectividade aos refúgios estivais, e de efeitos de vizinhança, na variação das comunidades piscícolas durante o período de continuidade hidrológica. Estes estudos foram desenvolvidos à escala do segmento de rio, em dois sistemas tipicamente mediterrânicos do Sudoeste de Portugal.

A avaliação dos efeitos do contexto espacial, morfologia e características físico-químicas dos pegos estivais na variação da estrutura das comunidades piscícolas foi conduzido na ribeira de Odelouca (Bacia Hidrográfica do Rio Arade, Algarve). O estudo foi desenvolvido em 21 pegos estivais, em setembro de 2005, permitindo avaliar quais as características determinantes na estruturação das comunidades em condições de seca extrema. A morfologia de cada pego foi descrita com base no volume da massa de água, e as variáveis físico-químicas selecionadas incluíram o grau de cobertura por canópia, a granulometria e heterogeneidade de substrato, e o pH. O contexto espacial de cada pego foi descrito com base na distância à foz e ao refúgio mais próximo. Globalmente, a estrutura das comunidades apresentou maior associação com a morfologia e características físico-químicas dos pegos do que com o seu contexto espacial. A riqueza específica global apresentou uma tendência de aumento com o volume do pego, enquanto a riqueza e abundância de espécies nativas demonstrou uma associação positiva com a canópia. No entanto, verificou-se uma elevada variabilidade nas associações entre diferentes espécies e classes de tamanho e as características dos pegos em termos de canópia, granulometria do substrato, e localização espacial.

A análise dos padrões de movimento das espécies piscícolas foi efetuada na ribeira do Torgal (Bacia Hidrográfica do Rio Mira, Alentejo). O estudo foi desenvolvido entre julho de 2007 e julho de 2008, num segmento de rio de 3.6 km, e incidiu sobre a espécie dominante neste sistema, o escalo-do-mira, S*qualius torgalensis*. Esta espécie foi selecionada para análise por ser uma espécie abundante e amplamente distribuída no sistema em estudo, e apresentar características de história de vida representativas e típicas das espécies nativas em rios mediterrânicos. O estudo baseou-se na utilização de técnicas de marcação-recaptura, e incluiu dois componentes de esforço de marcação distintos: um primeiro, efetuado durante a época seca, incidindo sobre indivíduos refugiados em pegos estivais; e um segundo, efetuado após o restabelecimento do caudal, incidindo sobre indivíduos distribuídos ao longo do gradiente longitudinal do sistema lótico. Em ambos os períodos, os indivíduos foram marcados com transmissores passivos integrados (PIT-tags), cuja adequabilidade à espécie em estudo foi previamente testada em ensaios laboratoriais. Globalmente, verificou-se uma considerável variabilidade na taxa e amplitude de movimentos do escalo-do-mira durante o período de estudo. Uma grande parte dos indivíduos marcados em pegos estivais deslocou-se para outros habitats, enquanto a maior parte dos indivíduos marcados na época húmida se manteve no habitat de marcação. Em geral, os movimentos correspondentes a abandonos dos pegos tenderam a ser mais amplos do que as deslocações efetuados na época húmida. Em ambos os casos, contudo, os movimentos foram efetuados quer para habitats efémeros quer para habitats persistentes, e a sua amplitude variou entre as dezenas e as centenas de metros. Verificou-se ainda que a taxa de movimento a partir dos refúgios estivais, variou em função da localização espacial do pego e que durante o período húmido os indivíduos maiores foram os que mais frequentemente abandonaram o local de marcação.

A análise do papel da qualidade do habitat, da conectividade aos refúgios estivais e dos efeitos de vizinhança, na variação espacio-temporal de distribuição e abundância das espécies piscícolas durante o período húmido foi também conduzido na ribeira do Torgal. O estudo foi desenvolvido entre fevereiro de 2006 e julho de 2007, em 60 locais distribuídos, por 2 segmentos de 2 km. A qualidade do habitat local foi avaliada com base em variáveis descritoras das características hidro-morfológicas, granulometria e heterogeneidade do substrato, tipo e percentagem de cobertura do leito, e percentagem de cobertura arbórea e arbustiva das margens. A conectividade aos refúgios estivais foi quantificada com base num índice que integra a distância aos refúgios, um indicador do número potencial de colonizadores no refúgio, e uma medida da capacidade de movimento dos organismos. Especificamente, para o cálculo deste índice, a área de cada refúgio foi utilizada como indicador de abundância local de colonizadores, e a distância média de deslocação a partir de refúgios do escalo-do-mira (292.7 m), como indicador da capacidade de movimento dos indivíduos. Os efeitos de vizinhança foram avaliados usando um termo de autocovariância, que pondera a distância entre os locais. A distribuição das espécies piscícolas apresentou associações fortes e consistentes com a qualidade do habitat e apenas episodicamente foram verificadas associações com a conectividade aos refúgios e efeitos de vizinhança. As características de habitat determinantes na ocorrência de diferentes espécies e classes de tamanho apresentaram uma variação sensível, e flutuaram ao longo do período de estudo. Contudo, a velocidade da corrente revelou associações consistentes com a distribuição de várias espécies nativas, as quais demonstraram uma tendência para ocorrer fundamentalmente em locais com baixa velocidade de corrente. A abundância total e particularmente a abundância de espécies nativas apresentou uma tendência de diminuição com o grau de cobertura arbórea.

No seu conjunto os resultados obtidos vêm confirmar estudos prévios que associam a estruturação das comunidades piscícolas à qualidade e ao contexto espacial dos habitats lóticos. Todavia, os resultados salientam que em rios mediterrânicos, à escala do segmento de rio, a influência das características locais de habitat pode ser mais determinante para a distribuição das espécies piscícolas do que o seu contexto espacial. Em particular, a heterogeneidade do habitat surge como fundamental na estruturação das comunidades piscícolas, tendo-se encontrado diferentes padrões de uso de habitat para diferentes espécies e classes de tamanho, e elevada variabilidade temporal nos referidos padrões. Apesar do menor relevo dos fatores de contexto espacial para os padrões encontrados, as relações espaciais entre os habitats parecem desempenhar um papel na dinâmica das populações e comunidades piscícolas, tendo o movimento dos peixes um papel relevante nessas relações. Em particular, o movimento pode contrariar o isolamento e fragmentação

sazonais, impostos pela seca estival, promovendo a redistribuição de indivíduos a várias distâncias dos refúgios estivais, a colonização de habitats previamente secos, e o contacto entre indivíduos que estiveram isolados durante o período estival. Globalmente, os resultados obtidos parecem indicar que, à escala do segmento de rio, a configuração espacial dos habitats não constrange a distribuição das espécies piscícolas de acordo com as características locais do habitat, refletindo provavelmente a adaptabilidade da fauna nativa às flutuações naturais da disponibilidade e conectividade de habitat típicas dos rios mediterrânicos.

Os resultados obtidos apresentam implicações importantes para a conservação e gestão dos ecossistemas lóticos mediterrânicos. Os refúgios estivais são habitats críticos para a persistência das populações e a recolonização dos habitats efémeros, sendo por isso determinante preservar a sua heterogeneidade para manter a diversidade da fauna piscícola nativa. Do mesmo modo, a preservação da heterogeneidade de habitat durante o período húmido apresenta-se como importante para assegurar os requisitos das diferentes espécies e classes de tamanho. Por último, garantir a manutenção de níveis naturais de conectividade entre os habitats é fundamental para permitir os movimentos entre os diferentes habitats e a distribuição dos organismos de acordo com os seus padrões de uso de habitat.

Esta tese constituíu uma primeira abordagem à quantificação das relações espaciais entre habitats e populações, e sua importância na estrutura e funcionamento de rios mediterrânicos. O aprofundamento das linhas de investigação aqui desenvolvidas será fundamental para a identificação dos processos que regulam a dinâmica das populações e comunidades de peixes nestes sistemas. Em particular, sugere-se o desenvolvimento das seguintes linhas de investigação: i) identificação dos habitats críticos de reprodução e alimentação, que permita esclarecer as relações espaciais entre os habitats de período húmido e os refúgios estivais; ii) análise do papel das relações bióticas na estruturação das comunidades piscícolas, sobretudo nos refúgios estivais; iii) análise da estrutura espacial das populações, incluindo avaliações ecológicas e genéticas que permitam testar a estrutura populacional a larga escala; iv) desenvolvimento de estudos demográficos que permitam clarificar as dinâmicas de colonização e extinção locais, sobretudo em habitats efémeros, e esclarecer as relações espaciais prevalecentes entre habitats.

Palavras-chave: peixes dulçaquícolas; rios temporários; habitat; estrutura espacial; refúgios estivais; recolonização; Península Ibérica.

ABSTRACT

Patterns of fish distribution and abundance in streams are currently thought of as a product of multi-scale factors. Local habitats, spatial relationships and movement are increasingly emerging as drivers of population and assemblage dynamics, though the way in which these factors may interplay remains poorly addressed, particularly in temporary streams. This dissertation addressed the role of multiple environmental and spatial factors and movement on patterns of fish distribution and abundance in dry and wet-season habitats in seasonally-drying mediterranean streams, at the segment scale. Overall, we found that assemblage structure of fish was more consistently associated with local habitat characteristics than spatial context. Specifically, species richness in dry-season pools was primarily associated with pool morphology, whereas abundance was mostly determined by physicochemical factors. During the wet-season, species richness, abundance and occurrence was consistently associated with habitat quality, whereas connectivity to dry-season refugia and neighbouring effects were episodically important. There was considerable variation in fish–habitat relationships among species and size classes, and over time, highlighting the importance of habitat heterogeneity. Moreover, patterns of fish movement uncovered complex ecological connections among habitats. Fish moved more often and displaced further away from dry-season pools than during the wet-season, though in both cases movements directed to persistent and ephemeral habitats and ranged from tens to hundreds of meters. We concluded that current patterns of fish distribution and abundance at the segment scale were not severally constrained by the spatial arrangement of habitats, probably reflecting the adaptability of native fish species to seasonal changes in habitat availability and connectivity prevailing in mediterranean streams. These results suggest that maintaining natural habitat heterogeneity and connectivity, and the processes that driven them (e.g. floods), may play a critical in promoting the persistence of native fish species in mediterranean streams.

Keywords: freshwater fish; temporary streams; local habitat; spatial structure; dry-season refugia; recolonization; Iberian Peninsula

Chapter 1

GENERAL INTRODUCTION

1. GENERAL INTRODUCTION

Understanding how and why organisms are distributed in space is a central tenet in ecology. Specifically, ecologists aim to identify how the spatial-temporal dynamics of habitat heterogeneity, influences the distribution and abundance of organisms and shapes biotic patterns and processes in natural landscapes (Hanski & Gaggiotti, 2004; Lovett *et al.*, 2005; Molles, 2008).

This thesis addresses the patterns and processes in fish assemblages in mediterranean streams, a particularly heterogeneous and dynamic type of riverscape. Specifically, it aims to increase current knowledge about the role of habitat heterogeneity and spatial context in population and assemblage dynamics of fish, particularly focusing on the use of refugia and movement patterns of fish at the stream segment scale.

1.1. THE RIVERSCAPE ORGANIZATION

The way ecologists conceptualize and describe riverine systems has evolved and increased in complexity in recent years, targeting a broader, holistic view of these systems (e.g. Vannote *et al.*, 1980; Pringle *et al.*, 1988; Junk *et al.*, 1989; Montgomery, 1999; Wiens, 2002; Benda *et al.*, 2004; Thorp *et al.*, 2006; Poole, 2010). Streams and rivers are currently interpreted as complex and highly dynamic landscapes, or riverscapes, which are spatially organized in three dimensions: i) the longitudinal dimension, structured along the upstream-downstream gradient; ii) the lateral dimension, involving interactions between the main channel and marginal structures such as riparian and flooodplain elements, and iii) the vertical dimension, regarding interactions between surface waters and the hyporheic and phreatic zones (Ward, 1989; Ward *et al.*, 2002; Wiens, 2002). Across all dimensions, natural flow variability is a fundamental driver of physical habitat and ecological patterns and processes, determining habitat structure, controlling the fluxes of energy and matter and the cycles of erosion and deposition, influencing floodplain connectivity and other elements of floodplain channels (e.g. meander loops and side channels), and making water-land interface complex and dynamic (Vannote *et al.*, 1980; Newbold *et al.*, 1982; Junk *et al.*, 1989; Ward, 1989; Poff *et al.*, 1997). The resultant, spatially complex and dynamic mosaic of habitat types and environmental gradients constitute the riverscapes.

Streams can thus be defined as hierarchically organized systems incorporating, on successively lower levels, basin, segment, reach, macrohabitat (e.g. riffles, pools and runs), and microhabitat units (Frissell *et al.*, 1986). At each level in the riverscape hierarchy, habitat heterogeneity, development, and persistence are controlled by processes acting at specific spatial and temporal scales (Frissell *et al.*, 1986; Ward, 1998; Montgomery, 1999). At the basin and segment levels, processes generating heterogeneity generally act over large spatial scales, but may operate at distinct temporal scales. For instance, tectonical uplift drives variation in regional geology, and in physiographic characteristics such as slope, while climatic processes define regional temperature and rainfall patterns that influence hydrologic regimes (Montgomery, 1999). Episodic events of high magnitude such as fires or hurricanes may also impact stream systems at broad spatial scales (e.g. massive sediment input derived from erosion after fire; Reeves *et al.*, 1995; Schlosser & Angermeier, 1995). At the reach to microhabitat levels, factors such as seasonal discharge variation, flood pulses, local erosion and sediment deposites, process at smaller spatial and temporal scales, promote longitudinal variation in depth, substrate, and current velocity, lateral heterogeneity in stream margins, and isolated pool backwaters, and variation in surface-hyporeich water exchange (Schlosser & Angermeier, 1995; Boulton *et al.*, 2010).

The riverscape hierarchy is spatially nested, that is, a system at one level forms the environment of its subsystems at lower levels (Frissell *et al.*, 1986; Poole, 2002). Consequently, at any spatial scale, streams can be perceived as a *discontinum* of discrete parts with unique attributes, that is temporally dynamic (Poole, 2002). Moreover, the different processes driving habitat heterogeneity are not independent but interact across scales, with large scales factors such as climate and geology, creating the setting for processes that ultimatlly shape stream habitat structure at smaller scales (Frissell *et al.*, 1986; Montgomery, 1999; Poole, 2002; Ward *et al.*, 2002).

1.2. STREAM FISH ECOLOGY

In agreement with the hierarchical structure of stream systems, the structure of biotic assemblages, and particularly of fish assemblages, is the result of multiple interacting processes, operating at distinct spatial and temporal scales that define the riverscape. These multi-scale processes can be viewed as *landscape filters*, ranging from microhabitat to basin scales, that sequentially limit fish species according to the functional significance of their biological traits (e.g. life history, body size, mobility, physiological tolerances) relative to qualitative and quantitative environmental conditions, ultimately determining local assemblage composition and structure (Tonn, 1990; Poff, 1997; Quist *et al.*, 2005). The pool of species in a particular basin, i.e. the regional species pool, is primarily shaped by continental scale-filters, such as high order climatic (e.g. glaciations) and geo-tectonic events (e.g. mountain building), and long-term biological responses such as speciation (Tonn, 1990; Abell *et al.*, 2008). Additionally, climatic and physiographic patterns, imposing, for instance, physiological (e.g. through temperature range and water chemistry) and dispersal constrains (e.g. barriers between neighbouring basins) to fish, contribute to shape the regional species pool (Poff, 1997). Contemporary patterns of species distribution, at lower spatial scales, are primarily influenced by processes shaping habitat characteristics and spatial arrangement, though constrains caused by some historic events (e.g. natural barriers such as waterfalls caused by historic geological events) may also play an important role (Crivelli, 1995; Pringle, 1997; Pusey *et al.*, 1998; Lake *et al.*, 2007). For instance, at the segment and reach scales, differences in stream slope and width, that can change from year to year due to depositional or erosive mechanisms (Poole, 2002), can cause variable habitat stability and flood susceptibility, and filter species according to their flood resistance, and contingent on their body size and mobility (Poff, 1997). Following the same rationale, at the macrohabitat and microhabitat scales, differences in habitat structure and complexity in terms of current velocity, depth, substrate size, wood debris that can change from hours to days, may create variability in flood refugia that will likely differ in suitability depending on fish body size and swimming ability (Poff, 1997; Quist *et al.*, 2005). The nature and importance of factors acting as landscape filters is expected to differ among riverscapes according to their range of variation in each riverscape, and the sensitivity of fish species in the regional pool (Angermeier & Winston, 1999).

In this dynamic perspective of the riverscape, the distribution, abundance, and the structure of local fish assemblage will depend on interacting species-specific habitat requirements, life histories, and dispersal abilities, and the shifting mosaic of stream habitats (Pringle *et al.*, 1988; Townsend, 1989; Schlosser, 1991; 1995; Schlosser & Angermeier, 1995; Robinson *et al.*, 2002). Spatial habitat heterogeneity is a key riverscape feature, as the placement of different habitats providing resources required by different fish species and their life stages will influence population spatial dynamics (Poole, 2002; Wiens, 2002). Specifically, fish assemblages are expected to be highly influenced by the spatial arrangement of spawning, feeding and rearing habitats, and as well as refugia from disturbing events such as floods and droughts (Schlosser, 1991; 1995). The spatial scale, at which fish may explore habitat heterogeneity to complete their life cycle, will depend on its life history (Schlosser, 1995). With the exception of highly migratory species, stream fish were traditionally considered to display restricted movement, completing their life cycle within the same channel unit or short stream reach (Gerking, 1959). This view referred as the Restricted Movement Paradigm has been increasingly challenged (Gowan *et al.*, 1994), and is now known that stream fish may exhibit different movement ranges, from meters to several kilometres (e.g. Gowan & Fausch, 1996; Smithson & Johnston, 1999; Lucas & Baras, 2001; Rodriguez, 2002; Petty & Grossman, 2004).

Habitat connectivity, the degree to which movement is facilitated or impeded across habitats, is also considered to be a critical riverscape feature influencing fish distribution

(Pringle, 2003; Fullerton *et al.*, 2010). Connectivity is highly dependent on flow conditions, habitat spatial arrangement and boundaries, the structure and distance of stream corridors, and artificial barriers (Fullerton *et al.*, 2010). The movement capability of fish also defines the riverscape connectivity (Fullerton *et al.*, 2010), as different species and sizes may respond differently to corridor structure (e.g. habitat complexity, corridor lengh, predator distribution Lonzarich *et al.*, 1998; Schaefer, 2001; Roberts & Angermeier, 2007b), and the range of movements limits the scale at which habitat heterogeneity is perceived and exploited to complete a life cycle (Schlosser & Angermeier, 1995; Albanese *et al.*, 2004; Roberts & Angermeier, 2007a).

The importance of multi-scale factors and processes in shaping the distribution and assemblage structure of stream fish is now well documented (e.g. Matthews, 1998; Hughes *et al.*, 2006). However, increasing evidence that factors driving assemblage dynamics may vary across riverscapes (Diana *et al.*, 2006; Stanfield *et al.*, 2006) calls for additional research in less studied riverine systems (e.g. Larned *et al.*, 2010), and on the use specific habitats (e.g. refugia, Magoulick & Kobza, 2003). Also, the consideration of spatial habitat relationships and connectivity measures on stream fish studies is still limited (but see Lonzarich *et al.*, 1998; Rich *et al.*, 2003; Davey & Kelly, 2007; Isaak *et al.*, 2007; Hitt & Angermeier, 2008; Woodford & McIntosh, 2011), demanding for further research in streams with highly variable flow regimes, and encompassing multiple spatial-temporal scales (Fullerton *et al.*, 2010; Peres-Neto & Cumming, 2010; Winemiller *et al.*, 2010). Improving knowledge on fish movement is also critical for understanding assemblage structuring, as it may explicitly establish the connections between habitat use and spatial habitat connectivity (Fausch *et al.*, 2002; Fullerton *et al.*, 2010).

Understanding the multi-scale, species–environment relationships is also important from an applied perspective. Human demands (e.g. water abstraction, damming, fisheries) on freshwater ecosystems have dramatically risen over the last century leading to large and growing threats to aquatic biodiversity worldwide (Malmqvist, 2002; Dudgeon *et al.*, 2006; Strayer & Dudgeon, 2010). Particularly, an increasing number of fish species are being considered imperilled due to human exploitation of freshwater resources (Xenopoulos *et al.*, 2005; Freyhof & Brooks, 2011). Understanding human impacts on stream fish requires previous knowledge of relationships between species and their environment, in which to base the design of adequate conservation and management measures to counteract the declining of fish diversity (Bond & Lake, 2003; Rosenfeld, 2003). Specifically, there is a need to determine the processes influencing populations and assemblages at relevant ecological temporal (i.e. shorter than evolutionary and long-term geological processes) and spatial scales (macrohabitat to segment) at which critical life history events for many species are expected to take place (Fausch *et al.*, 2002).

1.3. FISH ECOLOGY IN TEMPORARY STREAMS

Temporary streams are among the most hydrologically dynamic freshwater ecosystems (Uys & O'Keeffe, 1997; Larned *et al.*, 2010). Although this classification comprises systems with distinct flow regimes, it generally applies to streams in which surface flow ceases for some periods, in most years (Uys & O'Keeffe, 1997; Kennard *et al.*, 2010). Intermittency generates complex dynamics in the longitudinal dimension of streams (Stanley *et al.*, 1997; Larned *et al.*, 2010). Longitudinal dynamics, such as advancing and retreating wetted fronts, attenuation of floods and flow pulses, hydrological connection and disconnection of persistent stream reaches, and gradients in flow permanence, has significant influence on biological assemblages (Larned *et al.*, 2010).

Although temporary streams are worldwide distributed, from alpine to arid regions, and represent a high proportion of the global discharge, its ecology and functioning remain largely unexplored, especially compared to perennial rivers (Tooth, 2000; Larned *et al.*, 2010). However, improving ecological knowledge about temporary rivers is increasingly important in face of global climatic change and intensification of water abstraction for socio-economic uses (Huntington, 2006; Beniston *et al.*, 2007; Christensen *et al.*, 2007; Kundzewicz *et al.*, 2008; Vorosmarty *et al.*, 2010; von Schiller *et al.*, 2011). These issues are likely to further disrupt hydrologic processes in many temporary streams, by increasing the frequency of intermittency episodes, and even cause shifts in flow regimes from perennial to temporary in temperate streams (Arnell, 1999a; b; Murphy & Timbal, 2008; Pusey & Kennard, 2009; Larned *et al.*, 2010).

Temporary cessation of flow has pervasive ecological effects, promoting a sequence of changes in hydrological connectivity and spatial arrangement of stream habitats. Contingent on the frequency and intensity of the drought, hydrological connections and disconnections may occur at multiple spatial scales (Stanley *et al.*, 1997; Labbe & Fausch, 2000; Lake, 2003; Scheurer *et al.*, 2003; Larned *et al.*, 2010): at the basin scale, side channels and tributaries disconnect from the main river at low flows, reconnecting with floods; at the segment scale, perennial reaches disconnect when intervening reaches dry, and are reconnected by flow pulses; at the reach scale, persistent aquatic habitats, such as pools, disconnect and reconnect in response to drying and inundation of intervening areas.

The loss of hydrological connectivity has obvious implications for the stream biota, associated with the reduction of aquatic habitats and dispersal routes, and fragmentation of populations across the riverscape (Lake, 2003). As stream habitat reduces, organisms may become stranded in persistent habitats or trapped in ephemeral habitats and die (Filipe *et al.*, 2002; Magalhães *et al.*, 2002b; Cucherousset *et al.*, 2007; Pratchett *et al.*, 2011). In persistent aquatic habitats, individual fitness and survival may be reduced, as higher densities result in increasing competition and predation (Zaret & Rand, 1971; Matthews, 1998; Schlosser & Kallemeyn, 2000). Moreover, under isolation and water stagnation, organisms may experience increasing abiotic extremes, with high temperature and low oxygen causing physiological stress, and ultimately leading to death (Lake, 2003; Magoulick & Kobza, 2003). Nevertheless, organisms that find refugee in persistent stream reaches and pools, and the hyporheic zone, should experience greater survival than those in surrounding, drying areas (Sedell *et al.*, 1990; Magoulick & Kobza, 2003). Refugia are therefore crucial for the persistence of populations, by providing resistance to temporary droughts, via reduction of effective losses, and favouring resilience, by serving as sources of colonists for areas severely affected by drought, after hydrological reconnection (Sedell *et al.*, 1990; Magoulick & Kobza, 2003; Bunn *et al.*, 2006). Whether habitats serve as effective refugia will depend on the interplay between refugia characteristics and species biological traits (Lake, 2003; Magoulick & Kobza, 2003; Davey *et al.*, 2006).

Fish inhabiting temporary streams are expected to have acquired adaptations to cope with flow cessation and drying, including life-history traits, physiological mechanisms and behaviours (Spranza & Stanley, 2000; Magalhães *et al.*, 2003; Vasiliou & Economidis, 2005; Davey *et al.*, 2006). In particular, fish in temporary streams are highly dependent on drying and rewetting cycles, more than other taxonomic groups with aerial (e.g. macroinvertebrates) or dormant phases (e.g. algae) (Larned *et al.*, 2010). Apart from few species displaying adaptations to resist water loss (e.g. lungfishes) or to deal with short-term flow reductions (e.g. burrow habits), fish in temporary streams generally use persistent aquatic patches as refugia during drought (Magoulick & Kobza, 2003; Davey *et al.*, 2006; Williams, 2006; Sheldon *et al.*, 2010). With the onset of drying but prior to isolation, a net movement of fish to persistent habitats may occur, though there is still limited empirical evidence on active refugia seeking and selection (Labbe & Fausch, 2000; Magoulick & Kobza, 2003; Davey *et al.*, 2006; Larned *et al.*, 2010; Hodges & Magoulick, 2011). Occupation of refugia is constrained by colonization opportunities, depending on the interplay between refugia arrangement across the riverscape, hydrological connectivity, fish dispersal abilities, and habitat quality (Magalhães *et al.*, 2002b; Magoulick & Kobza, 2003; Arthington *et al.*, 2005; Arthington *et al.*, 2010; Larned *et al.*, 2010). During drought, populations become patchily distributed, with survival in persistent refugia depending on species- and size-specific traits and refugia characteristics (Magoulick & Kobza, 2003; Scheurer *et al.*, 2003; Balcombe & Arthington, 2009; Arthington *et al.*, 2010; Beesley & Prince, 2010). Composition of fish assemblages in each refugia may vary over the drying period, dependent on species- and size-specific mortality rates, with more severe changes likely occurring in less favourable refugia (Labbe & Fausch, 2000; Lake, 2003; Scheurer *et al.*, 2003; Balcombe *et al.*, 2006; Love *et al.*, 2008; Balcombe & Arthington, 2009; Rayner *et al.*, 2009; Arthington *et al.*, 2010; Larned *et al.*, 2010). After hydrological reconnection, fish are expected to disperse from refugia, redistributing across the stream network (Magoulick & Kobza, 2003; Scheurer *et al.*, 2003), contingent on species- and size-specific habitat requirements (Pires *et al.*, 1999; Birnbaum *et al.*, 2007; Clavero *et al.*, 2009), but also on spatial arrangement of habitats and connectivity to refugia (Scheurer *et al.*, 2003; Davey & Kelly, 2007; Larned *et al.*, 2010).

Overall, spatial dynamics of fish in temporary streams is still poorly understood, with few empirical evidences on the way assemblages are structured during the connected and disconnected hydrological phases, and habitats from each phase are spatially related. Understanding processes regulating population and assemblage dynamics of fish in temporary streams will greatly benefit with the identification of factors driving the use of persistent habitats as refugia, the redistribution of individuals after hydrological reconnection, and the evaluation of the roles of habitat connectivity and spatial arrangement on assemblage structure.

1.4. FISH ASSEMBLAGES IN SEASONALLY-DRYING, MEDITERRANEAN STREAMS

Streams in mediterranean[1]-climate regions, are shaped by strongly seasonal, and highly predictable periods of intermittency (Gasith & Resh, 1999). Areas with mediterranean-climate locate in the Mediterranean Sea basin, and in parts of western North America (California), south-western South Africa, west and south Australia, and central Chile (Gasith & Resh, 1999; Alvarez Cobelas *et al.*, 2005). The principal attribute of the mediterranean-climate is the seasonally and interannual variability in rainfall patterns (Gasith & Resh, 1999). Between 65% to 80% of the annual rain falls over the winter months, with no or negligible rainfall occurring in late spring to early fall (Gasith & Resh, 1999; Merenlender *et al.*, 2008; Grantham *et al.*, 2010). However, contrasting with the highly predictable seasonal pattern of rainfall, annual rainfall can be highly variable from year to year in some regions, and deviations of 30% or more from a multiannual average

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[1] mediterranean- written with a small m, is used in connection with climate or ecological region and is distinguished from Mediterranean that is used in a geographical context, referring to the Mediterranean basin (Elron *et al*., 2006).

are not uncommon (Gasith & Resh, 1999; Merenlender *et al.*, 2008). The flow regime of mediterranean streams generally follows that of the rainfall pattern and, therefore, exhibits strong seasonal and annual variability (Gasith & Resh, 1999). Typically, flows start or increase abruptly in fall or early winter and floods occur in late fall, winter, and early spring (Gasith & Resh, 1999). Declining of flow and drying is subsequent, and processes gradually through summer, ending abruptly in fall or early winter. High annual flow variability is also characteristic, leading to distinct year-to-year fluctuations in intensity and duration of floods and droughts; in wet years, floods tend to be more common an intense, and the drying period is shorter and less severe, while in dry-years significant floods are eliminated and the drying period is extended (Gasith & Resh, 1999).

Variable patterns of habitat drying may, however, occur in mediterranean streams, mainly reflecting local climatic settings but also variation in drainage area and geomorphology (Gasith & Resh, 1999; Argyroudi *et al.*, 2009; Mas-Marti *et al.*, 2010; Belmar *et al.*, 2011). For instance, in wetter areas, mediterranean streams usually maintain permanent flow or at least hold surface water throughout the year, whereas in the drier ranges streams are intermittent and have sections that lack continuous flow, or even dry entirely (Gasith & Resh, 1999; Argyroudi *et al.*, 2009; Mas-Marti *et al.*, 2010; Belmar *et al.*, 2011). In intermittent streams, drying leads to aquatic habitat reduction, and fragmentation in a series of isolated, segments, reaches and pools.

Stream fish assemblages in mediterranean streams generally exhibit low to medium species diversity, with most regional, basin pools including less than 20 native species (Smith & Darwall, 2006; Ferreira *et al.*, 2007; Reyjol *et al.*, 2007; Abell *et al.*, 2008; Hermoso & Clavero, 2011). However, levels of endemicity are usually high (i.e. up to 70% in some basins), and most species have restricted distribution ranges (Smith & Darwall, 2006; Reyjol *et al.*, 2007; Abell *et al.*, 2008; Hermoso & Clavero, 2011). Moreover, species native to mediterranean streams are expected to have developed biological traits to cope with the prevailing hydrological regime, as consistent landscape filters (Magalhães *et al.*, 2007) may have been operating throughout evolutionary time for about 5 Ma (Blondel *et* *al.*, 2010). Actually, mediterranean fish species usually present short life-spans, small sizes, early maturity and high reproductive investment, and generalist habits, traits which are consistent with patterns described for harsh environments (Magalhães, 1993; Fernández-Delgado & Herrera, 1995; Coelho *et al.*, 1997; Pires *et al.*, 2000; Magalhães *et al.*, 2003; Vasiliou & Economidis, 2005). Nevertheless, there is still considerable species-specific variability in life history traits, and on patterns of population fluctuation with floods and droughts (Magalhães *et al.*, 2003; Magalhães *et al.*, 2007). It has been suggested that at least some species spawning early in the year may be more susceptible to winter and spring floods, due to loss of adult spawners, whereas species spawning later in the season may be more susceptible to summer drought, due to stranding of juveniles in shrinking habitats (Magalhães *et al.*, 2002b; Magalhães *et al.*, 2003; Magalhães *et al.*, 2007). However, population responses to natural droughts and floods are not spatially homogeneous, and resistance and resilience of populations appear to rely on local habitat stability, and movement and dispersal opportunities (Magalhães *et al.*, 2007; Pires *et al.*, 2008; Bêche *et al.*, 2009).

