UNIVERSIDADE DE LISBOA

FACULDADE DE CIÊNCIAS

DEPARTAMENTO DE BIOLOGIA ANIMAL

ACTIVITY, DISTRIBUTION AND MICROHABITAT USE BY THE IMPERILLED MIRA CHUB *SQUALIUS TORGALENSIS*

Joana Maria Sofio Martelo Callapez Martins

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Tese orientada pela Professora Maria Filomena Magalhães e pelo Professor Gary Grossman, especialmente elaborada para a obtenção do grau de doutor em Biologia, especialidade Ecologia

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Preliminary notes

According with the Article 41 no 1 of the Post-Graduate Studies Regulation of the University of Lisbon (Diário da República II série nº 209 de 30 de Outubro de 2006), the current dissertation includes papers published or in preparation in co-authorship. As doctoral candidate presenting this dissertation, I was responsible for the study designs, data collection, statistical analyses and writing. The advisors of the thesis were deeply involved in the research conceptual framework and in all stages of the studies, being co-authors of the papers.

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Resumo

1. Os fatores e mecanismos que determinam a distribuição e abundância de peixes dulçaquícolas são temas centrais em Ecologia Aquática. A pequenas escalas, os padrões de distribuição e uso do habitat por peixes encontram-se relativamente bem documentados, evidenciando a importância quer da variabilidade espacial e temporal das características físicas do habitat, quer de fatores biológicos. A compreensão dos mecanismos subjacentes à complexidade e dinamismo destes padrões, requer o desenvolvimento de novos modelos de índole mecanística que permitam quantificar a seleção e qualidade do microhabitat em termos de fitness individual. No obstante, desconhece-se ainda em que medida estes modelos podem ser aplicados a ciprinídeos em geral, e a espécies Mediterrânicas em particular. Os estudos disponíveis até ao momento incluem apenas análises comparativas do microhabitat disponível e utilizado, quantificados através de métodos indiretos e sem informação sobre comportamento dos indivíduos, pelo que os padrões de atividade e mecanismos subjacentes à distribuição e uso do habitat das espécies Mediterrânicas permanecem largamente desconhecidos.
2. A presente dissertação incide sobre a atividade, distribuição e uso do microhabitat por ciprinídeos em rios Mediterrânicos, usando como caso de estudo, o Escalo do Mira *Squalius torgalensis*, uma espécie criticamente ameaçada, endémica do sudoeste de Portugal. Esta espécie foi selecionada para o estudo por ser abundante na região e apresentar características de história de vida representativas e típicas das espécies nativas em rios Mediterrânicos. Especificamente, o estudo foi estruturado para clarificar a importância relativa de diferentes características físicas do habitat e identificar os mecanismos subjacentes ao uso do habitat. Nesse sentido foram desenvolvidas três atividades de investigação distintas, visando: 1) descrever os padrões de atividade com base na análise do tempo despendido em diferentes atividades durante o dia, e quantificar a influência de fatores extrínsecos e intrínsecos nestes comportamentos, 2) quantificar a distribuição espacial relativamente à distribuição das características físicas do habitat, e analisar a forma como estas se associam ao uso do microhabitat, e 3) examinar os mecanismos subjacentes ao uso do microhabitat, com base na relação entre o sucesso alimentar e o uso individual da velocidade, frequentemente considerada determinante no uso do habitat por peixes

dulçaquícolas. Estes estudos envolveram uma componente de quantificação do uso de habitat no rio Torgal (SW Portugal) realizada em 2009 e 2010, sob condições reais de caudal reduzido, e uma componente laboratorial de quantificação do sucesso alimentar sob condições controladas de velocidade, realizada na Faculdade de Ciências, Universidade de Lisboa.

3. A análise dos padrões de atividade envolveu a quantificação do tempo despendido em orientação, natação, alimentação e exploração durante o dia, através da observação debaixo de água do comportamento de 231 indivíduos. A variação na atividade, quantificada com base numa Análise de Componentes Principais, foi posteriormente relacionada com fatores extrínsecos (i.e., temperatura e ano) e intrínsecos (i.e., comprimento do corpo e tamanho do grupo), utilizando Modelos Lineares Generalizados. A atividade apresentou variações acentuadas entre indivíduos, associadas a fatores extrínsecos e intrínsecos. O modo de alimentação variou anualmente entre a captura de organismos na coluna de água e a captura de organismos bentónicos, possivelmente em resposta à variação na disponibilidade de presas. Ocasionalmente verificou-se também variação no modo de alimentação com o tamanho do grupo. Além disso, o tempo despendido em natação versus exploração foi afetado pela temperatura e tamanho do corpo, refletindo provavelmente variações nos requerimentos energéticos dos indivíduos.
4. A distribuição espacial e o uso do microhabitat foram avaliados em 606 e 236 quadrados de 1m^2 dispostos sobre dois setores de estudo, e os padrões comparados entre anos, setores e classes dimensionais. A distribuição foi quantificada com base na variação espacial da abundância, e o uso do microhabitat com base nas relações entre a presença e abundância de peixe e as características do habitat, usando modelos *Hurdle*. Em ambos os sectores e anos de estudo, o padrão de distribuição foi agregado, provavelmente em resposta à variação espacial e autocorrelação de características do habitat, tais como, detritos lenhosos, raízes e vegetação aquática. No entanto, é possível que relações bióticas, nomeadamente de atração social, possam também ter estado envolvidas, particularmente entre indivíduos pequenos. Quer em termos de presença quer em termos de abundância, verificaram-se associações marcadas com a composição do substrato, detritos lenhosos, raízes, vegetação aquática e profundidade. A importância destas características variou entre setores, presumivelmente devido a alterações na disponibilidade de microhabitat, não se

detetando variações marcadas entre anos. A variação ontogenética no uso do microhabitat foi reduzida, possivelmente devido às baixas velocidades presentes nos setores de estudo ou à reduzida competição entre indivíduos.

5. A investigação do mecanismo subjacente ao uso do microhabitat envolveu a análise do uso do microhabitat a nível focal de 187 indivíduos em dois sectores, e a elaboração de modelos experimentais de sucesso alimentar primariamente baseados na velocidade. Para a elaboração destes modelos seguiram-se duas etapas. Primeiro, quantificou-se a variação na proporção de indivíduos que capturaram presas em relação à velocidade, e ainda à densidade de peixe e composição do grupo (i.e., grupos compostos por indivíduos pequenos, por indivíduos grandes ou por indivíduos pequenos e grandes). Segundo, examinou-se em que medida esta variação poderia refletir diferenças individuais na captura de presas e na distância de reação, em relação à velocidade, e também à densidade, comprimento do peixe e composição do grupo (i.e., grupos compostos por indivíduos pertencentes à mesma classe dimensional ou por indivíduos pertencentes a diferentes classes dimensionais). A análise de uso do microhabitat focal, em geral, indicou uso aleatório do habitat, nomeadamente em relação à velocidade, e independente do comprimento dos indivíduos. No laboratório, a proporção de indivíduos que capturaram presas diminuiu com a velocidade e, ainda, com a densidade e em grupos formados por indivíduos grandes. A velocidade afetou também negativamente o sucesso de captura, o qual tal como a distância de reação tendeu ainda a diminuir com a densidade. Estes resultados sugerem que o uso focal do microhabitat foi pouco influenciado pela velocidade provavelmente porque o sucesso de captura de presas tende a ser elevado sob as baixas velocidades prevalecentes em condições de reduzido caudal.
6. Globalmente, os resultados obtidos evidenciam uma elevada flexibilidade quer a nível comportamental quer a nível de seleção do microhabitat, que pode ser importante para a persistência de ciprinídeos em rios Mediterrânicos, onde as condições físicas do habitat são extremamente variáveis. O estudo realizado revela ainda que substratos heterogéneos, raízes, vegetação aquática e profundidade intermédia possivelmente favorecem a persistência de ciprinídeos em rios Mediterrânicos. Por último, é evidenciada a relevância do uso de abordagens mecanísticas, baseadas na observação direta e em critérios relacionados com a fitness dos indivíduos (e.g., sucesso

alimentar), para melhorar o conhecimento dos processos de seleção do habitat por ciprinídeos Mediterrânicos.

7. A abordagem aplicada neste estudo, utilizada tradicionalmente em rios temperados da América do Norte, pode ainda ser melhorada e mais profundamente explorada de forma a permitir uma melhor compreensão dos processos subjacentes à distribuição e seleção do microhabitat por espécies de ciprinídeos, e respetiva dinâmica em rios Mediterrânicos. Em particular, seria importante: 1) determinar a energia obtida em função da velocidade, não só a partir do sucesso de captura de presas, mas também do valor energético das presas disponíveis no campo, 2) incluir o custo energético de natação e captura de presas no modelo através do cálculo de taxas de respiração a diferentes velocidades, 3) incluir parâmetros relativos à competição intra e interespecífica, e 4) analisar a validade deste tipo de modelos para diferentes espécies e sob as diferentes condições de velocidade prevalecentes num conjunto alargado de rios Mediterrânicos, sob diferentes contextos hidrológicos, nomeadamente em anos húmidos.

Palavras-chave: seleção de habitat, comportamento, recursos físicos de habitat, abordagens mecanísticas, ciprinídeos, rios Mediterrânicos.

Abstract

Understanding habitat selection and use by stream fishes increasingly requires the analysis of the relationships between fish and their environments in terms of individual fitness. However, such mechanistic approaches are still uncommon for cyprinids, and remain to be evaluated and tested for Mediterranean species. This thesis addressed activity, distribution and microhabitat use by Mira chub *Squalius* torgalensis, an imperilled cyprinid native to Mediterranean streams in SW Portugal. This was analysed using a mechanistic approach, including direct observations of fish and habitat in two reaches of the Torgal stream, in spring 2009 and 2010, and laboratory experiments on foraging success under variable velocities. Daily activity patterns were variable at the individual level and shaped by extrinsic and intrinsic factors. Foraging changed from drift to benthic on an annual-basis and with shoal size, and time spent cruising vs. searching was influenced by temperature and body size. Chub displayed an aggregated distribution apparently in association with patchily distributed and autocorrelated physical resources, such as debris, roots and aquatic vegetation. Microhabitat use was influenced by substrata, cover and depth, but random at the focal level, and little variable throughout ontogeny. Contrary to expectations, microhabitat use was generally unrelated to velocity, probably because prey capture success was high at the low velocities prevailing in study reaches. Nevertheless, the proportion of fish capturing prey and capture success decreased at high velocities. Results indicate that chub may display high flexibility in activity and habitat use, which may be important to cope with the high variability of physical conditions in Mediterranean streams, with maintenance of patches with variable substrata, cover and depth likely favouring local persistence. Moreover, this thesis shows that mechanistic fitness-based criteria may be crucial for understanding habitat selection by Mediterranean cyprinids.

Keywords: habitat selection, behaviour, physical habitat resources, mechanistic approaches, cyprinids, Mediterranean streams.

Chapter I

General introduction

I. General introduction

I.I. Setting the scene

Understanding drivers and mechanisms that generate the spatial distributions and abundance of organisms over a range of scales is a central theme in ecology (e.g., Kearney & Porter 2004; Karlson et al. 2011; Dormann et al. 2012; Trainor et al. 2013; Boucher-Lalonde et al. 2014). This is a challenging task that requires addressing multiple potentially influential factors including habitat characteristics (Mendo et al. 2014), food availability (Folmer et al. 2012), conspecifics (Gibson & Koenig 2012), competition (Razgour et al. 2011), predation risk (Thaker et al. 2010) and phylogenetic constraints (Strauß et al. 2013). Because these multiple factors can be scale dependent (Van Beest et al. 2010; Chave 2013; Wisz et al. 2013), ecologists examining spatial distributions should explicitly consider the link between the spatial and temporal scales at which research is conducted and at which the processes to be quantified actually operate (Huston 1999; Chave 2013).

At small scales, legions of studies have attempted to understand the factors influencing the distribution and abundance of organisms (Cassini 2013). However, the question of why organisms prefer certain habitats and avoid others is still intriguing ecologists (Railsback et al. 2003; DeCesare et al. 2014; Piccolo et al. 2014; Potts et al. 2014). Studies of habitat selection traditionally are correlative and compare habitat characteristics at organisms locations with characteristics at unused or at random locations (Johnson 1980; Manly et al. 2002; Strickland & McDonald 2006). However, this traditional approach provides little or no information on the processes and mechanisms that generate habitat use (Kearney & Porter 2009; Bogosian III et al. 2012). This can be circumvented by mechanistic models that allow to unravel the causal basis of habitat selection through an understanding of how environmental characteristics interact with the organism itself (i.e., its behavioural, morphological and physiological traits) to affect key fitness elements such as growth, survival and reproductive success (Morris & Davidson 2000; Petty & Grossman 2010; Chapperon & Seuront 2011; Latif et al. 2011; Osbourn et al. 2014). Obtaining a more biological realistic understanding of habitat use patterns as well as of the factors determining them, is particularly important given that habitat loss is generally a crucial factor in declines in biodiversity (Dudgeon et al. 2006; Shochat et al. 2006; Vorosmarty et al. 2010).

I.2. Spatial distribution and microhabitat use of stream fishes

Streams exhibit pronounced heterogeneity in physical conditions and biological communities at multiple spatial and temporal scales (Pringle et al. 1988; Hildrew & Giller 1994). Currently, streams are defined as hierarchically nested systems incorporating, on successively lower levels, basin, segment, reach, riffles, pools and runs, and microhabitat units defined as patches of relatively homogeneous substratum, water velocity and depth (Frissel et al. 1986; Kotliar & Wiens 1990). This hierarchical organization is such that ecological processes at large scales, create, maintain and destroy habitats, thereby setting the context for physical and biological processes operating at smaller scales (Frissel et al. 1986; Schlosser & Angermeier 1995). Moreover, at any spatial scale, streams can be perceived as a *discontinum* of discrete parts with unique attributes that are temporally dynamic (Poole 2002).

Several studies have demonstrated the usefulness of work at the microhabitat scale in understanding the distribution and habitat use of stream fishes (e.g., Thompson et al. 2001; Einum et al. 2008; Petty & Grossman 2010; Donaldson et al. 2013). These studies generally allow ecologists to study fish-microhabitat relationships as a consequence of direct interactions both between individuals and between individuals and physical microhabitats (Rosenfeld 2003; Lee & Suen 2012). However, the distribution and microhabitat use of fishes may be strongly affected by both spatial and temporal variability in physical and biological characteristics. For instance, individuals are seldom distributed randomly over suitable microhabitats, often showing aggregated distributions (Freeman & Grossman 1993; Torgersen & Close 2004; Argentina et al. 2010). Non-random distributions may arise because the physical and biological resources needed for survival and reproduction are themselves patchily distributed over space or as a result of biological interactions (Freeman & Grossman 1993; Einum et al. 2008; Argentina et al. 2010). Similarly, temporal patchiness in resource availability due to variations in flow also may produce seasonal and annual shifts in the distribution and microhabitat use by fishes (Grossman & Ratajczak 1998; Thompson et al. 2001). Moreover, fishes have complex life histories and the importance and effects of spatial and temporal resource variability may change during ontogeny, which is presumably related to differences among size classes in resource utilization capabilities, energetic demands and predation risk (Rosenberger & Angermeier 2003; Ayllón et al. 2010; Henderson & Johnston 2010). Addressing such complex and dynamic patterns may thus require powerful and sophisticated approaches that provide a deep understanding of the mechanistic links between fishes and their environments.

1.3. Mechanistic approaches to microhabitat use of stream fishes

Traditionally, physical habitat is considered one of the most important factors influencing microhabitat use by stream fishes (Grossman 2014). Nevertheless, numerous studies have found that although fishes seldom occur in habitats with inadequate physical characteristics, the occurrence of fishes in areas with apparently suitable physiognomy varies significantly

(Grossman & Ratajczak 1998). For example, mottled sculpin *Cottus bairdii* Girard frequently select patches with high velocities, great amounts of erosional substrata, and high substratum stability (Grossman & Ratajczak 1998; Petty & Grossman 2007), but there are seasons in which sculpin randomly use habitat (Grossman & Freeman 1987; Petty & Grossman 1996; Grossman & Ratajczak 1998). This lack of microhabitat specialization represents a paradox in habitat use by stream fishes, and suggests that 1) descriptive approaches based on correlational analyses may be of limited use in determining the mechanisms of microhabitat use, and 2) experimental, mechanistically based studies may be necessary to unravel the causes underlying microhabitat use (Hill & Grossman 1993; Thompson et al. 2001; Grossman 2014).