Approximately 70 % of the fish species in mediterranean streams, for which adequate data are available, are threatened with extinction, a proportion that is a least three times that of reptiles (14%), amphibians (26%) and mammals (21%) (Smith & Darwall, 2006; Hermoso & Clavero, 2011). Mediterranean streams are heavily impacted by human activities that degrade water quality, reduce habitat availability and connectivity, and change habitat functionality. Among the principal threats, are water pollution, water diversion, damming, and the spread of non-native species (Smith & Darwall, 2006; Grantham *et al.*, 2010; Hermoso & Clavero, 2011). Moreover, under altered future climate scenarios, mediterranean regions in Europe will likely experience extended dry-periods, and increased natural stress, which combined with the rising human pressure on water resources, may increase the vulnerability of native fish faunas (Beniston *et al.*, 2007; Iglesias *et al.*, 2007; Magalhães *et al.*, 2007; Grantham *et al.*, 2010). Under these circumstances, more comprehensive knowledge of fish assemblage dynamics in mediterranean streams is needed, to base effective conservation and integrative management actions, encompassing social and ecological needs (Alvarez Cobelas *et al.*, 2005; Magalhães *et al.*, 2007; Grantham *et al.*, 2010; Hermoso & Clavero, 2011).

Previous studies in mediterranean streams highlighted multi-scale influences on local assemblage structure of fish. At the basin scale, drainage area, rainfall and temperature regimes, and lithology, shape patterns of species richness and abundance (Corbacho & Sanchez, 2001; Magalhães *et al.*, 2002a; Mesquita *et al.*, 2006; Filipe *et al.*, 2010), while at the reach scale, channel hydro-morphology, substrate and streambed cover, and bank and riparian cover influence fish distribution (Pires *et al.*, 1999; Godinho *et al.*, 2000; Magalhães *et al.*, 2002b; Morán-López *et al.*, 2005; Mesquita *et al.*, 2006; Clavero *et al.*, 2009). Spatial arrangement of dry-season refugia across the riverscape was also associated with assemblage dynamics, as core areas for fish tend to concentrate in larger streams, and downstream sections, where persistent dry-season habitats are more likely to occur (Magalhães *et al.*, 2002a). Besides, movement was pointed as an important process contributing to assemblage patterns, as colonization of formerly dewatered areas likely depends, at least to some extent, on the characteristics and distance to dry-season refugia, the pool of colonists, and species- and size-specific dispersal abilities (Gowan & Fausch, 1996; Skalski & Gilliam, 2000; Magalhães *et al.*, 2002a). Dry-season refugia are expected to play a key role in fish persistence in mediterranean streams, but may vary in suitability and functional importance for different species and life-stages (Magalhães *et al.*, 2002b; Morán-López *et al.*, 2005; Morán-López *et al.*, 2006). However, factors promoting this variation are still poorly understood, and little or no attempts were made to evaluate the importance of spatial connections between dry-season and wet-season habitats and clarify fish movement patterns among habitats (but see Rodriguez-Ruiz & Granado-Lorencio, 1992; Aparicio & de Sostoa, 1999). Therefore, clarification of fish population and assemblage dynamics in mediterranean streams will require explicit analyses of patterns of dry-season refugia availability and use, and quantification of the extent to which this is associated with the distribution and abundance of fish across the riverscape, over the annual cycle.

1.5. STUDY AIMS AND APPROACH

As described in the previous sections, there are still major gaps in fish ecology in temporary streams. Recent developments in stream ecology call for a shift in perspective, as local assemblage structure is increasingly thought of as a product of multi-scale factors, and spatial habitat relationships and fish movement emerge as critical processes driving population and assemblage dynamics.

The current dissertation is framed in this conceptual framework, aiming to contribute to advance knowledge of population and assemblage dynamics of fish in seasonally-drying, mediterranean streams.

Specifically, the study was designed to evaluate the role of multiple spatial and environmental factors on patterns of fish distribution and abundance at the stream segment scale, and to quantify movement patterns of fish. For that purpose, three separated research activities were conducted targeting at:

i) Characterizing fish assemblage patterns in dry-season refugia, and assessing the relative contributions of morphological, physicochemical, and spatial factors to variation in species richness and abundance, and population size structure;

ii) Describing fish movement patterns, and quantifying the rate and extent of displacements out of dry-season pools and among wet-season habitats;

iii) Quantifying variation in fish assemblage structure during hydrological connection, over space and time, and assessing the role of habitat quality, connectivity to dry-season refugia, and neighbourhood effects in shaping this variation.

Research activities were conducted in two mediterranean streams in Southwest Portugal: the Torgal and the Odelouca (see Chapter 2). These streams were selected for study because they are typically intermittent, reducing to isolated pools at the end of the dry-season. Also, these streams share a similar fish fauna, including some endemic but closely related species (Coelho *et al.*, 1998; Coelho *et al.*, 2005). Finally, fish and habitat surveys have been conducted in the Torgal and Odelouca streams since 1991 and 1997, respectively (e.g. Magalhães *et al.*, 2002a; Magalhães *et al.*, 2002b; Magalhães *et al.*, 2003; Pires *et al.*, 2004; Mesquita *et al.*, 2006; Magalhães *et al.*, 2007; Pires *et al.*, 2008; Santos & Ferreira, 2008) and constituted an important basis for the development of the current study. A more detailed description of these streams and its fish assemblages will be presented in the subsequent chapters.

Assessment of variation in fish assemblages in dry-season refugia was undertaken in the Odelouca stream during the dry-season of 2005. This was as particularly dry year in SW Portugal compared to the long term annual mean of the region, allowing the identification of factors most likely to influence fish distribution when dry-season conditions turn rigorous. The Odelouca stream encompassed a wide range of conditions of dry-season pools, which was judged most adequate to evaluate the suitability and functional importance of refugia for different species and life stages

Analysis of fish movement was undertaken between September 2007 and July 2008 in the Torgal stream. The study focused on movements by the endemic, highly endangered, but locally dominant species, the chub *Squalius torgalensis.* This species was selected for study based on the likelihood that it would provide sufficient sample sizes for analyses, its suitability for marking, and its ability to represent ecological traits of mediterranean fish. The study involved individual fish tagging with Passive Integrated Transponder tags, which was previously considered viable for the focal species in laboratorial essays.

Assessment of spatio-temporal patterns in fish distribution and assemblage structure during the hydrological connection was further conducted, also in the Torgal stream, between February 2006 and July 2007.

Overall, this thesis followed a landscape ecology perspective (Schlosser, 1991; Wiens, 2002; Fahrig, 2005), in the sense that patterns of fish distribution were expected to be influenced not only by local habitat characteristics but also by higher scales processes such as habitat arrangement across the riverscape. Therefore habitats were characterized by measures reflecting local attributes and its position and spatial context within the riverscape. Local stream habitat was characterized by means of hydro-morphological and physicochemical descriptors. Spatial context was described by measures of location in and isolation of dry-season refugia in the riverscape and connectivity and neighbourhood metrics of base flow habitats. Characterization of fish assemblage structure was made via estimates of species richness, composition and abundance, and size-structure.

In all surveys fish assemblages were sampled by electrofishing, with permission of concerning Portuguese authority, the Forestry Authority (permit nº 10 / 2008). Handling, manipulation, and detention of fish in the field, and associated laboratory essays (see below), were conducted with the permission of the concerning Portuguese authority, the Institute for Nature Conservation and Biodiversity (permit nº 316 / 2007).

1.6. THESIS OUTLINE

The thesis is composed by 7 chapters and 2 Appendixes.

The current chapter, Introduction, places this research in context, by synthesizing background knowledge, and relevant topics in ecology of the stream fish, and clarifying its pertinence in the study of mediterranean fish assemblages, before describing the study aims and thesis outline.

Chapter 2 presents the study area, describing the main characteristics of the Odelouca and Torgal streams and its fish assemblages.

Chapter 3 addresses factors influencing fish assemblages in dry-season pools. The study was conducted in the Odelouca stream, and specifically quantified the contributions of spatial context, pool morphology and physicochemical factors to variation in fish species richness and abundance, and life-stage abundance. The results of this chapter have been previously published in Ecology of Freshwater Fish.

Chapter 4 addresses patterns of fish movement in the Torgal stream. The study aimed to quantify the rate and the extension of movements of the *Squalius torgalensis*, both out of dry-season pools and among wet-season habitats.

Chapter 5 addresses variation in fish distribution during the wet-season in the Torgal stream. The study aimed, to identify the local habitat attributes influencing spatial and temporal variation in species richness, overall abundance and individual occurrence, and assess the role of habitat quality relative to connectivity to dry-season refugia and neighbourhood effects in shaping perceived patterns.

In chapter 6 the main results from each chapter are integrated and discussed, and their theoretical and applied implications to the functioning and conservation of fish assemblages in mediterranean streams are explored.

Finally, chapter 7 highlights the relevant achievements of this study and guidelines for future research that would contribute to advance population and assemblage ecology of fish and conservation planning in mediterranean streams.

In Appendix I the results of the laboratory essays assessing the efficacy of the tagging technique used in the study of fish movements are presented. These results have been previously published in Journal of Applied Ichthyology.

In Appendix II data on variation of fish abundance in dry-season pools in the Torgal stream are shown.

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Chapter 2

STUDY AREA

2. STUDY AREA

The current study was undertaken in two mediterranean streams in Southwest Portugal (Figure 2.1): the Odelouca (37º20'N, 8º21'W), a tributary of the Arade river, and the Torgal (37º38'N, 8º39'W), a tributary of the Mira river. The Arade and Mira basins have been recently included in two distinct ichthyogeographic provinces in the Iberian Peninsula, namely the Southwest and Mira province respectively (Filipe *et al.*, 2009). Nevertheless, the two drainages are located in neighbouring geographic areas, and the study streams exhibit several similarities in environmental settings and fish faunas. A detailed description of the topography, climatic and hydrological regimes, geology and landscape cover, and fish assemblages in the Odelouca and Torgal streams is presented below.

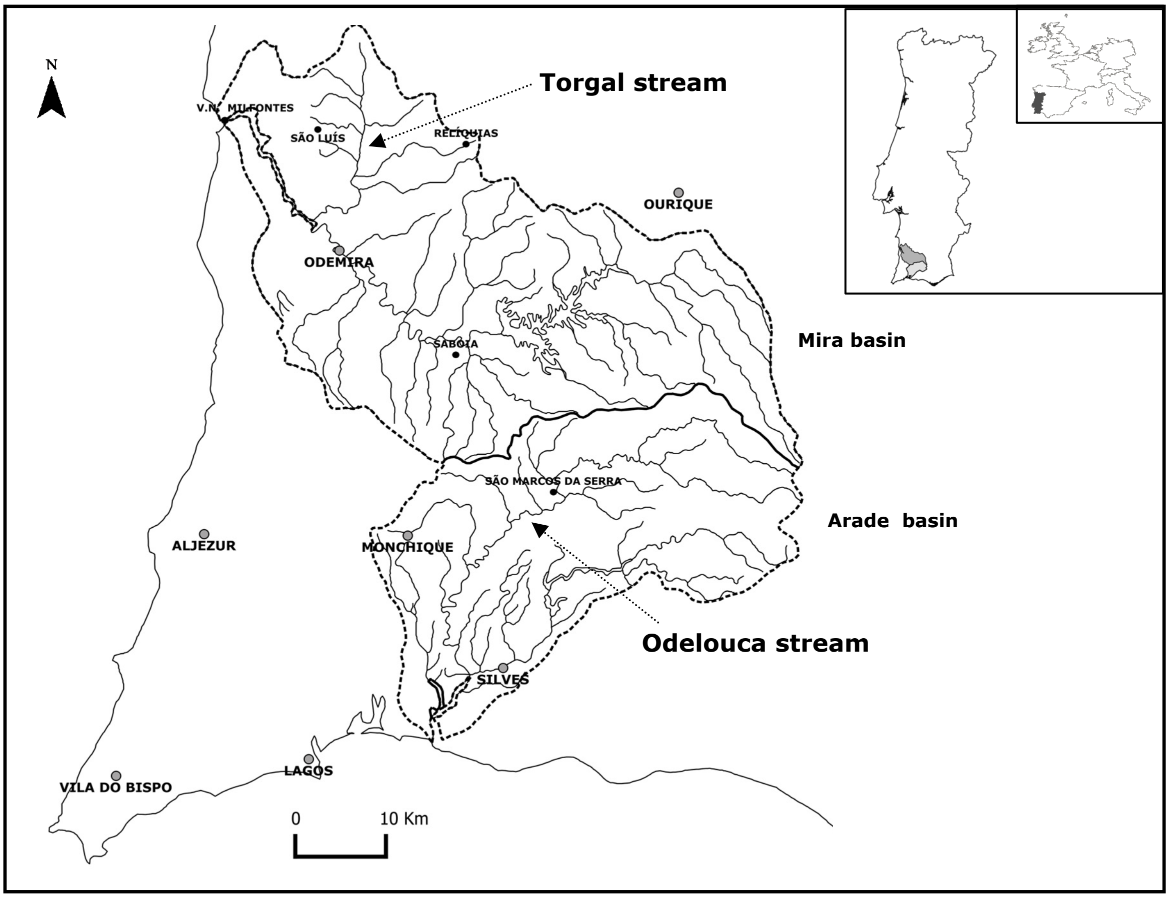

Figure 2.1 Map of Southwest Portugal showing the limits of the Arade and Mira basins and the location of the Odelouca and Torgal streams.

2.1. ENVIRONMENTAL SETTINGS

The Odelouca stream is the major tributary of the Arade river, draining about 520 km^2 and flowing for 83 km, from the Caldeirão mountains (509 m a.s.l), discharging at about sea level into the estuary of river Arade (Figure 2.2). Its major tributaries are the Azilheira (24 km, 63 km^2) and Monchique (18 km, 53 km^2) streams, both flowing in the left bank.

The Torgal is the major tributary of the Mira river, draining about 238 km^2 and flowing from the hills of Cercal (300 m a.s.l.) for 28 km, discharging into the estuary of the river Mira at about sea level (Figure 2.3). Its major tributaries are the Capelinha (17 km, 48 km^2) and Vale Ferro (18 km, 46 km^2) streams, in the western part of the basin.

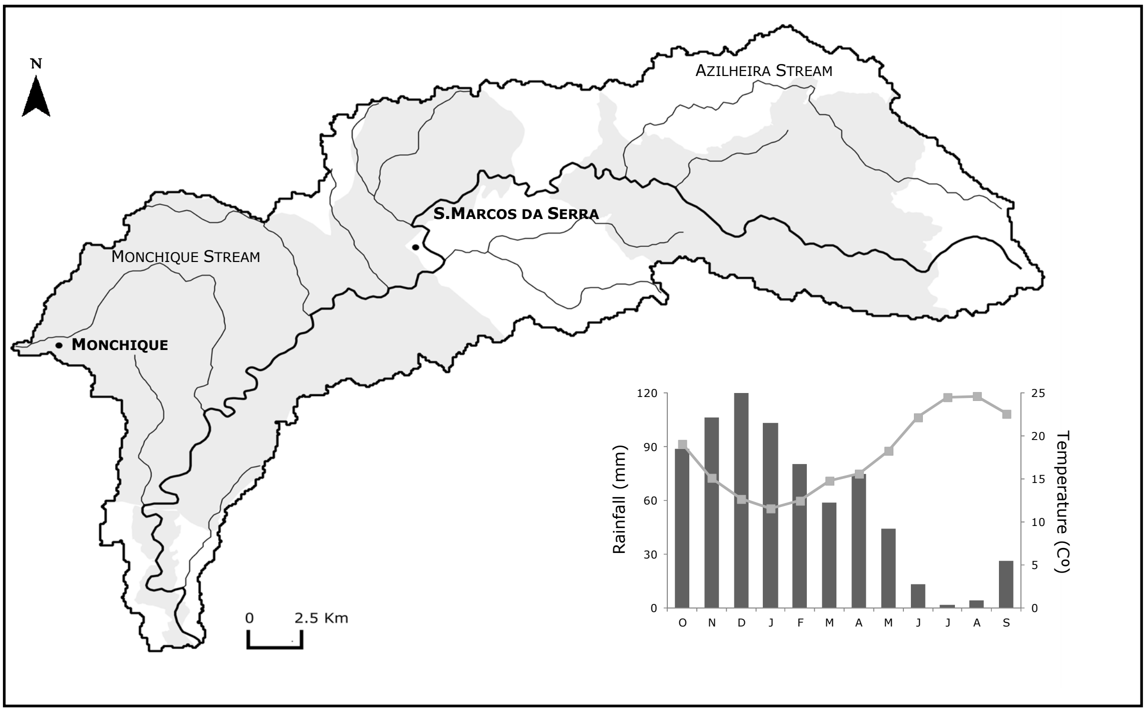

Figure 2.2 Limits of Natura 2000 sites in the Odelouca basin (grey shadows) and ombrothermic diagram for the region. Data were obtained in gauging stations of the Instituto Nacional da Água (available at http://snirh.pt/).

The geology of both basins is dominated by complexes of schist and graywackes from the Carbonic period (Coba, 1998; Hidroprojecto, 2008). However, syenite, an igneous rock is found in the Odelouca basin across the Monchique sub-basin, and siliceous igneous rocks are also present in western tributaries of the Torgal stream.

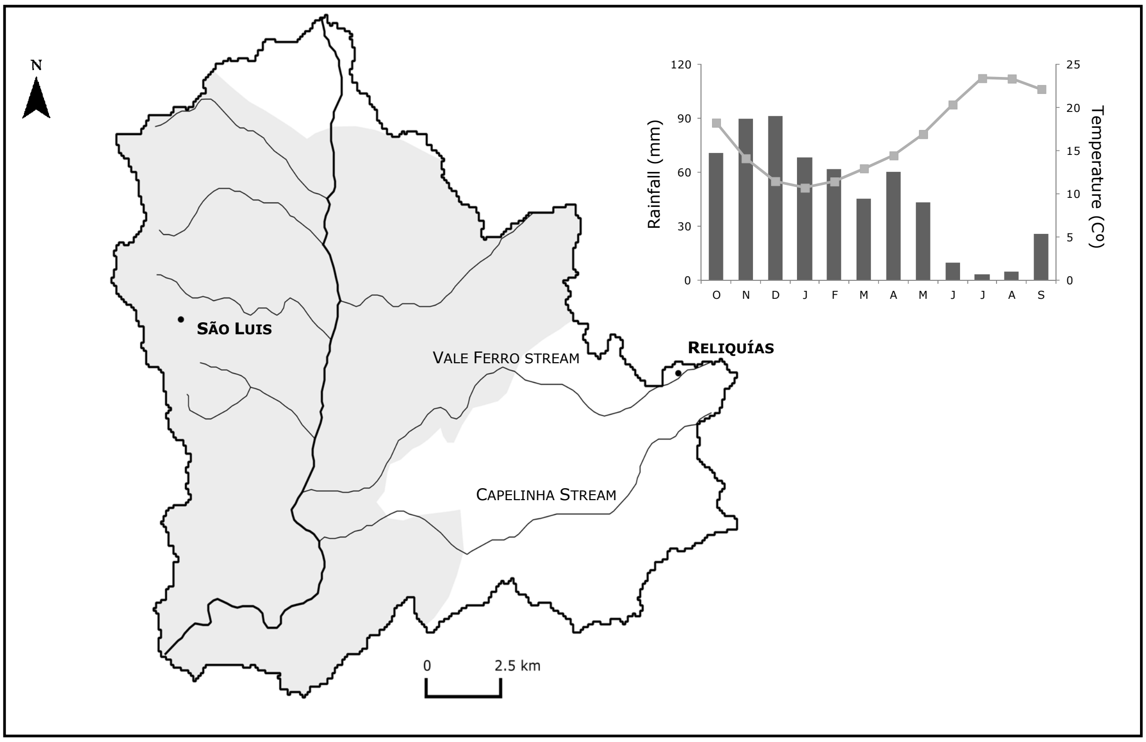

Figure 2.3 Limits of Natura 2000 sites in the Torgal basin (grey shadows), and ombrothermic diagram for the region. Data were obtained in gauging stations of the Instituto Nacional da Água (available at http://snirh.pt/).

The Odelouca stream runs through rounded hills and elevations, typical of schist formations, from deep and narrow valleys in the Monchique and Caldeirão mountains to more open and smooth valleys in the area of São Marcos da Serra (Coba, 1998). In the Torgal basin, the relief is globally gentle and the stream runs mostly through open and smooth valleys (Consórcio Hidroprojecto *et al.*, 2000).

The climate in both basins is mediterranean with oceanic influence, with warm to hot, dry summers, and cool, wet winters. Annual rainfall varies markedly from year-to-year but follows a predictable seasonal pattern, with about 80% of the rainfall occurring in October to March and virtually none falling in July and August (INAG, 2011a). Annual rainfall ranges from 294.5 to 1313.5 mm in the Odelouca basin and from 237.8 to 1121.2 mm in the Torgal basin (INAG, 2011a). Mean monthly temperature ranges from 11.6º C (January) to 24.7º C (August) in the Odelouca basin and from 10.7ºC (January) to 23.4º (August) in the Torgal basin (INAG, 2011a).

The flow regimes of both streams are highly dependent on rainfall patterns. Headwaters generally flow only after heavy rains, while the main stream and the largest tributaries typically dry to isolated pools during the summer. In dry years, there are no significant floods, the drying period is extended, and surface water is restricted to the deepest pools. In wet years there may be major flash floods, and flows may persist through the dry-season in downstream reaches.

The majority of both the Odelouca and Torgal basins is included in the fitogeographic region of the sub-sector Baixo-Alentejano–Monchiquense, where the potential vegetation is composed by species and associations belonging to the *Myrto-Quercinus suberis* series (Costa *et al.*, 1998). This series has as relevant succession stages, cork oak *Quercus suber* Linnaeus, 1753, woods (climacic stage), strawberry tree *Arbutus unedo* Linnaeus, 1753, woods, and mediterranean sclerophyllous and evergreen scrubs (*Cistus sp*. and *Erica sp.*). Nowadays, landcover is composed by mosaics of natural cork oak woods, mediterranean sclerophyllous and evergreen scrub woods, and cork oak, eucalyptus *Eucaliptus globulus* Labill, 1800, plantations in the more steep areas (Nemus, 2007; Hidroprojecto, 2008). In flatter and alluvial areas there are more intensive land uses, including mainly cereal and horticulture fields and rough pastures.

Both the Odelouca and Torgal streams are forested and include well preserved riparian galleries. The upstream reaches of the Odelouca stream are mainly dominated by alders *Alnus glutinosa* (L.) Gaertner, 1790, willows *Salix sp*. and ashes *Fraxinus angustifolia* Vahl, 1804, but large extensions of giant-reed *Arundo donax,* Linnaeus, 1753 dominate downstream. In the Torgal stream, banks show particularly well-developed riparian galleries, dominated by alder *Alnus glutinosa* and ash *Fraxinus angustifolia*.

Human settlement in both basins is generally low, and has diminished due to rural exodus in the last decades. Human occupation is largely restricted to the small villages of Monchique and São Marcos da Serra in the Odelouca basin (Figure 2.2), and São Luis and Relíquias in the Torgal basin (Figure 2.3) (Nemus, 2007; Hidroprojecto, 2008). During the field surveys conducted in this thesis (2005-2008) and until recently, both basins where free from major anthropogenic impacts such as urban pollution, impoundments, angling or other recreational activities. However, in 2009 a reservoir was constructed in the middle reaches of the Odelouca stream, flooding an area of about 7.5 km^2.

Great extensions of both the Odelouca and the Torgan basins are listed under legal protection and conservation categories (Figures 2.2 and 2.3). In the Odelouca basin there are three Natura 2000 sites (ICNB, 2011): the Caldeirão site (Resolução do Conselho de Ministros nº76/00 de 5 de Julho), located upstream, the Monchique site (Resolução do Conselho de Ministros nº142/97, de 28 de Agosto) in the intermediate part of the basin, and the Arade/Odelouca site (Resolução do Conselho de Ministros nº76/00 de 5 de Julho) including the stream mouth. The entire mainstem is also classified under the Water Law (Lei nº 58/2005 de 29 de Dezembro; INAG, 2011b) as cyprinid waters adequate for pisciculture, including areas where species with economic and management interest occur and require protection. The majority of the Torgal basin is included the Natura 2000 site Costa Sudoeste (Resolução do Conselho de Ministros nº142/97, de 28 de Agosto), with downstream reaches overlapping with the Natural Park of the Sudoeste Alentejano and Costa Vicentina (Resolução do Conselho de Ministros nº142/97, de 28 de Agosto). The area shelters numerous species with high conservation value associated with stream environments, besides fish (see below), including the white willow *Salix salvifolia* Brot. subsp. *australis* Franco, 1971, the Iberian newt *Lissotriton boscai*, Lataste, 1879, the Iberian midwife toad *Alytes cisternasii* Bósca, 1879, the Iberian painted frog *Discoglossus galganoi* Capula, Nascetti, Lanza, Bullini & Crespo, 1985, the Iberian green lizard *Lacerta schreiberi*, Bedriaga, 1878, the freshwater turtles *Emys orbicularis*, Linnaeus, 1758, and *Mauremys leprosa*, Schweiger, 1812, and the European otter *Lutra lutra*, L. 1758, (ALFA, 2004).

2.2. FISH FAUNA

Fish assemblages in the study streams include a reduced set of species (Table 2.1), as generally found in mediterranean streams (Smith & Darwall, 2006; Reyjol *et al.*, 2007). The Odelouca stream shelters 5 native and 6 non-native species whereas the Torgal stream is inhabited by 6 native and 4 non-native species. Overall the two streams share 4 native and 4 non-native species. Among the sets of native species in these streams there are 3 species endemic to Portugal, with high conservation value (Coelho *et al.*, 1998; Mesquita & Coelho, 2000; Coelho *et al.*, 2005; Rogado *et al.*, 2005): the Southwestern arched-mouth nase Iberoc*hondrostoma almacai* (Coelho, Mesquita & Collares-Pereira, 2005), that occurs in both basins, and the Arade chub *Squalius aradensis* (Coelho, Botgutskaya, Rodrigues & Collares-Pereira, 1998), and the Mira chub *Squalius torgalensis* (Coelho, Botgutskaya, Rodrigues & Collares-Pereira, 1998), which restrict to the Odelouca and Torgal basins, respectively. These three species are currently listed as Critically Endangered in the Portuguese Red Data Book, as result of their limited distribution ranges and degradation of its habitats (Rogado *et al.*, 2005). Other native species in the study streams are the Southern Iberian spined loach *Cobitis paludica* (De Buen, 1930), and Southern Iberian barbel *Luciobarbus sclateri* (Ghünter, 1868), the three-spined stickleback *Gasterosteus gymnurus*, Cuvier 1829, and the catadromous European eel *Anguilla anguilla (*L., 1758). Barbel, three-spinned stickleback and eel are currently listed as Endangered in the Portuguese Red Data Book (Rogado *et al.*, 2005). Overall, major threats to native species across their distribution ranges include changes in habitat structure and flow regime and the introduction of non-native species (Mesquita & Coelho, 2000; Rogado *et al.*, 2005). Non–native species occurring in both the Odelouca and the Torgal streams include the mosquito fish *Gambusia holbrooki* Girard, 1859, the pumpkinseed *Lepomis gibbosus* (L., 1758), the largemouth bass *Micropterus salmoides* (Lacepède, 1802), and the carp *Cyprinus carpio* L., 1758*.* Two other non-native species are found in the Odelouca stream, the chameleon cichlid *Australoheros facetus* Jenyns, 1842 and the straight-mouth nase *Pseudochondrostoma polylepis* (Steindachner, 1864). *S*pecies of the families

Atherinidae and Mugilidae also have been occasionally reported in the downstream reaches of both the Odelouca and Torgal streams (Coba, 1998; Magalhães, 2002; Pires *et al.*, 2004).

Table 2.1 Fish species list for the Odelouca (O) and Torgal (T) streams, based on records obtained between 2005 and 2008. Scientific and common names for species follow Kottelat & Freyhof (2007) and Leunda *et al.* (2009). Origin: N – native species, I – introduced, non-native species; Type: P – primary; Ph – peripheral, A – Amphidromous, C – catadromous; Endemicity: P - Portugal, IP – Iberian Peninsula; Red List Portugal: CR-critically endangered, EN – endangered, LC – least concern.

	Basin	Origin (1)	Type (2)	Endemicity (1)	Red List Portugal (1)
ANGUILLIDAE					
Anguilla Anguilla, eel	O, T	N	Ph, C		EN
CYPRINIDAE					
Cyprinus carpio, carp	O, T	I	P		
Iberochondrostoma almacai, Southwestern arched-mouth nase	O, T	N	P	P	CR
Luciobarbus sclateri, Southern Iberian barbel	O, T	N	P	IP	EN
Pseudochondrostoma polylepis, straight-mouth nase	O	I	P	IP	LC
Squalius aradensis, Arade chub	O	N	P	P	CR
Squalius torgalensis, Mira chub	T	N	P	P	CR
COBITIDAE					
Cobitis paludica, Southern Iberian spined loach	O, T	N	P	IP	LC
POECILIIDAE					
Gambusia holbrooki, mosquito fish	O, T	I	P		
GASTEROSTEIDAE					
Gasterosteus gymnurus, three-spined stickleback	T	N	Ph, A		EN
CICHLIDAE					
Australoheros facetum, chameleon fish	O	I	P		
CENTRARCHIDAE					
Lepomis gibbosus, pumpkinseed	O, T	I	P		
Micropterus salmoides, largemouth bass	O, T	I	P		

(1) Rogado *et al.*(2005)
(2) Darlington (1957)

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Chapter 3

VARIATION IN FISH ASSEMBLAGES ACROSS DRY-SEASON POOLS IN A MEDITERRANEAN STREAM: EFFECTS OF POOL MORPHOLOGY, PHYSICOCHEMICAL FACTORS AND SPATIAL CONTEXT

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 stream: effects of pool morphology, physicochemical factors and spatial context.

Ecology of Freshwater Fish, 19, 74-86.