During the last two decades, ecologists have developed new mechanistic models quantifying microhabitat quality and use in terms of individual fitness (Hughes & Dill 1990; Hill & Grossman 1993; Nislow et al. 1999; Grossman et al. 2002). These models are based on optimal foraging theory and are anchored on the ideal that natural selection will favour individuals that choose microhabitats that maximize their fitness. However, because fitness has proved difficult to measure in fishes due to their high individual fecundities (Wootton 1990) and difficulties in marking young fish individually (Olsen & Vøllestad 2001), fitness surrogates such as net energy intake have often been used (Hill & Grossman 1993; Piccolo et al. 2008). It can then be examined whether individuals are behaving in concordance with the predictions of optimal foraging theory by exploring whether they occupy microhabitats that yield high net energy intake.

Mechanistic models based on net energy intake have demonstrated that focal point velocity (i.e., velocity at the position of the fish; Grossman & Freeman 1987) is a critical component of optimal habitat selection for several drift-feeding fishes, mainly salmonids (Hughes & Dill 1990; Hill & Grossman 1993; Nislow et al. 1999; Hughes et al. 2003; Grossman 2014). Water velocity determines prey encounter rate, the probability that a fish will capture a prey and the swimming costs for a fish at a given position (Fausch 1984; Hill & Grossman 1993; Hughes et al. 2003). To date, results indicate that drift foraging models are more sensitive to changes in benefits (i.e., capture success) than they are to changes in costs (i.e., swimming costs; Hughes & Dill 1990; Hill & Grossman 1993, but see Hughes et al. 2003 for a discussion of prey capture costs). Consequently, most studies have focused on measuring capture success to quantify the effect of water velocity on microhabitat use (Grossman 2014).

Emphasis on mechanistic, fitness-based, microhabitat use models represents a significant advance over classical approaches, which focus solely on quantifying physical habitat characteristics and comparing microhabitat availability to use (Grossman 2014). Nevertheless, to date, fitness-based models of habitat use are still limited in geographic context and taxonomic application and correlative habitat use models remain the dominant approach for studying fishes distribution and abundance.

1.4. Activity patterns of stream fishes

Accounting for the behaviour of organisms may contribute for developing a better mechanistic understanding of habitat use (Roever et al. 2014; Wirsing & Heithaus 2014). Variation in habitat can strongly influence activity patterns of stream fishes (e.g. time spent foraging, resting and swimming), with individuals in different microhabitats displaying variable behaviours as they cope with distinct constraints (Baras & Nindaba 1999; David & Closs 2003; Clavero et al. 2005; Schrandt et al. 2012). Interactions between activity and habitat are illustrated by observations that fishes use different focal velocities and depths for feeding and hiding (Hill & Grossman 1993; Heggenes et al. 1999; Meyer & Gregory 2000). Although activity and habitat use may be intimately related, habitat use is often investigated without a behaviour context, which may limit our ability to generate more realistic process-based microhabitat use models.

Stream fish activity has been shown to vary with intrinsic factors such as energetic requirements, body size, condition and group size (Alanärä et al. 2001; Metcalfe & Steele 2001; White & Warner 2007; Busch & Mehner 2012) and with extrinsic factors such as, temperature, light intensity and food availability (Fraser & Metcalfe 1997; Metcalfe et al. 1999; Bremset 2000; Linløkken et al. 2010). Behavioural responses may, however, be extremely labile as individual activity may be shaped differently by the specific conditions each fish experiences (Reebs 2002; David & Closs 2003; Breau et al. 2007). However, the extent to which this variability may be associated with habitat use by stream fishes remains uncertain.

The majority of studies on activity patterns concerns salmonids, with information on cyprinids behaviour restricting to a few studies on the effects of season and temperature (Lucas & Batley 1996; Greenwood & Metcalfe 1998). Cyprinids differ significantly from salmonids in evolutionary history, ecological requirements and population dynamics (Rochet et al. 2000). Hence, it is difficult to infer general patterns of behaviour solely from information on salmonids, and thus studies including cyprinids are needed for a more comprehensive understanding of the relationship between habitat use and activity in stream fishes.

Moreover, there is conflicting evidence regarding the influence of extrinsic and intrinsic factors on salmonids activity. For example, the effect of temperature on activity may vary, with some species allocating more time to foraging or showing faster swimming and attach rates when temperatures increase (Weetman et al. 1998; Marchand et al. 2002; Linløkken et al. 2010), whereas the activity of other species are not affected by temperature (Gries et al. 1997; David & Closs 2003; Baktoft et al. 2012). Similarly, although the general pattern is that small fish spend more time foraging than larger individuals (e.g., Metcalfe et al. 1998; Bradford & Higgins 2001), there are species in which the opposite occurs (Gries & Juanes

1998). Factors that shape individual variability in activity patterns by fishes thus need to be further explored, especially given the increasingly recognized importance of behavioural information for animal conservation planning (Buchholz 2007; Bennett et al. 2013).

I.5. Fishes microhabitat use and activity in the context of Mediterranean streams

Because of their inherent variability, Mediterranean streams are ideal systems for examining the relationship between variation in physical habitat characteristics and distribution, microhabitat use and activity patterns of fishes. As a result of the Mediterranean climate, streams experience sequential, seasonally predictable events of flooding (late autumn–winter) and drying (late summer–early autumn) that vary substantially in intensity over the years (Gasith & Resh, 1999). In these circumstances, fishes are frequently subjected to spatial and temporal variation in physical habitat characteristics during their lifetime, and consequently, their distribution, microhabitat use and activity patterns may vary substantially. Moreover, Mediterranean streams harbour many endemic fish species that are largely threatened (Smith & Darwall 2006), and which conservation may require scientifically-based knowledge on essential habitat and patterns of behaviour (Hermoso & Clavero 2011; Hermoso et al. 2011).

At present, there is still a dearth of data about fish responses to spatial and temporal variation in physical microhabitat characteristics in Mediterranean streams. Several studies analyse microhabitat use of different size/age classes in distinct seasons, but often pool data from different rivers and/or years (Santos et al. 2004; Santos & Ferreira 2008; Martínez-Capel et al. 2009; Santos et al. 2010), and report average patterns, largely ignoring how microhabitat use may vary both spatially and annually (but see Grossman & de Sostoa 1994a, b).

Moreover, microhabitat use is typically assessed using indirect methods, such as comparisons between habitat availability and use (Santos et al. 2004; Santos & Ferreira 2008; Martínez-Capel et al. 2009; Santos et al. 2010), with no attempt to quantify the causes underlying microhabitat use, though mechanisms such as predation risk, competitive interactions and prey availability are often implied (e.g., Grossman & de Sostoa 1994a, b; Santos et al. 2004; Santos & Ferreira 2008; Boavida et al. 2011). In particular, there is a lack of studies based on direct observations that allow data on habitat associations to be easily collected together with data on individual or group behaviour, and mechanisms determining microhabitat use to be quantified (Almeida & Grossman 2012).

There is considerable evidence that light intensity may influence activity patterns in some Mediterranean stream fishes (Santos et al. 2002; Clavero et al. 2005; Santos et al. 2005; Maia 2006; Santos et al. 2008), however, effects of other extrinsic factors such as temperature, and intrinsic factors such as body length and shoal size are largely unknown. Typically, fishes in temperate streams exhibit substantial flexibility in the amount of time devoted to feeding, resting and exploring, involving rapid and reversible adaptive responses to extrinsic and intrinsic factors (Reebs 2002; David & Closs 2003; Breau et al. 2007), that may result in contrasting activity patterns among species and populations (Fraser et al. 1995; Valdimarsson et al. 2000), age cohorts in the same population (Gries et al. 1997), and in the same individuals at different conditions (Fraser et al. 1993). It may therefore be expected that species found in Mediterranean streams, also show great flexibility in behaviour, as physical and biological conditions can change markedly, for example, among years and across space.

I.6. Study objectives and approach

The primary objective of this dissertation is to advance knowledge on the activity, distribution and microhabitat use by the imperilled Mira chub *Squalius torgalensis* Coelho, Bogutskaya, Rodrigues & Collares-Pereira, a species endemic to Mediterranean streams in SW Portugal. Specifically, the study was primarily designed to clarify links between fish and physical habitat characteristics and mechanisms underlying habitat use. In order to meet this goal three lines of research were pursued, aiming at:

1. Describing activity patterns by quantifying the time chub spend orienting, cruising, foraging and searching and the effects of extrinsic and intrinsic factors on these behaviours;
2. Exploring chub spatial distribution and its relation to patchiness in physical habitat characteristics and how these associate with microhabitat use;
3. Examining the mechanics underlying microhabitat use, by focusing on the role of foraging success in determining individual velocity use.

In practice, the overall approach developed for this thesis involved a fieldwork component addressing chub activity, distribution and microhabitat use in the wild and a laboratory component aiming at exploring the functional mechanisms driving patterns of microhabitat use. This is an innovative approach that has seldom been applied to cyprinids, which combines visual observations of behaviour and microhabitat use with experimentally derived foraging success-based models to provide a more realistic and accurate quantification of microhabitat selection by chub.

Fieldwork was conducted in the Torgal stream, a tributary of the river Mira, located in the Natural Park of the *Sudoeste Alentejano e Costa Vicentina*, Southwest Portugal. The Torgal was selected for study because is a typically Mediterranean stream, relatively undisturbed by anthropogenic activities, maintains nearly pristine habitats (Magalhães et al. 2007), and consequently presents an ideal setting for studying fish-habitat relationships. In addition, fish and habitat are being surveyed in the Torgal since 1991 (Magalhães 2002; Magalhães et al. 2002a; Magalhães et al. 2002b; Magalhães et al. 2003; Magalhães et al. 2007; Pires 2012), thus providing important ecological information that was used as basis for this research. A detailed description of the study area and its fish assemblages is presented in Chapters 2 to 4. Fieldwork was conducted in May 2009 and May-June 2010. Rainfall data for the region indicated that the first year of study was extremely dry whereas the second year was normal, and hence the Torgal was characterized by low flow conditions.

The laboratorial component was conducted at the Faculty of Sciences, University of Lisbon, in October 2008, March-May 2009 and October-June 2010. Experiments were made in a 2.0 x 1.0 x 0.6 m (length x width x height) fiberglass tank equipped with a 25 Kg thrust electric trolling motor that generated flow (Zamor & Grossman 2007; Hazelton & Grossman 2009). A detailed description of the tank is presented in Chapter 4. Experiments conformed to the Portuguese legislation regarding animal capture (ICNB), manipulation and experimentation for scientific purposes, and IAUCUC guidelines of the University of Georgia.

Even though the approach was illustrated using a specific fish species, it may be widely applied to other stream fishes, especially cyprinids inhabiting Mediterranean streams. The study focused on the Mira chub (henceforth chub), which is the most abundant species in the Torgal stream (Magalhães et al. 2007; Pires 2012), and currently listed as critically endangered in the Portuguese Red Data Book due to habitat loss and degradation (Rogado et al. 2005). In addition, chub exhibit life-history attributes representative of many Mediterranean cyprinids, including small body size (maximum 16 cm, total length), early maturation (age 2 – 3 years), and short-life span (age of 5 – 6 years) (Doadrio 2001; Magalhães et al. 2003; Vila-Gispert et al.

2005). Moreover, they are relatively easy to distinguish from other fishes underwater (personal observation), prey upon drifting and benthic invertebrates during the day (Chapter 2), and occupy a range of habitats throughout the life cycle, (Magalhães et al. 2002b; Pires 2012), which make them particularly suited for studies on activity and habitat use.

To date, several ecological studies on chub have focused on the role played by environmental and spatial factors on their distribution, abundance and movements at the reach scale (Magalhães et al. 2002a; Magalhães et al. 2002b; Magalhães et al. 2003; Magalhães et al. 2007; Pires 2012; Pires et al. 2014). However, small scale studies addressing the effect of habitat characteristics and their spatial heterogeneity on chub activity, distribution and habitat use are inexistent. In addition, there is no information regarding the functional mechanism determining chub microhabitat use, though this may be considered crucial for developing scientific-based conservation and management actions for this imperilled species.

1.7. Thesis organization

This thesis is organized in 5 chapters. Chapter 1 includes a general introduction that sets the background, context and significance of the topics to be addressed, followed by a description of the aims and approach, and organization of the thesis. Chapters 2 to 4 correspond to scientific papers already published (2-3) or in the preparation stage (4). Chapter 5 summarizes and discusses main results, their implications for the understanding of the habitat selection by Mediterranean cyprinids, and highlights suggestions for further studies.

In Chapter 2 variation in daily activity patterns is analysed. The time chub spend orienting, cruising, benthic and drift foraging and searching during the day is quantified using instantaneous sampling of focal individuals, and variation in activity is related to extrinsic (i.e., temperature and year) and intrinsic factors (body size and shoal size).The general aim is to identify factors that influence the amount of time individuals devote to each specific activity. Information about activity was used in planning laboratory (Chapter 4) and habitat studies (Chapter 3 and 4), and hence, improving our ability to produce more realistic habitat use models.

Chapter 3 examines the spatial distribution and microhabitat use of chub in two stream reaches. Spatial variation in chub abundance is characterized and microhabitat use is quantified based on the relationship between fish presence and abundance and physical habitat characteristics. The general aim is to understand the effect of patchiness and availability of physical resources on the distribution and microhabitat use and determine essential habitat for small and large chub.

Chapter 4 combines field data on focal microhabitat use with laboratorial data on individual foraging success, and quantifies the influence of water velocity, fish density and length and group composition on foraging success. Overall, the aim is to elucidate the mechanism determining microhabitat selection by chub.

Chapter 5 summarizes and integrates main results, interprets and discusses them in the context of the complexity and dynamism of distribution and microhabitat use of Mediterranean stream fishes, and addresses their general implications for the understanding of the process of habitat selection by these fishes. It also highlights future studies that may be important to advance distribution and habitat selection ecology of fishes in Mediterranean streams and elsewhere.

In Appendix abundance of benthic and drift macroinvertebrates in study reaches, in spring 2009 and 2010, is presented.

1.8. References

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Chapter 2

Extrinsic and intrinsic factors influence daily activity of a Mediterranean cyprinid

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2. Extrinsic and intrinsic factors influence daily activity of a Mediterranean cyprinid

2.1. Abstract

Factors shaping individual behavior remain poorly understood for European cyprinids. We examined the effects of extrinsic (temperature and year) and intrinsic factors (body size and shoal size) on the daily activity of chub *Squalius torgalensis*, a critically endangered cyprinid found in the Torgal stream, Portugal. We quantified chub behaviors while snorkeling, using instantaneous sampling of focal individuals, in spring 2009 and 2010. We used Principal Component Analysis to describe individual behavior and assessed competing models relating individual behavior to explanatory variables using Akaike's Information Criterion. Year had a strong influence on chub behavior, with individuals spending more time drift foraging and orienting in 2009, whereas in 2010 they spent more time benthic foraging and cruising. Shoal size did not influence behaviors in 2009, but in 2010 individuals in small shoals foraged benthically and searched with greater frequency than those in large shoals. Individuals increased cruising and decreased searching as they grew in length, but the opposite trend was observed as temperature increased. Our results indicate that both extrinsic and intrinsic factors influence daily activity of chub, with individual behaviors varying between years, and depending upon shoal size, body size and temperature. This suggests that chub display high levels of individual behavioral flexibility, which may be important for fish inhabiting harsh and highly variable Mediterranean streams. Conservation efforts for this species will benefit by integrating behavioral information into management strategies.

2.2. Introduction

The amount of time an animal devotes to different daily activities (e.g. feeding, resting, exploring, and courtship) may be influenced by extrinsic factors such as temperature, light intensity, food availability and predators (Metcalfe et al. 1999; Belovsky et al. 2011; Nowicki et al. 2012), and by intrinsic factors such as body size, sex, physical condition and group size (Sharpe & Rosell 2003; Pelletier & Festa-Bianchet 2004; White & Warner 2007). Extrinsic and intrinsic factors may operate independently or in concert to influence individual behavior (Belovsky & Slade 1986; Alanärä et al. 2001; David & Closs 2003), though the nature and strength of these influences remains poorly understood. Clarifying the factors that influence behavior may be particularly important for endangered species for it may provide information required for proper and effective conservation (Buchholz 2007).

Despite recent advances in the study of fish activity patterns, very little is known about individual behaviors of non-game species, especially cyprinids. This is unfortunate because cyprinids represent one of the most diverse taxa of freshwater fishes worldwide (Nelson 2006). Most studies of activity patterns have focused on sport or commercially important species such as salmonids (e.g. Fraser et al. 1993; Bradford & Higgins 2001; Breau et al. 2007; Baktoft et al. 2012), which differ substantially from most cyprinids in life history attributes, ecological requirements and population dynamics (Rochet et al. 2000). Consequently, it is difficult to infer general patterns of behavior solely from information on these species. Moreover, there is a lack of information on the factors that influence the amount of time individuals spent on any specific activity, even for commercial fishes. Hence, there is a great need for studies that help advance our understanding of freshwater fish behavior.

Because fishes are ectotherms, they generally become more active when temperatures increase, either spending more time foraging or exhibiting faster swimming and attack rates (Weetman et al. 1998; Marchand et al. 2002; Linløkken et al. 2010). However, the effects of temperature on activity may vary, with some species shifting from nocturnal to diurnal foraging as temperatures rise (Fraser et al. 1993; Greenwood & Metcalfe 1998; Breau et al. 2007), although many species retain fairly constant activity patterns regardless of temperature (Gries et al. 1997; David & Closs 2003; Baktoft et al. 2012). Besides temperature, other extrinsic factors may influence fish activity. Many streams experience strong and unpredictable fluctuations in physical and biological conditions. Variation may occur in the form of both seasonal and annual changes in flow as well as the frequency and intensity of extreme events such as floods and droughts (Moyle & Vondracek 1985; Grossman & Ratajczak 1998; Lake 2000). This may cause fish activity to vary both seasonally and annually (Thompson et al. 2001), although most studies have focused only on analyzing seasonal variation in behaviors within a single year (e.g. Lucas & Batley 1996; Bremset 2000; David & Closs 2003; Reeves et al. 2010).

Intrinsic factors such as body and shoal size also are important factors to include in analyses of activity patterns of fishes. Because metabolic rates and the scope for growth decline with increasing body size (Brett & Groves 1979), smaller individuals may spend more time foraging than larger individuals (Bradford & Higgins 2001; Imre & Boisclair 2004; Busch & Mehner 2012). Shoal size may influence an individual's perception of potential competitors (Pitcher & Parrish 1993; Grand & Dill 1999), with fish in large shoals experiencing a reduction in food intake and typically allocating more time to foraging and searching than fish in smaller shoals (Pitcher et al. 1982; Pitcher & Parrish 1993; White & Warner 2007; Ward 2012). Concomitantly, because of risk dilution and collective threat detection, per capita predation risk frequently is lower for fish in large than small shoals, and may result in less time spent in threat-sensitive activities (Pitcher & Parrish 1993; Grand & Dill 1999; Stenberg & Persson 2005;

Magnhagen & Bunnefeld 2009). Nevertheless, the manner in which body size and shoal size interact among themselves and with other factors remains poorly understood.

Here, we examine the effects of extrinsic and intrinsic factors on the daily activity patterns of the Mira chub *Squalius torgalensis* (henceforth chub) in a Mediterranean stream in SW Portugal, in spring 2009 and 2010. The chub is a critically endangered species (Rogado et al. 2005), and behavioral information should aid in the development of conservation strategies for this species (Buchholz 2007). We also chose chub because it is the most abundant fish in the study site and exhibits life-history attributes representative of many Mediterranean fishes, i.e., small body size, fast growth, early maturation, and spring spawning (Doadrio 2001; Magalhães et al. 2003; Vila-Gispert et al. 2005). The specific aims of our study were to: (1) quantify daily activity patterns of individual chub, (2) determine whether these patterns varied between years, and (3) quantify the effects of extrinsic (i.e., water temperature, year) and intrinsic factors (i.e., body size and shoal size) on activity patterns.

2.3. Methods

Study site

Our study site was an 80 m long section in the downstream portion of the Torgal stream (SW Portugal, 37°38’N, 8°39’W), a small, undisturbed Mediterranean stream that has been previously described in detail in Magalhães et al. 2007). Briefly, the Torgal stream is strongly influenced by the Mediterranean climate type characterized by cool, wet winters and hot, dry summers (Gasith & Resh 1999). Mean monthly air temperature ranges from 11°C (December) to 24°C (August). About 80% of annual rainfall occurs in October-March, and virtually none in the hot summer months (July-August). Although the seasonal timing of rainfall is predictable, the total amount of rain varies considerably among years (205-1121 mm). By contrast, annual variation in mean air temperature is relatively low (15-18°C). The flow regime is greatly dependent on annual rainfall; headwaters are ephemeral and downstream reaches typically dry to isolated pools and runs in summer. In dry years, there are no significant floods and surface water persists in the deepest pools only. Conversely, in wet years there are major floods, and flows may persist in downstream reaches through summer. The underlying bedrock is siliceous igneous rock and the vegetation is dominated by cork oak *Quercus suber* woodlands and eucalyptus *Eucaliptus globulus* plantations interspersed with pasture and dry grain fields. Riparian vegetation is dominated by well developed galleries of alder *Alnus glutinosa* and ash *Fraxinus angustifolia*, with an understory of Mediterranean scrub.

The study site encompassed a relatively shallow run (<80 cm deep), dominated by a bedrock outcrop with a mixture of boulder, cobble and fine substrata. We choose this site because it typically retained water over summer, which reduced potential confounding effects of emigration/extinction and colonization processes on fish activity patterns. Chub dominated the fish assemblage, but co-occurred with native Southwestern arched-mouth nase *Iberochondrostoma almacai* (Coelho et al. 2005), Western three-spine stickleback *Gasterosteus gymnurus*, Southern Iberian spined-loach *Cobitis paludica* and the invasive pumpkinseed *Lepomis gibbosus* (Fox et al. 2007).

Environmental data and daily activity patterns

We analyzed the daily activity of individual fish in May 2009 and May-early June 2010. October 2008 to September 2009 was the driest 12 month period on record in the Torgal stream since 1931 (205 mm), and rainfall in the following year, October 2009 to September 2010, also was substantially lower than the long-term annual median (446 versus 643 mm). Mean air temperature in 2009 (16.7 °C ± 1.4, mean ± SE) and 2010 (17.4 °C ± 1.6) were similar to the long term mean (1972 - present, 16.9 °C ± 0.2). Air temperature in May 2009 was 19.0 °C, identical to the long-term monthly 75% quartile (18.7 °C), and in May-early June 2010 increased to 19.5 °C, slightly below the long-term 75% quartile for those months (21.0 °C). We obtained air temperature and rainfall data from two gauging stations located near the study site (37°30'N, 8°24'W and 37°42'N, 8°32'W respectively).

We conducted behavioral observations using the methods of Grossman & Freeman 1987) and Grossman & Ratajczak 1998). We entered the site at its lower-most border, snorkeling from downstream to upstream, in a zigzag fashion to minimize disturbance. Both the substratum and the water column were scanned to locate fish, and undisturbed individuals identified for observation. Disturbed fish were recognized by their characteristic escape responses (i.e. fleeing and sustained darting movements). We identified each subject to species and visually estimated their standard length (SL). Fish less than 5 cm (SL) were not observed because they were not reliably identifiable to species. We also visually estimated the number of individuals in each shoal and identified them to species. A shoal was defined as individuals that were within approximately four body lengths of each other (Pitcher & Parrish 1993). We divided the study site in three equal sections and observed one section per day, for 1.5h, at dawn (07:02h ± 00:00 to 08:34h ± 00:01, mean ± SE), mid-day (12:16h ± 00:01 to 13:56h ± 00:01) and dusk (18:35h ± 00:00 to 20:02h ± 00:01). We selected these observation times to cover a broad range of water temperatures, but restricted observations to day light hours because chub appeared quiescent at night (Martelo et al., personal observations). Efforts were made to not resample the same individuals by observing less than 50% of the total during a sampling period. Hence, although it is possible that some chub were observed more than once during a sampling period, the majority of observations are likely independent. We measured temperature at the water surface every 20 min during observations, using a HI 198129 probe (Hanna Instruments, Woonsocket, RI, USA).

We used instantaneous sampling (Altmann 1974) to record the behavior of individual fish, every 30 sec for 3 min. Observational times were recorded by an assistant. We made observations until we lost sight of a fish and only analyzed data that included at least three minutes of continuous observations (84 % of all observations). During observations we recorded the following specific behaviors: (1) orienting (holding in the current or towards a shelter, e.g. tree roots, branches), (2) cruising (swimming movements not oriented towards an object or food), (3) benthic foraging (feeding on tree roots, branches, boulders, cobbles or submerged macrophytes), (4) drift foraging (feeding on organisms carried by the current), (5) searching (approaching and orienting towards an object in search for food), and (6) other, which included non-oriented holding (generally holding position but not oriented towards the current or shelter) and agitated (short abrupt movements typical of fright, caused by the observer, other fishes or the invasive red swamp crayfish *Procambarus clarkii*).

Statistical analyses

Our statistical approach focused on explaining variation in daily activity patterns for individual chub by creating a suite of explanatory models that included both extrinsic (water temperature and year) and intrinsic (fish size and shoal size) explanatory variables. Year was coded as a dummy independent variable. Although shoal size may be influenced by extrinsic factors, we classified it as intrinsic for it also may integrate intrinsic factors, such as social interactions or behavioral motivations that may depend on individual properties (Flierl et al. 1999). Shoal size included solitary individuals, and when mixed-species shoals were observed (74% of shoals), it was determined by the number of chub in the shoals. Mixed-species shoals were always composed of more than 50% of chub, with one exception, in which chub comprised 30% of the individuals in the shoal. We used the total number of times that each individual performed a given behavior during a 3 minute observation period to derive our response variables.

Prior to analyses, both explanatory variables and counts of behavioral acts were log transformed for normality and to reduce kurtosis (Legendre & Legendre 1998). We calculated pairwise correlation coefficients between explanatory variables to identify multicollinearity. Analyses were conducted in the R 2.14.1 software using MuMIn (Barton 2011) and STATISTICA 10.0 with significance at $P < 0.05$. We found no evidence of strong correlations between explanatory variables (Pearson's $r \leq |0.30|$), thus all were retained in analyses. We tested for significant differences in explanatory variables between years using Mann-Whitney and Fisher's F tests on the mean and variance of each variable, respectively (Zar 1996). To quantify variation in chub activity patterns we conducted a Principal Components Analysis (PCA) on the counts of behavioral acts, pooling 2009 and 2010 data. We used PCA because several behaviors were significantly correlated and PCA is designed to handle intercorrelated data (Grossman et al. 1991; Legendre & Legendre 1998). To avoid unstable ordination results and spurious patterns, we excluded behaviors representing less than 5 % of the total from analyses, and the only category that this represented was other behaviors (Legendre & Legendre 1998). Following Grossman & de Sostoa 1994a, b) we tested the null hypothesis that activity patterns did not differ between 2009 and 2010 by calculating mean component scores and 95% confidence ellipses for these years. A lack of overlap of the 95% confidence ellipses demonstrated significance at the 0.05 level (Grossman & de Sostoa 1994a, b). We tested for significant differences in activity patterns between years to decide whether year should be included as an explanatory variable in our modeling exercise.

We then developed 13 *a priori*, mechanistic regression models including extrinsic and intrinsic explanatory variables (Table 1) to explain variation in activity patterns based on PCA scores, using generalized linear models with a normal error distribution (Crawley 2007). All explanatory variables were treated as fixed effects. Even though year may be considered a random effect conceptually, pragmatically we must treat it as a fixed effect because we only had two treatment levels (2009 and 2010), which would lead to imprecise estimates of variance (Douglas Bates, personal communication). The set of candidate models included four models evaluating the individual effects of temperature, year, fish size and shoal size. The set also included additive models to describe the combined effects of pairs of the extrinsic and intrinsic variables. In addition, two-way interaction terms were included in some models to account for possible differences in responses based on fish size and shoal size due to temperature and year effects (e.g. shoal size x temperature or shoal size x year). Finally, a global model with all main effects and interactions, and a null model including the intercept only were evaluated.

We used an information-theoretic approach as a model selection procedure, whereby the plausibility of each candidate model was assessed using Akaike Information Criteria adjusted for small sample sizes (AICc; Burnham & Anderson 2002). We assessed the relative performance of candidate models using ΔAICcs and Akaike weights (w_i). Akaike weights quantify the amount of information lost in the original data through use of a given model (Burnham & Anderson 2002). We only interpreted models with Akaike weights >10% of the model with the best fit (Burnham & Anderson 2002; Grossman et al. 2006). To quantify model selection uncertainty, we calculated model-averaged coefficient estimates and evaluated the precision of these estimates using 85 % confidence intervals (Arnold 2010). Estimates whose confidence intervals included zero were viewed as having lower explanatory power. Finally, we estimated the relative importance of explanatory variables by summing their Akaike weights over all interpretable models where the variable was included (Burnham & Anderson 2002).

Table 1 - A priori mechanistic explanatory models relating daily activity patterns of chub to extrinsic and intrinsic factors.

Hypothesis	Model
Activity pattern is explained by changes in temperature	y=temperature
Activity pattern is explained by year	y=year
Activity pattern is explained by changes in fish size	y=fish size
Activity pattern is explained by changes in shoal size	y=shoal size
Activity pattern is explained by changes in extrinsic variables	y=temperature+year
Activity pattern is explained by changes in intrinsic variables	y=fish size+shoal size
Activity pattern is explained by changes in extrinsic and intrinsic variables	y= temperature+year+ fish size+shoal size
Activity pattern is explained by temperature and changes with fish size	y=temperature+fish size+ temperaturexfish size
Activity pattern is explained by temperature and changes with shoal size	y=temperature+shoal size+ temperaturexshoal size
Activity pattern is explained by year and changes with fish size	y=year+fish size+yearx fish size
Activity pattern is explained by year and changes with shoal size	y=year+shoal size+yearx shoal size
Activity pattern is explained by to all main factors and interactions	
Activity pattern is independent of extrinsic and intrinsic variables	

2.4. Results

Daily activity patterns

We obtained activity data for 231 chub, 120 in 2009 and 111 in 2010, with an average of 40 (SD = 5) and 37 (SD = 5) individuals per observation period in 2009 and 2010, respectively. During the day chub allocated their time to a variety of behaviors, although foraging behaviors were most common. Individuals spent half of the time foraging, either on benthic (27%) or drifting (23%) organisms, but also often oriented towards the current or shelter (21%). Cruising (16%) and searching for food (11%) were less frequent, whereas other behaviors were rare (2%).

Both explanatory variables and activity patterns differed significantly between 2009 and 2010. Temperature was lower, and both fish size and shoal size were larger and more variable in 2009 than in 2010 (Table 2). The PCA identified three behavioral patterns with eigenvalues >1, however, we only interpreted the first (PC1) and second (PC2) components because they explained most of the variation in the data, 39 and 26% respectively, whereas the third component (PC3) explained 20% and added little new information. Principal component 1 contrasted drift foraging and orienting from benthic foraging and searching, and PC2 discriminated cruising from searching (Fig. 1a). PC1 scores indicated that chub drift foraged and oriented more and benthic foraged and searched less in 2009 than in 2010, whereas PC2 scores underlined that in 2009 chub tended to display more searching and less cruising than in 2010 (Fig. 1a).

Table 2 – Variation in temperature, fish size and shoal size in 2009 and 2010; values are the mean ± SD and the range (minimum–maximum). Statistical significance calculated via Mann-Whitney (U) and Fisher's F tests (F).

	2009		2010			
	Mean	Range	Mean	Range	U	$F_{1,229}$
Temperature	14.9 ± 1.0	13.4-15.7	16.6 ± 0.8	15.5 - 17.5	1634.00 ****	1.73 **
Fish size	6.3 ± 1.2	5.0-9.0	5.6 ± 0.8	5.0-8.0	4533.00 ****	1.81 **
Shoal size	18.8 ± 12.8	1.0-45.0	4.9 ± 3.1	1.0-13.0	1996.50 ****	1.58 *

*P< 0.05; **P< 0.01; ***P< 0.001;****P< 0.0001

Extrinsic and intrinsic influences on behaviour

Variation in activity patterns for chub was affected by both extrinsic and intrinsic factors. The analysis revealed two interpretable models for PC1; the model with the greatest explanatory power included year, shoal size, and the year x shoal size interaction (AIC_C = 624.69, w_i = 0.78), whereas the second included temperature, shoal size and their interaction (AIC_C = 627.49, ΔAIC_C = 2.80, w_i = 0.19) and was 4.1 times less likely to be true given the data, than the best model. There were variations in the relative importance of extrinsic and intrinsic variables in explaining activity patterns data for chub (Table 3). There was strong support (w_i = 0.80) for chub to spend more time benthic foraging and searching than drift foraging and orienting in 2010. In addition, there was strong support (w_i = 1.00) for individuals in small shoals to spend more time benthic foraging and searching and less time drift foraging and orienting than those in large shoals (Table 3). However, shoal size had a model-averaged coefficient estimate that overlapped zero, and consequently this result should be interpreted with caution. The effect of shoal size varied strongly with year (year x shoal size, w_i = 0.80), and less time was allocated to benthic foraging and searching as shoal size increased in 2010, however this pattern was not present in 2009 (Fig. 1b). Finally, there was weak support for the effects of temperature (w_i = 0.20) and the temperature x shoal size interaction (w_i = 0.20), indicating a tendency for benthic foraging and searching to increase slightly with temperature, but only when shoals were small (Fig. 1c). This effect was evident only when temperature was above 14 °C, and was strongest at 18 °C (Fig. 1c).