3. VARIATION IN FISH ASSEMBLAGES ACROSS DRY-SEASON POOLS IN A MEDITERRANEAN STREAM: EFFECTS OF POOL MORPHOLOGY, PHYSICOCHEMICAL FACTORS AND SPATIAL CONTEXT

3.1. INTRODUCTION

Droughts in streams disrupt hydrological connectivity, and have direct and indirect impacts on the resident biota (Bond *et al.*, 2008). Direct effects of droughts centre on loss of water and flow, and habitat reduction and reconfiguration (Lake, 2003). Resident biota experience unique challenges when drought is severe enough to cause complete drying of parts of the stream bed (Humphries & Baldwin, 2003). As a consequence, remnant habitats become critical to the survival of many species, acting as refuge and functioning in various ways as sources of recruits or colonists for the entire stream after the drought has passed (Sedell *et al.*, 1990; Lancaster, 2000; Humphries & Baldwin, 2003). Although refuges are a key component in resistance, resilience and recovery from drought, mechanisms and patterns of refuge use by stream biota remain poorly understood (Matthews & Marsh-Matthews, 2003; Boulton & Lake, 2008).

Pools have long been considered important refugia for fish during droughts (see review in Matthews, 1998). However there is growing evidence that assemblage structure of fish in dry-season pools may vary substantially both spatially and temporally (Magoulick, 2000; Davey & Kelly, 2007; Dekar & Magoulick, 2007). This variation has been associated with changes in the morphology, physicochemistry and spatial context of pools (e.g. Capone & Kushlan, 1991; Taylor, 1997; Labbe & Fausch, 2000; Magoulick, 2000; Davey & Kelly, 2007; Dekar & Magoulick, 2007), but the relative influence of these factors on different assemblage attributes remains unclear (see review in Magoulick & Kobza, 2003). A more comprehensive understanding of this issue would be important from both theoretical and applied perspectives, to assure the quality of pools as refuge for fish during droughts, and devise more adequate conservation management and restoration frameworks for drought prone streams (e.g. Bond & Lake, 2005; Lake *et al.*, 2007).

Understanding the mechanisms and patterns of use of dry-season pools is particularly important in the case of mediterranean stream fish assemblages, which include a large number of steadily declining endemic species that are likely to face major challenges in surviving extreme droughts expected under altered future climates (Santos & Miranda, 2006; Smith & Darwall, 2006; Magalhães *et al.*, 2007). Droughts are seasonally predictable events in mediterranean streams, occurring each year in summer-early fall, but varying markedly in intensity among years (Gasith & Resh, 1999). Direct effects of droughts range from reductions in flow to complete drying, usually resulting in a highly patchy and heterogeneous mosaic of remnant pools, embedded in dry channels. Fish persistence is dependent upon such dry-season pools, which seem to vary in suitability and functional importance for different species and life stages (Magalhães *et al.*, 2002b). Factors promoting this variation are still poorly understood, and it remains important to evaluate the range of heterogeneity in dry-season pools that needs to be maintained across the landscape to sustain its characteristic set of species under present day and altered future drought regimes.

This study addressed these issues by examining variation in fish assemblages among dry-season pools in a typical mediterranean stream, in Southwest Portugal. Specifically, the study assessed the relative contributions of variables reflecting pool morphology, physicochemistry, and spatial context to variation in: i) species richness and overall abundance, ii) relative abundances of individual species, and iii) relative abundances of individual length classes within the most abundant species. This information was then used to discuss conservation management recommendations to contend with drought in Mediterranean streams.

3.2. STUDY AREA

The study was conducted in the Odelouca stream, in Southwest Portugal (Figure 3.1), which is a seasonally-drying mediterranean stream in its flow regime and fish fauna. The Odelouca basin and its fish assemblages were previously described in detail in Chapter 2, so that only features relevant to the present investigation are given here. The climate is mediterranean with oceanic influence, with warm to hot, dry summers and cool, wet winters. Mean monthly temperature ranges from 10.5º C (January) to 22.3º C (July). Annual rainfall varies markedly (294.5-1313.5 mm), but follows a predictable seasonal pattern. About 81% of the annual rain occurs in October - March and virtually none falls in the hot, dry months (July - August). The flow regime is highly dependent on rainfall patterns. Headwaters flow only after heavy rains, while the main stream and the largest tributaries typically dry to isolated pools during the summer. In dry years, there are no significant floods, the drying period is extended, and surface water is restricted to the deepest pools. In wet years there may be major flash floods, and flows may persist through the summer in downstream reaches.

Fish assemblages include a pool of native species that is dominated by loach *Cobitis paludica* and Arade chub *Squalius aradensis*; eel Anguilla *anguilla* and Southwestern arched-mouth nase *Iberochondrostoma almacai* are widespread but less abundant whereas *Luciobarbus sclateri* occurs sparsely and in low numbers. Non-native species include the highly abundant mosquito fish *Gambusia holbrooki*, and the relatively rare pumpkinseed *Lepomis gibbosus*, largemouth bass *Micropterus salmoides*, chameleon cichlid *Australoheros facetus*, carp *Cyprinus carpio*, and straight-mouth nase *Pseudochondrostoma polylepis*.

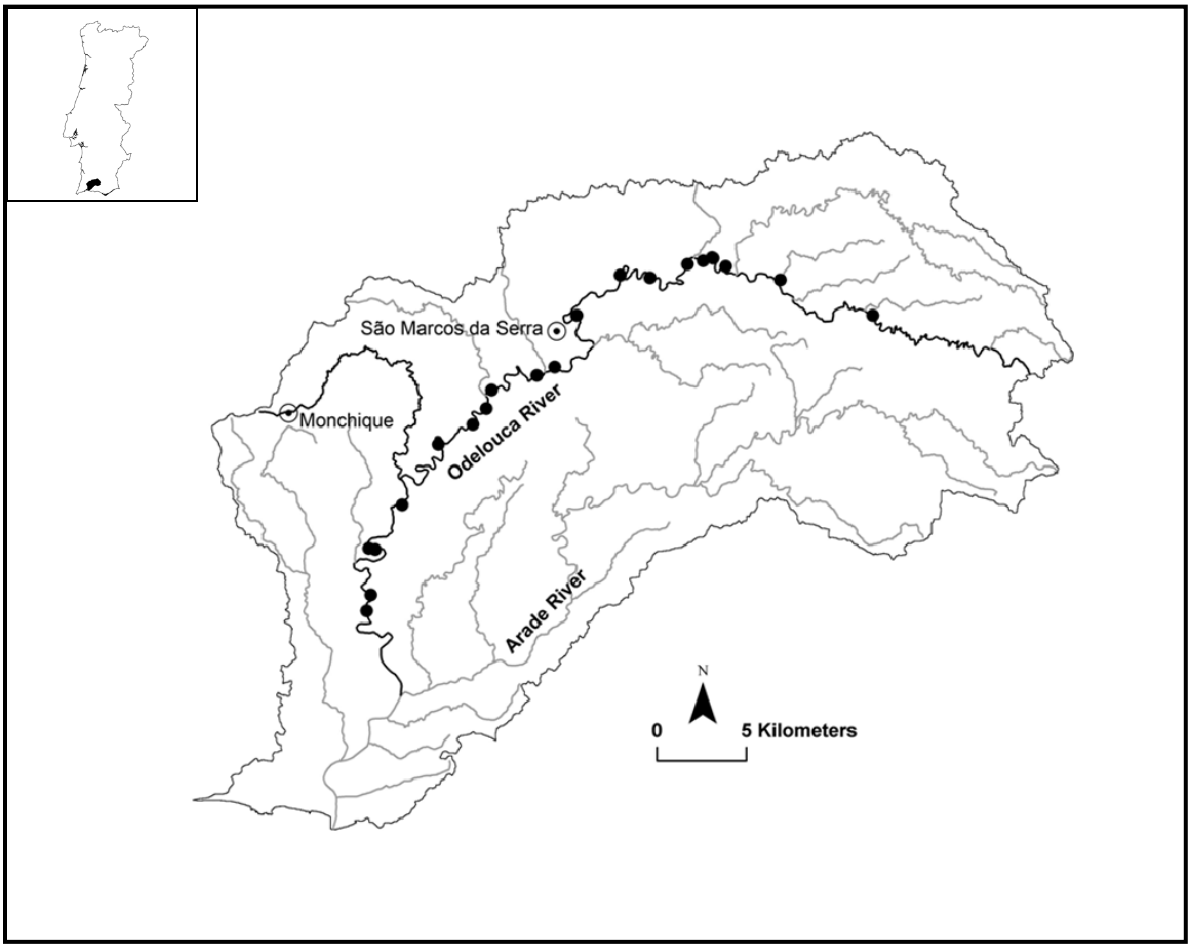

Figure 3.1 Map of the study area, showing the location of the 21 pools in the Odelouca stream, sampled for fish in September 2005.

3.3. METHODS

3.3.1. *Fish sampling*

Fish were sampled in 21 pools in September 2005, at the end of the dry-season (Figure 3.1). The study year was dry relative to the long-term annual mean (295 mm *vs.* 693 mm), causing dry-season environmental conditions to be particularly severe. Sampling locations were chosen in the field, based on the natural distribution of permanent pools and accessibility, so that they encompassed the range of variability in pool size and riparian cover in the study area. Overall, sampling pools were scattered throughout a 68 km segment of the stream, ranged in length from 7 to 214 m, and encompassed from open to well-developed canopies (Table 3.1).

Table 3.1 Characteristics of pools in the Odelouca stream, during September 2005. Values are the mean ± SD (standard deviation) and the range (minimum-maximum).

	Code	Mean ± SD	Range
Morphology			
Maximum length (m)	MLNT	58.0 ± 56.7	6.9 - 214.3
Maximum Width (m; $\log_{10}$)	MWDT	9.0 ± 4.8	3.5 - 18.9
Mean Channel Depth (m)	MCDE	0.4 ± 0.2	0.1 -0. 8
Maximum Depth (m; $\log_{10}$)	MDEP	0.7 ± 0.4	0.2 - 2.0
Volume (m^3; $\log_{10}$)	VOL	177.3 ± 255.8	3.4 - 834.9
Physicochemistry			
Canopy (%)	CAN	54.3 ± 17.7	23.3 - 100.0
Conductivity (µS cm^{-1}; $\log_{10}$)	COND	586.8 ± 133.9	412.0 - 910.0
Oxygen (ppm)	OXI	6.3 ± 3.6	1.8 - 13.6
pH	pH	7.1 ± 0.6	6.3 - 8.7
Substrate coarseness	SUBC	4.0 ± 0.4	3.3 - 5.0
Substrate heterogeneity	SUBH	0.7 ± 0.4	0.2 - 1.8
Temperature (ºC)	TEMP	23.4 ± 3.4	19.4 - 31.4
Turbidity (m)	TUR	0.3 ±0.2	0.04 - 72.0
Spatial context			
Distance to the stream mouth (km)	DMTH	42.0 ± 21.0	8.0 - 76.0
Distance to the nearest pool (m; $\log_{10}$)	DPOOL	72.7 ± 76.0	7.8 - 300.0

Sampling was carried out using a standard electrofishing gear (Hans Grassl EL62 IIGI), fitted with a single 30 cm anode, and discharging 300V, 4-6 A, pulsed D.C. Each pool was divided into stretches for sampling, with limits of stretches coinciding with discontinuities in depth and substrate. Water depth exceeded 1.2 meters in one short stretch only, which was excluded from analysis, to allow for more consistent sampling across pools. The number of stretches sampled per pool ranged between 1 and 5, contingent on the size and heterogeneity of the pools. Each stretch was single-pass electrofished for 5 minutes, by wading in an upstream direction, and covering all detectably distinctive microhabitats. The same person operated the electrofishing gear across stretches, and there were always two netters present to pick up stunned fish. Fish were identified, measured for fork (FL) or total length (TL) depending on species, and returned to the pool. The sampling effort was primarily chosen to balance time expenditure at each pool and the need to sample all pools before early-fall rains. Previous experience in other Mediterranean streams showed that the sampling procedure yielded in average 92% (SD=10.2%, n=7, range= 80–100%) of the species recorded in three consecutive fishing passes, and there were high correlations between the first and the overall sample in species ranks (R_s; Mean = 0.87, SD = 0.16, n = 7, range = 0.60-1.00) and relative abundances (R; Mean = 0.95, SD = 0.04, n = 7, range = 0.87-1.00) (D.F. Pires & M.F. Magalhães, unpublished data).

3.3.2. Explanatory variables

Factors influencing assemblage structure were analysed using three sets of explanatory variables reflecting the morphological, physicochemical and spatial contexts of each pool (Table 3.1).

Morphological context was described from five variables reflecting pool size and shape. The volume of pools was calculated from its maximum length, maximum width, and middle channel depth, by approximation to a half ellipsoid. Width was measured along three to five equally spaced transects, perpendicular to the long axis of each pool; middle channel depth was derived from measures taken at the middle-point of each transect. A total of three to six additional measures taken at the deepest points in each pool were used to quantify maximum depth.

Physicochemical context was described from eight variables reflecting water quality, substrate composition, and riparian canopy development. All variables were measured along three to five middle-channel points in each pool. Turbidity was assessed using a Secchi disk. Conductivity and pH were measured at the surface with a HI 198129 probe (Hanna Instruments, Woonsocket, RI, USA), and surface temperature and dissolved oxygen were measured with an YSI DO200 oxygen meter (Yellow Spring Inc., OH, USA). Substrate coarseness and heterogeneity were quantified with a 1-meter lead core divided into 10-centimem sections, using the frequency size class method of Bain (1999). Specifically, the segment rope was stretched at the pool bottom, and the dominant substrate in each 10-cm section was recorded, as: 0 - <0.06 mm; 1 - 0.06-1 mm; 2 - 2-15 mm; 3 - 16-63 mm; 4 - 64-256 mm; and 5 - >256 mm. The mean and standard deviation of dominant substrate values were used as indices of substrate coarseness and heterogeneity respectively. Canopy was quantified as the proportional area shaded by riparian vegetation, using a Konustar Professional Compass clinometer (Konus Optical & Sport System, Italy).

Spatial context was described from two variables reflecting pool location and isolation in the stream network. The position of each pool was logged with a Magellan 200 XI, GPS (Global Positioning System) receiver (MiTAC International Corporation), and plugged in a Geographic Information System (GIS; ArcView 3.1 for Windows, Environmental Systems Research Institute). Distance of each pool from the stream mouth was then calculated in the GIS, using digital cartography in the scale 1:25,000 (http://www.igeoe.pt). Distance to nearest pool was directly measured in the field.

All variables but distance from the stream mouth were determined immediately after fish sampling, always by the same person.

3.3.3. Data analysis

Analyses were designed to quantify variation in fish assemblages among pools, and identify variables explaining this variation. Fish and habitat data per stretch were combined to derive species richness and relative abundance, and average habitat conditions at each pool. Species detectability was considered to be constant across pools. The relative abundance of each species in each pool was expressed in terms of catch-per-unit-effort (CPUE; mean number of individuals per fishing pass). The most abundant species were divided into three length-classes (small: < 50 mm, medium: 50-79mm, and large ≥ 80mm), because previous studies showed size-related variation in the use of dry-season (e.g. Capone & Kushlan, 1991; Magalhães *et al.*, 2002b). To reduce daily influences in temperature and oxygen measurements residuals from linear regressions on time of day were used instead of the original variables. Prior to analysis, species richness and catch data, and explanatory variables showing skewed distributions were log-transformed to approach normality and to reduce the influence of extreme values (Table 3.1).

Species richness was quantified using rarefaction methods to account for variation in total catches among pools (Gotelli & Colwell, 2001). Rarefaction generates the number of species expected for a small collection of *n* individuals drawn randomly from a large sample (*N*), allowing for meaningful standardisation and comparison of data sets with similar species-occurrence distributions (e.g. Cao *et al.*, 2007). Sample size to rarefaction (*n*) was defined as the lowest number of fish captured across pools (n=45). The Ecosim 7 software (Gotelli & Entsminger, 2009) was used to randomly select individuals from samples of each pool and record the number of species; the randomisation was repeated 1000 times, and the mean number of species was used as the expected species richness.

Relations between fish species richness and abundance, and variables describing pool characteristics were investigated using linear regression (Legendre & Legendre, 1998). Analyses were carried out separately for morphological, physicochemical and spatial sets of variables, starting with a preliminary screening of univariate relationships between each pair of response and explanatory variables. To reduce collinearity problems in subsequent multivariate modelling, Pearson's correlation (R) was computed between each pair of explanatory variables, dropping from further analyses one variable from each pair with $|R| > 0.60$. The variable dropped from each pair was always the one least associated with the dependent variable, as judged from univariate analysis. Multivariate modelling was then carried out for each response variable and set of explanatory 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. In each case, the first step involved the fitting of a full model relating the response variable to all the explanatory variables in a given set (full model), carrying to subsequent forward selection only sets of variables producing statistically significant full models. Forward selection was then carried out using the double stopping criteria of Blanchet *et al.* (2008), based on the alpha significance level and the adjusted coefficient of multiple determination (adj-R^2). However, maximization of adj-R^2 was used as stopping criterion instead of the adj-R^2 of the full model, because the later tends to over-correct the forward selection procedure when the number of variables and the sample sizes are relatively small (Guillaume Blanchet & Daniel Borcard, pers. communication). In common with other studies (e.g. Gutzwiller & Barrow, 2001; Moreira *et al.*, 2005), significance of statistical tests was assessed at $\alpha = 0.10$, to reduce the incidence of Type II errors and thereby avoid missing eventual relationships with ecological relevance (Buhl-Mortensen, 1996; Underwood, 1997).

Morphological, physicochemical and spatial models were used in variation partitioning to isolate the amount of variation of the response variable that can be attributed to one set of explanatory factors once the effects of other sets is taken into account (Legendre & Legendre, 1998). Analyses were based on multivariate models built with the forward selection procedure, except when the control of collinearity reduced the set to a single variable and when lack of significance of the full models precluded the development of multivariate models, in which cases the variables yielding the best significant univariate relationship were used. Partial linear regressions were used in partitioning the explained variation among the sets of explanatory variables. Variation partitioning with two sets of explanatory variables followed Borcard *et al.* (1992), and the extension of the method to three sets of explanatory variables by Anderson & Gribble (1998) was used to isolated seven components of explained variation: (i) pure morphological variation; (ii) pure physicochemical variation; (iii) pure spatial variation; (iv) combined morphological/physicochemical variation; (v) spatial component of morphological variation; (vi) spatial component of physicochemical variation; and (vii) spatial component of morphological/physicochemical variation.

Associations between the matrix of species abundances and pool characteristics were analysed taking a similar approach, using redundancy analysis (RDA) and partial-RDA (Legendre & Legendre, 1998). A linear model of ordination was used, because preliminary detrended correspondence analyses showed turnovers along the first axes <2 SD, which is the recommended criterion for choosing linear vs. uni-modal ordination models (Ter Braak, 1995). RDA was carried out on covariance matrices to weight each species by the variance of its log-transformed abundance, thus preventing the undue influence of rare species (Ter Braak, 1995). Species occurring in less than 5 pools were excluded from the analysis. RDAs were carried out with the Canoco 4.5 software (Ter Braak & Smilauer, 2002) using the forward selection procedure by Blanchet *et al.* (2008), whereby significant variables were added to the model in order of the greatest contribution to the adj-R^2. To avoid collinearity problems and enhance model interpretability, variables causing variation inflation factor to exceed 2.0 were not considered in model building. Statistical significance of variables and RDA models were assessed from Monte Carlo permutation tests (10^4 permutations).

3.4. RESULTS

3.4.1. Fish assemblage composition

A total of 7,494 fish of 10 species were captured (Table 3.2). The number of species recorded per pool ranged from 4 to 9 and the mean expected species richness per pool from 2.1 to 7.2. Mean total catches per pool ranged from 26.5 to 884.0. Mosquito fish was the most caught species (37% numbers), occurring at 76% of the pools. Loach, chub and arched-mouth nase were also often captured (>67% of pools) but in smaller numbers (10-21%). The frequency of occurrence was still high for eel (95%), barbel (67%), and pumpkinseed (48%), but their catches were even lower (< 6%). Other species were both sparse (< 25% of pools) and rare (< 3% numbers).

3.4.2. Variation in species richness and abundance

Preliminary univariate analyses underlined several associations between species richness and abundances and morphological, physicochemical, and spatial variables. Volume was the morphological variable showing the strongest relations with species richness and abundances (R^2; Mean = 0.29, SD = 0.07, n = 6, range = 0.21-0.42 *vs.* Mean = 0.23, SD = 0.08, n = 29, range = 0.14-0.48). Volume was also highly correlated with all other morphological variables (|R|; Mean = 0.82, SD =0.07, n = 5, range = 0.72-0.89), and so it was the only retained further. Analysis of physicochemical variables, revealed some strong associations between species abundances and canopy (R^2; Mean = 0.33, SD = 0.13, n = 9, range = 0.17-0.54), temperature (R^2; Mean =0.22 , SD =0.05, n = 8, range = 0.14-0.29), oxygen (R^2; Mean =0.23 , SD =0.08, n = 8, range = 0.14-0.36), and pH (R^2; Mean = 0.24, SD = 0.07, n = 6, range = 0.15-0.32). Canopy was highly correlated with temperature (|R|=0.62) and oxygen (|R|=0.67), and so was the variable retained in multivariate modelling, as it was also associated with native species richness (R^2=0.26). Other significant associations were found between species abundance and substrate coarseness (R^2; Mean =0.16, SD = 0.00, n = 2, range = 0.16-0.16), and conductivity (R^2=0.26, n=1). The latter two variables were highly correlated (|R|=0.61), and so only substrate was retained in subsequent analyses. Distance from the stream mouth showed stronger associations with species richness and abundance than distance to the nearest pool (R^2; Mean = 0.22, SD =0.09, n =10, range = 0.14-0.40 *vs.* Mean = 0.16, SD =0.01, n = 2, range = 0.16-0.17). However, as these spatial variables were independent (|R|=0.02), they were both retained in multivariate modelling.

Table 3.2 Composition of fish assemblages in pools in the Odelouca stream during September 2005, quantified using the percentage occurrence (% Occ), the mean (± SD), and range of catches per fishing pass for each species. FL: furcal length; TL: total length.

Species	Code	% Occ	Mean ± SD	Range
Anguilla anguilla, eel	*Ang*	95.2	7.7 ± 7.2	0.0 - 26.8
Cobitis paludica, loach		100.0	52.4 ± 116.9	1.0 - 406.0
Small (< 50 mm, TL)	*Cob 1*	33.3	6.7 ± 21.6	0.0 - 91.9
Medium (50 - 79 mm, TL)	*Cob 2*	95.2	31.2 ± 67.7	0.0 - 266.7
Large (> 80 mm, TL)	*Cob 3*	100.0	14.6 ± 31.9	0.3 - 130.2
Luciobarbus sclateri, barbel	*Bar*	66.7	2.5 ± 3.2	0.0 - 11.5
Iberochondrostoma almacai, arched-mouth nase		66.7	16.7 ± 21.0	0.0 - 76.0
Small (< 50 mm, FL)	*Ich1*	61.9	4.0 ± 7.5	0.0 - 32.0
Medium (50 - 79 mm, FL)	*Ich2*	57.1	7.2 ± 10.8	0.0 - 39.0
Large (> 80 mm, FL)	*Ich3*	47.6	5.4 ± 8.0	0.0 - 23.3
Squalius aradensis, chub		71.4	34.1 ± 43.6	0.0 - 146.0
Small (< 50 mm, FL)	*Squ1*	71.4	17.1 ± 33.1	0.0 - 128.0
Medium (50 - 79 mm, FL)	*Squ 2*	66.7	10.7 ± 14.6	0.0 - 57.0
Large (> 80 mm, FL)	*Squ 3*	57.1	6.3 ± 7.9	0.0 - 27.5
Pseudochondrostoma polylepis, straight-mouth nase	*Pch*	23.8	0.9 ± 3.6	0.0 - 16.8
Australoheros facetum, chameleon fish	*Aus*	23.8	2.5 ± 6.5	0.0 - 26.0
Lepomis gibbosus, pumpkinseed	*Lep*	47.6	2.9 ± 5.5	0.0 - 20.8
Micropterus salmoides, largemouth bass	*Mic*	9.5	0.3 ± 0.8	0.0 - 3.5
Gambusia holbrooki, mosquito fish	*Gam*	76.2	86.5 ± 166.7	0.0 - 590.0
Total fish			209.2 ± 253.0	26.5 - 884.0
Native fish			113.4 ± 128.8	13.2 - 482.0
Total species			5.8 ± 1.3	4.0 - 9.0
Native species			4.0 ± 1.0	2.0 - 5.0

Relationships between species richness and abundance and morphological, physicochemical, and spatial models varied greatly in type and strength (Table 3.3). Morphological models underlined a tendency for overall species richness to be highest in large pools. Conversely, total catches and those of natives tended to decrease with pool volume, with small chub and nase being less caught in large pools where catches of pumpkinseed increased. Physicochemical models, showed a trend for more native species occurring in pools well covered by riparian canopy. Catches of arched-mouth nase and chub also increased in well shaded pools but those of small loach, eel, and mosquito fish tended to be lower therein. Total catches, and those of chub and mosquito fish decreased with substrate coarseness, whereas eel were more caught in pools with coarser substrates. Catches of arched-mouth nase and chub decreased with water acidity, and those of medium-size arched showed a positive association with substrate heterogeneity. Spatial models reflected a tendency for overall species richness to be highest in downstream pools. Catches of barbel and pumpkinseed also increased downstream, but those of arched-mouth nase and chub were highest upstream. Medium and large-sized arched-mouth nase tended to be more caught in more isolated pools.

Variation partitioning among morphological, physicochemical and spatial sets of variables showed that relatively large proportions of variation in overall species richness and total catches were accounted for uniquely by pool morphology (Table 3.4). By contrast, only physicochemical context showed unique significant contributions to explained variation in the catches of chub and medium-sized arched-mouth nase. Although no unique contributions were found for spatial context, there were relatively large proportions of variation shared among sets of variables, particularly between physicochemical and spatial contexts (>20%).

Table 3.3 Summary results of linear regression (richness and abundance) and RDA (abundance matrix) models for fish in pools in the Odelouca stream during September 2005. Directions of association (+ or -) and P-levels are given for variables significantly related to the response variables. Variables in bold were included in forward regression models; variables yielding the best univariate relationships are underlined. The amount of explained variation (R2) is given for each model. Codes for explanatory variables and fish species are in Tables 3.1 and 3.2, respectively.

	Morphology		Physicochemistry					Spatial context		
	R^2	VOL	R^2	CAN	PH	SUBC	SUBH	R^2	DMTH	DPOOL
Species Richness										
Overall	33.2	0.006(+)						27.7	**0.014(-)**	
Natives			25.8	0.019(+)						
Abundance										
Overall	41.6	0.002(-)	15.6			0.077(-)				
Natives	24.5	0.022(-)								
Eel			35.2	**0.021(-)**		**0.044(+)**				
Loach										
Small			36.0	**0.004(-)**						
Barbel								39.5	**0.002(-)**	
Nase			30.9	0.025(+)	**0.009(-)**			27.0	**0.016(+)**	
Small	28.2	0.013(-)	19.6		0.045(-)			31.6	**0.008(+)**	
Medium			51.4	**0.001(+)**			**0.018(+)**	33.4	**0.042(+)**	**0.060(+)**
Large			17.0	0.063(+)				30.6	**0.074(+)**	**0.056(+)**
Chub			47.9	**0.002(+)**	0.007(-)	**0.026(-)**		17.6	0.059(+)	
Small	21.3	0.035(-)	18.8		0.050(-)			14.6	0.087(+)	
Medium			64.5	**0.000(+)**	0.017(-)	**0.036(-)**		14.6	0.088(+)	
Large			46.6	**0.001(+)**	0.083(-)					
Pumpkinseed	24.5	0.022(+)						15.2	0.081(-)	
Mosquito fish			36.1	**0.027(-)**		**0.097(-)**				
Matrix of fish abundances										
Overall			28.4	**0.000**	0.036	**0.073**		11.2	**0.042**	

Table 3.4 Partitioning among the pure and combined effects of morphological (M), physicochemical (PC), and spatial (S) sets of variables, of explained variation in linear regression (richness and abundance) and abundance matrix (RDA) models for fish in pools in the Odelouca stream, during September 2005. P-levels for pure components are given in brackets.

	Pure components			Shared components				Unexplained
	Morphology	Physicochemistry	Spatial context	M + PC	M + S	PC + S	M + PC +S	
Species richness								
Overall	15.2 (0.080)		9.7 (ns)		18.0			57.1
Abundance								
Overall	30.5 (0.009)	4.4 (ns)		11.2				53.9
Nase		8.1 (ns)	4.2 (ns)			22.8		64.9
Small	10.0 (ns)	0.5 (ns)	6.4 (ns)	0.5	7.6	6.6	11.1	57.3
Medium		25.0 (0.075)	7.1 (ns)			26.4		41.5
Large		8.1 (ns)	21.7 (ns)			8.9		61.3
Chub		35.5 (0.019)	5.2 (ns)			12.4		46.9
Small	9.8 (ns)	4.7 (ns)	0.1 (ns)	1.3	4.3	1.6	8.7	69.5
Medium		51.4 (0.002)	1.5 (ns)			13.1		34.0
Pumpkinseed	13.3 (ns)		3.9 (ns)		11.2			71.6
Matrix of fish abundances								
Overall		23.5 (0.001)	6.4 (ns)			4.9		65.2

ns - not significant

3.4.3. Variation in assemblage structure

The redundancy analysis showed significant associations between assemblage structure and physicochemical and spatial sets of variables (Table 3.3, Figure 3.2). The first RDA axis (RDA1) of the physicochemical model was primarily correlated with riparian canopy, reflecting the tendency for catches of chub and arched-mouth nase increasing in well-shaded pools relative to pools with poorly developed canopies, where eel, pumpkinseed and small loach were more caught. Along the RDA2, increasing substrate coarseness was associated with decreasing catches. After controlling for the effects of spatial context, both variables were still significant, with patterns of assemblage variation remaining largely the same.

The spatial model reflected a gradient of distance from the stream mouth, contrasting downstream pools where barbel and pumpkinseed were more caught, with upstream pools where catches of chub and arched-mouth increased (Table 3, Figure 3.2). This spatial trend lost significance after accounting for the effects of physicochemical context.

Partial redundancy analysis indicated that the two sets of variables together accounted for 34.8% of variation in the fish species data matrix (Table 3.4). However, only the unique contribution of physicochemical variables was significant (23.5%). The proportion of variation shared among sets of variables was small (<5.0%).

3.5. DISCUSSION

3.5.1. General patterns

Assemblage structure of fish in dry-season stream pools has long been associated with pool morphology, physicochemistry, and spatial context, (see reviews in Matthews, 1998; Magoulick & Kobza, 2003), yet there have been few attempts to isolate the unique contribution of these different factors to variation in species richness and abundance. The variation partitioning approach adopted herein yielded explicit measures of such unique contributions, showing variation in species richness to be primarily associated with pool morphology, but relative species abundances to be mainly related to physicochemical contexts. Furthermore, this study highlighted considerable functional heterogeneity in the use of dry-season pools, with different species favouring pools with different sizes and physicochemical characteristics, and variable locations.

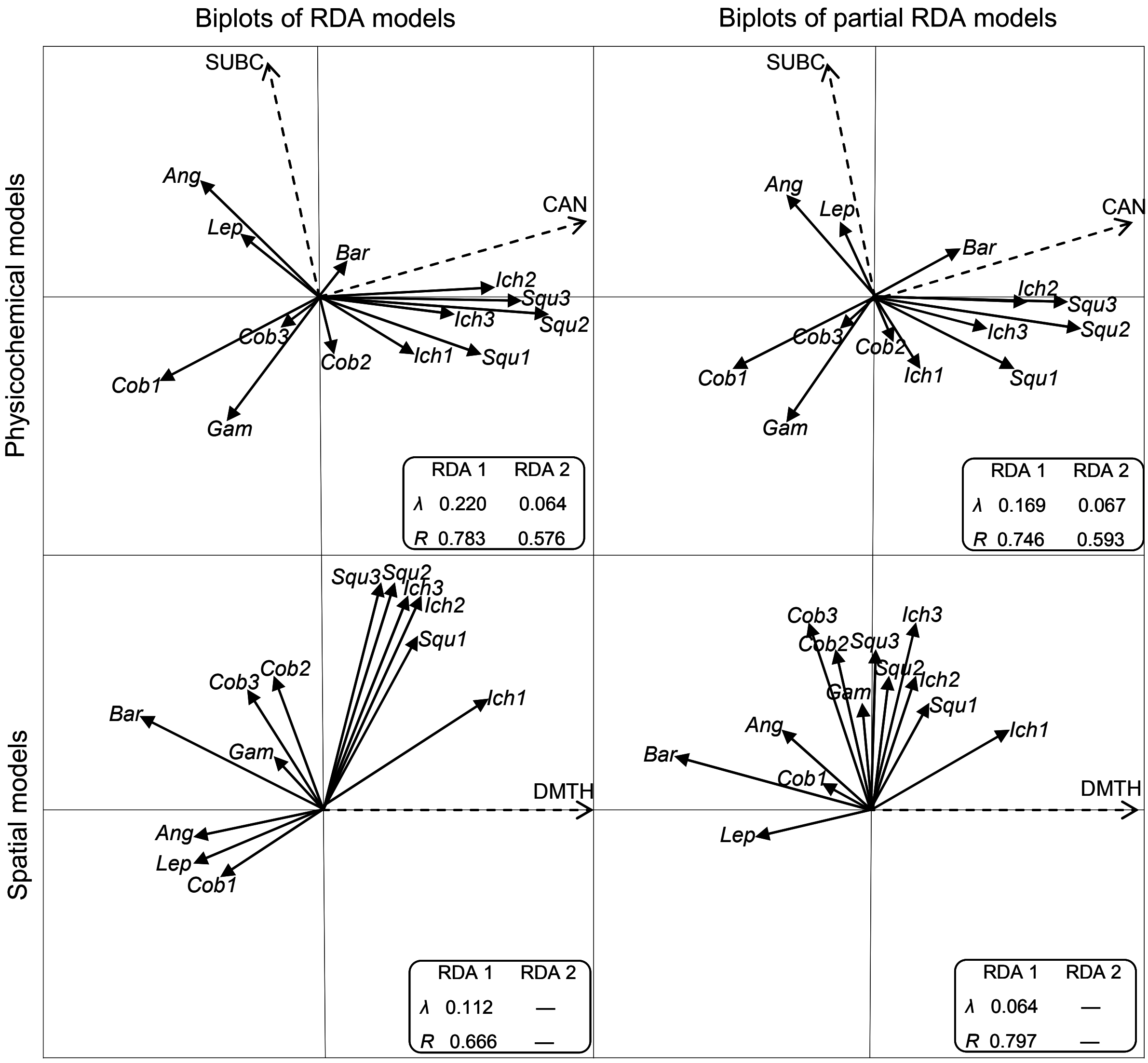

Figure 3.2 Results from redundancy analysis (RDA1/RDA2) depicting the effects on fish assemblage structure of sets of variables describing the morphology and spatial context of pools in the Odelouca stream during September 2005. Diagrams show the effects of each set of variables when considered alone (RDA models), and after partialling out the effects of the remaining two sets of variables (partial RDA models). Ordination axes were rescaled to range from –1.0 to 1.0. Dashed-line arrows are the explanatory variables and full-line arrows are the fish species. The eigenvalues (λ) and the species-environment correlations (R) are indicated for each canonical axis. Codes for explanatory variables and fish species are in Tables 3.1 and 3.2, respectively.

These general patterns are unlikely to be shaped by methodological artefacts or shortcomings. Although sampling locations have been constrained by the uneven distribution of pools and accessibilities throughout the landscape, biases resulting from this were probably small, because spatial turnover of species in streams in SW Portugal is generally low (Magalhães *et al.*, 2002a), making it unlikely that assemblage structure changes markedly among similar habitats. Also, previous investigation has shown that the sampling effort used at each pool was sufficient to record most species present and estimate their relative abundances (D.F. Pires & M.F. Magalhães, unpublished data), consistent with previous evidence that species poor assemblages, lacking abundant but discontinuous species, may require less effort to be characterized adequately than diverse assemblages where such species do occur (Angermeier & Smogor, 1995; Cao *et al.*, 2001). The potential effects of sampling conditions and worker's skills on capture efficiency of the electrofishing gear were controlled by sampling in wadeable pools, with the same field crew operating throughout the study. Electrofishing biases against small and less conspicuous fish were not likely to have affected the results much, because both small and inconspicuous fish (i.e. mosquito fish and loach) were well represented in samples taken in large pools, where under-sampling was potentially more serious (Zalewski & Cowx, 1990). Even if some bias still occurred for some species (see Bayley & Austen, 2002), they were probably systematic because electrofishing was not severely complicated by different biological contexts and environmental conditions, and thus among pools comparisons remain valid. Although failing to account for imperfect detectability was a limitation with potentially more serious consequences in estimating occurrence and relative abundance for rare species (e.g. MacKenzie & Kendall, 2002; MacKenzie *et al.*, 2002), there was a tendency for species enrichment and increased abundance of rare species (i.e. pumpkinseed and barbel, data not shown) in large pools, suggesting that the patterns observed were not an artefact of the sampling protocol. Finally, conducting the study in a single, dry year, despite the strong temporal variability of mediterranean streams, may have contributed primarily to an increase in the proportion of unexplained variation in the data. Spatial patterning of mediterranean stream fish assemblages tends to change with temporal rainfall variability (Magalhães *et al.* 2007; D.F. Pires & M.F. Magalhães, unpublished data), with associations with local habitats being weaker and less diverse in dry than in wet years (Mesquita *et al.*, 2006), in contrast to patterns found in other intermittent streams (Dekar & Magoulick, 2007). Possibly, results of the variation partitioning approach would have been stronger if average estimates of assemblage attributes over several years were available. Nevertheless, this snapshot study was worth to identify factors most likely to limit fish when drought conditions turn rigorous, though it may be short to evaluate the full set of factors accounting for assemblage dynamics.

3.5.2. Relative contributions of morphological, physicochemical and spatial factors to assemblage variation

The three sets of explanatory variables considered here explained relatively high proportions of the total variation in species richness (25.8 to 42.9%) and abundances (24.5 to 66.0%). These results are exceptional compared to what has been reported in earlier studies of mediterranean stream fish assemblages (e.g. Godinho *et al.*, 1997; Pires *et al.*, 1999; Pires *et al.*, 2004; Mesquita *et al.*, 2006; Morán-López *et al.*, 2006; Ferreira *et al.*, 2007). Nonetheless, there were still considerable amounts of unexplained variation, which might be associated with unmeasured local factors, landscape influences, biotic and stochastic processes, and sampling variability.

In variation partitioning, morphological and physicochemical factors had major unique contributions to variation in overall species richness and abundance, and relative species abundances, respectively. These results seemed to reflect some hierarchy in assemblage organization (see Gaston & Blackburn, 2000), with pool size influencing the set of species and total individuals in each pool but physicochemical conditions determining their relative abundances and actual assembly structure. Clearly, further information is needed to clarify the extent to which perceived patterns might be dependent on the number and ecological requirements of species, and geographic and climatic settings, given different mechanisms of assemblage organization may imply different conservation options.

Spatial context alone showed a rather small contribution to assemblage variation, contrary to what has been suggested in other studies (e.g. Taylor, 1997; Filipe *et al.*, 2002; Magalhães *et al.*, 2002a; Arthington *et al.*, 2005; Davey & Kelly, 2007). Conversely, the amount of shared contributions between spatial context and morphological and physicochemical factors, and between all three sets of factors was notable (Arthington *et* *al.*, 2010). Shared contributions could be due to different reasons, as for instance correlations among sets of explanatory variables (Legendre & Legendre, 1998). Specifically, spatially structured variation in pool size and riparian cover may have caused overlaps between spatial context and the morphological and physicochemical sets of variables, respectively. Another potential explanation to this overlapping, not necessarily exclusive, may be related to unmeasured external or biotic factors that covary in space with the measured variables (Legendre & Legendre, 1998). Whatever its ultimate causes, the large amount of shared contributions suggests that the relative importance of spatial context may have been insufficiently scrutinized and additional work is needed to detail the spatial conditions associated with assemblage variation.

3.5.3. Morphological, physicochemical and spatial correlates of assemblage variation

Species richness was generally highest in the largest pools, as found in many studies in intermittent streams (Capone & Kushlan, 1991; Taylor, 1997; Magalhães *et al.*, 2002b; Dekar & Magoulick, 2007). This may be due to large pools providing a most favourable habitat to pool specialists, as they usually show higher habitat diversity, and are the most likely to persist through the dry-season (Labbe & Fausch, 2000). This may explain the associations of pumpkinseed with large pools, given this species tend to favour lentic and deep habitats, respectively (Aparicio & de Sostoa, 1999; Moyle, 2002).

There was a tendency for overall abundance and that of natives declining in large pools. Negative associations between fish abundance and pool size may be regarded as expected (e.g.Capone & Kushlan, 1991; Balcombe *et al.*, 2006; Dekar & Magoulick, 2007), though positive relationships have also been found in intermittent streams (Magoulick, 2000). This negative trend seemed to reflect a shift in the size structure of assemblages, from relatively large abundances of small fish (i.e. small chub and arched-mouth) towards reduced abundances, of larger fish (i.e. pumpkinseed), similar to observations by Schlosser (1987) and Capone & Kushlan (1991). This may be a consequence of several factors, including local habitat resources and food availability, predation, and colonization and dispersal processes (Power, 1987; Schlosser, 1987). However, this trend can also simply indicate that fish got more congregated as pool size decreased (Dekar & Magoulick, 2007). Additional work is needed to investigate which of these alternatives, if any, underlined the perceived patterns in fish abundance.

Overall abundance was negatively related to substrate coarseness, through associations of mosquito fish and chub with fine substrates. However, positive associations with substrate coarseness and heterogeneity were found for eel and arched mouth, respectively. These results were in line with previous evidence implicating substrate in the structuring of mediterranean fish assemblages, and highlighting considerable variation among species in substrate use (Godinho *et al.*, 1997; Pires *et al.*, 1999; Mesquita *et al.*, 2006; Morán-López *et al.*, 2006; Ferreira *et al.*, 2007). Likewise, the relationships uncovered for chub, eel and mosquito fish seemed consistent with their known habitat requirements (Mesquita *et al.*, 2006; Santos & Ferreira, 2008), suggesting that fish may tend to concentrate in the pools with their most favourable substrate grounds.

Native species were strongly associated with riparian canopy, with well shaded pools holding more species, the highest relative abundances of chub and arched-mouth nase, and the lowest of small loach and eel. Relationships between native fish and riparian canopy have been frequently reported in mediterranean streams, likely reflecting particularly favourable sheltering and feeding conditions (e.g. Collares-Pereira *et al.*, 1995; Morán-López *et al.*, 2006; Ferreira *et al.*, 2007). This seemed also to be the case herein, as for instance, water temperature decreased in well shaded pools, probably resulting in generally benign environmental conditions for fish. Furthermore, inputs of terrestrial insects from well developed canopies (Baxter *et al.*, 2005), probably improved prey supply for at least some water-column insectivores, such as chub (Magalhães, 1993a; b). Likewise, canopy openness likely promoted primary productivity in little shaded pools (Schiller *et al.*, 2007), and this may have resulted in more suitable conditions for eel and loach, as found for other species elsewhere (Dekar & Magoulick, 2007).

Assemblage structure varied along the stream network, as relative abundances of barbel and pumpkinseed increased and those of chub and nase decreased downstream. These changes were consistent with longitudinal gradients found in other mediterranean streams (Godinho *et al.*, 1997; Pires *et al.*, 1999; Magalhães *et al.*, 2002b; Morán-López *et al.*, 2006), likely reflecting unmeasured spatially structured environmental influences, and other geographical or biotic factors independent of environmental conditions.

3.5.4. Implications for conservation

Taken together, results from this study highlighted that during severe droughts fish may refuge in pools with the most favourable morphological and physicochemical conditions, contingent on their location in the stream network, but independently on whether there are other pools nearby. Large pools, and pools with well developed canopies held the richest assemblages and the highest overall abundances of native species. However, they seemed not to cover the entire functional diversity associated with dry-season pools, as there was considerable among species variability in pool use. It appeared unlikely that species with stringent habitat requirements such as barbel would persist if no large pools were available, but there was some indication that small pools may still be needed to refuge small chub and nase. Likewise, pools with well developed canopies may be critical for native cyprinids, but pools with open canopies may be important in maintaining other native species, such as loach.

Although lack of data on fish movements and recolonization dynamics limits the inferences that may be drawn from the patterns recorded in this study, these results suggest that conservation efforts targeted at large pools, with well developed canopies are particularly important, especially considering that these pools may be under-represented and highly susceptible to human impacts during more severe droughts expected under altered future climates. However, there appears also to be a need for incorporating other pools in conservation strategies, to guarantee the long-term persistence of diverse native fish fauna in mediterranean streams. Specifically, the presence of networks of heterogeneous pools, representative of the entire gradients in pool size and physicochemical conditions might be essential to provide adequate conditions for a complement of species with different habitat requirements. Future research should thus focus on the way patterns of use of dry-season pools translate into population and community dynamics at the landscape scale, which is critical for predicting the number and spatial configuration of pools to be included in such networks, to maximize its effectiveness for biodiversity conservation.

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Chapter 4

MOVEMENTS OF THE ENDANGERED CHUB *Squalius torgalensis* IN A MEDITERRANEAN STREAM: DRY- AND WET-SEASON PATTERNS

4. MOVEMENTS OF THE ENDANGERED CHUB *Squalius torgalensis* IN A MEDITERRANEAN STREAM: DRY- AND WET-SEASON PATTERNS

4.1. INTRODUCTION

Movement is a key mechanism underlying important ecological processes, including habitat use (Bélanger & Rodríguez, 2002), population dynamics (McMahon & Matter, 2006) and predator-prey interactions (Gilliam & Fraser, 2001; Fraser *et al.*, 2006). In highly dynamic and spatially complex stream environments, movement links fish to habitats over a broad range of spatial scales. Short-scale movements within the home range, allow fish to track changes in local habitat conditions (Albanese *et al.*, 2004), food resources (Gowan & Fausch, 2002; Gowan, 2007) and predation risk (Roberts & Angermeier, 2007a). Large-scale movements allow the use of specific habitats by different life-history stages (Schtickzelle & Quinn, 2007), the colonization of unoccupied habitats (Adams & Warren, 2005; Roghair & Dolloff, 2005), and individual and genetic flow among spatially separated populations (Schlosser & Angermeier, 1995; Henriques *et al.*, 2010). Therefore solid knowledge of movement is critical to improve understanding of stream fish ecology and inform conservation efforts for endangered fish.

The view conceptualized by Gerking (1959) and later referred by Gowan *et al.* (1994) as the Restricted Movement Paradigm (RMP) has long been considered the norm in stream fish movement. This view maintains that adult fish are sedentary, often spending their entire life cycle within short stream reaches (20-50 m). However, this has been increasingly contested, and claimed to result from sampling bias favouring detection of short movements (Gowan *et al.*, 1994). A growing body of evidence points to a high degree of variability on movement patterns by stream fish (e.g. Skalski & Gilliam, 2000; Albanese *et al.*, 2004; Roberts & Angermeier, 2007a; Breen *et al.*, 2009) with some species being largely sedentary (Aparicio & de Sostoa, 1999; Petty & Grossman, 2004), whereas other have more heterogeneous movement patterns, with some individuals moving long distances (Smithson & Johnston, 1999; Skalski & Gilliam, 2000; Rodriguez, 2002; Roberts *et al.*, 2008; Breen *et al.*, 2009). Variability in movement patterns appears to result from differences in fish ecology (Albanese *et al.*, 2004; Roberts & Angermeier, 2007a), local habitat suitability (Roberts & Angermeier, 2007a), and reach-scale factors, such as predator distribution (Gilliam & Fraser, 2001; Roberts & Angermeier, 2007a), corridor complexity (Gilliam & Fraser, 2001; Roberts & Angermeier, 2007b), habitat isolation (Roberts & Angermeier, 2007a), and distance to main stem (Albanese *et al.*, 2004). Moreover, fish may change movement behaviour over its life-cycle, dependent on environmental stimulus (Gowan & Fausch, 1996; Skalski & Gilliam, 2000; Fausch *et al.*, 2002; Rodriguez, 2002; Albanese *et al.*, 2004; Roberts & Angermeier, 2007b), morphological and physiological conditions (Gowan & Fausch, 1996; Albanese *et al.*, 2004; Roberts & Angermeier, 2007a), and boldness in some systems (Fraser *et al.*, 2001). Consequently, it is difficult to infer general patterns of movement by stream fish, and more research is needed on different species and streams to advance understanding of movement causes and consequences.

Temporary streams experience recurrent droughts that disrupt hydrological connectivity and reduce stream habitat to a few persistent refugia (Gasith & Resh, 1999; Magalhães *et al.*, 2002; Meyer *et al.*, 2007; Larned *et al.*, 2010). Fish that survive drought in dry-season refugia may redistribute after flow resumption, colonizing previously dewatered habitats along the stream network (Gasith & Resh, 1999; Magoulick & Kobza, 2003; Wigington *et al.*, 2006). In these circumstances, movement is likely to play a critical role in fish population dynamics and persistence, though few studies have addressed this issue (Aparicio & de Sostoa, 1999; Labbe & Fausch, 2000; Albanese *et al.*, 2004). Nevertheless, information on fish movement patterns in temporary streams is critical to clarify ecological links between persistent and ephemeral habitats, establish spatial boundaries of populations, and define appropriate scales for ecological research and conservation management (Gowan *et al.*, 1994; Fausch *et al.*, 2002).

In this study we addressed the movements of chub *Squalius torgalensis* Coelho, Bogutskaya, Rodrigues & Collares-Pereira, a highly endangered cyprinid, endemic to seasonally drying mediterranean streams, in SW Portugal. Our objectives were to quantify the rate, directionality, and extension of movements out of dry-season refugia, and over the wet-season, and to identify factors that may influence these attributes. Results were then used to explore the spatial dynamics of fish populations in temporary streams.

4.2. STUDY AREA AND FOCAL SPECIES

The study was carried out in the Torgal stream, in Southwest Portugal (Figure 4.1), which is a typical mediterranean stream in its flow regime and fish fauna. The Torgal basin and its fish assemblages were previously described in detail in Chapter 2, so that only features relevant to the present investigation are given here. The stream is forested, are there are well developed riparian galleries. Human settlement is sparse, and there are no major human impacts such as impoundments, urban pollution, angling or other recreational activities. Climate is mediterranean, with annual rainfall varying markedly from year-to-year (238–1121 mm), but following a predictable seasonal pattern. About 80% of annual rainfall occurs in the wet-season (October to March) and only about 3.0% in the hot, dry-season (June to August). Mean monthly temperature is 11 ºC in the coldest month (January) and 23 ºC in the hottest (August). The flow regime is highly dependent on rainfall patterns. Flow generally stops between July and September, though in dry years the drying period may be extended. Dry-season habitat for fish largely restricts to isolated pools in the mainstream and aquifer fed runs in the tributaries, with hydrological connectivity persisting only in an intermediate section of the mainstream. During wet years, major floods may occur between October and April, and flows may persist through the dry-season in downstream reaches.

Fish assemblages include a reduced pool of native species, dominated by chub *Squalius torgalensis*. Nase *Chondrostoma almacai,* eel *Anguilla anguilla* and loach *Cobitis paludica* are also abundant, whereas barbel *Luciobarbus sclateri* Gunther and stickleback

Gasterosteus gymnurus occur sparsely and at low numbers. Non-native species are relatively rare and include mosquito fish *Gambusia holbrooki*, pumpkinseed *Lepomis gibbosus* and largemouth bass *Micropterus salmoides*.

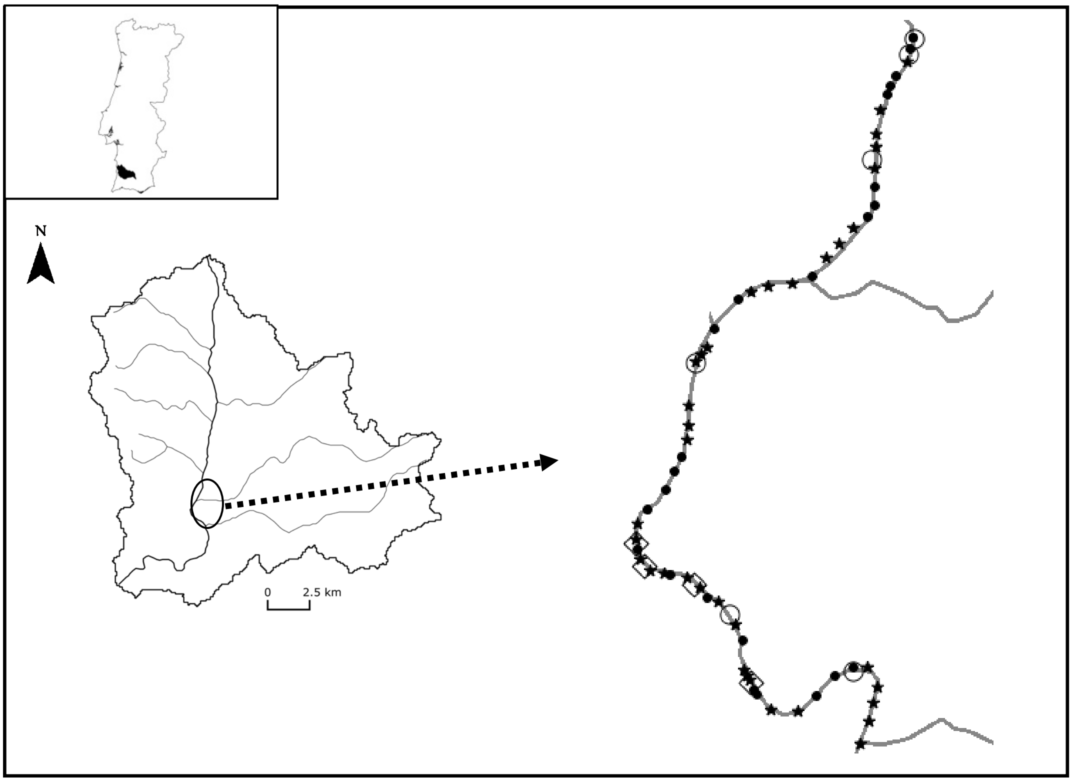

Figure 4.1 Maps of the Torgal catchment, study segment, and marking and recapture sites. Open squares represent dry-season pools that generated recaptures and open circles dry-season pools that did not generated recaptures. Black stars indicate wet-season marking sites that generated recaptures and black circles those that did not generated recaptures

We selected chub as our focal species based on the likelihood that it would provide sufficient sample sizes for analyses, its suitability for marking (see Appendix I), and its ability to represent ecological traits of Mediterranean stream fish. In brief, chub are widespread and abundant throughout the entire study area, occupying a range of habitats throughout the life cycle, but generally favouring moderate flows (Magalhães *et al.*, 2002; see also Chapter 5). Chub are small sized (< 150 mm, FL), breed in spring, mature in 1-2 years, and live to a maximum age of 6 years (Magalhães *et al.*, 2003). Moreover, chub are highly constrained by habitat loss and degradation, and currently listed as Critically Endangered in the Portuguese Red Data Book (Rogado *et al.*, 2005).

4.3. METHODS

4.3.1. *Study design*

We used mark-recapture techniques to quantify chub movements, between July 2007 and July 2008, across a 3.6 km intermittent segment in the Torgal mainstream (Figure 4.1). During the wet-season this segment comprised a complex mosaic of riffles, runs, and pools of varying size, but long stream sections dried by late summer confining fish to a few, persistent pools.

The first marking bout was carried out in July and September 2007, in persistent, wadeable pools (mean depth < 1.0 m), to allow efficient sampling. Seven pools were sampled in both July and September 2007, and 3 additional pools were sampled in September 2007. Recapture sampling was carried out after flow resumption, in five occasions: December 2007, February, April, June and July 2008. We defined 60 recapture sites, 15 m long, spread across the study segment, at least 20 meters apart from each other. Recapture sites were selected to cover the range of habitat diversity, and included 26 sites that persisted in September 2007, 18 of which in the marking pools. In July 2008 the study segment was partially dry, and sampling restricted to 37 sites only. Secondary marking bouts were conducted in December 2007 and February 2008 in all recapture sites, aiming to quantify movements over the wet-season.

We sampled fish using a standard electrofishing gear (Hans Grassl EL62 IIGI), fitted with a single 30 cm anode and discharging 300 V, 4-6 A, pulsed D.C., and there were always two netters present to pick up stunned fish. Electrofishing was generally conducted by wadding upstream, and covering the entire wet area, except in sites deeper than 1.0m (N=11) where fishing during the wet-season restricted to the area reachable from the banks. Single-pass electrofishing was carried throughout the study, except in 7 marking pools where multiple passes were conducted in July and September 2007 to increase sample sizes.

During each marking event, we measured chub for fork length (FL), and adult fish (>60 mm) showing no tag were marked with Passive Integrated Transponders (PIT) tags (Trovan ID-100A, 2.12x11.5 mm). PIT tags allowed for individual recognition, and exhibited high retention (80%), and low mortality effects (8%) on adult chub in laboratorial trials (see Appendix I). Fish were anaesthetised with clove oil, and PIT tags were inserted into its body cavity through an incision made just off the midventral line of the body, anterior to the vent, using a sharpened needle. Incisions were not sutured but clinical grade cyanocaricle adhesive (3M™ Vetbond) was applied to close the wound. Tagged fish were allowed to recover in an aerated vat until they regained balance and swimming, and then returned to the stream.

We logged the marking and recapture locations of fish with a Magellan 200 Xl, GPS (Global Positioning System) receiver (MiTAC International Corporation, Taiwan,China), and plugged these in a Geographic Information System (GIS; ArcView 3.1 for Windows, Environmental Systems Research Institute Redlands, CA,USA).

4.3.2. Data analysis

Analysis focused on evaluating the probability, distance, and directionality of movements from dry-season refugia and over the wet-season, and identifying factors that may influence these attributes. Except were indicated otherwise, analyses were conducted using first recapture data and the statistical package Statistica 10 (StatSoft, 2011). Significance of statistical testing was assessed at $P<0.05$.

For analysis, fish recaptured in their original marking site or adjacent sites within their marking pool were classified as stayers, and attributed a movement distance of 0. Fish recaptured beyond marking or adjacent sites were assigned as movers, and attributed the distance along the stream network between the midpoints of mark and recapture sites. Fish shifting from movers to stayers or vice-versa between recaptures were classified as alternates. Movements towards downstream were arbitrarily classified as negative and those directed upstream as positive.

Because mark-recapture methods generally generate movement data biased downward (Gowan *et al.*, 1994; Koenig *et al.*, 1996), we evaluated the degree of distance-weighting in our dry- and wet-season marking designs following Albanese *et al.* (2003). For each design, we determined the proportion of total possible movements sampled, using 100 m increments to avoid generating too narrow or too broad distributions. First, we calculated the number of times that each distance was sampled for each marking site, weighted by the total number of individuals marked in that site. Summing across sites thus provided the total number of times each distance was sampled. Secondly, we calculated the number of times each distance was possible by counting the times each distance class was sampled and not sampled for each marking site, weighted by the number of individuals marked. Summing across sites provided the total number of times that each distance was possible. The proportion of total possible movements sampled for each distance was finally obtained by dividing the number of times each distance class was sampled by the number of times each distance was possible. We then tested the null hypothesis that movement distance distributions were not different from those expected by chance, assuming that if each movement distance had an equal likelihood of being observed, the distributions of observed and total possible movement distances would be similar (Roberts & Angermeier, 2007a; Roberts *et al.*, 2008). Comparisons between distributions were made using pair-wise non parametric randomization t tests in the program Resampling Procedures (Howel, 2001). At each of 100, 000 randomizations, distance observations were randomly assigned to groups, group means were compared, and the resultant t-value was compared with an ordinary critical t-value. The proportion of times the randomized t exceeded the critical t equalled the probability that there was no effect of group on movement distance. To account for eventual variations in movement direction, comparisons were made between null and upstream movement, null and downstream movement, and null and unsigned movement (i.e. ignoring direction) data.

We analyzed the associations between the probability of movement and distance moved by fish and four explanatory variables: i) the location of marking site, derived as the distance along the stream network to the downstream edge of the study segment, ii) the marking effort, quantified as the total number of individuals marked in each site, iii) the mark-recapture time lag (days), and iv) the size (FL, mm) of fish at marking. We selected these variables for analysis because they have been generally considered to affect movement by stream fish (Albanese *et al.*, 2003; Albanese *et al.*, 2004; Hudy & Shiflet, 2009). Prior to analysis, explanatory variables were log transformed to reduce skewness and effects of extreme values. As there was no evidence of strong pairwise correlations (Pearson's correlation $|R| < 0.50$) among explanatory variables, they were all retained for analysis.

We used Generalized Linear Models (Dobson, 2002) with binomial distribution and logit link function to evaluate the influence of explanatory variables on the probability of fish moving beyond marking sites. Relationships between distance moved and explanatory variables were assessed using Linear Regression (Quinn & Keough, 2002).

We evaluated the relationships among dependent and explanatory variables based on the Information Theoretic Approach (Burnham & Anderson, 2002), using the set of models obtained from all possible combinations of explanatory variables. The ITA looks for simplicity, parsimony and informative proprieties of several working hypotheses and it is based on the strength of evidence of each candidate predictive model. Loss of information of each candidate model was measured through the Akaike Information Criteria, with the best informative model having lower AIC values. Absolute differences (Δi) between each model and the best model AIC were used to rank candidate models. Because the number of parameters was large relative to the sample sizes available, the Akaike Information Criteria adjusted for small samples (AICc) was used. Strength of evidence of each model was evaluated by Akaike weights (Wi), which measure the posterior probability that a given model is true, given the data and the entire set of competing candidate models (Burnham & Anderson, 2002).

We used Multi-model inference (MMI) to assess the relative importance of each variable, within the set of explanatory variables considered. The probability that a variable is included in the best approximating models, was estimated by summing the Wi of all candidate models were the variable was included (Burnham & Anderson, 2002). Additionally, effects of explanatory variables were assessed by calculating averaged coefficients and confidence intervals weighted by Akaike weights of models having strong support ($\Delta i < 2$) (Burnham & Anderson, 2002). Modelling was performed in the R 2.12.0 software (R Development Core Team, 2010) using glm for and lm function from stats package, respectively for GLM an LR analyses, and functions from MuMIn package (Barton, 2010) for ITA model ranking and MMI.

Multiple recaptures were used to evaluate plasticity in movement behaviour, by comparing the observed numbers of fish that maintained and that changed behaviour between recaptures to those expected by chance, under equal probabilities, using one-way χ^2 tests (Zar, 1999).

Preference in movement directionality was tested using also one-way χ^2 tests, comparing the observed numbers of individuals that moved upstream and downstream with those expected under equal probability for both directions. The Mann-Whitney U test (Zar, 1999) was used to assess differences between distances moved up and downstream.