Table 3 - Model averaged parameter estimates, 85% confidence intervals, and Akaike weights for parameters in interpretable models explaining variation in daily activity patterns of chub in 2009 and 2010. Year was coded as 1 in 2009 and 0 in 2010. See figure 1a for results of behavioral PCA.

	PC1				PC2			
		85% CI				85% CI		
Parameter	Estimate	Lower	Upper	wi	Estimate	Lower	Upper	wi
Year	-1.31	-1.8	-0.81	0.8	-0.86	-1.18	-0.54	1
Temperature	6.05	3.29	8.81	0.2	-3.58	-5.13	-2.04	1
Shoal size	0.62†	-3.12	4.37	1	0.07†	-0.05	0.19	0.8
Fish size					0.96	0.39	1.54	0.8
Temperature x shoal size	-2	-3.3	-0.7	0.2				
Year x shoal size	0.51	0.27	0.74	0.8				

† A lower confidence parameter (85% confidence intervals, CI, include zero).

Results for PC2 scores were similar to those for PC1, with two interpretable models also differing about 4.0 times in explanatory evidence given the data. The best model included the additive effects of temperature, year, fish size and shoal size (AIC_C = 643.45, w_i = 0.77), whereas the second best model included temperature and year (AIC_C = 646.23, ΔAIC_C = 2.78, w_i = 0.19). All variables had strongly supported associations with PC2 scores ($w_i > 0.80$, Table 3). In 2010, and as fish size increased and temperature decreased, chub cruised more and searched less. Individuals in larger shoals also cruised more and searched less than those in small shoals, though the model-averaged coefficient estimate for shoal size overlapped zero and needs to be interpreted with careful.

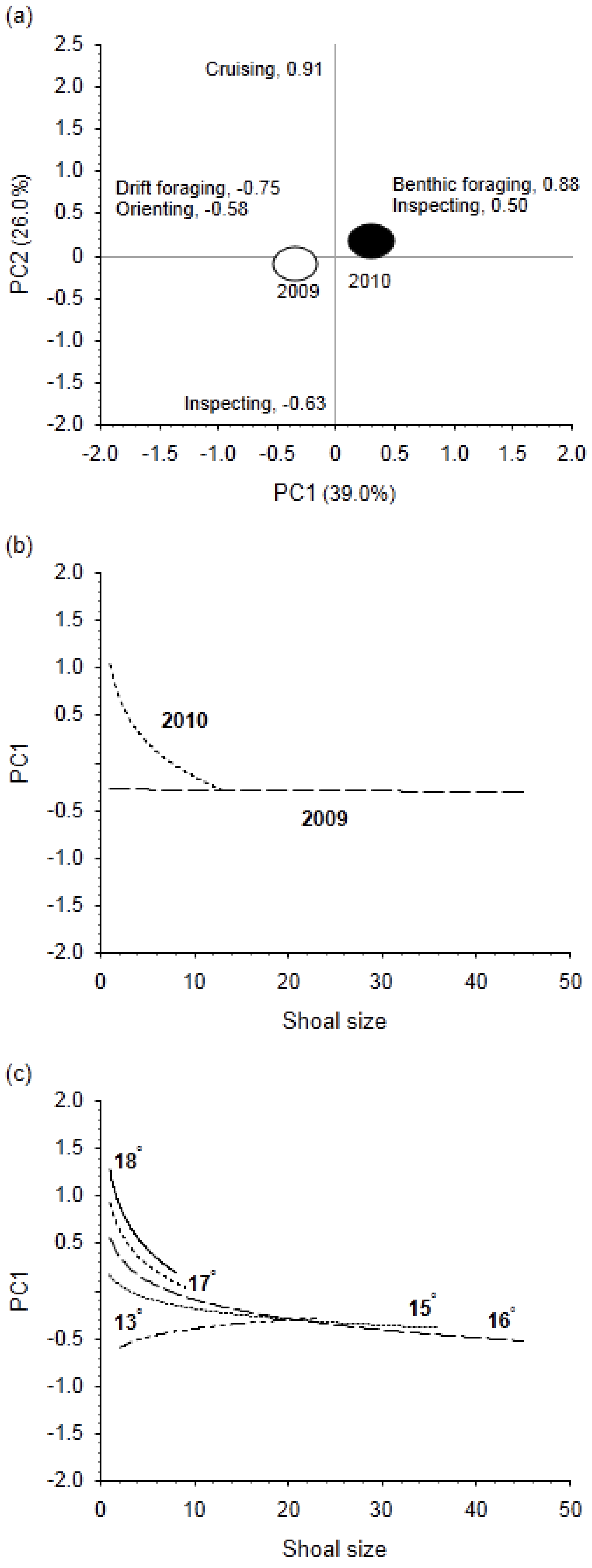

Fig. 1. Ordination diagram of Principal Component Analysis (PCA) of behavioral data for chub, in 2009 and 2010 (a), and generalized linear model fits of relationships between PC1 scores and shoal size, for 2009 and 2010 (b), and for water temperatures (°C) observed during the study (c). The ordination diagram (a) presents 95% confidence ellipses. Behaviors with loadings ≥0.40 are indicated for each axis.

2.5. Discussion

Our results demonstrate that daily activity patterns of chub in the Torgal stream varied on both an individual and annual basis and that both extrinsic and intrinsic factors and their interactions affected the behavior of this species. In 2009, chub allocated more time to drift foraging and orienting towards the current and shelter whereas in 2010 chub spent more time benthic foraging and cruising. Behavioral differences also were related to shoal size in 2010, with smaller shoals allocating more time to benthic foraging and searching. In addition, we observed that chub spent more time cruising and less time searching when temperatures were lower and chub length was greater. Overall, our results suggest that activity patterns of chub are flexible at an individual level, which may be of particular adaptive value in highly heterogeneous Mediterranean streams.

Cyprinidae is the most diverse family of fishes, having an almost worldwide distribution, with many ecologically important species(Nelson 2006). Despite these facts, very few studies have examined the daily activity patterns of cyprinids. To our knowledge, this study is one of the first to quantify the effects of extrinsic and intrinsic factors on activity patterns of European cyprinids in natural habitats, and provides important ecological information that may be useful to develop conservation plans for highly endangered Mediterranean species. Our findings add to a body of evidence that activity patterns of fishes are likely to be influenced by complex interactions of both extrinsic and intrinsic factors that act at an individual level (Greenwood & Metcalfe 1998; Metcalfe et al. 1999; Ovidio et al. 2002; David & Closs 2003).

Although our study provides valuable empirical evidence on activity patterns for a cyprinid in the wild, our results were potentially affected by several factors. For example, data collection occurred in a single site and season (spring) although we did observe chub in two years. Observations in other seasons were not possible due to high flows and turbidities (Martelo et al. personal observations). In addition, during the two years of observations conditions were dry, and thus our results may not be completely relevant to wet or perhaps average water years (Magalhães et al. 2007). Even though inter-annual variability in activity patterns was evident, it might have been greater if behavioral data also had been collected in wet years, because variation in flow may affect fish movement and foraging behavior (Petty & Grossman 2004; Kemp et al. 2006). Furthermore, we excluded fish less than 5 cm (SL) from analysis, which may have weakened the association between fish size and activity patterns. However, this was unavoidable because we could not identify small fish to species underwater. Finally, we never observed reproductive behaviors, probably because sampling occurred at the end of the spawning season (see Magalhães et al. 2003); consequently, it is unlikely that our results were influenced by reproduction processes.

Chub displayed variable behaviors between years, which may have been affected by differences in food availability. Chub allocated more time to drift foraging in 2009 and largely shifted to benthic foraging in 2010. The abundance of drifting macroinvertebrates declined in 2010 in the study site, whereas the abundance of benthic organisms remained very similar between years (Martelo et al., unpublished data). Variations in prey availability produce changes in foraging modes for several species (e.g. Nakano et al. 1999; Katano 2011), and also affect the diet of many Mediterranean cyprinids (e.g. Magalhães 1993; Gomes-Ferreira et al. 2005). Nonetheless, it also is possible that random processes played a role in the differences observed between 2009 and 2010.

Chub spent more time feeding on and searching for benthic organisms when in small groups than in large groups in 2010. This suggests that intraspecific interactions may affect activity patterns of this species (Pitcher & Parrish 1993; Grand & Dill 1999). It is curious, however, that shoal size did not affect activity patterns in 2009, when shoals showed greater variation in size. Reduced in shoal size in 2010 was apparently related to the decrease in abundance of small adults (Martelo et al., unpublished data), which probably reflected the influence of the severe drought in 2009 (Magalhães et al 2007).

Temperature also influenced the activity patterns of chub, probably through its effect on metabolism. It is likely that increased temperatures resulted in an increase in the metabolic rate of chub and hence they spent more time searching for prey and less time cruising. These results are concordant with previous studies of fish activity patterns that demonstrate a relationship between diel activities and water temperature (Fraser et al. 1993; Greenwood & Metcalfe 1998; Bremset 2000), however, few of these studies examined foraging behaviors in the field. In laboratory studies, search, capture rate and swimming characteristics (i.e., speed and angle of turn) may be positively related to temperature (Persson 1986; Marchand et al. 2002; Linløkken et al. 2010). Despite the strongly supported effect of temperature on searching behavior, its effect on foraging was weak, which may have been related to the low range of temperature values observed during the study.

Smaller individuals spent more time searching for prey and less time cruising than larger individuals. It is possible that this occurred because individuals with smaller body sizes have higher energetic demands than larger members of the same species (Brett & Groves 1979). Another potential explanation for these differences is that intraspecific competition between small and large adults limited small adult access to high-quality prey, forcing them to spend more time searching for food. The influence of intraspecific competition on daily activity and habitat use has been documented in studies of salmonids (Hughes & Dill 1990; Nakano 1995; Alanärä et al. 2001) cyprinids (Hazelton & Grossman 2009b) and cottids (Petty & Grossman 2007), and on foraging success in cyprinids (Hazelton & Grossman 2009a). Nonetheless, small and large chub occurred in the same shoal without intraspecific aggression.

In addition, small and large chub occupy similar habitat patches and changes in abundance did not affect habitat use in this system (Martelo et al., unpublished data).

Little is known about the behavior of European cyprinids, especially members of the widespread genus *Squalius*. Extant studies focus on patterns of movement, habitat use and feeding, and, in accordance with our findings, have shown that individual behavior can be highly variable and affected by multiple factors, including temperature and fish size. Changes in habitat use in relation to temperature may include increasing use of shallow or low-velocity habitats in periods of high temperatures (Santos et al. 2004; Santos & Ferreira 2008), and use of vegetation as shelters as temperatures drop (Baras & Nindaba 1999). Size-related differences in habitat use may be small (Martelo et al. unpublished data), or, substantial, with larger individuals generally associating with deep, high velocity habitats with little cover (Grossman & de Sostoa 1994a, b; Santos et al. 2004; Santos & Ferreira 2008). Movements may increase with both temperature (Santos et al. 2002, 2005) and fish size (Pires 2012). Finally, size-related shifts in feeding behavior also have been reported, with larger fish preying on a higher percentage of hard-shelled prey than smaller fish with a concomitant decrease in dietary breadth (Magalhães 1993). Although temperature and fish size seem to influence the behavior of *Squalius spp*., the effects of annual environmental variation and shoal size variation require further study.

Daily activity patterns of chub were affected by both extrinsic and intrinsic factors, individual behaviors varied between years, and were dependent on shoal size, body size and temperature. Similar to other fish species (e.g. Reebs 2002; David & Closs 2003; Breau et al. 2007), activity was variable at an individual level indicating that the behavior of chub display substantial behavioral flexibility. This likely is of adaptive value given that Mediterranean streams are highly variable and heterogeneous. Despite this flexibility, given their rarity it is possible that chub will be vulnerable to the long and intense droughts (Magalhães et al. 2007), predicted for the Mediterranean region by climate change models (Santos & Miranda 2006). In conclusion, our results suggest that conservation and management efforts for chub should include the maintenance of natural temperature and flow regimes and continued evaluation of environmental change on shoal and fish size distributions. Moreover, given that behavioral studies are increasingly recognized as important in species conservation (Buchholz 2007), a better understanding of the factors influencing the behavior of chub is needed. We believe that our results provide one of the first efforts to remedy this shortcoming.

2.6. References

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2.7. Corrigendum

Martelo, J., Grossman, G.D., & Magalhães, M.F. 2013. Extrinsic and intrinsic factors influence daily activity of a Mediterranean cyprinid. Ecology of Freshwater Fish 2013: 22: 307–316.

Our article “Extrinsic and intrinsic factors influence daily activity of a Mediterranean cyprinid” used rainfall measurements to classify years by flow with data from the website of *Sistema Nacional de Informação de Recursos Hídricos (SNIRH) (http://snirh.pt)*. Unfortunately, this agency has just revised the rainfall estimates for one of our study years, and this has resulted in some minor changes in the wording of our paper as follows:

1. The fifth sentence below the subtitle ‘Study site’ on p. 308 should read: Although the seasonal timing of rainfall is predictable, the total amount of rain varies considerably among years (129–1121 mm).
2. The second sentence below the subtitle ‘Environmental data and daily activity patterns’ on p. 309 should read: October 2008 to September 2009 was the driest 12-month period on record in the Torgal stream since 1931 (129 mm), and rainfall data in the following year, October 2009 to September 2010, also were close to the long-term annual median (690 vs. 658 mm).
3. The fourth sentence of the third paragraph on p. 312 should read: In addition, during the two years of observations, conditions were dry to normal, and thus, our results may not be completely relevant to high flow years.

Chapter 3.

Habitat patchiness affects distribution and microhabitat use of endangered Mira chub *Squalius torgalensis*

Martelo, J., Porto, M., Grossman, G.D. & Magalhães, M.F. 2014. Habitat patchiness affects distribution and microhabitat use of endangered Mira chub *Squalius torgalensis*. Hydrobiologia, 732: 93-109.

3. Habitat patchiness affects distribution and microhabitat use of endangered Mira chub *Squalius torgalensis*

3.1. Abstract

We hypothesized that distribution and microhabitat use by imperilled chub *Squalius torgalensis* in the Torgal stream, Portugal, during low flows, were related to spatial patchiness in physical resources and shifts in ontogenetic preferences. We mapped fish abundance and sampled microhabitat use and availability via snorkelling. We used the coefficient of dispersion in abundance and spatial autocorrelation analyses to characterize chub distribution, and Hurdle models to relate chub presence and abundance to habitat characteristics. We showed that chub displayed an aggregated distribution, apparently in association with patchily distributed and autocorrelated physical resources, such as debris, roots and aquatic vegetation. Microhabitat use generally was unrelated to velocity, and ontogenetic differences in microhabitat use were not substantial. Nevertheless, sometimes small chub preferred low velocity patches with coarse substrata, debris and roots whereas large chub preferred deeper patches with roots and aquatic vegetation. Results suggest that, in low flow conditions, chub respond to resource patchiness by congregating in favourable microhabitats, and that maintenance of mosaics of patches with variable substrata, cover and depth may be important for the persistence of fish in Mediterranean streams.

3.2. Introduction

Resource patchiness both within and among habitats affects a variety of ecological processes, such as the spatial distribution and microhabitat use of individuals (Palmer & Poff 1997; Einum et al. 2008; Winemiller et al. 2010). For example, the spatial distribution of oviposition sites (i.e., rocks) by aquatic insects constrains their dispersal, and hence their spatial distribution (Lancaster et al. 2003). Similarly, patchiness in prey distributions strongly affects microhabitat use by stream fishes (Thompson et al. 2001; Petty & Grossman 2010; Grossman 2013). The identification of patchiness in physical and biological resources and quantification of their effects on the distribution and resource use of organisms will greatly aid our understanding of the dynamics of natural systems.

Patchiness in biological and physical resources is particularly common in streams (Hildrew & Giller 1994), which may exhibit substantial spatial and temporal variability at multiple scales due to seasonal and annual variations in flow (Pringle et al. 1988; Grossman et al. 1995; Thompson et al. 2001). Small-scale spatial patchiness in physical resources may influence the distribution and abundance of stream fishes, often promoting aggregation around preferred resources (Freeman & Grossman 1993; Torgersen & Close 2004; Argentina et al. 2010). Similarly, temporal patchiness in resources may produce seasonal and annual shifts in the distribution and abundance of fishes (Grossman & Ratajczak 1998; Lake 2000; Thompson et al. 2001). Moreover, fishes have complex life histories and the importance and effects of spatial and temporal resource variability may change through ontogeny (Wootton 1990). Despite increasing recognition that resource use by fishes is influenced by resource patchiness, and that resource use varies over ontogeny, many studies ignore both the distribution of resource points in space and time and their effects on different size/age classes of fishes. This likely limits our ability to identify the factors influencing the distribution and resource use patterns of stream fishes (Keitt et al. 2002; Lancaster 2006).

At present, there is little information on the distribution and abundance of fishes in habitats with high habitat and hydrologic variability, such as Mediterranean streams (but see Santos et al. 2004; Santos & Ferreira 2008; Martínez-Capel et al. 2009). A knowledge of these patterns and the factors influencing them is particularly important given the high degree of imperilled fishes in local assemblages (Smith & Darwall 2006) and the expected increase in intensity and frequency of droughts in the Mediterranean region due to climate change (Millán et al. 2005; IPCC 2008). This information likely will contribute for management actions focused on conserving Mediterranean stream fishes.

We examined the influence of patchiness in physical resources on the spatial distribution and microhabitat use of the critically endangered Mira chub *Squalius torgalensis* Coelho, Bogutskaya, Rodrigues & Collares-Pereira in a Mediterranean stream using spatially explicit methods. During the study, the stream was characterized by low flows, thus providing important information for the conservation of the species under altered future environments. Specific objectives were to: 1) quantify the spatial distribution of chub, 2) determine the physical characteristics related to chub microhabitat use, 3) explore relationships between chub distribution and microhabitat use patterns and patchiness in physical characteristics, and 4) compare these patterns between size classes, reaches and years. Results were then used to explore conservation implications for chub, and possibly, other Mediterranean stream fish.

3.4. Methods

Study species

Mira chub *S. torgalensis* (henceforth chub) are endemic to Mediterranean streams in the SW of Portugal. Although this species may be locally abundant, it is thought to have declined due to habitat loss and degradation, and is currently listed as Critically Endangered in the Portuguese Red Data Book (Rogado et al. 2005). Chub are small (less than 16 cm total length) invertivores (Magalhães et al. 2003) that occur in runs and pools (Magalhães et al. 2002), and occupy mid-water column microhabitats from which they prey upon drifting and benthic invertebrates during the day (Martelo et al. 2013). Chub typically live up to 5 years, mature in 1-2 years and spwan between March and May (Magalhães et al. 2003). This species generally shows recruitment failures in dry years (Magalhães et al. 2003), thereby being highly vulnerable to dry spells lasting for 6 years or more (Magalhães et al. 2007).

Study reaches

We conducted our study in two reaches of the Torgal stream (SW Portugal, 37°38’N, 8°39’W), a small (28 km) relatively undisturbed Mediterranean stream that has been described in detail in Magalhães et al. (2007). In brief, the Torgal primarily drains a landscape of siliceous igneous geology, dominated by native open cork oak *Quercus suber* L. woodlands and introduced eucalyptus *Eucaliptus globules* Labill plantations. Riparian galleries are generally well preserved, and there are few human impacts. The climate is Mediterranean, with rainfall varying markedly from year-to-year (205-1121 mm), but following a predictable seasonal pattern. About 80% of annual rain occurs in October-March, but rainfall is almost completely lacking during summer months (July-August). Mean monthly temperatures ranges from 11°C (December) to 24°C (August). The flow regime of the Torgal is highly dependent on rainfall patterns. Headwaters flow only after heavy rains, while the mainstream and the largest tributaries typically dry in variable extents each summer. In dry years, there are no significant floods, the drought is intense and last for longer periods, with water persisting in a few isolated pools and aquifer-fed runs. Conversely, in wet years large floods are common and flows may persist through the summer in downstream reaches.

Our study reaches were approximately 2 km apart, in the downstream segment of the Torgal. They consisted of persistent but relatively shallow runs (< 80 cm deep in spring), with clear water and intact riparian vegetation. We selected persistent reaches to reduce potential confounding effects of emigration/extinction and colonization processes, which may result in weak fish-habitat relationships in streams (Angermeier & Schlosser 1989). Reach boundaries were determined by discrete channel units with little suitable habitat for underwater observations. The upstream reach (Reach 1) was 80 m in length, and bounded downstream by a deep pool and upstream by a shallow rapid with boulders. The lower reach (Reach 2) was 40 m in length, and bounded downstream by a deep pool and upstream by a gravel bar. Because neither pools nor riffles are barriers to chub movement (Pires 2012), chub were probably able to move in and out of the reaches. However, this potential bias was constant across reaches, and consequently unlikely to have substantially influenced our results.

Fish assemblages in the study reaches were dominated by chub (72-87%), although the endemic Southwestern arched-mouth nase *Iberochondrostoma almacai* Coelho, Mesquita & Collares-Pereira (4-12%) also occurred in both reaches and years. Native Western three-spine stickleback *Gasterosteus gymnurus* L. (7%), European eel *Anguilla anguilla* L. (0.3%) and Southern Iberian spined-loach *Cobitis paludica* De Buen (0.3%) occurred only in Reach 1 in 2009, and Southern Iberian barbel *Luciobarbus sclateri* Ghünter (23%) occurred only in Reach 2 in 2010. The exotic pumpkinseed *Lepomis gibbosus* L. occurred throughout the study, but in low numbers (0.3-1%).

Sampling regime

We sampled Reach 1 in spring 2009 (13-15 May) and spring 2010 (31 May-2 June) and Reach 2 only in spring 2010 (22-23 June). We restricted sampling to spring because increased flow or water turbidity prevented underwater observation of fish and habitat assessment in other seasons. In addition, our sampling was conducted after the peak spawning period of chub, to avoid biases produced by reproductive behaviour. Rainfall data from a nearby gauging station (37°42'N, 8°32'W), indicated that October 2008 to September 2009 was the driest 12 month period on record since 1931 (129 mm), and October 2009 to September 2010, also was close to the long-term annual median (690 versus 658 mm). During our study, flow persisted in both reaches, though the wetted channel in Reach 1 was narrower in 2009 than in 2010 (3-9 m versus 6-10 m) and Reach 2 also was narrower (4-7 m) than Reach 1. Water temperatures varied between 15 to 17° C during sampling.

Fish and habitat sampling

We quantified fish distribution and microhabitat use, and microhabitat availability via snorkelling during daylight hours because this was when fish actively foraged, whereas they were quiescent at night (Martelo et al. 2013). We sampled each reach in 2-3 days, and both reaches were sampled within 3 weeks of each other. Preliminary investigation showed that snorkelling on consecutive days yielded similar fish counts (< 7% difference) within reaches, suggesting that sampling on any given day yielded comparable results.

We mapped the number of chub in 1 m^2 quadrats using the methods of Freeman & Grossman (1993). Specifically, we established a 1 x 1 m grid over the wetted channel of the study reach using transect ropes and numbered spikes. Spikes were driven into the banks at the ends of transect lines, and marked at 1 m intervals with coloured tape. We also mapped the wetted channel for each reach in each sampling period. We quantified microhabitat use of chub using the methods of Grossman & de Sostoa (1994a, b). We first entered the reach from the downstream boundary and snorkelled slowly upstream in a zigzag manner to maximize spatial coverage and minimize fish disturbance. We scanned both the substratum and the water column to locate fish, and only recorded undisturbed individuals. Disturbed fish were recognized by their characteristic escape responses (i.e., fleeing and sustained darting movements). No reproductive behaviours were observed during sampling. We identified fishes to species, and visually estimated standard length (SL, cm) and abundance in each quadrat. Fish less than 5 cm were excluded because they were not reliably identifiable to species underwater. To minimize observer and detection bias, a single observer (J. Martelo) conducted sampling throughout the study.

We quantified physical habitat in all quadrats occupied by chub, and in approximately 10% of the remaining, non-occupied quadrats. This resulted in relatively similar numbers of occupied and unoccupied quadrats, as recommended for minimization of modelling bias (Hosmer & Lemeshow 1989). We stratified non-occupied quadrats by location, and randomly selected 80% of quadrats in the mid channel and 10% along each margin of the reach. Stratified random sampling was used to obtain a statistically reliable sample of microhabitat availability (Grossman & Skyfield 2009). Overall, we sampled 27 occupied and 47 unoccupied quadrats in Reach 1 in 2009, 51 occupied and 55 unoccupied quadrats in Reach 1 in 2010, and 26 occupied and 18 unoccupied quadrats in Reach 2 in 2010.

We measured seven physical variables commonly used in fish microhabitat use studies (e.g., Grossman & de Sostoa 1994a, b; Carter et al. 2004; Santos et al. 2004; Martínez-Capel et al. 2009). Water depth (meter stick) and average velocity (Global Water FP101 electronic velocity meter) were measured in the centre of each quadrat. We quantified average velocity at 60% depth in quadrats ≤ 75 cm deep, and at 20% and 80% depth in quadrats > 75 cm deep. We visually estimated the percentages of organic plant debris (i.e., leaves, limbs and sticks) and the percentage of inorganic substrata. Inorganic substrata were scored for maximum diameter as 1: ≤ 0.2 cm, 2: 0.3 - 2.5 cm, 3: 2.6 - 15 cm, 4: 16 - 30 cm, and 5: > 30 cm, and coarseness was then calculated as the weighted average of all inorganic substratum categories (Joy & Death 2002). We also estimated visually the percentages of roots, aquatic vegetation and overhanging vegetation (< 50 cm above water surface).

3.5. Data analysis

Analyses were carried out separately for data collected in Reach 1 in 2009 and 2010 and Reach 2 in 2010 to compare chub distribution and habitat relationships across the two years and reaches of study. In addition, because size-related variation in microhabitat use is common in stream fishes (e.g., Grossman & de Sostoa 1994a, b; Santos & Ferreira 2008), we conducted separate analyses for small (5.0 – 5.9 cm, SL) and large (≥ 6.0 cm, SL) chub, with the former generally being immature individuals (Magalhães et al. 2003). Large fish in Reach 1 averaged 6.9 cm (± 1.3) in 2009 and 6.5 cm (± 1.0) in 2010 and those in Reach 2 averaged 7.1 cm (± 1.5) in 2010. Prior to statistical analyses, we inspected histograms of fish abundance and habitat data for skewness. Skewed habitat variables were transformed using the angular transformation for proportional data and the logarithmic transformation ($\log_{10}$) for continuous variables. We then standardized habitat variables to a mean of 0 and a standard deviation of 1 to facilitate interpretation of relative parameter effect sizes (Crawley 2007; Hazelton & Grossman 2009). We tested for multicollinearity among habitat variables using correlation analysis (Pearson's r), and found no strong correlations ($|r| < 0.50$) thereby retaining all variables in analyses. Simple regressions between habitat variables and chub abundance only yielded linear relationships, and consequently we used linear models for data analyses. Except where indicated, analyses were conducted using the R 2.15.2 environment and the STATISTICA vs.10.0 software, and the significance of statistical testing was assessed at $P < 0.05$.

Variation in habitat characteristics

We used two approaches to quantify variability in habitat characteristics. First, we quantified habitat patchiness by calculating the coefficient of variation (CV) for each habitat variable among quadrats, in each reach and year (Palmer et al. 1997; Thompson et al. 2001). We classified habitat conditions based on the variability criteria of Grossman et al. (1990) : CV < 40% = low variability, 40% ≤ CV ≤ 70% = moderate variability and CV > 70% = high variability. Second, we used Principal Component Analysis (PCA) on habitat variables to summarize temporal and spatial changes in microhabitat availability, pooling all data. We tested the null hypotheses that (1) habitat variables did not differ between 2009 and 2010 in Reach 1, and (2) habitat variables did not differ between Reach 1 and Reach 2 in 2010, by calculating mean component scores and 95% confidence ellipses for each year and reach. A lack of overlap of the 95% confidence ellipses demonstrated significance at the 0.05 level (Anglin & Grossman 2013).

We examined whether habitat variables were spatially autocorrelated, using Moran's I (Legendre & Legendre 1998) under the null hypothesis of random spatial distribution of habitat characteristics among quadrats. Moran's I usually takes values in the interval -1.0 and 1.0, with significant values of I indicating that habitat characteristics in quadrats at a given distance are more similar (positive autocorrelation) or less similar (negative autocorrelation) than expected for randomly associated pairs of quadrats. We calculated Moran's I using 1 m classes for pair wise distances because our quadrats were 1 m^2 and this class width provided a good compromise between the resolution of the correlogram and the power of the test of significance. We evaluated the significance of I with Monte Carlo permutation tests (1000 permutations), using the progressive Bonferroni method to correct for multiple testing (Legendre & Legendre 1998).

Spatial patterns in chub abundance

Spatial patterns in chub abundance were analysed using the coefficient of dispersion (CD, Krebs 1989; Lancaster et al. 2003) and Moran's I coefficients. We first compared the distribution of chub abundance per quadrat to a Poisson distribution using a two-tailed χ^2 test on CD data, thus testing the null hypothesis that chub were randomly distributed among quadrats. Values for the CD: 1) approach one when spatial distributions are essentially random, 2) are greater than one if chub are aggregated, and 3) are less than one if their distribution is uniform. Because our sample sizes were large we used a normal approximation for χ^2 value (Krebs 1989). Given that the CD is not spatially explicit, we then assessed autocorrelation in chub abundance per quadrat using Moran's I coefficients under the null hypothesis of random spatial distribution of chub among quadrats. Finally, to illustrate spatial patterns, we mapped chub abundance per quadrat.

Microhabitat use by chub

Because our data sets were zero inflated, we used hurdle models to assess relationships between chub abundance and habitat characteristics (Cunningham & Lindenmayer 2005; Potts & Elith 2006), which have proven successful in previous studies of fish habitat use (Wenger & Freeman 2008). The hurdle model typically consists of two components, the first of which is a binary (presence/absence) model and the second is an abundance model without zeroes. For independent abundance data, the two components of the hurdle model are orthogonal, and can be fitted and interpreted separately (Cunningham & Lindenmayer 2005; Wenger & Freeman 2008). We built hurdle models using binomial distributions and logit links for the binary presence/absence component (henceforth presence component), and Poisson distributions and log link functions for the positive count component (henceforth abundance component).

We constructed 20 candidate microhabitat models using as explanatory variables, velocity, depth, and two sets of variables that characterized substrata and cover (Table 1). Specifically, substrata included substratum coarseness and percentage of debris, while cover included percentage of roots, aquatic vegetation and overhanging vegetation. To avoid the production of trivial models, overhanging vegetation in Reach 1 in 2009 and 2010, and aquatic vegetation in Reach 2 in 2010 were excluded from analysis because they included a large number of zeroes (> 90%). For simplicity, interaction terms were not incorporated in candidate models.

We used an information-theoretic approach based on the Akaike's Information Criterion corrected for small sample sizes (AICc) to evaluate the plausibility of each candidate model, given the data (Burnham & Anderson 2002). We evaluated the relative performance of each candidate model by calculating the ΔAICc, and scaling them according to their Akaike weight (w_i). We only interpreted models with Akaike weights >10% of the model with the best fit (Burnham & Anderson 2002). To quantify model selection uncertainty, we calculated model-averaged parameter estimates (β) and evaluated the precision of these estimates using 85% confidence intervals; estimates whose confidence limits included zero were not interpreted (Burnham & Anderson 2002). The relative importance of each explanatory variable (w_+) was estimated by summing Akaike weights of all interpretable models in which the variable occurred (Burnham & Anderson 2002).

Because AIC may be sensitive to the presence of spatial autocorrelation, generating unstable and over fitted minimum adequate models (Diniz-Filho et al. 2008) we assessed whether the residuals of the average models were spatially autocorrelated, using Moran's I (Legendre & Legendre 1998) under the null hypothesis of random spatial distribution of the residuals.

Table 1 – Candidate models used to evaluate relationships between chub presence and abundance and habitat variables in the study reaches of the Torgal in spring 2009 and 2010. Vegetation (Ve) includes aquatic vegetation (Av) in Reach 1 in 2009 and 2010, and overhanging vegetation (Ov) in Reach 2 in 2010.

Model description	Model notation
Velocity	V
Depth	D
Substratum coarseness	Sc
Debris	De
Roots	R
Vegetation	Ve
Substrata (substratum coarseness & debris)	Sc+De
Cover (roots & vegetation)	R+Ve
Velocity & depth	V+D
Velocity & substrata	V+Sc+De
Velocity & cover	V+R+Ve
Depth & substrata	D+Sc+De
Depth & cover	D+R+Ve
Substrata & cover	Sc+De+R+Ve
Velocity, depth & substrata	V+D+Sc+De
Velocity, depth & cover	V+D+R+Ve
Velocity, substrata &cover	V+Sc+De+R+Ve
Depth, substrata & cover	D+Sc+De+R+Ve
Velocity, depth, substrata & cover	V+D+Sc+De+R+Ve
Intercept	Intercept

3.6. Results

Variation in habitat characteristics

Microhabitat availability displayed substantial variability among quadrats within reaches. All variables showed high coefficients of variation (94% ≤ CV ≤ 600%) in both reaches and years, except substratum coarseness and depth (Table 2). Substratum coarseness displayed low variability in Reach 1 in 2009 (CV = 18%) and in 2010 (CV = 28%), but in Reach 2 it exhibited high variability (CV = 100%). Depth also exhibited low variability in both reaches and years (30% ≤ CV ≤ 38%).