4.4. RESULTS

We marked 1138 chub, 60-132 mm, during the dry-season and 521 chub, 63-143 mm, during the wet-season (Table 4.1). However, recapture rates were lower for chub marked in the dry-season than for those marked in the wet-season (2.9% vs. 13.0%).

The recaptures from the dry-season marking were from 4 pools located downstream in the study segment (Figure 4.1, Table 4.2), where 72 % of marking effort was deployed. The recaptures from the wet-season marking were from 37 sites spread across the entire segment, where 79 % of marking effort was applied. Double recapture rates were higher for chub marked during the dry- than the wet-season (21% *vs.* 13%, respectively). Conversely, triple recaptures were only seldom observed (3%) in the wet-season. Recapture rates were the highest in the first sampling following marking, for both fish marked during the dry- (47% vs. 0-26%) and the wet-season (78% vs. 0-21%).

Average time lag between marking and recapture was higher in the dry-season (130.6 days) than in the wet-season (81 days) design.

Table 4.1 Mark-recapture results for chub sampled in the Torgal stream, between July 2007 and July 2008. Mean, standard deviation (SD), and range (minimum, maximum) of distance moved (m) were estimated only from individuals that moved.

			Dry-season	Wet-season
Number of marked fish			1138	521
Number of recaptures (%)			34 (2.9)	68 (13.0)
Number of movers (%)			18 (53.0)	14 (21.0)
Distance Moved	Upstream			
		Mean±SD	336.0 ±281.7	313.2 ±484.1
		Range	26.7 -932.7	42.7 -1463.2
	Downstream			
		Mean±SD	167.0 ±130.2	45.2 ±24.6
		Range	55.5 -379.1	22.0 -85.7
	Overall			
		Mean±SD	260.9 ±237.6	198.3 ±381.3
		Range	26.7 -932.7	22.0 -1463.2

The average size of recaptured chub was similar in the dry- and wet-season designs (86.1 mm and 86.5 mm, respectively).

Although our sampling designs were unbalanced, we had high probabilities (>0.70) of detection from -1200 to 2300 meters in the dry-season design, and from -1400 to 1600 meters in the wet-season design (Figure 4.2).

Table 4.2 Location of marking sites (distance downstream, m), marking effort at each marking site (number of marked fish), mark-recapture time lag (days), and size at marking (FL, mm) of chub recaptured in the Torgal stream between December 2007 and July 2008. Values for each variable are the mean (± SD) and range (minimum, maximum). Numbers and proportions of mobile and sedentary individuals are in Table 4.1

	Dry-season		Wet-season	
	Movers	Stayers	Movers	Stayers
Location of marking sites				
Mean±SD	1255.3 ±176.5	1061.4 ±271.5	1842.9 ±989.2	1680.6 ±1059.5
Range	775.5 -1454.6	775.5 -1454.6	566.5 -3307.5	12.5 -3449.5
Marking effort				
Mean±SD	263.7 ±135.9	293.6 ±92.2	7.9 ±3.6	10.9 ±5.7
Range	58.0 -392.0	58.0 -392.0	3.0 -15.0	3.0 -25.0
Mark-Recapture Time Lag				
Mean±SD	134.8 ±61.4	125.8 ± 64.9	88 ±35.4	79.2 ±30.7
Range	68.0 -255.0	67.0 -255.0	57.0 -185.0	57.0 -187.0
Fish size				
Mean±SD	89.2 ±13.8	82.7 ± 8.6	95.6 ±19.9	84.2 ±8.2
Range	70.0 -125.0	68.0 -97.0	75.0 -134.0	66.0 -102.0

4.4.1. Movement behaviour

The proportion of movers was much higher among fish marked in the dry- than in the wet-season (53% *vs.* 21%, respectively, Table 4.1).

The low recapture sizes precluded the analysis of movement behaviour plasticity in each marking season. Overall, the majority of fish maintained behaviour between the first and second recapture, though this proportion was similar to what would be expected by chance (χ^2=3.556, df= 1, P=0.059).

Multi-model inference indicated that the probability of fish marked in the dry-season moving out of pools was moderately associated with pool location, increasing upstream (Table 4.3). Conversely, fish size was signed as having a strongly supported influence on the probability of movement of fish marked in the wet-season, with larger fish being the most likely to move.

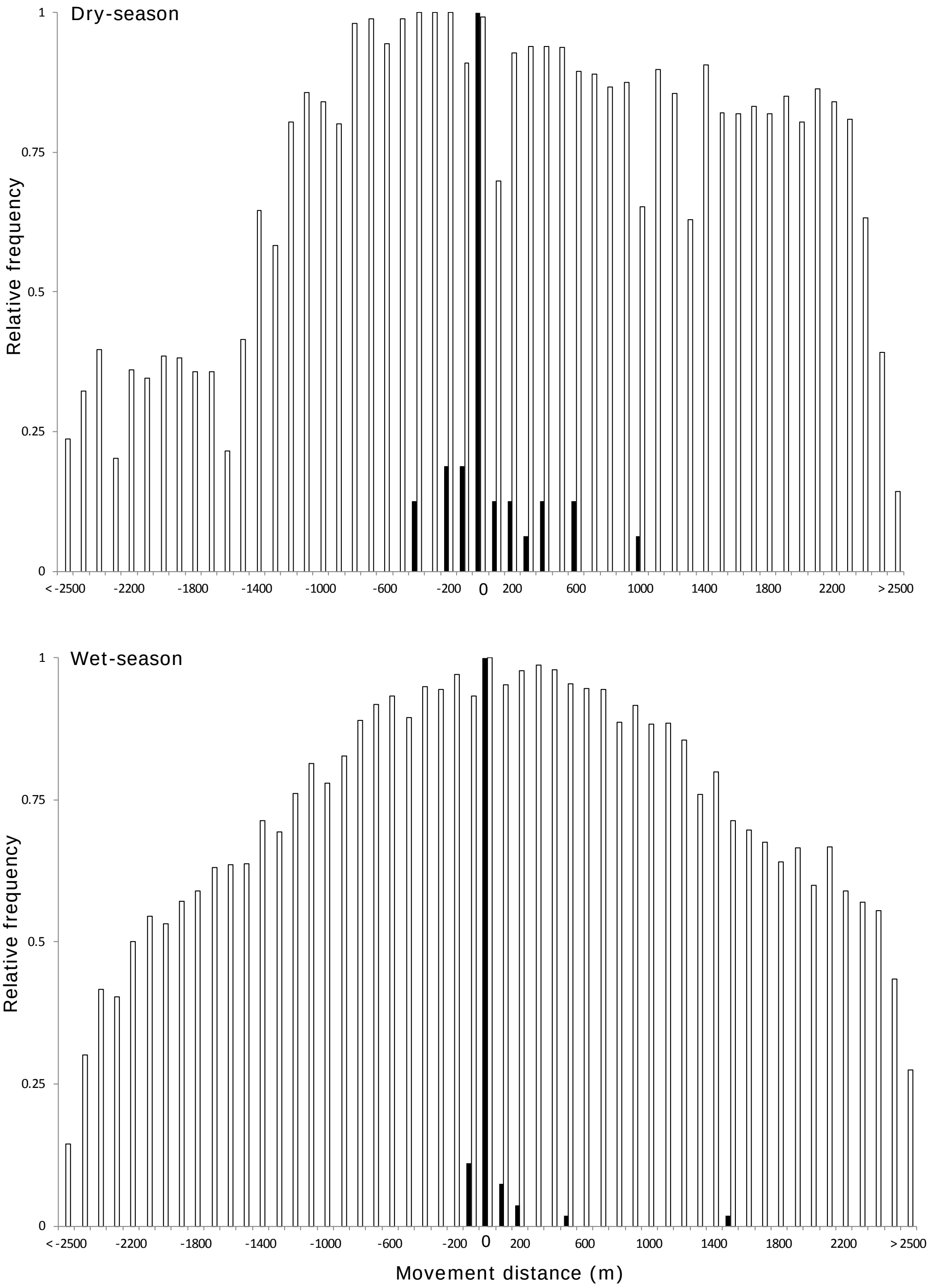

Figure 4.2 Relative frequency of possible movements sampled (white bars) and observed movements (black bars) for each distance class, in the dry- and wet-season mark-recapture designs. Values for distances longer than 2500 m are averaged frequencies. Upstream and downstream movements were arbitrarily assigned as positive and negative, respectively.

Table 4.3 Summary results from information theoretic multi-model inference for the relationships between probability of movement by chub and explanatory variables in each marking season. Range of Akaike weights of strongly supported models (ΔAICi <2) is provided. For variables having moderate (selection probability > 0.7; underlined) and strong support (selection probability >0.9; in bold) the averaged coefficient estimates ($\bar{\boldsymbol{\beta}}$) and Lower (LCI) and Upper (UCI) 95% Confidence Limits are showed.

		Explanatory Variables							
		Location of marking site		Marking effort		Mark-Recapture Time Lag		Fish Size	
	Wi range of Δ AIC <2 models	Wi	$\bar{\beta}$ (LCI; UCI)	Wi	$\bar{\beta}$ (LCI; UCI)	Wi	$\bar{\beta}$ (LCI; UCI)	Wi	$\bar{\beta}$ (LCI; UCI)
Dry-Season	0.21-0.24	0.82	9.57 (0.69; 18.4)	0.31	- -	0.25	- -	0.50	- -
Wet-season	0.10-0.23	0.46	- -	0.35	- -	0.28	- -	**0.92**	14.1 (2.89; 25.4)

4.4.2. *Extension and direction of movements*

Chub marked in dry-season pools moved to both persistent (7) and ephemeral (11) habitats, as distant as 371.1 m and 932.7 m, respectively. Chub marked in the wet-season moved more often to ephemeral (10) than to persistent habitats (4) as far as 1463.2 m and 460.2 m respectively.

Overall, displacements from dry-season pools tended to be longer than wet-season movements (Table 4.1), with displacements shorter than 100 m being occasional in the dry-season (28%, , Figure 4.2) but the most common in the wet-season (71%). However, the maximum distance moved was higher in the wet- than in the dry-season (1463.2 m *vs.* 932.7 m, respectively, Table 4.1).

There was no preference in the direction of movements by fish marked in the dry- (χ^2=0.222, df=1, P=0.637) and the wet-season (χ^2=0.285, df=1, P=0.593) Also, the distance moved up and downstream was not significantly different for chub marked in the dry-season (336.0 m *vs.* 167.0 m; U = 22.000, n_1=10, n_2=8, P=0.119), but those marked in the wet-season tended to move longer distances upstream (313.2 m *vs.* 45.2 m; U =

7.500, n_1=8, n_2=6, P=0.038). However, graphical analysis of the data showed that two distance records were clearly detached from the others (>260 meters) taken in the wet-season, and both were in the upstream direction. Excluding these records, there were no significant differences in the distances moved up and downstream (U = 7.500, n_1=6, n_2=6, P=0.109).

Accounting for differences in detectability, observed signed distances moved were shorter than expected from random, for fish marked in both the dry- (upstream: t=10.652, n=35, P<0.0001; downstream: t=9.860, n=35, P<0.0001;) and the wet-season (upstream: t=12.977, n=35, P<0.0001; downstream: t=10.235, n=35, P<0.0001). Similarly, unsigned distances moved were shorter than expected in the dry- (t= 11.415, n=35, P<0.0001) and wet-season (t= 11.622, n=35, P<0.0001).

Multi-model inference indicated that time lag between marking and recapture had a moderate, negative effect on the distance moved by fish marked in the dry-season (Wi=0.86). However, graphical analysis of the data showed that this effect was mainly caused by four records over 60 days, which were associated with small distances moved. If these records were excluded from the analysis the negative trend loosed support, but there was no effect on the remaining relationships. According to multi-model inference none of the explanatory variables had unequivocal support in influencing movement during the wet-season (Wi <0.53).

4.5. DISCUSSION

Our results underlined considerable variability between seasons in the movement patterns of chub, with many recaptured fish moving out of dry-season pools but the majority of fish marked in the wet-season remaining in the marking sites. Also, we found that only a small proportion of fish changed movement behaviour through time. Overall, our findings corroborate other studies on small stream fish highlighting the incompleteness of the Restricted Movement Paradigm (Smithson & Johnston, 1999; Rodriguez, 2002; Knaepkens *et al.*, 2005; Roberts *et al.*, 2008; Breen *et al.*, 2009; Hudy & Shiflet, 2009), and adds to increasing evidence pointing to temporal plasticity in movement behaviour (Fausch *et al.*, 2002; Belica & Rahel, 2008; Alldredge *et al.*, 2011) and individual variation in movement propensity (Skalski & Gilliam, 2000; Fraser *et al.*, 2001; Rodriguez, 2002; Petty & Grossman, 2004).

4.5.1. Study bias and limitations

Our mark-recapture designs assured high detection probabilities up to 2300 m away of the marking sites, allowing the detection of long movements irrespective of direction in both seasons. Nevertheless, there were still some minor methodological limitations that could not be avoided. Mobile fish could have moved to unsampled areas within or outside the study segment biasing estimates of the proportion of movers and distance moved downward (Gowan *et al.*, 1994; Gowan & Fausch, 1996; Skalski & Gilliam, 2000; Albanese *et al.*, 2003).

We also obtained low recapture rates which may be at least partially associated with tag loss and fish mortality (Gowan *et al.*, 1994; Smithson & Johnston, 1999; Roberts & Angermeier, 2007a). Although PIT tag loss and mortality registered for adult chub in laboratorial experiments were low (see Appendix I), these are likely to increase in the wild. However, we do not expect tag retention and mortality to differ between movers and stayers and between seasons, significantly affecting the perceived movement patterns.

4.5.2. Movement patterns

The proportion of chub moving out of dry-season pools was much higher than that of movers previously recorded for other small-sized stream fish inhabiting temperate streams (Knaepkens *et al.*, 2005; Roberts & Angermeier, 2007a; Roberts *et al.*, 2008; Breen *et al.*, 2009; Hudy & Shiflet, 2009). Only during the wet-season a large proportion of individuals were sedentary suggesting that at least some chub may use relatively small river stretches during this season. Although distances moved were generally shorter than expected from random, most displacements of fish marked in the dry-season were greater than 100m, thus over-passing the movement range usually associated with the RMP (Gerking, 1959). This was not the case in the wet-season, when distances moved were mostly within the range predicted by the RMP, though some long displacements were also detected. Taken together these results highlight that chub may display considerable variability in movement behaviour and displacement extent, in contrast with the RMP (Gerking, 1959), and reinforce previous evidence indicating that movements from dry-season refugia may be critically important in shaping the distribution and dynamics of fish in temporary streams (Bravo *et al.*, 2001; Medeiros & Maltchik, 2001).

The variety of displacements exhibited by chub is likely to reflect different ecological processes. The majority of the small movements detected are likely to reflect tracking of environmental conditions in proximate habitats, as previously reported (Gowan & Fausch, 2002; Albanese *et al.*, 2004; Gowan, 2007). However, small movements out of dry-season pools may also contribute to counteract local isolation and promote the mixing of neighbour individuals that were spatially separated during drought. In complement, longer movements from dry-season pools may allow for colonization of distant and previously dewatered habitats, thus promoting the flow of individuals across the riverscape. Even if less common than in the dry-season, this flux may also continue throughout the wet- season with at least some individuals moving long distances.

Multiple recaptures indicated that only a small part of fish changed the movement behaviour across recapture events. Nevertheless, full evaluation of the extent to which individual fish may change movement behaviour in response to stream conditions and resources variation as suggested elsewhere (Smithson & Johnston, 1999; Fausch *et al.*, 2002; Belica & Rahel, 2008; Alldredge *et al.*, 2011), will probably require a close tracking of individuals at smaller temporal scales than the one used in the present study.

4.5.3. Factors influencing movement

Correlates of movement behaviour changed between seasons, with pool location and fish size appearing the most influential factors in shaping whether fish remained in or moved out of dry-season pools and wet-season marking sites, respectively. Fish marked in pools located upstream in the study segment were the most likely to move, probably reflecting differences in habitat quality across the stream network as suggested by Albanese *et al.* (2004). Dry-season pools in mediterranean streams are highly heterogeneous in local conditions and corridor contexts (Magalhães *et al.*, 2002; see Chapter 3), and this may significantly influence movement patterns of fish as found elsewhere (Gilliam & Fraser, 2001; Albanese *et al.*, 2004; Breen *et al.*, 2009; Alldredge *et al.*, 2011).

Large chub were the most likely to move during the wet-season as found for other stream fish (Skalski & Gilliam, 2000; Gilliam & Fraser, 2001; Albanese *et al.*, 2004; Roberts & Angermeier, 2007a). Although processes underlying this pattern are still controversial, possible explanations include the superiority of large relative to small fish in searching and settling in foraging sites (Gowan & Fausch, 2002) and in mobility related capabilities (Jones *et al.*, 1974; Lonzarich *et al.*, 1998; Aedo *et al.*, 2009).

Correlates of distance moved by chub remain uncertain, and should be the subject of further evaluation.

4.6. CONCLUDING REMARKS

Our results highlight that movement may be a key mechanism for fish in temporary streams, promoting redistribution of individuals stranded in dry-season pools, and colonization of ephemeral habitats at a variety of distances, and favouring the flux of individuals across the stream network over the annual cycle. Despite the majority of recorded movements were of short distance, some individuals displayed long displacements both in the dry- and wet-seasons, counteracting seasonal isolation at the segment scale. These long displacements must be viewed as key components of population processes (Rodriguez, 2002), stressing the importance of maintaining habitat connectivity across several spatial scales in temporary streams.

Progress in fish ecology in temporary streams will greatly benefit from further understanding of the factors promoting individual and temporal variability in movement patterns. Extending movement studies to young life-stages will be critical to achieve a complete view of population movement patterns. Future research should aim at (i) tracking individual movement over short time frames, and defining home ranges and extent of explorative movements; (ii) quantifying individual flux and colonization across a broad range of spatial and temporal scales; iii) identifying other biotic and abiotic correlates of fish movement, and (iv) encompassing multiple life-stages and species, and different intermittency contexts.

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Chapter 5

FISH ASSEMBLAGE VARIATION ACROSS A MEDITERRANEAN STREAM: THE ROLE OF HABITAT QUALITY, CONNECTIVITY TO REFUGIA, AND NEIGHBOURHOOD EFFECTS

5. FISH ASSEMBLAGE VARIATION ACROSS A MEDITERRANEAN STREAM: THE ROLE OF HABITAT QUALITY, CONNECTIVITY TO REFUGIA, AND NEIGHBOURHOOD EFFECTS

5.1. INTRODUCTION

Streams are highly heterogeneous and dynamic systems, including a shifting and complex mosaic of habitats, dependent on processes that operate over a broad range of spatial and temporal scales (Ward *et al.*, 2002; Wiens, 2002). Besides depending on local habitat quality (e.g. Matthews & Hill, 1979; Taylor *et al.*, 1993; Grossman & de Sostoa, 1994; Fischer & Paukert, 2008), stream fish often show higher-scale habitat relationships (Fausch *et al.*, 2002; Wiens, 2002), as they may require multiple habitats to complete their life cycles, which are often dispersed throughout the riverscape (Schlosser, 1991; Schlosser & Angermeier, 1995). Therefore, processes such as neighbourhood effects, the quality, arrangement and boundaries of surrounding habitats, and connectivity between habitats, may also play a critical role in population persistence (Schlosser, 1995; Fausch *et al.*, 2002; Fullerton *et al.*, 2010). However, knowledge of the effects of spatial and temporal variation in habitat quality and connectivity on the distribution and abundance of fish across the riverscape remains general (Fausch *et al.*, 2002; Fullerton *et al.*, 2010).

Droughts are increasingly frequent events worldwide (Huntington, 2006; Kundzewicz *et al.*, 2008), and may have significant impacts on stream habitat and fish dynamics (Humphries & Baldwin, 2003; Magoulick & Kobza, 2003; Matthews & Marsh-Matthews, 2003). Over drying events stream hydrological connectivity is generally disrupted with habitat retracting to a few, isolated refugia (Stanley *et al.*, 1997; Lake, 2003; Bunn *et al.*, 2006; Bond *et al.*, 2008). Particularly, in temporary streams habitat contraction and expansion are recurrent, connectivity is often broken and re-established, and availability of refugia is highly fluctuating in time and space (Stanley *et al.*, 1997; Gasith & Resh, 1999; McMahon & Finlayson, 2003). Fish in temporary streams strongly rely on persistent refugia during adverse drought conditions, becoming patchily distributed until flow resumes, stream connectivity is restored, and dispersal and colonization processes of ephemeral habitats may proceed (Magoulick & Kobza, 2003). Under these circumstances, spatial arrangement of refugia is expected to play a critical role in fish population and assemblage dynamics, as movements to and from refugia may be constrained by connectivity between ephemeral and persistent habitats, contingent on distance and species-specific movement abilities (Schlosser 1991; Magoulick & Kobza 2003; Pringle 2003; Fullerton, Burnett *et al.* 2010). However, the way in which fish respond to temporal and spatial fluctuations in habitat quality and connectivity, and spatial processes interact with habitat selection, remain largely unexplored in temporary rivers (Larned *et al.*, 2010).

Numerous studies on local fish-habitat relationships in temporary rivers highlight the importance of channel hydro-morphology (Pires *et al.*, 1999; Magoulick, 2000; Birnbaum *et al.*, 2007; Dekar & Magoulick, 2007; Arthington *et al.*, 2010), streambed substrate and cover (Godinho *et al.*, 2000; Mesquita *et al.*, 2006; Morán-López *et al.*, 2006; Clavero *et al.*, 2009) and bank and riparian cover (Magalhães *et al.*, 2002b; Dekar & Magoulick, 2007; Clavero *et al.*, 2009). There is also increasing evidence on spatial processes affecting fish, with habitat isolation (Labbe & Fausch, 2000; Scheurer *et al.*, 2003), and characteristics of surrounding floodplains (Arthington *et al.*, 2010) shaping variation on local persistence and assemblage structure, while connectivity to refugia appears key in colonization of ephemeral habitats at the catchment scale (Davey & Kelly, 2007). However, comparative assessments of habitat quality and connectivity influences on fish have seldom been conducted (but see Scheurer *et al.*, 2003; Arthington *et al.*, 2010), and thus the way in which these processes could interact with each other remains poorly known, although this has been considered critical for advancing population and community ecology of fish and conservation planning (Larned *et al.*, 2010; Winemiller *et al.*, 2010).

This study address this issue, by analyzing variation in fish distribution and abundance in relation to habitat quality, connectivity to refugia, and neighbourhood effects in a mediterranean stream in Southwest Portugal. Mediterranean streams are highly dynamic systems, shaped by seasonally predictable flooding and drying events, which vary markedly in intensity over the years (Gasith & Resh, 1999). Therefore, analyses were based on repeated surveys of fish distribution, in order to provide more representative views of habitat use and capture eventual temporal changes in the effects of the different habitat components. Specific objectives of this study were to: (i) assess the role of habitat quality, connectivity to refugia, and neighbourhood effects in determining local fish species richness, overall abundance, and occurrence, and (ii) examine whether these change over time.

5.2. STUDY AREA

The study was carried out in the Torgal stream, in Southwest Portugal (Figure 5.1), which is a typical mediterranean stream in its flow regime and fish fauna. The Torgal basin and its fish assemblage were previously described in detail in Chapter 2, so that only features relevant to the present investigation are given here. The stream is forested and free from major human impacts. The stream valleys are largely pristine, there are well-developed riparian galleries, and no urban pollution, impoundments, angling or other recreational activities. Climate is mediterranean, with annual rainfall varying markedly from year-to-year (237.8–1121.2 mm), but following a predictable seasonal pattern. About 80% of annual rainfall occurs in the wet-season (October to March) and only about 3.0% in the hot, dry-season (June to August). Mean monthly temperature is 11 ºC in the coldest month (January) and 23 ºC in the hottest (August). The flow regime is highly dependent on rainfall patterns, exhibiting strong seasonal and inter-annual variation. Flow generally stops between July and September, though in dry years the drying period may be extended. During this period, habitat for fish restricts to some isolated pools in the upstream and downstream reaches of the mainstream and aquifer feed runs in the largest tributary (Capelinha stream), with hydrological connectivity persisting only in a intermediate section near to this confluence (Figure 5.1). The drying pattern is spatially asymmetrical, with upstream reaches being the first to dry and more persistent pools concentrating downstream. During wet years, major floods may occur between October and April, and flows may persist through the dry-season in downstream reaches.

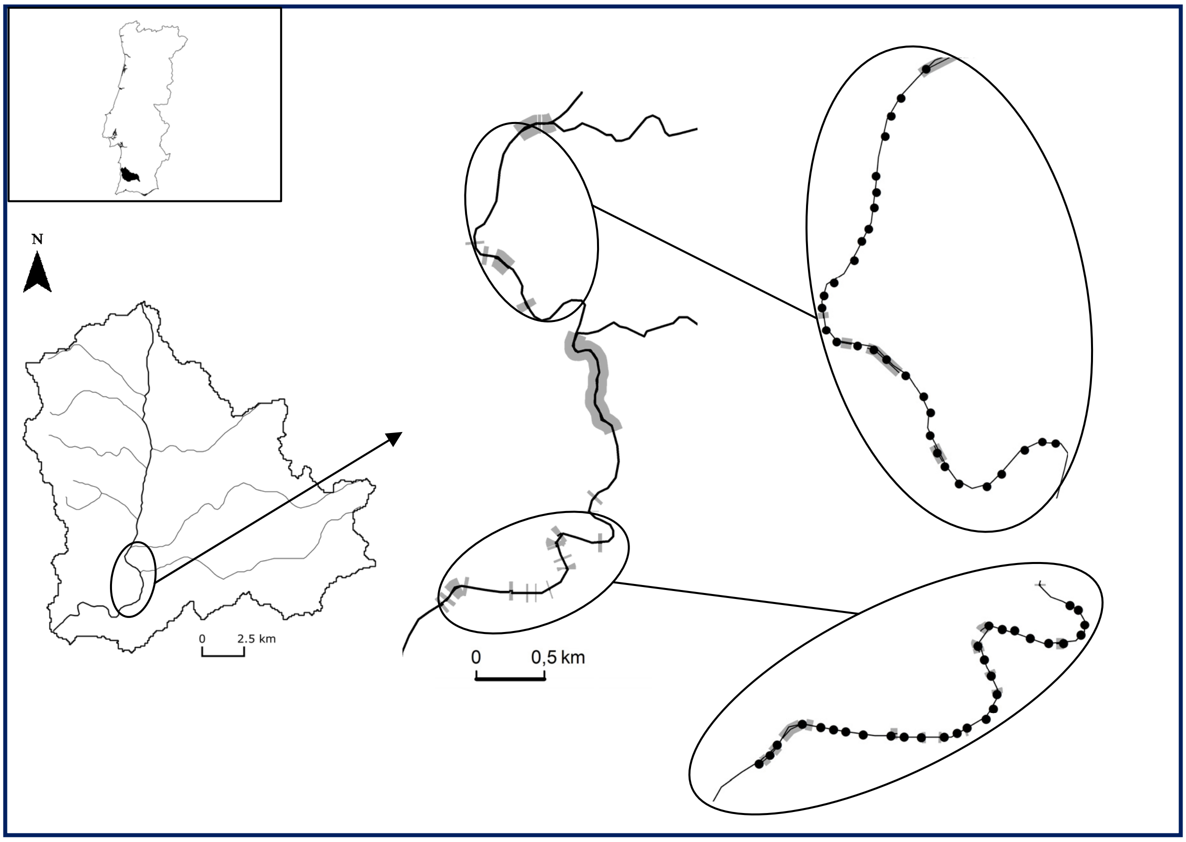

Figure 5.1 Map of the Torgal catchment, showing locations of the sites sampled for fish between February 2006 and July 2007 (black dots) and persistent dry-season pools in September 2005 (grey bands).

Fish assemblages in the Torgal stream are dominated by native chub *Squalius torgalensis* Coelho, and eel *Anguilla anguilla*, with loach *Cobitis paludica*, nase *Iberochondrostoma almacai*, barbel *Luciobarbus sclateri* and the non-native pumpkinseed *Lepomis gibbosus* occurring sparsely and in lower numbers. Also present but rare are the native three-spined stickleback *Gasterosteus gymnurus*, and the non-natives, mosquito fish *Gambusia holbrooki*, large-mouth bass *Micropterus salmoides* and carp *Cyprinus carpio.*

5.3. METHODS

5.3.1. Study design

The study was carried out between September 2005 and July 2007 in the Torgal mainstream. The year of 2005 was dry relative to the annual mean (237.8 mm vs. 614.5 mm) leading to extreme dry-season conditions at the beginning of the study; conversely moderate dry conditions occurred in 2006 (662.2 mm vs. 614.5 mm). Extensive surveys of dry-season pools were conducted in September 2005 and 2006, by walking through the mainstream channel, logging the position of each pool, and measuring its length and maximum width. Fish and habitat surveys were further conducted in two segments of 2 km each (Figure 5.1), selected for being intermittent, covering the range of dry-season pools, and good accessibility. Each segment was divided into stretches of 200 meters, and 3 sampling sites of 10 meters were selected in each stretch. Sites were stratified according to macrohabitat type (i.e. pool, run or riffle), and kept apart at least 50 meters. Overall, 60 sites were selected for sampling. The position of each site and dry-season pool was logged with a Magellan 200 Xl, GPS (Global Positioning System) receiver (MiTAC International Corporation, Taiwan, China), and plugged in a Geographic Information System (GIS; ArcView 3.1 for Windows, Environmental Systems Research Institute, Redlands, CA, USA).

Sampling was carried out in seven occasions, during periods of steady flow: February, April and June 2006, following the 2005 dry-season, and November 2006, March, May and July 2007, following the 2006 dry-season. In each occasion stream segments were sampled consecutively during 6-days.

5.3.2. Fish data

Fish were sampled using a standard electrofishing gear (Hans Grassl EL62 IIGI), fitted with a single 30 cm anode and discharging 300V, 4-6 A, pulsed D.C. Each 10 meter site was single-pass electrofished for 5 minutes, by wading in an upstream direction, except in the 2 deepest sites (average depth > 1 meter) were sampling was made from the river bank.

The same person operated the electrofishing gear across sites, and there were always two netters present to pick up stunned fish. Fish were counted, measured for fork length (FL) in the case of chub, and returned to the stream. Preliminary studies showed that single-pass electrofishing yielded on average 91% (SD = 13.78, n = 12, range = 60-100%) of the species recorded in three consecutive fishing passes, and there were high correlations between the first and the overall sample in species ranks (Rs; mean = 0.94, SD = 0.01, n = 12, range = 0.98–1.00) and relative abundances (R; mean = 0.99, SD = 0.09, n = 12, range = 0.71–1.00), independent of the length of stream sampled (R=0.03, p=0.93; R=-0.127, p=0.69; R= 0.26, p = 0.42, respectively) (D.F. Pires & M.F. Magalhães, unpublished data).

5.3.3. Explanatory variables

Fourteen explanatory variables describing local habitat characteristics, connectivity to dry-season pools, and neighbourhood effects were quantified.