Table 2 – Habitat characteristics (HC) of the study reaches of the Torgal in spring 2009 and 2010. Values are the mean, standard deviation (SD), range (minimum-maximum), and coefficient of variation (CV); n is the sample size. Other abbreviations are as per Table 1.

	Reach 1						Reach 2		
	2009 (n = 74)			2010 (n = 106)			2010 (n = 44)		
HC	Mean ± SD	Range	CV	Mean ± SD	Range	CV	Mean ± SD	Range	CV
V	1.1 ± 3.6	0-16.0	327	7.2 ± 6.8	0-29.0	94	3.6 ± 5.3	0-19	147
D	37.5 ± 14.2	10.0-76.0	38	48.3 ± 16.1	10.0-81.0	33	44.4 ± 13.4	9.0-66.0	30
Sc	3.8 ± 0.7	1.6-4.9	18	3.2 ± 0.9	1.0-4.6	28	2.3 ± 0.4	1.2-3.1	100
Db	8.9 ± 18.3	0-100.0	206	10.7 ± 21.7	0-100.0	203	28.2 ± 20.4	5.0-90.0	100
R	9.3 ± 20.4	0-85.0	219	10.4 ± 20.2	0-100.0	194	3.4 ± 9.7	0-40.0	285
Av	11.4 ± 16.3	0-65.0	143	9.8 ± 15.8	0-80.0	100			
Ov	1.9 ± 11.4	0-95.0	600	3.9 ± 12.4	0-70.0	318	5.9 ± 17.8	0-95.0	302

The PCA did not detect significant inter-annual (2009-2010) differences in habitat within Reach 1, however, habitat differed significantly between Reaches 1 and 2 in 2010 (Fig. 1). The first PC axis accounted for 25% of the variation in the data, and represented a gradient involving debris, substratum coarseness and velocity. The second PC axis (18%) identified a gradient of aquatic vegetation and roots. The PCA demonstrated that in 2010, Reach 1 had higher water velocity, coarser substrata, and more aquatic vegetation and roots than Reach 2.

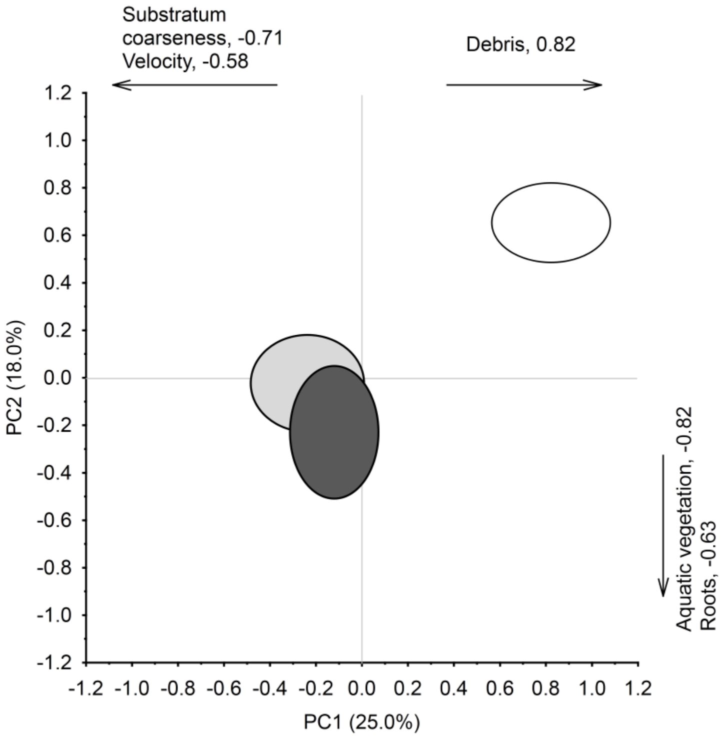

Fig. 1 – Ordination diagram of Principal Component Analysis (PCA) of habitat in the study reaches of the Torgal in spring 2009 and 2010. Variables with loadings > 0.40 and 95% confidence ellipses of scores are presented. Dark grey ellipse represents Reach 1 in 2009, light grey ellipse represents Reach 1 in 2010, and blank ellipse represents Reach 2 in 2010.

Spatial autocorrelation in habitat variables varied among reaches and years. In Reach 1 in 2009 only debris, roots and aquatic vegetation showed positive autocorrelations whereas all habitat variables were spatially correlated in 2010; in Reach 2, spatial correlations were found only for depth, substratum coarseness and overhanging vegetation (Fig. 2). In all cases, the highest positive spatial autocorrelations ($0.001 \leq P \leq 0.039$) generally occurred in the first distance class (1 m), decreasing at subsequent distances (2-5 m), and fluctuating up and down thereafter (Fig. 1-3 in the Supplementary Material).

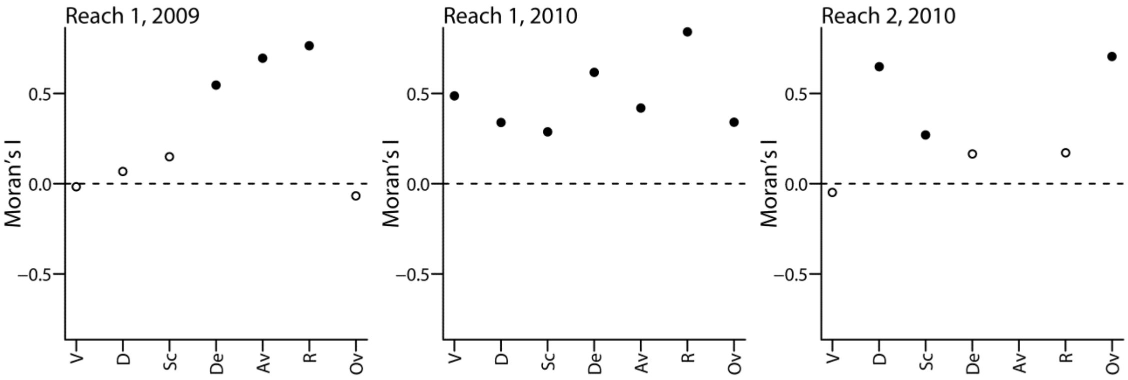

Fig. 2 – Summary Moran's I correlograms for habitat characteristics using 1 m classes for pair wise distances along the study reaches of the Torgal in spring 2009 and 2010. Dark symbols represent Moran's I statistics that are significant after progressive Bonferroni correction ($P < 0.05$). Positive significant values indicate positive autocorrelations, and thus that nearer quadrats are more similar in habitat characteristics than quadrats farther apart. Significant negative values have the opposite meaning. Abbreviations are as follows: V = velocity; D = depth; Sc = substratum coarseness; De = debris; R = roots; Av = aquatic vegetation; and Ov = overhanging vegetation.

Spatial patterns in chub abundance

There was considerable variation in the distribution and abundance of chub between years but not between reaches. The percentage of quadrats occupied by chub always was low (5-11%), but the numbers of individuals per quadrat varied considerably in Reach 1 between 2009 and 2010. In 2009, there were a maximum of 34 individuals per quadrat, and 91% of chub were in quadrats with more than 5 individuals (Fig. 3a). By contrast, in 2010 the maximum number of individuals per quadrat was 12, only 47% of chub were in quadrats having more than 5 individuals, and 9% were solitary (Fig. 4a). These differences were produced by changes in the abundance of small chub, whose mean number per quadrat decreased from 10 to 3 between 2009 and 2010, while large chub abundance remained relatively constant (3) between years (Table 3, Fig. 3b and 4b). Moreover, the distribution of size classes was highly variable in 2009, because 88% of small chub were in quadrats with more than 5 individuals and 1% were solitary whereas only 56% of large chub were in quadrats with more than 5 individuals and 12% were solitary (Fig. 3b, c). Conversely, in 2010 most small (59%) and large chub (60%) were in quadrats with 2-5 individuals and 15% of small and 40% of large chub were solitary (Fig. 4b, c).

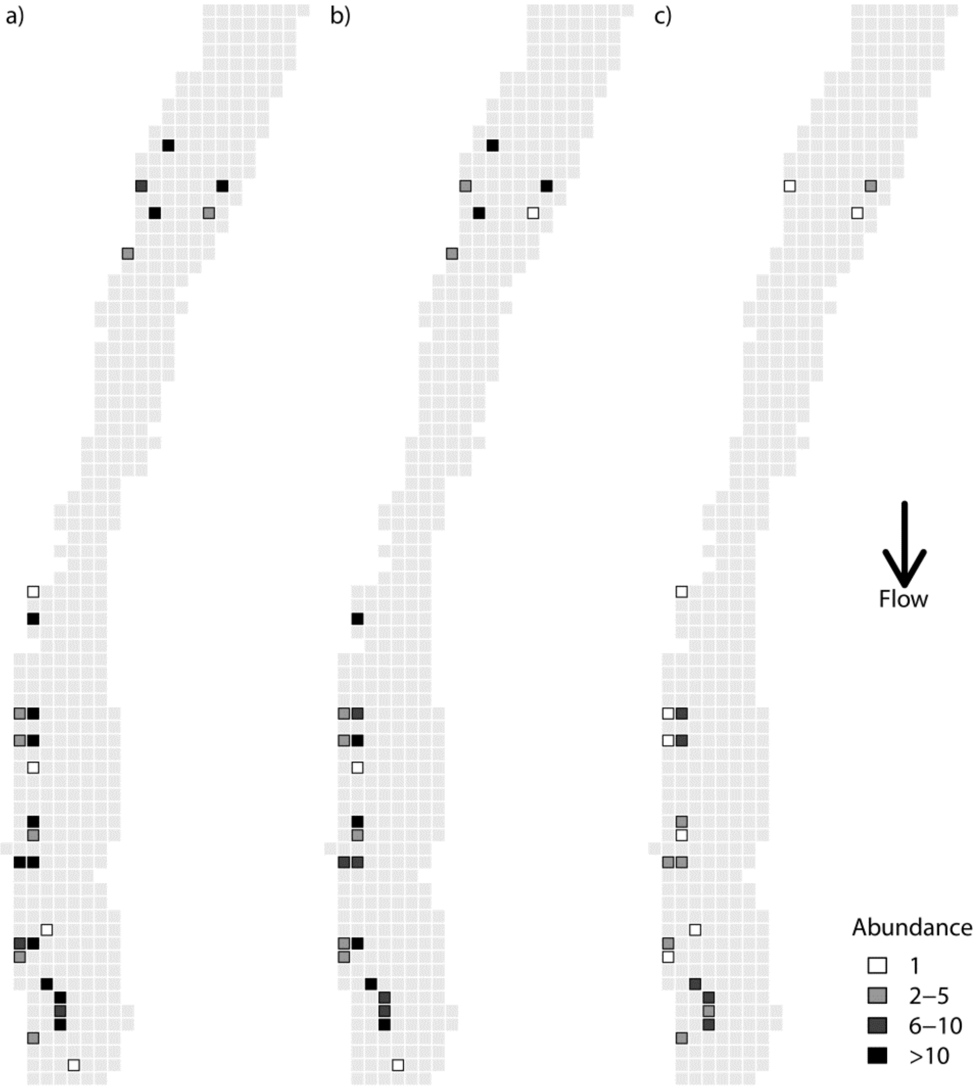

Fig. 3 – Representation of Reach 1 in 2009 showing the spatial distribution of (a) pooled, (b) small and (c) large chub abundance in 1 m^2 quadrats. Blank quadrats did not contain chub.

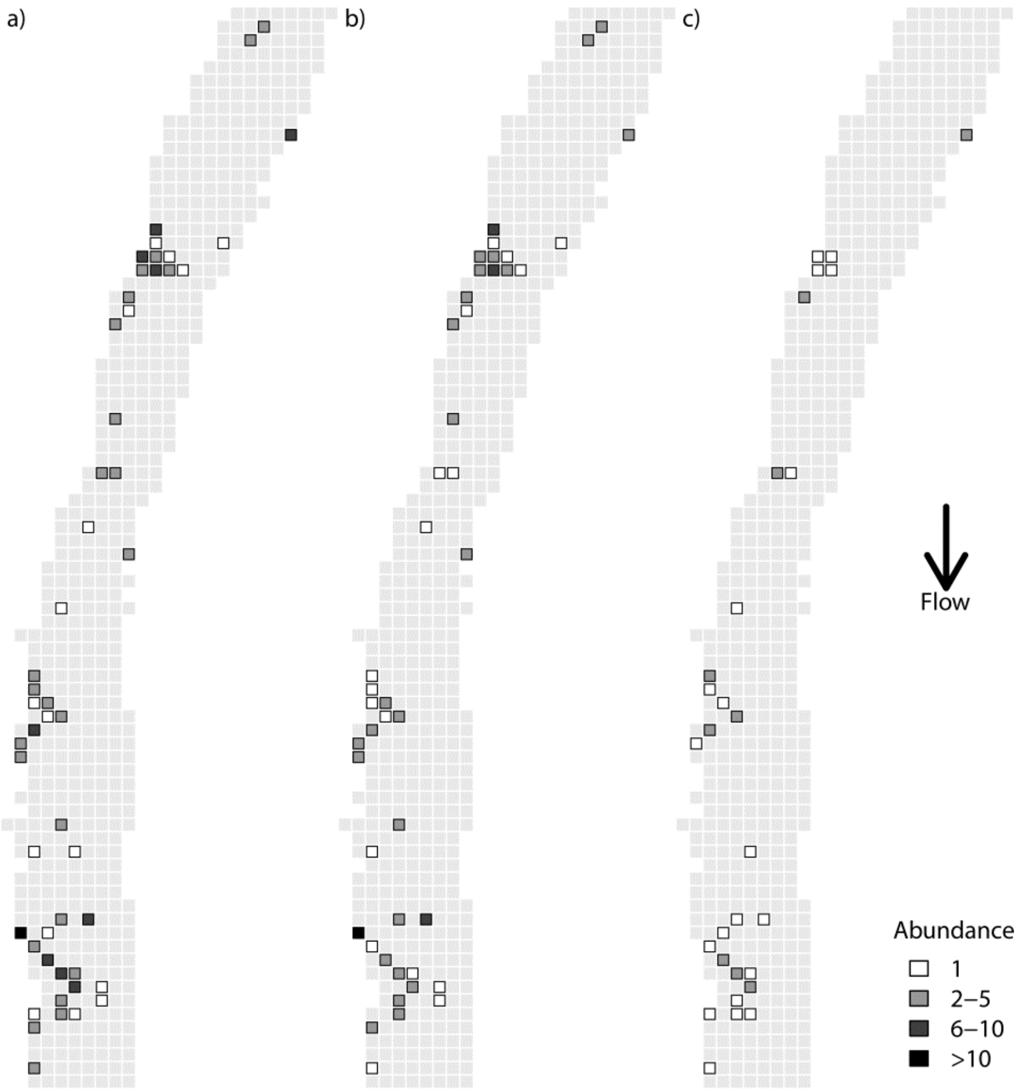

Fig. 4 - Representation of Reach 1 in 2010 showing the spatial distribution of (a) pooled, (b) small and (c) large chub abundance in 1 m^2 quadrats. Blank quadrats did not contain chub.

Table 3 - Spatial distribution of chub abundance in the study reaches of the Torgal in spring 2009 and 2010.

	Reach 1		Reach 2
	2009	2010	2010
No of quadrats	535	606	236
Pooled			
Total fish	300	174	73
No of quadrats occupied (%)	27 (5)	51 (8)	26 (11)
No of fish per occupied quadrat (mean ± SD)	11±9	3±3	3±2
Small			
Total fish	229	124	35
No of quadrats with fish (%)	24 (4)	46 (8)	19 (8)
No of fish per occupied quadrat (mean ± SD)	10±7	3±2	2±1
Large			
Total fish	71	50	38
No of quadrats with fish (%)	20 (4)	29 (5)	18 (8)
No of fish per occupied quadrat (mean ± SD)	4±3	2±1	2±1

This pattern was similar to Reach 2 where there was a maximum of 8 individuals per quadrat (Fig. 5a), most small (77%) and large chub (63%) were in quadrats with 2-5 individuals and 23% of small and 21% of large chub were solitary (Fig. 5b, c).

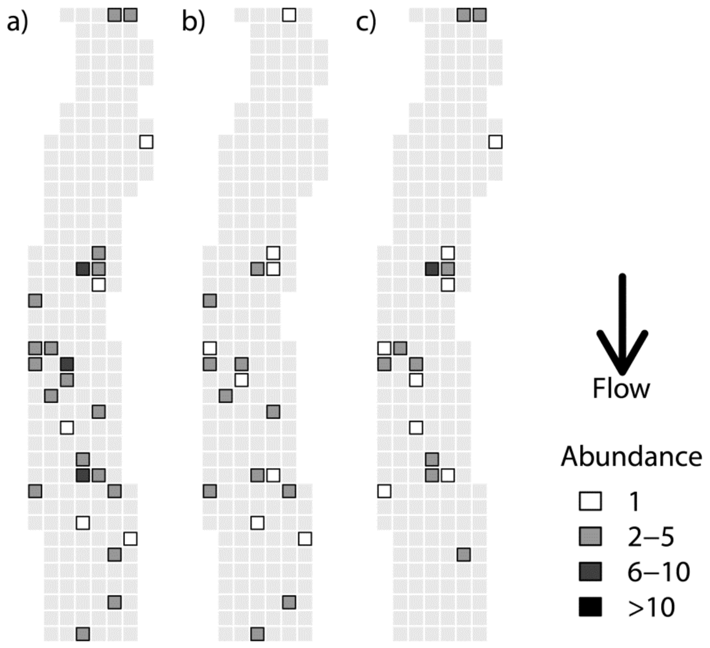

Fig. 5 - Representation of Reach 2 in 2010 showing the spatial distribution of (a) pooled, (b) small and (c) large chub abundance in 1 m^2 quadrats. Blank quadrats did not contain chub.

Although chub generally displayed non-random distributions in both years and reaches, spatial aggregation tended to be stronger in Reach 1 in 2009 and stronger for small than large individuals. The coefficient of dispersion for all chub was higher in Reach 1 in 2009 (CD = 17.6) than in 2010 (CD = 5.3), though both indicated aggregated distributions ($P < 0.0001$). Again, this variation was driven mostly by small chub, which displayed much higher CDs than large chub in 2009 (small chub: CD = 14.7, $P < 0.0001$; large chub: CD = 5.8, $P < 0.0001$) but not in 2010 (small chub: CD = 4.4, $P < 0.0001$; large chub: CD = 2.5, $P < 0.0001$). In Reach 2, the dispersion of all chub was slightly lower than in Reach 1 in 2010 (CD = 3.6, $P < 0.0001$), and similar between size classes (small chub: CD = 2.2, $P < 0.0001$; large chub: CD = 2.8, $P < 0.0001$).

Abundance of chub per quadrat always was spatially correlated for large but not for small chub. For both reaches and years, high positive spatial autocorrelations ($0.001 \leq P \leq 0.04$) for large and all chub generally occurred in the first distance class (1 m), decreasing at subsequent distances (2-4 m), and fluctuating up and down thereafter (Fig. 6a, c). By contrast, abundance of small chub only was spatially autocorrelated in Reach 1 in 2010, with the highest positive autocorrelation ($P = 0.001$) also occurring in the 1m distance class (Fig. 6b). This suggests that adjacent quadrats always hold more similar numbers of large chub than more distant quadrats, and that similar patterns are only occasionally observed for small chub.

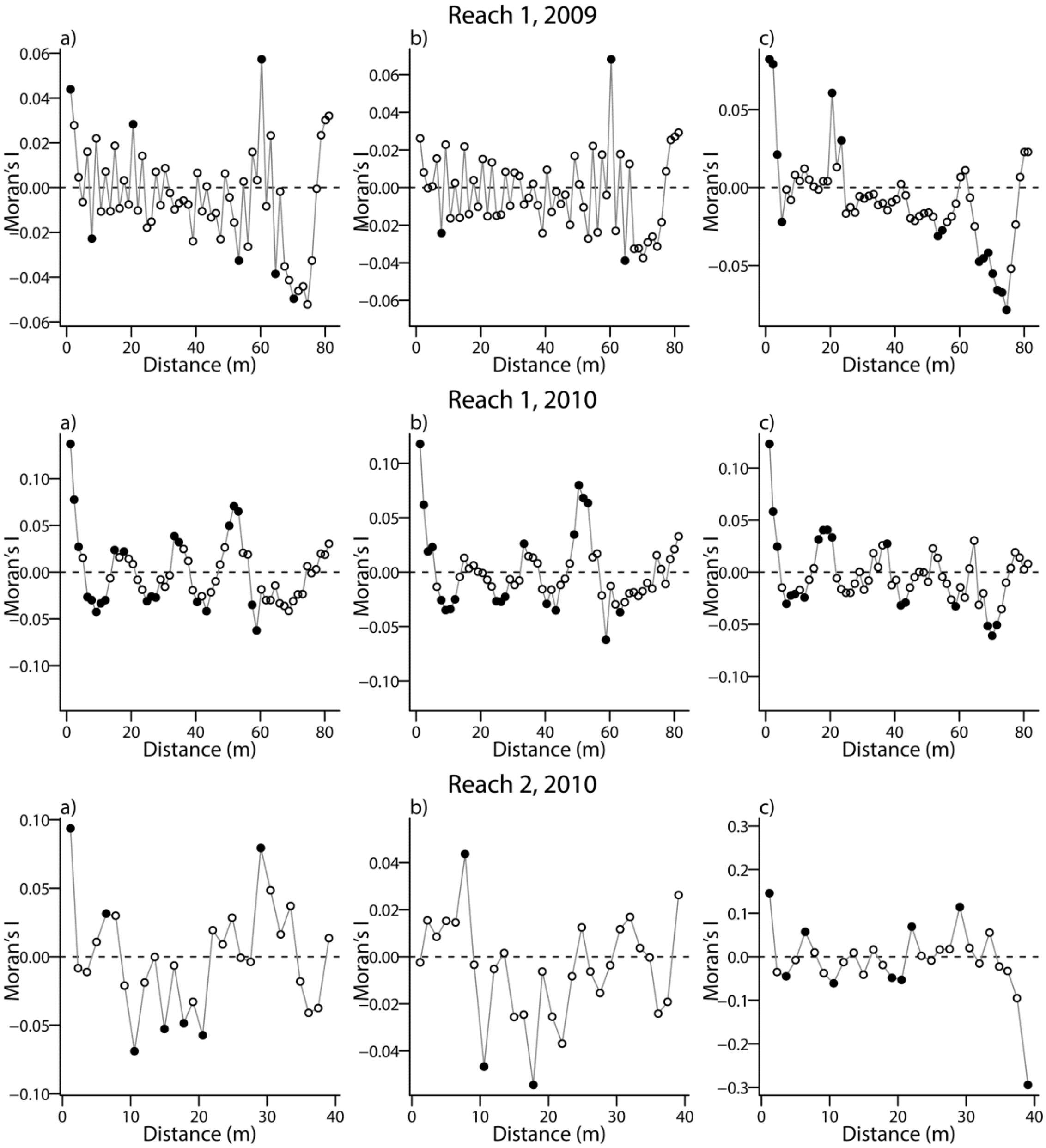

Fig. 6 - Moran's I correlograms for abundance of (a) pooled, (b) small and (c) large chub using pair wise quadrat distances along the study reaches of the Torgal in spring 2009 and 2010. Dark symbols represent Moran's I statistics that are significant after progressive Bonferroni correction ($P < 0.05$). Positive significant values indicate positive autocorrelations, and thus that nearer quadrats hold more similar abundances than quadrats farther apart. Significant negative values have the opposite meaning.

Microhabitat use by chub

The presence and abundance of chub were affected by multiple habitat characteristics that varied substantially between reaches but were similar between years (Tables 4 and 5). The average models for Reach 1 indicated that chub presence and abundance always increased with substratum coarseness and roots, but in 2009 they also increased with debris whereas in 2010 they increased with aquatic vegetation and abundance decreased with velocity (Table 5). Conversely, the average model for chub in Reach 2 in 2010 indicated a positive association between chub occurrence and abundance and depth (Table 5).

Table 4 – Summary results of model selection for the relationships between chub presence and abundance and habitat variables in the study reaches of the Torgal in spring 2009 and 2010. Values are the Akaike's Information Criterion (AICc), the change in AICc between subsequent models (ΔAICc) and the Akaike weights (w_i) for each interpretable model (models with Akaike weights >10% of the model with the best fit). Other abbreviations are as per Table 1.

	Interpretable model	AICc	ΔAICc	wi
Reach 1, 2009				
Pooled	Sc + De + Ve + R	324.99	0.00	0.64
	D + Sc + De + Ve + R	326.60	1.62	0.29
Small	Sc + De + Ve + R	275.58	0.00	0.61
	V + Sc + De + Ve + R	276.74	1.17	0.34
Large	D + Ve + R	143.25	0.00	0.84
Reach 1, 2010				
Pooled	Sc + De + Ve + R	374.09	0.00	0.38
	V + Sc + De + Ve + R	374.12	0.03	0.38
	D + Sc + De + Ve + R	377.32	3.22	0.08
	V + D + Sc + De + Ve + R	378.56	4.46	0.04
Small	V + Ve + R	317.63	0.00	0.47
	V + Sc + De + Ve + R	318.63	1.00	0.28
	V + D + Ve + R	320.74	3.11	0.10
Large	D + Ve + R	191.62	0.00	0.37
	Sc + De + Ve + R	192.92	1.30	0.19
	R	193.68	2.06	0.13
	D + Sc + De + Ve + R	194.84	3.22	0.07
	Ve + R	195.08	3.46	0.07
	D	195.40	3.78	0.06
	V + D + Ve + R	195.61	3.98	0.05

Table 4 continued

	Interpretable model	AICc	ΔAICc	wi
Reach 2, 2010				
Pooled	D	149.59	0.00	0.80
	V + D	153.26	3.75	0.13
Small	D	108.77	0.00	0.43
	Sc	110.97	2.20	0.14
	Intercept	111.18	2.42	0.13
	D + Sc + De	112.00	3.23	0.09
	Ve	112.36	3.59	0.07
Large	D	112.09	0.00	0.44
	D + Ve + R	114.09	2.00	0.16
	R	114.36	2.27	0.14
	V + D	115.49	3.40	0.08

Table 5 – Summary results of information theoretic multimodel inference for the relationships between chub presence and abundance and habitat variables in the study reaches of the Torgal in spring 2009 and 2010. Values are Akaike weights (w_+), model-averaged coefficient estimates (β) and 85% confidence intervals (CI) for each parameter in interpretable models. Parameters with regression coefficients including zero in both the presence and abundance components are not shown. † Regression coefficients with 85% confidence intervals including zero.

			Presence			Abundance		
				85% CI			85% CI	
	Parameter	w+	β	Lower	Upper	β	Lower	Upper
Reach 1, 2009								
Pooled	D	0.31	0.14	-0.36	0.64†	0.15	0.04	0.27
	Sc	1.00	1.46	0.66	2.26	0.60	0.45	0.74
	De	1.00	1.21	0.57	1.86	0.42	0.33	0.51
	R	1.00	1.48	0.81	2.15	0.28	0.19	0.36
	Av	1.00	0.65	0.17	1.13	-0.05	-0.15	0.06†
Small	V	0.36	-0.13	-0.74	0.49†	-0.43	-0.78	-0.09
	Sc	1.00	1.89	1.02	2.76	0.51	0.35	0.68
	De	1.00	1.34	0.69	1.99	0.39	0.29	0.49
	R	1.00	1.48	0.81	2.15	0.07	-0.03	0.18†
Large	D	1.00	0.65	0.13	1.17	0.69	0.43	0.95
	R	1.00	1.40	0.81	1.99	0.43	0.24	0.62
	Av	1.00	0.52	0.02	1.01	0.10	-0.11	0.32†
Reach 1, 2010								
Pooled	V	0.48	-0.32	-0.71	0.06†	-0.20	-0.36	-0.03
	Sc	1.00	0.71	0.31	1.10	0.18	0.00	0.36
	De	1.00	0.16	-0.24	0.56†	0.16	0.04	0.27
	R	1.00	0.72	0.35	1.10	0.20	0.07	0.33
	Av	1.00	0.40	0.06	0.74	0.22	0.10	0.34
Small	V	1.00	-0.28	-0.65	0.09†	-0.37	-0.57	-0.16
	Sc	0.33	0.61	0.22	0.99	0.20	-0.02	0.42†
	R	1.00	0.61	0.25	0.97	0.00	-0.18	0.17†
	Av	1.00	0.45	0.12	0.77	0.18	0.03	0.33
Large	D	0.58	0.51	0.13	0.90	0.51	0.07	0.94
	Sc	0.28	0.76	0.26	1.26	0.60	-0.01	1.20†
	R	0.94	0.67	0.31	1.03	0.40	0.01	0.79
	Av	0.80	0.37	0.02	0.72	-0.08	-0.42	0.26†

Table 5 continued

			Presence			Abundance		
				85% CI			85% CI	
	Parameter	w+	β	Lower	Upper	β	Lower	Upper
Reach 2, 2010								
Pooled	D	1.00	0.97	0.40	1.55	0.38	0.11	0.65
Small	D	0.60	0.97	0.37	1.57	-0.14	-0.61	0.32†
	Sc	0.27	-0.28	-0.78	0.22†	-0.38	-0.62	-0.13
Large	D	0.83	1.15	0.48	1.82	0.30	-0.15	0.75†
	R	0.37	1.12	0.23	2.01	-0.22	-0.52	0.08†

Size-related variation in microhabitat use was strong in Reach 1 in 2009, where the presence and abundance of small and large chub were influenced by different habitat characteristics, but low in both Reach 1 and 2 in 2010 (Tables 4 and 5). The average models for Reach 1 in 2009 demonstrated that presence and abundance of small chub increased with substratum coarseness, debris and roots, whereas those of large chub increased with depth, aquatic vegetation and roots. In addition, there was a negative association between abundance of small chub and velocity, which was not present for large chub (Table 5). Conversely, average models for Reach 1 in 2010 identified positive associations between presence of both small and large chub and substratum coarseness, roots and aquatic vegetation. The abundance of small chub was positively associated with aquatic vegetation and negatively associated with velocity whereas that of large chub associated positively with depth and roots (Table 5). Similarly, average models for Reach 2 indicated that presence of both small and large chub was positively associated with depth and roots (large chub only) whereas abundance of small chub decreased with substrate coarseness (Table 5).

Residuals of the average models were not spatially autocorrelated (0.096 ≤ P ≤ 0.468, Fig. 4 in the Supplementary Material), indicating that spatial autocorrelation in chub abundance was removed after incorporating the habitat variables into our models.

3.7. Discussion

During our study, chub microhabitat use primarily was affected by substratum coarseness, amount of debris, roots and aquatic vegetation and depth although these effects varied by reach and size class. In addition, chub showed an aggregated spatial distribution apparently associated with patchily distributed and spatially autocorrelated habitat resources. Overall, there was a stronger tendency for small chub to aggregate, although the abundance of small individuals was not always spatially autocorrelated. Chub microhabitat use generally was unrelated to water velocity, despite the fact that water velocity often has pervasive effects on microhabitat use by stream organisms (e.g., Dahl & Greenberg 1996; Hart & Finelli 1999; Donaldson et al. 2013). Nonetheless, during study years high velocities were uncommon in the reaches. Consequently, it is possible that velocity plays a more important role in chub distribution and microhabitat use in high flow years. However, if global climate change results in increased frequency of drought and decreased precipitation (IPCC 2008), our results may better depict microhabitat use and spatial relationships of chub than those measured in high flow years.

To our knowledge, this study is one of the first to examine both the spatial distribution and microhabitat use of a European riverine fish, incorporate habitat patchiness, and account for ontogenetic variation in microhabitat use. Incorporating habitat patchiness at small scales is difficult because: 1) organisms differ in their abilities to perceive patches, which renders the identification of patches by investigators somewhat subjective (Kotliar & Wiens 1990; Thompson et al. 2001), and 2) patches frequently occur on several hierarchical levels (i.e., larger patches contain several smaller patches) (Kotliar & Wiens 1990). Consequently, although many ecologists agree that explicit recognition of patchiness in resource availability is important to understanding the mechanisms controlling fish distribution and habitat use regardless of the spatial scale (Thompson et al. 2001 and references therein), there are still few small scale studies that include habitat patchiness. Here, by using a spatially explicit method, we were able to include the spatial arrangement of individuals and habitat variables in our analyses, which allowed us to better understand and interpret chub distribution. Nonetheless, our study was conducted in a single season (spring), and hence the transferability of our results to other seasons may be limited.

Chub displayed an aggregated spatial distribution, which may have been primarily influenced by the patchy distribution of physical habitat characteristics, such as debris, roots and aquatic vegetation. The effect of resource patchiness (e.g., physical microhabitat, food, breeding nests) on the spatial distribution of stream fishes has been documented for multiple species (Freeman & Grossman 1992; Thompson et al. 2001; Einum et al. 2008), which, together with our results, reinforce the importance of quantifying spatial variability in resources availability in habitat and distributional studies of stream fishes (Palmer et al. 1997).