Habitat variables reflected channel hydro-morphology, substrate, streambed and bank cover (Table 5.1), and have been previously associated with assemblage organization in mediterranean fish (Godinho & Ferreira, 1998; Pires *et al.*, 1999; Godinho *et al.*, 2000; Magalhães *et al.*, 2002b; Mesquita *et al.*, 2006; Morán-López *et al.*, 2006; Ferreira *et al.*, 2007; Santos & Ferreira, 2008). Habitat variables were determined immediately after fish sampling. Channel width was measured at four transects spaced along each site (1, 3, 6 and 9 meters); depth, and current velocity (FP101 Global Water Flow Probe, Gold River, CA, USA), were measured at the middle-points of each transect. Substrate and streambed cover were quantified at 1-meter transects spaced along each site. Substrate size was categorized as follow: 1, 1-2mm; 2, 2-5 mm; 3, 5-25 mm; 4, 25-50 mm; 5, 50-100 mm; 6, 100-250 mm and 7, >250mm). Each substrate category was visually estimated and scored as 3-dominant, 2-co-dominante, 1-residual, and 0-absent. Substrate coarseness was then derived as the weighted average of substrate size categories across transects, and the number of substrate categories was used to index substrate heterogeneity. The percentage of wood and leaf debris, algae, emergent and submersed vegetation covering the streambed was estimated visually. The percentage of shrub and trees covering each bank was also estimated visually.

Site connectivity to dry-season pools was quantified using an Incidence Function model measure, as Si= $\sum_i \exp(-\alpha d_{ij}) A_j$(Hanski, 1998), where α is the inverse of the average species movement distance, dij the distance of site i to dry-season pool j and A the area (length x maximum width) of each pool. Pool area was used as a surrogate of the potential of each pool as a source of colonists, given pilot-surveys conducted in the Torgal showed a tendency for the fish abundances increasing with pool area (Appendix II). The species movement distance was approached by using the average distance moved out of dry-season pools by chub (292.7m), estimated using mark-recapture techniques (Chapter 4); this single measure was used in calculations for all species, given there was no information on movement patterns for other species.

Neighbourhood effects were evaluated using an inverse distance weighted auto covariate term, as $\sum_{i \neq z} \frac{{d_{iz}}^{-2}}{\sum_{i \neq z} {d_{iz}}^{-2}} Y_z$ (Shepard, 1968), where d_{iz} is the distance from site i to neighbouring site and Y the value of the assemblage attribute at neighbouring site z.

Connectivity to dry-season pools and neighbourhood effects were estimated considering only the habitats available in the segment where each site was included. Moreover, only dry-season pools persisting in the year of 2005 were used to determine connectivity, because preliminary investigation in the study area showed that long-term persistent pools shelter the majority of fish, even in wet years (Pires & Magalhães, unpublished data). Distances were always calculated between the extremes of each site and pool in the GIS.

Table 5.1 Characteristics of sites in the Torgal stream sampled between February 2006 and July 2007. Values are the mean (± standard deviation) across all sites.

	Code	Feb-06	Apr-06	Jun-06	Nov-06	Mar-07	May-07	Jul-07	Overall
Channel hydro-morphology									
Channel Depth (cm)	D	58.9 ± 32.2	61.9 ± 30.8	50.0 ± 35.9	62.0 ± 33.6	66.1 ± 32.9	56.8 ± 33.9	49.2 ± 37.1	57.8 ± 34.1
Channel Width (m)	W	6.8 ± 2.7	7.1 ± 2.7	6.3 ± 3.0	6.6 ± 2.4	7.2 ± 2.6	6.9 ± 2.8	6.4 ± 2.9	6.8 ± 2.7
Current Velocity (m/s)	V	0.2 ± 0.2	0.2 ± 0.2	0.1 ± 0.1	0.3 ± 0.2	0.3 ± 0.2	0.2 ± 0.2	0.1 ± 0.1	0.2 ± 0.2
Streambed Substrate									
Substrate Coarseness	Sc	4.5 ± 1.7	4.6 ± 1.6	4.7 ± 1.9	4.6 ± 1.5	5.0 ± 1.7	4.6 ± 1.7	4.7 ± 1.7	4.7 ± 1.7
Substrate Heterogeneity	Sh	5.0 ± 0.8	5.0 ± 0.7	5.3 ± 0.6	5.1 ± 0.7	5.1 ± 0.6	5.0 ± 0.8	4.8 ± 1	5.0 ± 0.8
Streambed cover (%)									
Algae	A	3.0 ± 4.6	1.3 ± 2.3	0.3 ± 1.3	0.0 ± 0.2	0.2 ± 0.8	0.0 ± 0.1	0.9 ± 4.8	0.8 ± 2.9
Wood debris	wD	11.0 ± 7.0	8.8 ± 4.8	11.9 ± 4.3	8.0 ± 5.2	7.8 ± 7.5	6.8 ± 5.1	9.0 ± 4.2	9.1 ± 5.8
Leaf debris	lD	11.8 ± 14.4	5.0 ± 6.1	11.1 ± 2.6	11.5 ± 8.9	1.7 ± 4.1	3.6 ± 4.6	9.0 ± 4.2	7.7 ± 8.3
Submerged vegetation	sV	10.2 ± 13.1	8.6 ± 14.4	9.6 ± 21	3.4 ± 4.6	5.2 ± 8.1	4.6 ± 8.0	5.8 ± 13.1	6.8 ± 12.9
Emergent vegetation	eV	3.5 ± 3.5	4.6 ± 7.4	2.9 ± 6.1	1.3 ± 2.5	2.7 ± 3.1	2.7 ± 5.3	1.7 ± 5.8	2.8 ± 5.2
Bank cover (%)									
Arboreum cover	Ar	42.1 ± 20.4	54.8 ± 22.8	62.1 ± 23.3	58.1 ± 23.5	56.3 ± 23.6	56.8 ± 23.6	59.3 ± 23.0	55.6 ± 23.5
Shrub cover	Sb	42.8 ± 26.9	58.2 ± 21.5	71.0 ± 17	44.8 ± 21.6	37.8 ± 20.2	70.7 ± 18.9	72.8 ± 18.0	56.9 ± 24.9

5.3.4. Data analysis

Data analysis focused on quantifying associations between fish and habitat characteristics, in relation to connectivity to dry-season pools and neighbourhood effects, and the extent to which these change over time. Analyses were thus carried out individually for each sampling occasion, considering all sites, to encompass the gradient of spatial variability in pool persistence and connectivity prevailing in the study area.

Response variables included the richness and abundance of overall, native and non-native species and occurrence of individual species occurring in at least in 20 % of sampling sites. No modelling of individual species abundance was conducted, given counts of fish were generally low and highly correlated with occurrence (R, mean= 0.57, SD= 0.05, range= 0.52-0.66, n= 7; Table 5.2). Because previous studies have reported size-related variations in fish habitat use (Magalhães *et al.*, 2002b; Pires *et al.*, 2010), the dominant species in the study area was divided into three-length classes (small:<40 mm, medium: 40-59 mm and large: ≥60mm FL).

To reduce collinearity problems in subsequent multivariate modelling we computed Pearson’s correlation among each pair of habitat variables, dropping from analysis the variable of each pair with |R|>0.60 less associated with the response variables in univariate analyses.

Associations between response and explanatory variables were examined using Generalized Linear Models (GLM; Dobson, 2002), with Poisson functions corrected for over-dispersion in the case of species richness and overall abundance, and logistic functions in the case of species occurrences.

Modelling involved a two-stage procedure based on the Information Theoretic Approach (ITA; Burnham & Anderson, 2002). The ITA looks for simplicity, parsimony and informative proprieties of several working hypothesis and it is based on the strength of evidence of each candidate predictive model. Loss of information of each candidate model was measured through the Akaike Information Criteria, with the best informative model having lower AIC values. Absolute differences (Δ AIC) between each model and the best model AIC were used to rank candidate models. Because the number of parameters was large relative to the sample sizes available, the Akaike Information Criteria adjusted for small samples (AICc) was used in logistic regressions, and the correction for small samples and overdispersion (QAIc) was used in quasi-Poisson generalized modelling. Strength of evidence of each model was evaluated by Akaike Weights (Wi), which measure the posterior probability that a given model is true, given the data and the entire set of competing candidate models (Burnham & Anderson, 2002).

Multi-model inference was used to assess the relative importance of each variable, within the set of explanatory variables considered. The probability that a variable is included in the best approximating models was estimated by summing the Wi of all candidate models were the variable was included (Burnham & Anderson, 2002). Additionally, effects of explanatory variables were assessed by calculating averaged coefficients weighted by model akaike weights. Analyses were performed in R 2.8.1 software (R Development CoreTeam, 2008) using glm function from stats package and functions from MuMIn package (Barton, 2010).

The analysis started by modelling variation in response variables in relation to habitat variables. Candidate models were built considering all possible subsets of habitat variables, but only those with considerable support (ΔAIC <4) (Burnham & Anderson, 2002) were used to estimate predictor averaged coefficients. Averaged coefficients of predictors were used to calculate averaged predicted values of the response variables, which were further considered as an index of habitat quality for each site and sampling occasion.

The second set of analyses examined whether fish-habitat relationships as indexed by the habitat quality index were influenced by connectivity to dry-season pools, and neighbourhood effects. Connectivity and neighbourhood effects were modelled separately, to assess factors operating at different scales and avoid possible confounding effects. Candidate models were built considering all combinations of the habitat quality index, and connectivity or neighbourhood variables, and their interaction terms. Evaluation of the magnitude of connectivity and neighbourhood effects was made via Multi-model inference, as described above.

5.4. RESULTS

5.4.1. Fish assemblage composition

A total of 4338 fish of 9 species were sampled (Table 5.2). Overall species richness per site ranged from 1.9 in June 2006 and May 2007 to 2.7 in November 2006, and overall abundance per site ranged from 6.8 to 14.6 individuals in July and February 2006. The richness and abundance of native species were consistently higher than those of non-native species (2 vs. 0.3 species and 9.5 vs. 0.8 individuals per site).

Mean abundance per site was generally low for all species (< 1), but for chub and eel, which accounted for >75 % of total numbers, and occurred in >50% of the sites. Eel exhibited stable abundance (0.7-5.2) and occupation rate (40%—67%) over the study period, whereas chub tended to be less abundant (2.2—10.7) and more concentrated (50%—92%) in drier months (June 2006 and July 2007). Loach, nase, barbel and sunfish were less widespread (16%-26% sites), though were still caught in all sampling occasions. Among these species, loach showed little fluctuating abundances (0.4—0.9) and occupation rates (22-33%) while nase, barbel and pumpkinseed exhibited considerable temporal changes in both attributes, which prevented modelling of species individual occurrences in some months. Mosquito fish, three-spined stickleback, and largemouth bass were rare (0%—2,5% of overall abundance) and sparse (0-6% of sites), occurred in less than 20% of the sites in all sampling occasions, and were therefore discarded from further analysis.

Table 5.2 Composition of fish assemblages in the Torgal stream during February 2006 – July 2007, quantified using the mean abundance (± standard deviation) and percentage of occurrence (between brackets) for each species.

	Feb-06	Apr-06	Jun-06	Nov-06	Mar-07	May-07	Jul-07	Overall
Anguilla Anguilla	0.7 ± 1.0 (40)	1.3 ± 1.8 (60)	2.4 ± 4.6 (57)	0.7 ± 0.9 (45)	2.0 ± 4.1 (53)	3.7 ± 6.1 (67)	5.2 ± 8.1 (62)	2.3 ± 1.7 (55)
Cobitis paludica	0.4 ± 0.9 (22)	0.9 ± 1.8 (33)	0.7 ± 2.1 (23)	0.7 ± 1.7 (25)	0.6 ± 1.2 (25)	0.8 ± 1.7 (30)	0.6 ± 2.0 (25)	0.7 ± 0.2 (26)
Luciobarbus sclateri	0.2 ± 0.8 (8)	0.1 ± 0.5 (8)	0.4 ± 1.2 (10)	0.6 ± 1.0 (28)	0.6 ± 1.3 (22)	0.4 ± 1.3 (17)	0.3 ± 0.9 (17)	0.4 ± 0.2 (16)
Iberochondrostoma almacai	0.8 ± 2.1 (30)	0.3 ± 0.8 (17)	0.1 ± 0.5 (8)	0.7 ± 1.8 (32)	0.7 ± 2.0 (25)	0.7 ± 2.5 (20)	0.1 ± 0.5 (7)	0.5 ± 0.3 (20)
Squalius torgalensis	10.7 ± 11.6 (92)	4.8 ± 5.7 (73)	2.2 ± 3.9 (50)	9.0 ± 7.4 (88)	5.9 ± 5.5 (82)	4.2 ± 5.6 (73)	2.9 ± 3.8 (65)	5.7 ± 3.1 (75)
Small (<40 mm)	1.1 ± 4.1 (23)	0.1 ± 0.3 (7)	0.0 ± 0.1 (2)	2.8 ± 4.3 (63)	1.4 ± 2.2 (45)	0.4 ± 0.8 (27)	0.1 ± 0.2 (5)	0.8 ± 1.0 (25)
Medium (40 - 59 mm)	6.1 ± 7.7 (82)	2.3 ± 3.7 (52)	0.8 ± 1.5 (30)	1.5 ± 2.2 (53)	1.1 ± 1.6 (50)	1.2 ± 1.9 (47)	0.9 ± 1.5 (38)	2.0 ± 1.9 (50)
Large (> 60 mm)	3.6 ± 3.4 (75)	2.4 ± 2.7 (65)	1.4 ± 2.8 (40)	4.7 ± 4.2 (83)	3.4 ± 3.6 (72)	2.5 ± 4.3 (57)	1.9 ± 3.0 (60)	2.8 ± 1.1 (65)
Gasterosteus gymnurus	-	0.1 ± 0.3 (3)	0.1 ± 0.3 (5)	0.0 ± 0.2 (3)	0.2 ± 0.7 (10)	0.1 ± 0.2 (5)	0.0 ± 0.1 (2)	0.1 ± 0.1 (4)
Lepomis gibbosus	1.2 ± 2.6 (30)	0.4 ± 1.0 (17)	0.8 ± 2.5 (27)	1.0 ± 2.9 (27)	0.2 ± 0.5 (15)	0.1 ± 0.4 (12)	0.2 ± 0.5 (10)	0.6 ± 0.5 (20)
Micropterus salmoides	0.0 ± 0.2 (3)	-	-	-	-	-	-	-
Gambusia holbrooki	0.6 ± 2.7 (8)	0.2 ± 1.1 (5)	0.2 ± 0.7 (7)	0.7 ± 2.6 (17)	0.1 ± 0.2 (5)	-	0.0 ± 0.2 (3)	0.3 ± 0.3 (6)
Total species	2.3 ± 1.2	2.2 ± 1.3	1.9 ± 1.4	2.7 ± 1.2	2.4 ± 1.2	2.2 ± 1.1	1.9 ± 1.1	2.2 ± 0.3
Native species	1.9 ± 0.9	2.0 ± 1.1	1.5 ± 1.1	2.2 ± 1.0	2.2 ± 1.0	2.1 ± 1.0	1.8 ± 1.0	2.0 ± 0.2
Non-native species	0.4 ± 0.6	0.2 ± 0.4	0.3 ± 0.5	0.4 ± 0.7	0.2 ± 0.4	0.1 ± 0.3	0.1 ± 0.3	0.3 ± 0.1
Total fish	14.6 ± 14.1 (98)	8.0 ± 6.4 (93)	6.8 ± 7.3 (87)	13.4 ± 9.4 (98)	10.3 ± 7.3 (97)	10.1 ± 8.4 (97)	9.3 ± 8.6 (88)	10.3 ± 2.8 (94)
Native fish	12.8 ± 12.5 (98)	7.4 ± 6.4 (92)	5.8 ± 6.6 (85)	11.7 ± 8.8 (98)	10.0 ± 7.2 (97)	9.9 ± 8.4 (97)	9.1 ± 8.6 (88)	9.5 ± 2.4 (94)
Non-native fish	1.9 ± 4.1 (33)	0.6 ± 1.5 (22)	1.0 ± 3.0 (30)	1.7 ± 3.9 (33)	0.3 ± 0.6 (18)	0.1 ± 0.4 (12)	0.2 ± 0.5 (13)	0.8 ± 0.7 (23)

5.4.2. Fish-habitat associations

Preliminary screening of habitat variables revealed significant associations among channel hydro-morphological variables (velocity vs. depth, |R|, mean=0.65; SD=0.12, n=7; velocity vs. width |R|, mean=0.60; SD=0.10, n=7; depth vs. width |R|, mean=0.67; SD=0.07, n=7). Current velocity exhibited the strongest correlations with species richness (|R|, mean=0.26, SD=0.08, range:0.20-0.43, n=7, vs. mean=0.15, SD=0.11, range: 0.01-0.30, n=7, and mean=0.08, SD=0.09, n=36, range:0.00-0.27) and abundance (|R|, mean=0.28, SD=0.16, range: 0.05-0.50, n=7, vs. mean=0.23, SD=0.14, range: 0.03-0.38, n=7, and mean=0.20, SD=0.15, range: 0.00-0.43 n=7) and so it was the only retained for further analyses. Associations among the remaining habitat variables were weak |R|<0.60 and they were thus all considered for analyses.

Multi-model inference suggested several associations (selection probability >0.70) between fish and habitat variables (Table 5.3). Although there was considerable variation in habitat predictors of species richness and abundance through the study period, current velocity, substrate coarseness, submerged vegetation and arboreum cover, showed the most consistent associations with assemblage attributes. Overall species richness was higher in low flowing sites in November 2006, May 2007, and July 2007. Native species richness increased in sites with fine substrates in June 2006 and with coarser substrate in November 2006 and May 2007. More non-native species were found in sites less covered by submerged vegetation, in February, April and June 2006. Abundance of overall and native species peaked in sites with less arboreum cover in July 2006, and March, May, and July 2007. Substrate coarseness was positively associated with abundance of overall species, in March and May 2007, and that of native species in November 2006 and May 2007. Conversely, overall abundance of fish in June 2006, and abundance of native species in March 2007 and of non-native species in April, June and November 2006 were higher in sites with fine substrates.

Table 5.3 Summary results of information theoretic multi-model inference for the relationships between fish and habitat variables. Variables having moderate (selection probability > 0.7; in italic) and strong support (selection probability >0.9; in bold) in explaining variation in fish occurrence and abundance in each sampling month are displayed. Variables with negative averaged coefficients are underlined. Variables codes are described in Table 5.1. (*) - indicates that no variables had moderate support (-) – indicates months with insufficient data for analysis.

	Feb-06	Apr-06	Jun-06	Nov-06	Mar-07	May-07	Jul-07
Richness							
Overall	**ID**, **Ar**	**Sh**	**Sc**, **ID**	**V**, **eV**	*	**V**, *Sb*	*V*, *Sc*, *A*
Native Species	**V**, *sV*	*Sh*	**Sc**, *Sh*, **ID**	**V**, *Sc*, *eV*	*****	*Sc*, **Sb**	**A**
Non-native Species	**wD**, **ID**, **sV**, **Ar**, **Sb**	**sV**	**Sc**, *sV*	*Sc*, *wD*	-	-	-
Abundance							
Overall	**V**	**sV**	**Sc**, *Ar*	**V**, **eV**	*Sc*, **sV**, **Ar**	*Sc*, **Ar**	**Ar**
Native Species	**V,** *ID*	*sV*	*Ar*	**V**, **Sc**, **eV**	*Sc*, **sV**, **Ar**	**Sc**, **Ar**	**Ar**
Non-native Species	**Sh**,*wD* **ID**, **Ar**, *Sb*	*Sc*,	**V**, **Sc A**, **ID**	**Sc**, *Sb*	-	**-**	**-**
Species Occurrence							
Eel	**V**, **Ar**	*V*, **Sc**, *eV*, **ID**	*V*, *Sc*, *A*, *ID*, *Sb*	**V**, **Sb**	**V**, *Sc*	**V**	*V*, *sV*
Loach	*V*	**V**, **Sh**, **eV**, **sV**, *Ar*	***V***, **sV**, *Ar*	**V**, **wD** **sV**, **A**	**V**, **A**, **wD**, **eV**	**Sc**, eV	**A**, **eV**
Barbel	-	-	**-**	**sV**	**sV**	-	**-**
Nase	**V**, **Sc**			**V**, **Sb**	**Sc**, **Sh**, **A**, **sV**, **Sb**	**V**, **Sc**, **Ar**	
Chub	**V**, **wD**, **eV**,**sV**	**V**	**V**, **Sc**	**V**, **A**	**V**	**V**, *sV*, *Sb*	**V**
Small	*Sc*, **Sh**, **ID**, **sV**, **Sb**	-	**-**	*Sb*	**V**, *ID*, **Ar**	*V*, **Sh**	
Medium	**V**, *sV*, *Sb*	*V*	*Sc*	**V**, *Sc*	**V**, **Sc**, *sV*	*A*, **Sb**	**Sc**, **A**
Large	**V**, **eV**, **sV**	**V**	**V**, *Sc*, *sV*	**V**, *eV*	**V**, *Sc*, *wD*	**V**, **sV**, *Ar*	**V**, *Ar*
Pumpkinseed	**wD**, **Sb**	-	*V*, **Sc**	**V**, *wD*, **eV**	-	-	-

Current velocity was the factor most consistently associated with the occurrence of individual species and length classes (Table 5.3). All species and sizes groups tended to occur in low flowing sites, but eel that was associated with higher flows. Occurrence of eel was also associated with finer substrates in April and June 2006, and March 2007. Barbel was mainly associated with absence of submerged vegetation. Streambed vegetation was positively associated with the occurrence of loach, in all sampling occasions but in February 2006. Nase was systematically associated with coarser substrates whereas medium sized chub showed variable associations with substrate coarseness, occurring in sites with coarser substrate in November 2006 and March 2007 and with finer ones in June 2006 and July 2007. Increased submersed vegetation apparently favoured the occurrence of large chub in February and June 2006, and May 2007.

5.4.3. Habitat quality vs. connectivity to refugia

Multi-model inference supported that local habitat quality was more important than connectivity to dry-season pools in shaping fish species richness, abundance, and occurrence (Table 5.4). Nevertheless, there were also moderate to strong evidences (selection probabilities >0.70) on connectivity and habitat-connectivity interactions increasing the number of non-native species in November 2006, non-native species abundance in June 2006, and probability of occurrence of medium-sized chub in May 2007 (Figures 5.2 and 5.3). Moreover, there was strong evidence of positive effects of connectivity in high quality habitats, on the number of native species in July 2007, and on the occurrence of medium-sized chub in March 2007. Conversely, connectivity appeared to have a negative effect on the overall number of species and that of native species and in the occurrence of chub in March 2007 and nase in February 2006.

5.4.4. Habitat quality vs. neighbourhood effects

Multi-model inference provided evidence that local habitat quality was more important than neighbourhood effects in structuring fish assemblages (Table 5.5). Nevertheless, neighbourhood effects and habitat-neighbourhood interactions showed positive associations with non-native species richness in April and November 2006, non-native species abundance in November 2006, occurrence of barbel in November 2006 and March 2007, of nase in February and November 2006 and of medium sized chub and pumpkinseed in February 2006 (Figures 5.4 and 5.5). Neighbourhood effects were equivocal in February 2006, increasing non-native species richness only in less favourable habitats, and in July 2007, favouring the occurrence of chub in high quality habitats.

Table 5.4 Summary results from information-theoretic multi-model inference for the relationships between fish and habitat quality and connectivity to dry-season pools. Variables having moderate (selection probability > 0.7; in italic) and strong support (selection probability >0.9; in bold) in explaining variation in fish occurrence and abundance in each sampling month. H – habitat quality; C – connectivity to dry-season pools; HC – interaction between habitat and connectivity. Variables with negative averaged coefficients are underlined. (*) - indicates that no variables had moderate support. (-) – indicates months with insufficient data for analysis.

	Feb-06	Apr-06	Jun-06	Nov-06	Mar-07	May-07	Jul-07
Richness							
Overall	**H**	**H**	**H**	**H**	*C*	**H**	*****
Native Species	**H**	**H**	**H**	*H*	*C, HC*	**H**	*C, HC*
Non-native Species	**H**		**H**	*HC*	-	-	-
Abundance							
Overall	**H**	**H**	**H**	**H**	*	**H**	*H*
Native Species	**H**	**H**	*H*	*H*	*	**H**	*H*
Non-native Species	*H*	**H**	*H*, **HC**	*H*	-	-	-
Species Occurrence							
Eel	**H**	*H*	**H**	*H*	**H**	**H**	**H**
Loach	*	*H*	*H*	*	**H**	**H**	*
Barbel	-	-	-	**H**	**H**	-	-
Nase	**H, HC**	-	-	*	*H*	**H**	-
Chub	*	*H*	*H*	**H**	*C*	*H*	*H*
Small	**H**	-	-	*H*	*H*	*H*	-
Medium	*H*	**H**	*	**H**	*C, HC*	*HC*	**H**
Large	*H*	*H*	**H**	**H**	*H*	**H**	*H*
Pumpkinseed	*H*	-	*H*	*H*	-	-	-

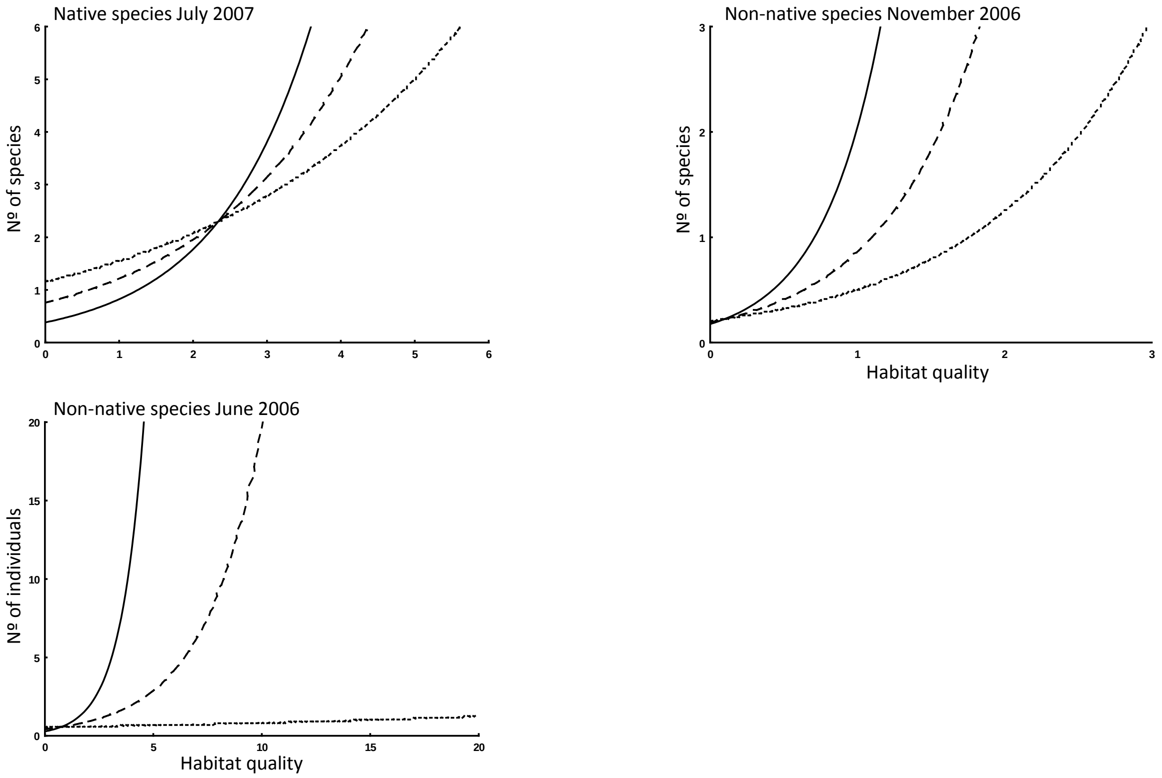

Figure 5.2 Variation in habitat response curves for native and non-native species richness, and non-native species abundance, as a function of connectivity to dry-season pools. Curves represented in each panel reflect averaged model fitted habitat responses for the 1st (···), 5th (- - -) and 9th (—) deciles of the distribution of connectivity to dry-season pools.

5.5. DISCUSSION

Our results suggested that habitat quality plays a major role in shaping variation in fish species richness, overall abundance, and occurrence across the Torgal stream, though connectivity to dry-season pools and neighbourhood effects may also be episodically important. Moreover, this study documented considerable variation in fish-habitat associations over time and among species and length groups, emphasizing the need to incorporate temporal dimensions (Schlosser, 1991; 1995; Fausch *et al.*, 2002) and species-specific analyses (Grossman & de Sostoa, 1994; Magalhães *et al.*, 2002b; Buisson *et al.*, 2008; Santos & Ferreira, 2008) in studies addressing the structuring of stream fish assemblages.

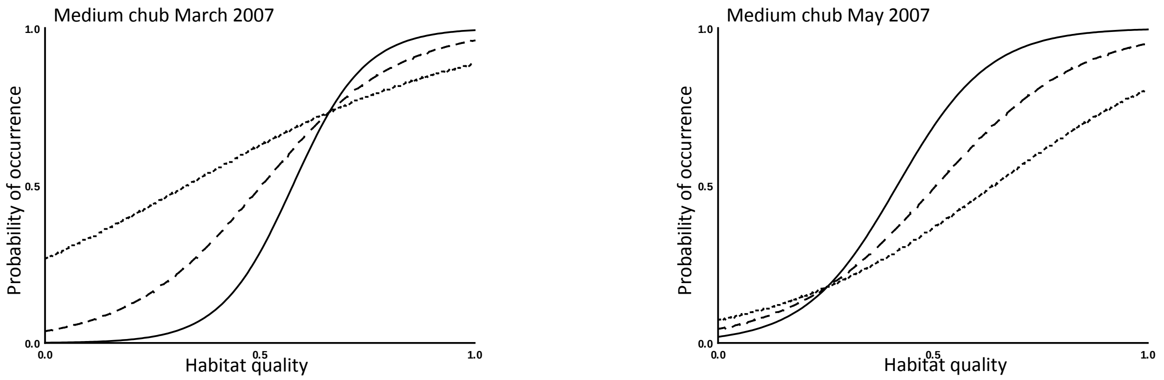

Figure 5.3 Variation in habitat response curves for occurrence of medium sized chub as a function of connectivity to dry-season pools Curves represented in each panel reflect average model fitted habitat responses for the 1st (····), 5th (- - -) and 9th (—) deciles of the distribution of connectivity to dry-season pools.

The perceived patterns are unlikely to be shaped by major methodological shortcomings. Previous studies have shown that the sampling effort used is suitable for detecting common species (Pires, D.F. & Magalhães, M.F. unpublished data), which were the main focus of our study. Moreover, potential drawbacks associated with electrofishing were minimized by the same person operating across sites and over time. Even if biases occurred for some species, these were probably consistent over the study, and thus across-site and over-time comparisons remain valid.

5.5.1. The roles of habitat quality, connectivity to refugia and neighbourhood effects

Associations between habitat quality and local fish assemblages were consistent over time, contrasting with episodic and species-specific associations with connectivity to refugia and neighbouring effects. These findings corroborate previous studies highlighting the importance of habitat quality in shaping fish assemblages during steady flows in temporary streams (e.g. Bond & Lake, 2003; Mesquita *et al.*, 2006), and its predominance over spatial context in dry-season habitat use (Balcombe *et al.*, 2006; Arthington *et al.*, 2010; see also Chapter 3).

Table 5.5 Summary results from information-theoretic multi-model inference for the relationships between fish and habitat quality and neighbourhood effects. Variables having moderate (selection probability > 0.7; in italic) and strong support (selection probability >0.9; in bold) in explaining variation in fish occurrence and abundance in each sampling month are displayed. H- habitat quality, N - neighborhood effects, HN - interaction between habitat and neighbourhood. Variables with negative averaged coefficients are underlined. (*) - indicates that none of the variables had moderate support in explaining variation of fish occurrence and abundance; (-) – indicates months with insufficient data for analysis.

	Feb-06	Apr-06	Jun-06	Nov-06	Mar-07	May-07	Jul-07
Richness							
Overall	**H**	*H*	*H*	**H**	**H**	*H*	*H*
Native Species	*H*	*H*	*H*	**H**	**H**	*H*	*H*
Non-native Species	**H, N,** *HN*	**N**	**H**	**H, N**	-	-	-
Abundance							
Overall	**H**	**H**	**H**	**H**	**H**	*	**H**
Native Species	**H**	**H**	**H**	**H**	**H**	*	**H**
Non-native Species	**H**	**H**	**H**	**H, N**	-	-	-
Species Occurrence							
Eel	**H**	**H**	**H**	**H**	**H**	*H*	**H**
Loach	**H**	**H**	**H**	**H**	**H**	*H*	**H**
Barbel	-	-	-	*H, N*	**HN**	-	-
Nase	**N, HN**	-	-	*H, HN*	**H**	**H**	-
Chub		**H**	*H*	**H**		*H*	*N, HN*
Small	**H**	-	-	*H*	**H**	*H*	-
Medium	**H,** *N*, *HN*	**H**	*H*	*H*	*H*	**H**	*H*
Large	**H**	*H*	**H**	**H**	**H**	*H*	*
Pumpkinseed	*H, N*	-	**H**	**H**	-	-	-

Connectivity to dry-season pools apparently did not limit site colonization, after flow resumption. This contrasts with other studies, indicating that proximity to dry-season refugia strongly contributes to fish persistence at the reach scale (Labbe & Fausch, 2000) and to increased speed and rates of recolonization of ephemeral habitats at the catchment scale (Davey & Kelly, 2007). However, fish inhabiting predictable, intermittent streams are generally expected to have evolved and adapted to the cyclic changes in habitat availability, with assemblages recovering rapidly after drought (Lake, 2003), and this may also be the case herein. In mediterranean streams fish density at the reach scale may decrease between the dry- and wet-seasons, indicating quick seasonal redistribution from refuge habitats (Bravo *et al.*, 2001). Also, in semiarid, Brazilian streams, reestablishment of stream connectivity for 20 days in a stream reach, leaded to higher fish diversity due to colonization by species absent in the dry-season, and to decreased fish dominance due to redistribution among habitats, from dry-season to wet-season (Medeiros & Maltchik, 2001). Moreover, experimental defaunation studies have shown that recolonization as few meters from sources may occur in hours to weeks (Peterson & Bayley, 1993; Sheldon & Meffe, 1995; Lonzarich *et al.*, 1998), with local fish species richness recovering rapidly to pre-defaunation levels. However, delayed assemblage recovery rates at higher distances from sources (>100 m) were also reported (130-560 m, Albanese *et al.*, 2009).

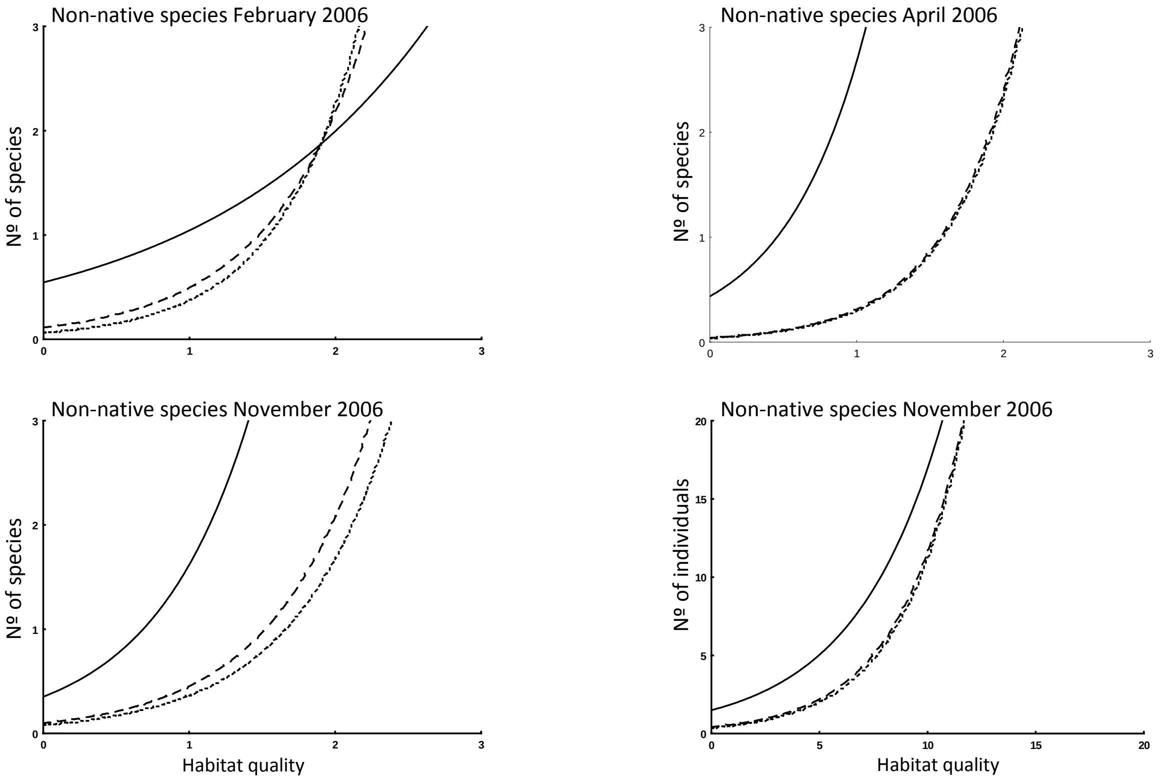

Figure 5.4 Variation in habitat response curves for non-native species richness and abundance, as a function of neighbourhood effects. Curves represented in each panel reflect average model fitted habitat responses for the 1st (·····), 5th (- - -) and 9th (—) deciles of the distribution of neighbourhood effects.

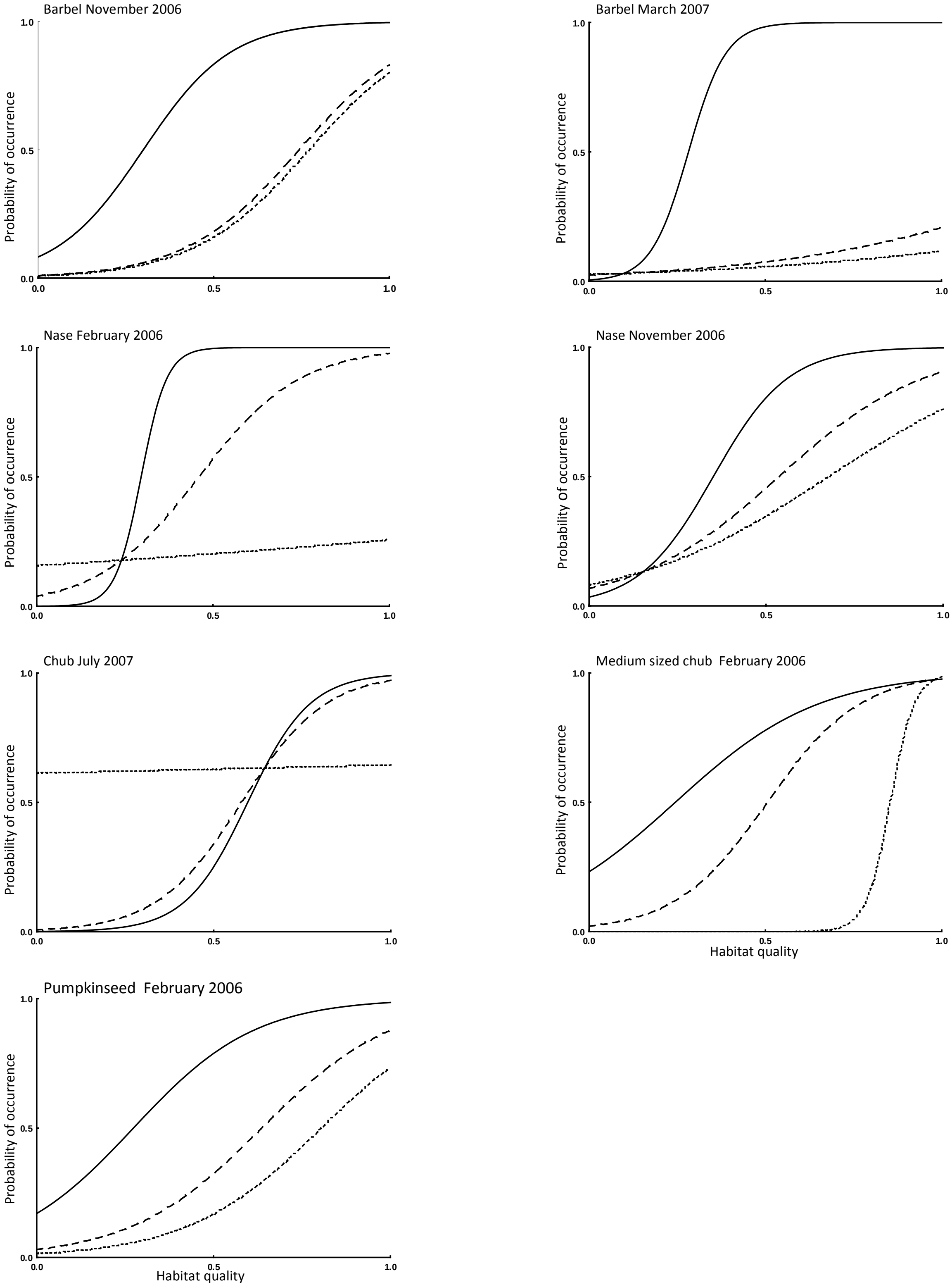

Figure 5.5 Variation in habitat response curves for the occurrence of barbel, nase, chub and pumpkinseed, as a function of neighbourhood effects. Curves represented in each panel reflect average model fitted habitat responses for the 1st··(), 5th (- - -) and 9th (—) deciles of the distribution of neighbourhood effects.

Contrasts in the importance of connectivity to dry-season refugia among stream fish assemblages may reflect differences in the spatial scale of observations and stream disturbance regimes. For instance, variation in habitat isolation may explain differences between our results and those of Davey & Kelly (2007), as site distances to permanent habitats were at most 0.5 km herein and at least 3 km in their studies. Moreover, intermittency episodes in the streams studied by Davey & Kelly (2007) and Labbe & Fausch (2000) seemed more frequent than in the Torgal stream, probably resulting in stronger disturbance (Davey & Kelly, 2007) and more pronounced variability in habitat connectivity.

Associations with connectivity to dry-season pools were not restricted to a single period of the hydrological cycle, probably reflecting different ecological processes. The increase in non-native species richness in November 2006, after flow resumption, suggests that connectivity to dry-season pools may favour pos-drought dispersal for at least some non-native species. Conversely, positive association of connectivity and non-native species abundance, in June 2006, and with native species richness, in July 2007 may reflect fish aggregation in the surrounding of dry-season pools. Indeed fish inhabiting temporary streams may respond to changes in flow (Davey *et al.*, 2006) and net immigration movements to pools are expected during the drying phase (Magoulick & Kobza, 2003; Davey & Kelly, 2007). The processes underlying associations for medium sized chub in March and May 2007, during flow conditions, were unclear, but are unlikely to be linked to recovery from drought. There appeared to be also some size-specific variation in the importance of connectivity, as associations with occurrence were found for medium chub but not for the remainder size-classes. Finally, we detected unexpected negative associations between connectivity and overall and native species richness, and occurrence of chub in March 2007, and nase in February 2006, suggesting that biological or spatial processes leading to the occupation of less connected sites may also be at play herein.

A better understanding of the role of habitat connectivity in shaping fish assemblage variation may require considering multi-scale interactions on habitat selection (Fullerton *et al.*, 2010). For instance, incorporating measures of connectivity to dry-season refugia at higher spatial scales of the landscape, and using a multi-temporal design would help accounting for species and life stages differences in the scale of response to connectivity. Moreover, due to logistical limitations, the potential of each pool as a source of colonists was approached from its area, which seemed reasonable given previous evidences of strong, positive correlations between fish abundance and pool size (Appendix II). Nevertheless quantification of fish numbers and sizes in each dry-season pool would likely improve connectivity estimates. Also, using species- and size-specific average movement distances would probably be beneficial, given inter-specific and ontogenetic differences in movement ability and colonization rate have been reported elsewhere (Lonzarich *et al.*, 1998; Albanese *et al.*, 2009).

Our study also provides evidence for occasional association between neighbourhood effects and the fish species richness, abundance and occurrence. This agrees with large-scale studies pointing to the positive influence of neighbouring effects on fish assemblage structuring (Magalhães *et al.*, 2002a; Rich *et al.*, 2003; Grenouillet *et al.*, 2004; Thornbrugh & Gido, 2010; Woodford & McIntosh, 2011). These associations are not straightforward interpretable as they may be related to several underlying processes, such as small-scale connectivity (Schlosser, 1995), spatial clusters of favourable habitat (Heikkinen *et al.*, 2005), spawning aggregations (Heikkinen *et al.*, 2004), and unmeasured local habitat features causing spatial autocorrelation (Keitt *et al.*, 2002). Herein, neighbouring effects in the occurrence of nase and medium size chub in February 2006, within the reproductive period of both species (Magalhães *et al.*, 2003) were probably related to spawning aggregations promoted by spawning habitat arrangement and biotic contagious processes. Small scale connectivity appears unlikely to shape this, as fish were spread across the stream segments, though lack of data for mature and immature individuals makes difficult to rule out which process was responsible for the observed patterns. Conversely, as no non-native species in the Torgal stream reproduce during winter and early spring (Pires, D.F. & Magalhães unpublished data), positive neighbouring effects found for non-native species richness in February and April 2006, and pumpkinseed occurrence in February 2006, are more likely to reflect small scale connectivity and clustering of favourable habitat. This same set of factors may shape patterns for barbel in November 2006 and March 2007, as this species was mainly found in the neighbourhood of dry-season pools. Small scale connectivity effects may also be reflected in patterns for non-native species richness and abundance and nase occurrence in November 2006, as flow was re-established early before, and redistribution processes could still be at play. Finally, neighbourhood effects in chub occurrence in July 2007 may reflect aggregation processes owing to immigration to pools, prior to drought (Magoulick & Kobza, 2003; Davey & Kelly, 2007).

A deeper insight about the relative roles of habitat quality, connectivity to dry-season refugia, and neighbourhood effects in shaping fish assemblage structure could be achieved by modelling species abundance instead of occurrence, as other studies indicate that the former may be more dependent on distance to sources (Peterson & Bayley, 1993; Sheldon & Meffe, 1995; Lonzarich *et al.*, 1998; Woodford & McIntosh, 2011). Also, tracking fish movements over multiple spatial scales would help clarify the importance of habitat connectivity and neighbourhood effects, by disclosing underlying dispersal and colonization processes (Bélisle, 2005; Fullerton *et al.*, 2010).

5.5.2. Fish-habitat relationships

Our results corroborate other studies in mediterranean streams, disclosing several associations between fish species richness, overall abundance, and occurrence and channel hydro-morphology, streambed substrate and cover, and bank cover (e.g. Grossman & de Sostoa, 1994; Godinho *et al.*, 1997; Pires *et al.*, 1999; Mesquita *et al.*, 2006; Morán-López *et al.*, 2006; Santos & Ferreira, 2008). Moreover, this study highlights considerable temporal variation in fish habitat-associations as suggested by previous research (Grossman & de Sostoa, 1994; Mesquita *et al.*, 2006; Santos & Ferreira, 2008), which may reflect circumstantial habitat requirements during post drought recovery, spawning, pre-drought movements, and refugia search (Schlosser & Angermeier, 1995). There was also considerable variation in habitat–associations among chub size classes, suggesting that fish may show variable requirements, and shift habitat throughout the life cycle (Schlosser & Angermeier, 1995).

Overall and native species abundances seemed to be consistently promoted by low bank arboreum cover. This contrasts with other studies revealing positive associations between native species and tree canopy in mediterranean systems (Collares-Pereira *et al.*, 1995; Morán-López *et al.*, 2006), although ambiguous associations of canopy and native species abundance have been previously found (Chapter 3). Given riparian corridors are generally well developed in the Torgal stream, causing considerable shading, our results may reflect a tendency for fish abundance increasing in habitats where primary production is promoted (Schiller *et al.*, 2007).

Current velocity appeared to have consistently strong associations with eel, loach, nase, and chub occurrence, in agreement with previous findings in other mediterranean streams (Perdices & Doadrio, 1997; Mesquita *et al.*, 2006; Santos & Ferreira, 2008). Temporal prevalence of the effects of current velocity suggests that this attribute may act as primary environmental filter (Poff, 1997) at the macrohabitat scale, that occasionally interacts with other habitat features, displaying variable importance throughout the hydrological cycle.

5.6. CONCLUDING REMARKS

Previous research on temporary rivers pointed to the importance of habitat quality (Bond & Lake, 2003), connectivity to dry-season refugia (Davey & Kelly, 2007) and neighbouring effects (Magalhães *et al.*, 2002a) in structuring fish assemblages. Our results highlight that habitat quality may be key in shaping assemblage structure of fish in mediterranean streams, as fish redistribute quickly after dry-season drought throughout stream segments, according to the characteristics of local habitats. Connectivity to dry-season pools and neighbourhood effects appeared less important than habitat quality, though call out for further investigation in different contexts of disturbance, i.e. frequency and intensity of drought. Moreover, our results highlight considerable temporal variation in fish-habitat associations, stressing the importance of adopting multi-temporal rather than snapshot approaches in assessing fish assemblage organization. Additional studies disclosing processes beyond fish habitat selection over the hydrological cycle are needed to advance knowledge about fish assemblage dynamics in seasonally-drying mediterranean streams and in other temporary streams.

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Chapter 6

GENERAL DISCUSSION

6. GENERAL DISCUSSION

In the current chapter the main findings of each research activity presented in the previous sections are summarized, discussed in an integrated manner, and explored in light of current theoretical framework and empirical knowledge about fish ecology in streams. The implications of these findings to the conservation management of fish and habitats in mediterranean riverscapes are also approached.

6.1. MAIN FINDINGS

This thesis has contributed to increase the understanding of fish assemblage structuring in seasonally-drying mediterranean streams, by examining associations between fish distribution and abundance and habitat quality, spatial context (Chapters 3 and 5), and movement patterns (Chapter 4). Taken together, the results of this thesis also improved empirical evidence on fish ecology in temporary streams, particularly in two key issues about which information remained scarce: the use of refugia during drought, and the factors and processes influencing fish distribution after flow resumption. Evaluation of the relative influence of morphology, physicochemical characteristics, and spatial location on the use of dry-season pools (Chapter 3) provided important data to assess the quality of pools as refuge for fish during drought. Analysis of movement patterns (Chapter 4) provided the first empirical evidence on fish displacements out of dry-season refugia in mediterranean streams, allowing the identification of pathways for flux of individuals to and from wet-season habitats. Finally, comparative assessment of the influences of local habitat quality, neighbourhood effects, and connectivity to dry-season refugia on fish distribution during the wet-season was essential to better understand ecological links between habitats (Chapter 5). This information is important from both theoretical and applied perspectives, to establish spatial boundaries of populations and assemblages and define appropriate scales for further ecological research but also to devise more adequate conservation management and restoration frameworks for drought-prone streams.

In brief, the main findings of this thesis can be summarized as follows:

i) Assemblage structure of fish in dry-season pools was more strongly associated with morphological and physicochemical characteristics of the pools than with their spatial context (Chapter 3). In general, large pools held the highest species richness, and the highest richness and abundance of native fish was found in pools well shaded by riparian cover. However, there was considerable variation in the use of dry-season pools between different species and size-classes, with relative abundances and assemblage structure presenting consistent changes contingent on canopy cover, substrate composition, and spatial location.

ii) Fish displayed considerable variability in movement behaviour and displacement extent out of dry-season pools and between wet-season habitats (Chapter 4). A large proportion of Mira chub moved out of dry-season pools, whereas during the wet-season most fish were sedentary. Pool location and fish size were the most influential factors shaping whether Mira chub remained in or moved out of dry-season pools and wet-season habitats, respectively. Displacements of Mira chub out of dry-season pools were generally larger than those out of wet-season habitats, though in both cases movements directed to persistent and ephemeral habitats, and ranged from ten to hundreds of meters.

iii) Fish distribution during the wet-season was strongly associated with habitat quality, and showed weaker and inconsistent associations with connectivity to dry-season refugia and neighbourhood effects (Chapter 5). Although there was considerable variation in fish-habitat associations over time and among species and size classes, current velocity emerged as the most important factor shaping the occurrence of native species. In general, native species tended to occur at low flowing sites, with low bank arboreum cover additionally promoting overall and native species abundances.

6.2. DRIVERS OF FISH DISTRIBUTION AND ABUNDANCE

The results of the present thesis clearly add to the growing evidence that contemporary patterns of distribution, abundance and assemblage structure of stream fish are driven by multi-scale processes (e.g. Angermeier & Winston, 1998; Magalhães *et al.*, 2002a; Bond & Lake, 2003; Scheurer *et al.*, 2003). The role of habitat quality has been previously linked to variation in fish assemblages among macrohabitats, across and among basins, and among ecoregions (e.g. Eros *et al.*, 2003; Eros & Grossman, 2005; Yamazaki *et al.*, 2006; Ferreira *et al.*, 2007; Fischer & Paukert, 2008; Rowe *et al.*, 2009). Similarly, previous studies have highlighted that spatial context, such as position in the riverscape and connectivity, contributed to assemblage patterns at the basin scale (Rich *et al.*, 2003; Grenouillet *et al.*, 2004; Smith & Kraft, 2005; Davey & Kelly, 2007; Isaak *et al.*, 2007; Hitt & Angermeier, 2008; Thornbrugh & Gido, 2010; Nislow *et al.*, 2011; Woodford & McIntosh, 2011). The current thesis enriches the existent knowledge of stream fish ecology by disclosing the role of local habitat characteristics and spatial context in assemblage structuring at the stream segment scale (Chapters 3 and 5). Moreover, by assessing the relative roles of both components, this thesis highlighted that local habitat may be the main driver of fish distribution and abundance in mediterranean streams, at this spatial scale.

Despite the minor importance of spatial relationships relative to local habitat in shaping fish distribution and abundance during the wet-season, patterns of Mira chub movement (Chapter 4) uncovered complex connections among habitats. Movement by fish clearly emerged as an important process contributing to assemblage patterns, with fish moving out of dry-season pools and redistributing at a variety of distances, contingent on pool location in the stream network. This agrees with the increasing number of studies showing that movement patterns of small bodied, non-migratory stream fish are much more complex than previously thought (Skalski & Gilliam, 2000; Rodriguez, 2002; Albanese *et al.*, 2004; Petty & Grossman, 2004; Roberts & Angermeier, 2007a; Roberts &

Angermeier, 2007b; Belica & Rahel, 2008; Roberts *et al.*, 2008; Skyfield & Grossman, 2008; Albanese *et al.*, 2009; Breen *et al.*, 2009; Alldredge *et al.*, 2011). Moreover, results herein revealed a pattern of variability in the rates and extension of Mira chub movement that does not comply with the assumptions of the Restricted Movement Paradigm (Gerking, 1959), that as long been considered the norm in stream fish ecology.

6.2.1. Habitat

The importance of local habitat in shaping fish assemblages suggested in the current thesis corroborates previous findings in mediterranean streams disclosing multi-scale associations between fish and habitat attributes (Godinho *et al.*, 1997; Pires *et al.*, 1999; Godinho *et al.*, 2000; Corbacho & Sanchez, 2001; Filipe *et al.*, 2002; Magalhães *et al.*, 2002b; Pires *et al.*, 2004; Clavero *et al.*, 2005; Morán-López *et al.*, 2006; Pires *et al.*, 2008; Filipe *et al.*, 2010). The results herein added up to the previous knowledge by: identifying habitat characteristics associated with assemblage structure of fish assemblages in dry-season pools; revealing temporal variation in habitat characteristics associated with fish distribution over the wet-season; highlighting the importance of habitat heterogeneity, by disclosing species- and size-specificity in fish-habitat associations, both in dry-season pools and wet-season habitats.

Functional heterogeneity in dry-season pools was strongly associated with variation in morphology and physicochemical conditions (Chapter 3). Species richness was higher in large dry-season pools as reported in other temporary streams (Capone & Kushlan, 1991; Taylor, 1997; Magalhães *et al.*, 2002b; Dekar & Magoulick, 2007). This might reflect an increase of habitat diversity and stability, as large pools are most likely to persist through the dry-season (Labbe & Fausch, 2000; Magalhães *et al.*, 2002b), probably resulting in more favourable habitat conditions to pool specialists like pumpkinseed or largemouth bass (Moyle, 2002). Variation of pool size seemed also to be linked with differences in assemblage size structure. Assemblages tended to shift from relatively large abundances of small fish (i.e. small Arade chub and arched-mouth nase) in small pools towards reduced abundances of large fish (i.e. pumpkinseed) in large pools, similar to reports for other systems (Schlosser, 1987; Capone & Kushlan, 1991). The importance of habitat diversity was also denoted by changes in relative abundances of different species and size classes, associated with variation in physicochemical characteristics. Specifically, species abundances were associated with substrate and canopy, characteristics that were previously found to be important correlates of fish distribution and abundance in mediterranean streams (e.g. Collares-Pereira *et al.*, 1995; Godinho *et al.*, 1997; Pires *et al.*, 1999; Mesquita *et al.*, 2006; Morán-López *et al.*, 2006; Ferreira *et al.*, 2007).

Fish distribution and abundance during the wet-season were associated with multiple habitat characteristics (Chapter 5), with habitat associations also varying among species and size classes, and some being time-specific. Overall and native species abundances appeared to be consistently promoted by low bank arboreum cover, in contrast with other studies revealing positive associations between native species and tree canopy in mediterranean systems (Collares-Pereira *et al.*, 1995; Morán-López *et al.*, 2006). However, ambiguous associations between canopy and native species abundance have also been found in dry-season pools (Chapter 3). Moreover, changes in fish-habitat associations are expected to occur among riverscapes, according to the interplay between the range of environmental variation and the sensitivity of fish species in the regional pool (Angermeier & Winston, 1998). Given riparian corridors in the study area are generally well developed relative to other mediterranean streams, current results may reflect a tendency for fish abundance increasing in less shaded reaches where primary production is promoted (Schiller *et al.*, 2007). Conversely, current velocity emerged as the main driver of species occurrence, in agreement with previous reports in other mediterranean streams (Perdices & Doadrio, 1997; Mesquita *et al.*, 2006; Santos & Ferreira, 2008). Associations with current velocity were prevalent over time, suggesting that this attribute may act as primary environmental filter (Poff, 1997) at the macrohabitat scale that interacts occasionally with other habitat features that display variable importance across the wet-season. There was also considerable variation in habitat–associations among size classes of

Mira chub, suggesting that fish may display life stage-specific patterns of habitat use (Schlosser, 1995; Labbe & Fausch, 2000). Taken together results obtained in this thesis (Chapters 3 and 5) point out that habitat heterogeneity may be critical to sustain species and size classes showing variable habitat requirements throughout the hydrological cycle (Schlosser & Angermeier, 1995).

6.2.2. Spatial context

Current conceptual frameworks in stream fish ecology give great emphasis to the role of spatial habitat arrangement in shaping assemblage and population dynamics. Fish may need to move across habitats that provide circumstantial resources, in order to meet their life cycle requirements, and the spatial arrangement and connectivity among habitats are considered critical riverscape properties that influence spatial dynamics of fish (Schlosser, 1991; 1995; Schlosser & Angermeier, 1995; Fausch *et al.*, 2002; Pringle, 2003). In temporary streams the spatial distribution of fish is expected to be particularly influenced by the distribution of refugia within the riverscape and connectivity among habitats that limit movements to and from refugia (Labbe & Fausch, 2000; Magoulick & Kobza, 2003; Larned *et al.*, 2010; Sheldon *et al.*, 2010; Arthington & Balcombe, 2011). The results gathered in this thesis suggest that at stream segment scale patterns of fish distribution and abundance in seasonally-drying mediterranean streams are related to, but not severely constrained by the spatial configuration of habitats.

The spatial context of dry-season refugia has been previously associated with the assemblage structure of fish in refugia, across stream segments (Taylor, 1997) and basins (Magoulick, 2000; Magalhães *et al.*, 2002b; Balcombe *et al.*, 2006; Love *et al.*, 2008; Arthington *et al.*, 2010; Beesley & Prince, 2010). Specifically, fish assemblage patterns were related to factors reflecting refugia location in the riverscape (Taylor, 1997; Magalhães *et al.*, 2002b), stream order (Beesley & Prince, 2010), proximity to other streams and location in the drainage (Magoulick, 2000), width of surrounding floodplains and distance to nearest refugia (Arthington *et al.*, 2005), and distance to floodplain edge

(Arthington *et al.*, 2010). This thesis also highlighted some associations among assemblage patterns and spatial location of dry-season pools, but the interpretation of these associations was not straightforward. For instance, spatial location may reflect local variability in habitat stability and diversity, and colonization opportunities (Schlosser, 1987; Taylor, 1997; Hitt & Angermeier, 2008; Love *et al.*, 2008), whereas as distance to near refugia or floodplain width evaluate indirectly the role of connectivity to local sources on assemblage patterning (Arthington *et al.*, 2005; Arthington *et al.*, 2010). Herein, perceived associations with spatial location are most likely to reflect structured spatial variation in habitat characteristics given there were significant shared contributions between spatial context and habitat attributes of dry-season pools. Distance to near refugia seemed to have minimal influence in the assemblage patterns, as found in intermittent Australian streams (Balcombe *et al.*, 2006; Beesley & Prince, 2010).

The minor importance of spatial context, relative to physicochemical and morphological characteristics in shaping fish assemblages patterns in dry-season pools detected in this thesis may reflect the existence of adaptative mechanisms to natural breaks in hydrological connection in native fish fauna (Lake, 2003). The long term exposition to substantial and total desiccation on a predictable, seasonal basis may impose a strong selective pressure on fish to emigrate from intermittent and ephemeral habitats as flow declines (Davey & Kelly, 2007). Laboratorial experiments seem to support this view, revealing that fish native to intermittent streams respond to flow recession by moving to deeper habitats (Davey *et al.*, 2006). This is also sustained by some preliminary field evidences. Triggering of fish movement has been linked to the loss of shallow microhabitats during channel dewatering (Roberts & Angermeier, 2007a), and the probability of some fish species emigrating from a habitat appears to be higher in intermittent than perennial sites (Albanese *et al.*, 2004). There are also some field indications that fish actively seek refugia (Davey & Kelly, 2007), and that refugia choice is species-specific (Hodges & Magoulick, 2011). However, mechanisms behind refugia selection, generating assemblage patterns in dry-season refugia remain unclear and poorly addressed. There is still no explanation for fish straying in dry habitats (Davey & Kelly, 2007), and there is evidence that not all species respond to site intermittency (Albanese *et al.*, 2004), and that fish may choose habitats where survival is diminished (Hodges & Magoulick, 2011). Further manipulative and field studies with several species are required to uncover fine movement triggers and pathways of dry-season refugia use.

The role of the refugia location in shaping fish distribution and abundance during periods of hydrological connection has rarely been explicitly approached, despite being widely highlighted as important to population dynamics (Labbe & Fausch, 2000; Magoulick & Kobza, 2003). Apart from the study in the thesis (Chapter 5), only one study has previously addressed this issue. In a New Zealand intermittent stream, Davey & Kelly (2007) found that proximity to refugia was linked to faster and higher rates of assemblage recovery from drought. Results in this thesis pointed to a less important role of connectivity to long term persistent refugia in driving wet-season assemblage patterns, thought comparison between studies is limited by the use of different connectivity metrics. Also there is considerable variability between studies in stream intermittency settings and scale of analysis. The stream studied by Davey & Kelly (2007) had a high intermittency degree, with some sites staying dry over six months, and suffering more than four intermittency episodes. Over a similar period of time, in the Torgal stream, sites remained dry at most four months, and hydrological disconnection occurred only twice. According to their own results, Davey & Kelly (2007) considered that the influence of connectivity to refugia was related to the frequency and duration of intermittency. Fish assemblages in less disturbed sites recovered fully during prolonged wetted periods, while in frequently dry sites recolonization was slow, and assemblages differed from those in neighbouring perennial reaches. Plus, Davey & Kelly (2007) focused in fish assemblage recovery at the basin scale, with sites locating at least 3 km from refugia. Herein, assemblage patterns were analysed at the segment scale, and maximum distance to dry-season pools was 0.5 km.

Overall, the minor effects of connectivity to refugia on assemblage structure found in this thesis (Chapter 5) may indicate quick and effective post-drought redistribution of native fish, corroborating evidence for other temporary streams. Other studies in mediterranean streams have shown that fish density at the reach scale may decrease between the dry- and wet-seasons, indicating quick seasonal redistribution from refuge habitats (Bravo *et al.*, 2001). In a semiarid, Brazilian stream, reestablishment of hydrological connectivity for 20 days in a stream reach, leaded to higher fish species diversity due to colonization by species absent in the dry-season, and to decreased species dominance due to redistribution among habitats, from dry-season to wet-season (Medeiros & Maltchik, 2001). In an Australian semi-arid floodplain river experiencing short periods of hydrological connectivity during flood pulses, fish species richness and total biomass were uniformly spread across the floodplain (Arthington & Balcombe, 2011). Rapid recovery of fish assemblages suggests that fish respond quickly to the reestablishment of hydrological connectedness. In a North-American semiarid stream fish were observed on the leading edge of flow, as it rewetted a stream segment, and were sampled in pools that were dry a few hours before (Scheurer *et al.*, 2003). Experimental defaunation studies also have shown that recolonization of vacant habitats at the segment and reach scales may occur in hours to weeks, with local fish species richness and abundances returning rapidly to pre-defaunation levels (Peterson & Bayley, 1993; Sheldon & Meffe, 1995; Lonzarich *et al.*, 1998).

Nevertheless, the current results do not exclude that refugia location might have effects on fish assemblage patterns at spatial scales other than the stream segment. Important effects of refugia location in patterns of fish assemblages in mediterranean streams have been highlighted in studies carried out at the basin scale, in the Mira and neighbouring drainages across Southwest Portugal (Magalhães *et al.*, 2002a). Plus, there is evidence of long lags in fish assemblage recovery in ephemeral habitats in the Guadiana basin (Pires *et al.*, 1999), and of differential recovery lags associated with spatial position across the Torgal basin after severe drought (Magalhães *et al.*, 2007). This calls for further assessment of effects connectivity to dry-season refugia on assemblage structuring, across multiple spatial scales and under various environmental contexts.

Fish assemblage patterns were also occasionally associated with neighbourhood effects (Chapter 5). This suggests that small scale, spatial configuration of habitats might play a role in fish distribution. Underlying processes generating the observed patterns were probably related to small-scale connectivity (Schlosser, 1995), spatial clusters of favourable habitat (Heikkinen *et al.*, 2005) or biotic contagious processes (e.g. spawning) (Heikkinen *et al.*, 2004). However, identifying the proper processes underlying perceived patterns would require tracking of fish movement at smaller spatial scales that the one used in this thesis.

In highly dynamic and spatially complex stream environments, movement is an important mechanism, that links fish to habitats and underlies important ecological processes such as habitat use and population dynamics (Schlosser & Angermeier, 1995; Bélanger & Rodríguez, 2002; McMahon & Matter, 2006). Fish movement in temporary streams remains poorly addressed despite the recognition of its role in fish population and assemblage spatial dynamics (Magalhães *et al.*, 2002a; Larned *et al.*, 2010; Arthington & Balcombe, 2011). In a pioneer study tracking fish movement, Labbe & Fausch (2000), highlighted the importance of habitat heterogeneity for population persistence and dynamics in an intermittent North-American stream. Fish used permanent spring pools over winter and summer harsh conditions, but moved across stream reaches when hydrological connectivity was restored to colonize habitats that were of poor quality as refugia but provided good feeding and spawning conditions. Herein, assessments of movements by the Mira chub (Chapter 4) seemed to reveal similar patterns of population spatial dynamics and complementary habitat use. A high proportion of chub moved out of dry-season pools to colonize ephemeral habitats, suggesting that a least some fish leaving dry-season pools may use ephemeral habitats for feeding and rearing during the wet-season. The uncovered patterns of movement by the Mira chub also may explain at least partially, why connectivity to dry-season pools had a minor role in fish distribution over the wet-season in the Torgal stream. Indeed, the Mira chub redistributed from dry-season pools to other habitats at variety of distances, and to a less extent continued to move among habitats during the wet-season. Moreover, there were no relationships between time lag since marking and movement probability and distance moved, indicating that fish redistribution from dry-season refugia may occur early in the wet-season. Conversely, limited movement rates during the wet-season, as suggested by the high proportion of sedentary recaptures, might be responsible for spatial structuring associated with neighbouring effects. Positive associations between fish body size and probability of movement may also explain why distribution of medium sized Mira chub but no large Mira chub was episodically related to connectivity to refugia and neighbouring effects.

Results from this thesis, clearly revealed important links between dry-season pools and ephemeral habitats allowing a first insight on the way populations and habitats might be spatially connected in mediterranean streams. However, further research on movement and population and assemblage structuring over multiple spatial and temporal scales will be needed to advance current knowledge on fish population and assemblage dynamics and the mechanisms that regulate them. Specifically, testing for emerging concepts in temporary stream ecology, as metapopulation dynamics and metacommunity structure (Larned *et al.*, 2010; Sheldon *et al.*, 2010), is likely to be valuable not only to basic fish ecology but also to multiple species conservation and management in mediterranean streams.

6.3. CONSERVATION AND MANAGEMENT IMPLICATIONS

A large number of freshwater fish species in mediterranean streams are threatened with extinction (i.e. they are *Critically Endangered, Endangered,* or *Vulnerable*) or are already extinct, due to human-induced habitat loss and fragmentation (Smith & Darwall, 2006; Hermoso & Clavero, 2011). Climate change is expected to accelerate habitat degradation in these systems, with current climate scenarios forecasting prolonged droughts and significant reductions in water availability, which may result in increased water-allocation conflicts (Beniston *et al.*, 2007; Iglesias *et al.*, 2007). To mitigate the conflicts between environmental water and human demand, proactive management and conservation measures are needed to maintain ecological processes and species diversity in seasonally drying mediterranean streams (Hermoso & Clavero, 2011).

According to the results obtained in this thesis, management and conservation measures for fish fauna in seasonally drying mediterranean streams must be based on a holistic, integrated, and broad approach that recognizes the multiple scale processes shaping the structure of contemporary assemblages. Management measures should focus on sustaining the diversity of habitats and connectivity among the habitats, and give particularly attention to habitats and pathways which are critical for population persistence and recovery.

The identification and protection of refugia from drought should therefore be an essential component of conservation management in mediterranean streams, particularly during periods of severe and extended drought. Conservation management should have the primary objective of evaluating the availability and the value of individual pools to the persistence of local populations, but most importantly, of sustaining a heterogeneous network of pools that assures effective refugia for different species and size classes, across the riverscape. In cases in which this might not be a realistic management perspective (Falke *et al.*, 2011), at least some selective refugia protection measures should be thought and implemented. For instance, managers should strive to maintain large pools that generally sustain high species diversity, and pools with well developed canopies that appeared as key refugia for native and endemic species that are currently highly endangered. Preventing and controlling water abstraction from pools during the dry-season is also of particular importance, to assure its persistence throughout the dry-season and maintain environmental conditions under the tolerance limits of native species.

Protection of dry-season pools must be associated with the maintenance of adjacent, diverse habitats (e.g. gradients of current velocity, wood debris, and heterogeneous substrates), that provide critical resources for fish of multiple species and sizes throughout the wet-season and pathways for fish movement between them. This mean to assure the natural processes responsible for creating (e.g. floods), and maintaining complex and diverse habitats (Gasith & Resh, 1999; Bond & Cottingham, 2008). In practice, maintenance of flow regime as close as possible to natural appears as the most effective measure to attain this goal (Poff *et al.*, 1997; Maltchik & Medeiros, 2006; Arthington & Balcombe, 2011). Natural flows will generally assure the levels of hydrological connectivity that trigger and promote movement by native fish, and underlie spatial distribution and dynamics according to local habitat characteristics. In addition, natural flows are likely to prevent or to hold the proliferation of non-native species, as these appear to be less able to cope with harsh hydrological events such as floods (Bernardo *et al.*, 2003; Magalhães *et al.*, 2007; Propst *et al.*, 2008). If maintenance of flow regime reveals unrealistic, the identification of flow requirements for highly valued species and ecological processes might be necessary (Larned *et al.*, 2010). This will imply the determination of flow components such as frequency, intensity, and duration of floods and droughts, necessary to maximize species and habitat diversity and maintain critical riverscape processes. Maintenance of stream channels clear from barriers that obstruct fish movement will also be crucial, especially during receding flows when fish must actively seek for refugia habitats.

The importance of good management practices and conservation of freshwater systems and associated biodiversity has been fully recognized in the European Union, with the development and application of the Water Framework Directive (WFD) and associated legal instruments (2000/60/European Council). Achieving the good ecological status of streams and rivers will require the development of pro-active actions, to be defined in specific management plans for each river basin, and the promotion of sustainable water use trough regulatory measures such as water pricing. Besides, the threats imposed to society and biodiversity by increasing water scarcity and droughts have already been recognized (EC, 2007), and specific guidelines to promote more sustainable water use and minimise drought effects have been defined (EEA, 2009). These include the reinforcement of water price policies, improvement of water use efficiency and conservation, changes of agricultural policies, promotion of water recycling, tackling of illegal water use, and the development of specific drought management plans. The effective implementation of the current mandatory measures would certainly help to protect aquatic biodiversity and ecosystem structure and function in seasonally-drying mediterranean streams. However, enforcing the effectiveness of the WFD and associated water management guidelines policies in these systems would also imply finding better water management approaches from the ecological perspective. This can only be achieved by recognizing the specificity of mediterranean streams relative to temperate streams, and that they might need distinct management approaches from perennial streams (Larned *et al.*, 2010). Currently, this appears not be fully guaranteed under the WFD as it does not fully account for the diversity of flow regimes across Europe, as for instance temporary and intermittent river categories are absent from the river classification systems (von Schiller *et al.*, 2011). This might result from the erroneous perception of the representativeness of these ecosystems and their importance within the European Union as well as from lack of sound scientific knowledge about its basic functioning (Larned *et al.*, 2010; von Schiller *et al.*, 2011). The continued development of studies on the ecology of seasonally drying mediterranean streams is therefore fundamental to provide a solid basis for the development, improvement and implementation of effective conservation management actions for aquatic biodiversity and sustainable water use.

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Chapter 7

CONCLUDING REMARKS

7. CONCLUDING REMARKS

The main aim of the current dissertation was to examine the role of habitat quality, spatial context, and movement on the patterns of fish distribution and abundance in seasonally-drying mediterranean streams, at the segment scale. The research conducted allowed the identification of morphological, physicochemical, and spatial factors contributing to the use of dry-season refugia, which are widely recognized as playing a major role in fish persistence in temporary streams. Moreover, to our knowledge, this was the first study in mediterranean streams that explicitly characterized relationships between dry-season refugia arrangement across the riverscape and patterns of species richness, abundance and occurrence during the wet-season, and quantified displacements of fish out of dry-season refugia and among wet-season habitats.

Most important results emerging from this study can be summarized as follows:

i) Heterogeneity in dry-season pool refugia may be critical for persistence of different species and size classes. Overall, species distribution and abundance were more related to dry-season pool morphology and physicochemical characteristics than to spatial context. Specifically, patterns in species and size-class abundances changed with pool size, canopy cover, and substrate composition. Larger pools appeared as important habitats as they shelter higher number of species, but pools well shaded by riparian canopy held the highest abundance of native species.

ii) Movement may be a key mechanism in fish population dynamics, promoting fish redistribution from dry-season pools, colonization of ephemeral habitats at a variety of distances, and favouring the flux of individuals at the stream segment scale, over the annual cycle. Fish moved more often and displaced further away from dry-season pools than during the wet-season, though in both cases movement directed to persistent and ephemeral habitats and ranged from ten to hundreds of meters.

iii) Local habitat characteristics constituted the most obvious driver of fish species richness, abundance, and distribution during the wet-season while connectivity to dry-season pools and neighbourhood effects were episodically important. Variation in fish-habitat associations over time and among species and size classes highlighted the importance of riverscape habitat heterogeneity to fish distribution. Local habitat characteristics, such as current velocity and bank arboreum cover were the most important factors shaping the occurrence of native species.

Finally, it was concluded that fish distribution was not severally constrained by the spatial arrangement of habitats across stream segments reflecting the adaptability of the native fish species to the annual cycle of habitat contraction and expansion and variation of hydrological connectivity.

7.1. FUTURE RESEARCH DIRECTIONS

A large proportion of freshwater fish inhabiting mediterranean streams are currently listed as endangered (Smith & Darwall, 2006; Hermoso & Clavero, 2011). Predictions of increasing human pressure on freshwater resources coupled with climate change projections indicating increased likelihood of droughts (Beniston *et al.*, 2007; Iglesias *et al.*, 2007) suggest that current threats are likely to maintain or to intensify in the future. In these circumstances, more comprehensive and detailed knowledge of fish ecology in mediterranean streams is needed, to base effective conservation and management actions (Alvarez Cobelas *et al.*, 2005; Magalhães *et al.*, 2007; Grantham *et al.*, 2010; Hermoso & Clavero, 2011). Results in this thesis emphasized the need to adopt ecologically sound, multi-scale frameworks that recognize the hierarchical nature of stream environments and the temporal dynamism of mediterranean streams habitats. Advances in ecology of stream fish in mediterranean streams would benefit from developing further the questions related to population and assemblage dynamics approached herein, in addition to other key issues that remain poorly addressed. Furthermore, it will be worthy to direct ecological research on fish in mediterranean streams towards more accurate and quantitative approaches.

Some important points for future research emerging from this thesis can thus be pointed, as follow.

Examining associations between habitat quality and fitness of individuals (e.g. growth, survival, reproduction success) and populations (e.g. production), from the microhabitat up to the macrohabitat scale (i.e. pools and runs), might provide a finer identification of critical habitat features that define optimal dry-season and wet-season habitats. This will allow the distinction between occupation of optimal habitat, due to true requirements, from occupation of sub-optimal habitat, derived by other processes (e.g. density dependent processes, predation) some of which may actually have negative fitness (e.g. sinks habitats). This kind of information will provide a more solid basis for identifying suitable habitats over a broad range of spatial scales, and may serve as a guide for advanced management practices, such as habitat restoration (Rosenfeld, 2003; Rosenfeld & Hatfield, 2006; Budy *et al.*, 2011).

Changing from descriptive correlational to mechanistic approaches in which predictions of distribution, abundance, and fitness are based on explicit biological mechanisms (e.g. bioenergetics models), will promote the identification of causal relationships between explanatory factors and biological responses instead of indirect causal associations. For instance, evaluating fish energetic budgets across current velocity gradients could reveal processes behind the prevalent fish-velocity associations found in the current thesis (Rashleigh & Grossman, 2005). This approach could also be useful to understand further the role of physicochemical factors pointed as influential in refugia use (e.g. oxygen and temperature), for individual and population fitness, allowing a more adequate evaluation of the quality of refugia habitats (Magoulick & Kobza, 2003; Rashleigh & Grossman, 2005; Budy *et al.*, 2011).

The identification of feeding and spawning habitats while crucial for the understating of fish population dynamics remains poorly addressed. For instance, many native species are generally considered to spawn in running waters and coarse substrates

(Doadrio, 2001; Santos & Ferreira, 2008), but this view lacks strong empirical support. Besides, there are evidences that some species spawning requirements might be rather flexible, as an adaptation to the strong environmental variability of mediterranean streams (Elron *et al.*, 2006). Proper delineation of critical spawning and feeding habitats would help clarify which spatial habitat relationships (e.g. habitat complementation, supplementation) are potentially influent in population dynamics (Schlosser, 1995). Moreover, this may also contribute to a more adequate assessment of spatial relationships among habitats, by generating, for instance, more refined measures of spatial context to be used in assessments of dry-season refugia use. Identifying key features of critical feeding and spawning habitats would be also important from an applied perspective, to devise more effective restorations of stream habitat.

Biotic interactions are known to influence fish distribution patterns (Jackson *et al.*, 2001) but its role in shaping fish assemblages structure in mediterranean streams are under-addressed relative to that of abiotic factors (but see Magalhães, 1993; Godinho *et al.*, 1997; Godinho & Ferreira, 2000; Magalhães *et al.*, 2002; Clavero *et al.*, 2004; Blanco-Garrido *et al.*, 2008). Specifically, there is still scarce information on biotic interactions among fish in dry-season refugia, despite both competition and predation risks are expected to increase during this period (Magoulick & Kobza, 2003). This is of particular importance, owing several non-native piscivorous fish are now part of the species pool in many mediterranean streams (Hermoso *et al.*, 2011), and interactions among native and non-native species may intensify as fish became stranded in disconnected refugia as the drying progress (Propst *et al.*, 2008).

The quantification of spatial relationships among stream habitats and fish populations and assemblages is increasingly considered key in ecology and management of stream fish (Schlosser & Angermeier, 1995; Moilanen *et al.*, 2008; Hermoso *et al.*, 2010).However, this will require the employment of different and complementary ecological approaches and tools (Gido *et al.*, 2006; Pease *et al.*, 2006; Cook *et al.*, 2007; Koizumi *et al.*, 2008; Durbec *et al.*, 2010; Zeigler & Whitledge, 2010; Koizumi, 2011).

Multi-scale analysis of fish movement can provide insights on spatial patterns of habitat use, flux of individuals, and colonization rates across a broad range of spatial and temporal scales. Continuous tracking of fish over short time frames would help define home ranges and the extent of explorative movements both during the wet- and the dry-seasons. Long term (1 to more years) fish tracking would disclose spatial relationships at larger spatial scales and provide useful information on habitat use and fidelity to refugia. Moreover, complementary use of population genetics markers would help disclosing large scale dispersal rates (e.g. basin), and advance understanding of long-term effects of seasonal fragmentation in dry-season refugia on population spatial structure (Henriques *et al.*, 2010; Huey *et al.*, 2011; Koizumi, 2011).

Assessing population demographic parameters would also be essential to clarify population structure and ecological processes that shape it over several scales. Evaluation of immigration and extinction parameters, at small spatial scales, would be essential to help understanding post-drought colonization dynamics of ephemeral habitats and pre-drought dispersal to dry-season refugia. Specifically, this will provide valuable empirical evidence on the order and timing of species arrivals and departures, the length of time that each habitat remains unoccupied, and how this relates to spatial location and connectivity (Taylor & Warren Jr, 2001; Love *et al.*, 2008). Moreover, immigration rates could also be interpreted as an indirect measure of habitat quality (Bélanger & Rodríguez, 2002). Finally, analysis of variation and synchrony in population structure and survival parameters would indicate the degree of demographic dependence among separated habitats, and serve as an indirect measure of dispersal (Koizumi *et al.*, 2008). Plus, it would allow the evaluation of fitness in habitat selection at the population level, and the identification of optimal (e.g. sources) and non-optimal habitats (e.g. sinks) (Labbe & Fausch, 2000).

From the results of this thesis, metapopulation and metacommunity concepts emerge as particularly suited frameworks for advancing ecological research and multispecies conservation and management planning in mediterranean streams. These are naturally multi-scaled and multidisciplinary frameworks (see Hanski & Gaggiotti, 2004; Holyoak *et al.*, 2005) which have been increasingly applied in fish stream ecology and conservation (Schlosser & Angermeier, 1995; Peres-Neto & Cumming, 2010; Brown *et al.*, 2011; Koizumi, 2011), and were recently considered a critical body of research in temporary streams, where fragmentation and connectivity disruptions are natural ecological components (Falke & Fausch, 2010; Larned *et al.*, 2010; Sheldon *et al.*, 2010; Huey *et al.*, 2011). Future work on the mechanisms regulating stream fish population and assemblages in mediterranean streams will likely benefit greatly from the incorporation of metapopulation and metacommunity models, and evaluation of testable hypotheses including stream fish life history attributes, and seasonal and inter-annual environmental variability, across multiple spatial and temporal scales.

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Appendixes

Appendix I.

Evaluation of PIT-tagging in an endangered Iberian cyprinid.

Pires, D., Ribeiro, F. & Magalhães, M.F. (2010)
Journal of Applied Ichthyology, 26, 612-613.

INTRODUCTION

Tagging has been increasingly used in freshwater fisheries science to gain information on fish growth, movement and behaviour and for mark-recapture population assessments (Lucas & Baras, 2001; Gibbons & Andrews, 2004). However, effects of tagging on fish are still equivocal, with studies reporting adverse effects on survival, growth and behaviour (e.g. Armstrong & Rawlings, 1993; Knights & Lasee, 1996; Jepsen *et al.*, 2002) contrasted by those showing no adverse effects (e.g. Connors *et al.*, 2002; Acolas *et al.*, 2007; Newby *et al.*, 2007). Therefore, thorough evaluations of tagging reliability are needed for previously unstudied species to assure fish welfare and unbiased data collection (Lucas & Baras, 2001; Gibbons & Andrews, 2004). This is especially critical in rare and endangered species, as adverse tagging effects can strongly impact declining populations and result in poor conservation prescriptions.

Passive Integrated Transponders (PITs) are among the best candidates for uniquely marking small, endangered fish. PIT tags are small, have millions of unique alphanumeric codes and unlimited functional life, and allow repeated non-destructive sampling (Gibbons & Andrews, 2004). Recent studies have documented the suitability of PIT-tagging for small fish (e.g. Skov *et al.*, 2005; Ruetz *et al.*, 2006; Acolas *et al.*, 2007; Knaepkens *et al.*, 2007; Bolland *et al.*, 2009), though evidence for endangered cyprinids remains scarce (but see Ward *et al.*, 2008). This study evaluated the reliability of PIT-tagging for the Mira chub *Squalius torgalensis* (Coelho, Bogutskaya, Rodrigues & Collares-Pereira), a critically endangered cyprinid endemic to the Iberian Peninsula. Specific objectives were to analyse the retention of PIT tags and their short-term effects on survival, growth, and swimming performance of the Mira chub.

MATERIALS AND METHODS

Fish were captured in the wild (Torgal stream, SW Portugal) using electrofishing, and transported in aerated vats to the site of experiment (University of Lisbon). Fish injured by sampling, in poor condition and with diseases were discarded. Overall, 80 fish, 66-124mm fork length, were stocked into aerated aquariums (50 x 30 x 25 cm), with gravel bottom and filtered water. The range of lenght used was typically encountered in the wild and appeared acceptable for PIT-tagging based on results in other small fish (Acolas *et al.*, 2007; Ward *et al.*, 2008). Fish were acclimated to experimental conditions for seven days. Aquariums were under ambient conditions of temperature and photoperiod, in an isolated room. Fish were fed daily with a ration of commercial dry food, using automatic feeders.

Three treatments were considered: control, sham-tagging, and PIT-tagging. Treatments were tested on groups of four fish per aquariam, in six aquariums (i.e. 3 treatments X 6 replicates X 4 fish). Fish were randomly assigned to treatments, and aquarium effects were assumed to be null. All fish were anaesthetised using clove oil, as this has minimal behavioural and physiological impacts (Anderson *et al.*, 1997). Initial fork length (mm) and total weight (0.1g) were recorded. PIT tags (Trovan ID-100A, 2.12x11.5 mm) were inserted into the body cavity of fish through an incision made just off the midventral line of the body, anterior to the vent, using a sharpened needle (Ruetz *et al.*, 2006). Sham-tagging involved the surgical procedure but no transponder implant. Incisions were not sutured but clinical grade cyanocaricle adhesive (3M™ Vetbond) was applied to close the wound. Tag-to-body weight ratio ranged between 0.4 and 2.9 % in air. After treatment, fish were allowed to recover until they regained balance and swimming, and then returned to the experimental aquariums.

Fish were held for observation for 30 days. Aquariums were inspected for mortalities and tag retention throughout the experiment. Fish were scanned for tag presence and function using a handheld detector (Trovan LID 571, EID Aalten B.V.). Lost tags were scanned in the bottom of the aquariums, and manually removed. Dead fish and lost tags were not replaced. At days 1, 3, 7, and 30 (D1, D2, D3, D7 and D30), fish were video recorded for 30 minutes, between 9.00-11.30 a.m. Swimming performance was considered as the number of cells in a reference grid (16X9 cm mesh) crossed by each fish per 30 seconds, in scans taken at 5, 15, 25 min of record. Finally, all fish were measured and weighted at D30.

Contingency analysis was used to compare cumulative mortalities per treatment. One-way analysis of variance was used to test for variation on length and weight among treatments, and repeated measures ANOVA was performed on swimming counts to test for variation depending on treatment and time. Prior to analysis data were log10-transformed to comply with ANOVA assumptions of normality and homecedasticity (Zar, 1999). Significance of statistical testing was assessed at $P < 0.05$.

RESULTS AND DISCUSSION

Survival was not different among treatments (92-100%; $Chi^2=0.000$, $P=1.000$), with only two PIT-tagged (65 and 76 mm) and one sham-tagged fish (85 mm) lost in D1, D6, and D15, respectively. Length and weight at D30 showed no variation among treatments ($F=0.030$, $P=0.968$ and $F=0.223$, $P=0.801$, respectively). Swimming performance did not differ among treatments ($F=0.989$, $P=0.409$) or over time ($F=2.528$, $P=0.078$), with tagged fish reducing swim in D1 and D3 only (Table 1). Tag retention was 80%, with losses occurring from D7 to D29, in fish 66-75 mm.

PIT tags showed little or no short-term adverse effects on survival, growth and swimming performance of Mira chub. PIT-tagging did not markedly increase mortality or decrease growth of chub, consistent with evidence for other small fish (Ruetz *et al.*, 2006; e.g. Acolas *et al.*, 2007; e.g. Ward *et al.*, 2008; Bolland *et al.*, 2009). Likewise, PIT-tagging had minimal impact on swimming performance, similar to what has been reported for bullhead (Knaepkens *et al.*, 2007) and juvenile rainbow trout (Newby *et al.*, 2007). Finally, tag retention was comparable

Table 1 Comparison of traits of Mira chub *Squalius torgalensis* in the control, sham-tagging and PIT-tagging treatments, at 0, 1, 3, 7, and 30 days post-marking. Values are the mean ± standard deviation

		Fork Length (mm)		Total Weight (g)		Swimming Performance (Nr. of cells crossed per 30 sec)			
	Day post-marking	D0	D30	D0	D30	D1	D3	D7	D30
Treatment	Control	81.3±12.8	81.5±12.6	7.4±4.3	7.2±3.9	9.5±5.6	7.8±5.1	8.3±6.0	8.5±5.5
	Sham-tagging	80.8±13.5	80.8±13.6	7.2±4.7	6.9±4.3	10.0±9.6	8.9±4.4	12.4±5.9	5.2±1.7
	PIT-tagging	81.3±13.9	81.3±13.9	7.3±4.6	6.7±4.2	3.1±1.2	4.2±2.3	7.6±2.9	5.5±2.2

to that found in other experimental studies (Acolas *et al.*, 2007; Ward *et al.*, 2008; Bolland *et al.*, 2009).

Ultimately, these findings suggest that PIT tags were effective on Mira chub, and should be considered when selecting a method for uniquely marking other Iberian cyprinids. Specifically, PIT tags appear a promising alternative to standard techniques such as Floy and Visible Implant tags which are plagued by high tag-to-body size ratios and poor retention, respectively (see Prenda & Granado-Lorencio, 1994; Aparicio & de Sostoa, 1999). Nevertheless, additional research on the long-term effects and reliability of PIT tags under natural conditions is needed prior to their large-scale use in behaviour and population assessments in endangered Iberian cyprinids.

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Appendix 2

Fish abundance in dry-season pools in the Torgal stream

Fish abundance was estimated in 10 dry-season pools in the Torgal stream, during September 2007. Fish were sampled using multiple catch removal methods (2 to 5 fish passes per pool), and the number of fish in each pool (mean and 95% confidence limits) was estimated by the Zippin removal method (Zippin, 1956) in the software Mark (White & Burnham, 1999). Youngs of the year and juveniles of Mira chub and nase were identified using length frequency distributions, and excluded from estimates. Associations between mean fish number and pool area were determined using the Pearson correlation coefficient (R). Species scientific names are given in Chapter 2, Table 2.1. * p<0.05, ** p<0.01.

Pool	Area (m2)	*Chub*	*Nase*	*Eel*	*Loach*
1	34.7	15.2	*2.0*	*3.0*	*3.0*
		(15-24.7)	*(2.0-2.0)*	*(3.0-3.0)*	*(3.0-3.0)*
2	70.1	20.0	*1.0*	*6.0*	*5.0*
		(20.0-20.0)	*(1.0-1.0)*	*(6.0-6.0)*	*(5.0-5.0)*
3	92.4	4.0	0.0	5.0	4.0
		(4.0-4.0)	-	(5.0-5.0)	(4.0-4.0)
4	122.6	1.0	0.0	0.0	22.2
		(1.0-1.01)	-	-	(15.8-84.1)
5	127.1	1.0	2.0	3.0	0.0
		(1.0-1.0)	(2.0-2.0)	(3.0-3.0)	-
6	150.9	46.0	27.7	8.4	41.9
		(46.0-46.0)	(22.9-59.5)	(8.0-21.3)	(37.2-64-7)
7	189.7	24.2	0.0-	*7.0*	12.1
		(223.3-39.5)	-	*(7.0-7.0)*	(11.1-27.7)
8	248.8	94.2	32.6	21.6	17.4
		(83.1-128.3)	(27.1-65.9)	(13.7-113.1)	(14.3-47.2)
9	253.6	63.2	16.0	8.0	0.0
		(63.0-70.4)	(16.0-16.0)	(8.0-8.0)	-
10	339.5	368.3	313.3	131.3	84.1
		(344.7-410.3)	(200.8-665.2)	(61.1-646.5)	(73.7-117.1)
R		0.79 **	0.71 *	0.722*	0.63 *

REFERENCES

White, G.C. & Burnham, K.P. (1999) Program MARK: survival estimation from populations of marked animals. . *Bird Study,* **46 (Supplement)** S120-S138.

Zippin, C. (1956) An evaluation of the removal method of estimating animal populations. *Biometrics,* **12,** 163-189.