Factors other than resource patchiness also may affect the spatial distribution of chub in reaches, for example, fish abundance. Indeed, aggregation was strongest when chub abundance was highest, with small chub in particular being more clumped and forming larger groups when they were more numerous. This result agrees with other studies showing that the density of conspecifics may be positively related to aggregation behaviour of fishes (Brown et al. 1992; Hensor et al. 2005), although it is unknown whether this is social attraction or the attraction of individuals to highly favourable microhabitats.

Although small chub generally were aggregated, their abundance was not always spatially autocorrelated among quadrats. Without using spatially explicit methods this variability would be impossible to detect, leading to erroneous inferences about the abundance patterns of small chub (e.g., similar abundance in nearby quadrats). Similarly, the use of a spatially explicit approach highlighted that spatial autocorrelation in abundance of large fish was likely due to habitat similarity among neighbor quadrats, because autocorrelation was not detected when fish abundance was related to autocorrelated habitat variables in our models. Distribution and microhabitat studies should include spatially explicit elements, for these will greatly improve our ability to detect and interpret associations between fish and patchily distributed and spatially correlated physical resources.

Factors influencing the presence and abundance of chub varied substantially between reaches, suggesting that this species may display flexible microhabitat use. Although our reaches were located within 2 km of each other, they had distinct physical characteristics, which may have contributed to promote differential microhabitat use by chub. For instance, suitable microhabitats may be inherently more limited in Reach 2 than Reach 1 due to its smaller size, resulting in microhabitats being used according to availability. Flexible microhabitat use strategies are common among fishes inhabiting Mediterranean streams (e.g., Grossman & de Sostoa 1994a, b; Santos et al. 2004; Santos & Ferreira 2008) and other systems with harsh environmental conditions (e.g., Sinnatamby et al. 2012), and might reflect long-term adaptations to the highly variable hydrological regimes (Moyle 1995).

We found that chub presence and abundance were largely unrelated to velocity. The sole exception was small chub in Reach 1 in 2009 and 2010, for which abundance was negatively associated with velocity. It is possible that small chub were affected by velocity because of their lack of ability to withstand the negative effects of high velocity compared to larger individuals (Hill & Grossman 1993; Flore & Keckeis 1998). Velocity is an important habitat use component for many drift or epibenthic feeding fishes, because it affects the cost of holding position, the supply of drifting prey and prey capture success (Facey & Grossman 1990; Hill & Grossman 1993; Piccolo et al. 2008; Hazelton & Grossman 2009). In dry and normal years, however, other factors may drive microhabitat use and velocity may have little effect on both fishes and invertebrates as suggested by Grossman & de Sostoa (1994a). Similar to our findings, Grossman & de Sostoa (1994a) found that substratum composition and depth were the most important variables for cyprinids microhabitat use in the River Matarrana in dry years.

Although size-related differences in microhabitat use were not substantial, there was some variation in microhabitat associations. This was especially evident in Reach 1 in 2009, when small chub preferred low velocity patches with coarse substrata, debris and roots whereas large chub preferred deeper patches with aquatic vegetation and roots. Several investigators have found that large fishes use deeper habitats often as a response to predation (e.g., Power 1984; Byström et al. 2003). Nevertheless, this is unlikely to be the case here given piscivorous fish were virtually absent in the study reaches, and otter *Lutra lutra* L., which is the main fish predator in the Torgal, largely preys on crayfish and eels from April to October, with cyprinids being a staple prey in winter only (Beja 1996). Although chub may display some variation in diet throughout ontogeny (Magalhães 1993), the extent to which this may contribute to size-related differences in microhabitat use remains uncertain.

Our study assessed the influence of patchiness in physical habitat characteristics on chub distribution and microhabitat use, however, other factors also may have played a role in the use of spatial resources by chub in the Torgal and deserve further study. Ontogenetic changes in foraging behaviour in association with prey availability should be further examined to help clarify the mechanisms behind size-related differences in chub microhabitat use. In addition, interactions between chub and other fish species should be examined given competition may affect microhabitat use in stream fishes (e.g., Hazelton & Grossman 2009; Crow et al. 2010). Finally, chub distribution and microhabitat use patterns also should be assessed in wet years to more rigorously assess the effect of low flows on these patterns.

Nevertheless, our findings may have important implications not only for chub conservation and management, but also for other fishes in Mediterranean streams, under alter future climates. Management models based on fish-habitat relationships often consider water velocity the driving physical factor (e.g., Maddock 1999), and frequently use pooled data from different reaches or streams (e.g., Gibbins & Acornley 2000; Vismara et al. 2001). We suggest that in dry and normal years, velocity may be a poor predictor of fish microhabitat use, and that variation among reaches should not be ignored in favour of the average patterns of pooled data sets. In addition, we argue that the maintenance of mosaics of patches with different physical characteristics, specifically, coarse substrata, cover and intermediate depths may greatly contribute to the persistence of vulnerable fish species in Mediterranean streams in the face of low flow conditions.

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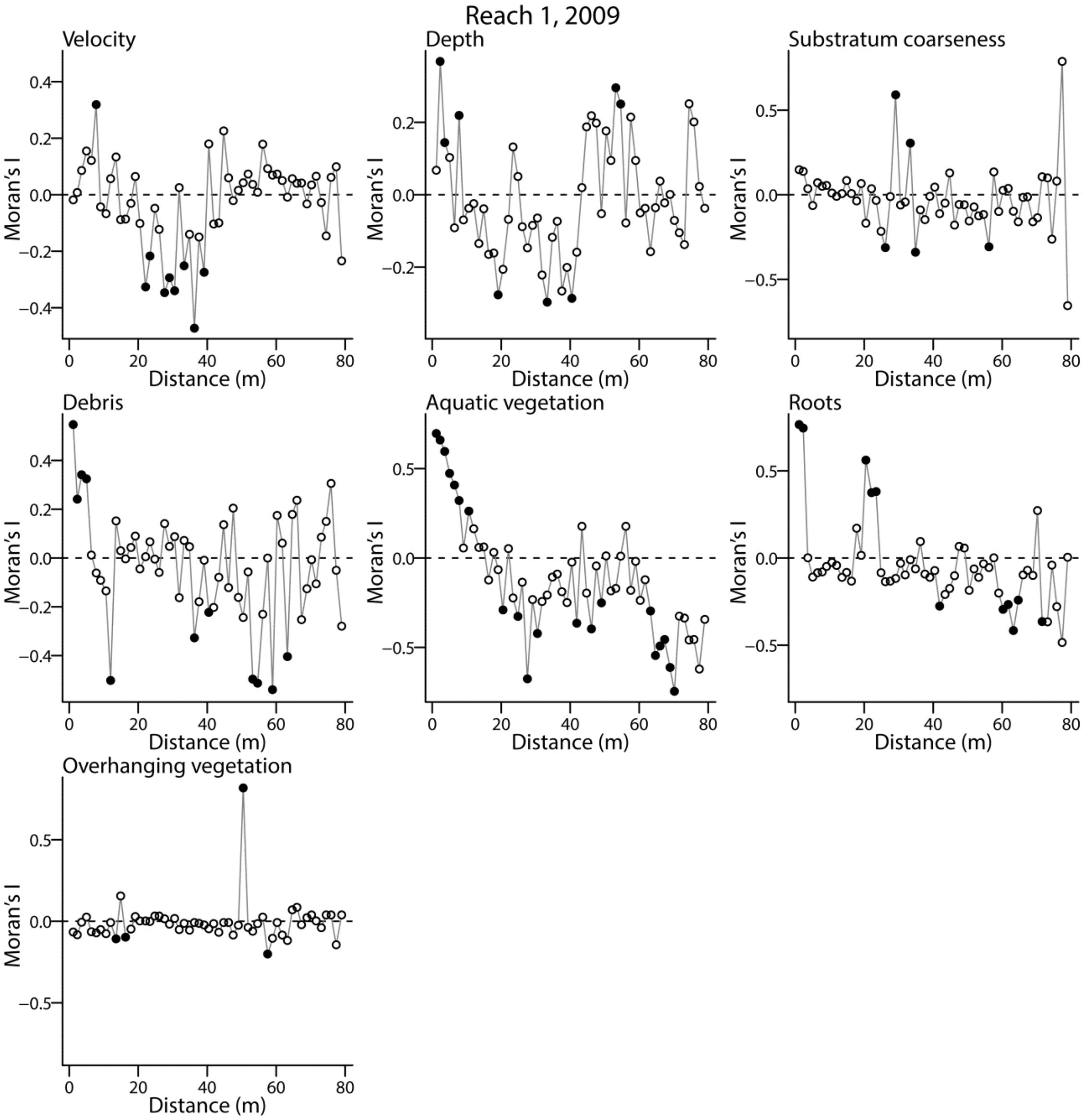
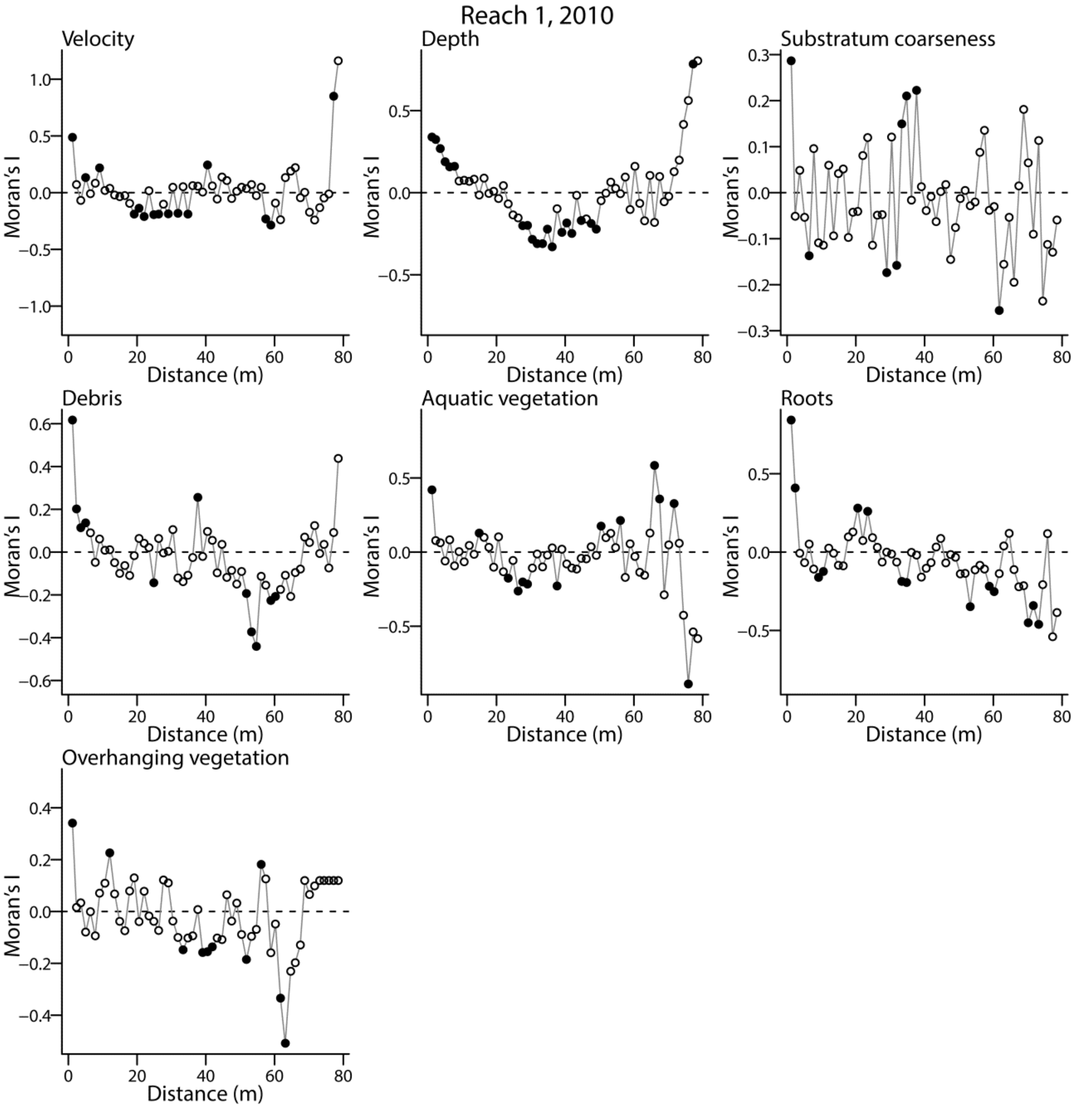
3.9. Supplementary material

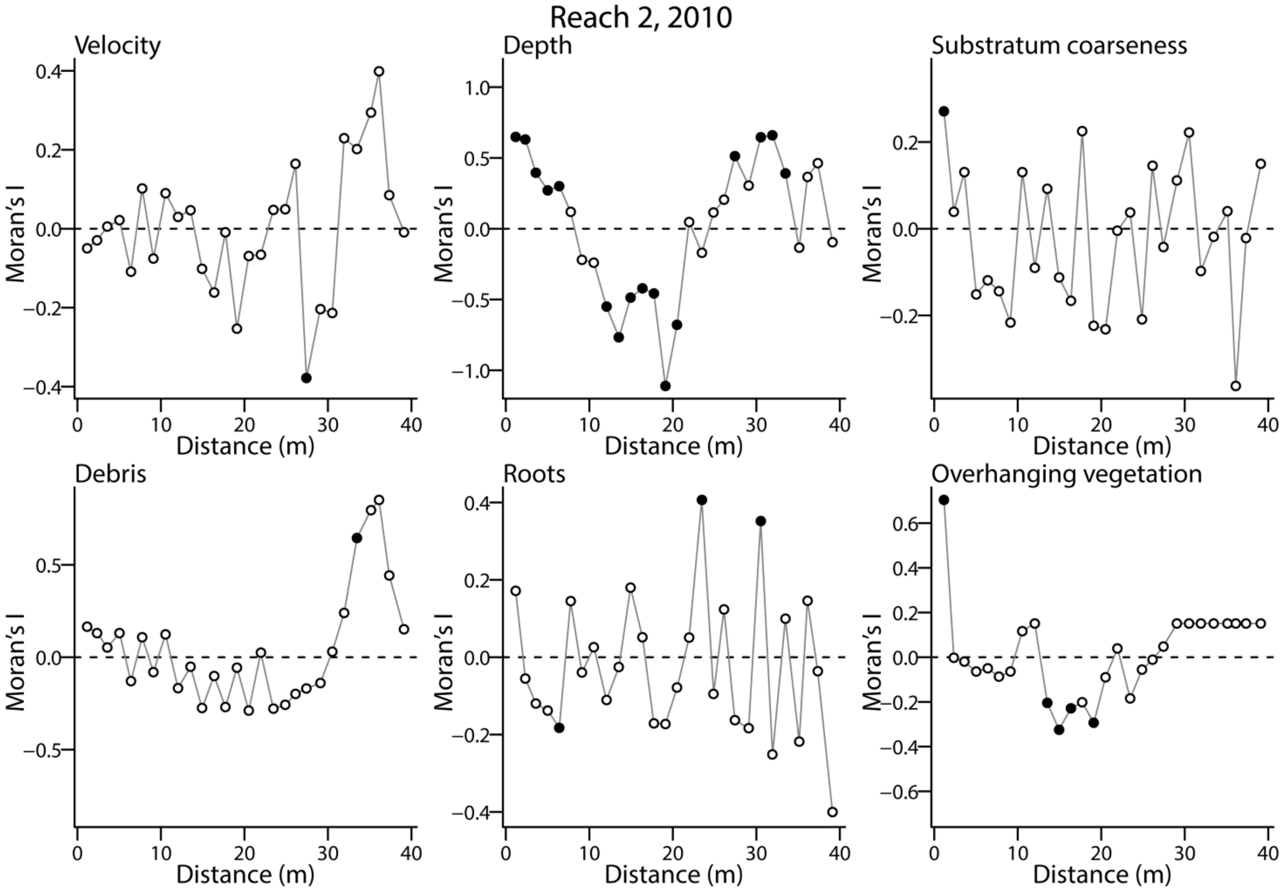
ESM. 1 –Moran's I correlograms for habitat characteristics using pair wise distances along Reach 1 of the Torgal stream in spring 2009. Dark symbols represent Moran's I statistics that are significant after progressive Bonferroni correction (P < 0.05). Positive significant values indicate positive autocorrelations, and thus that nearer quadrats are more similar in habitat characteristics than quadrats farther apart. Significant negative values have the opposite meaning.



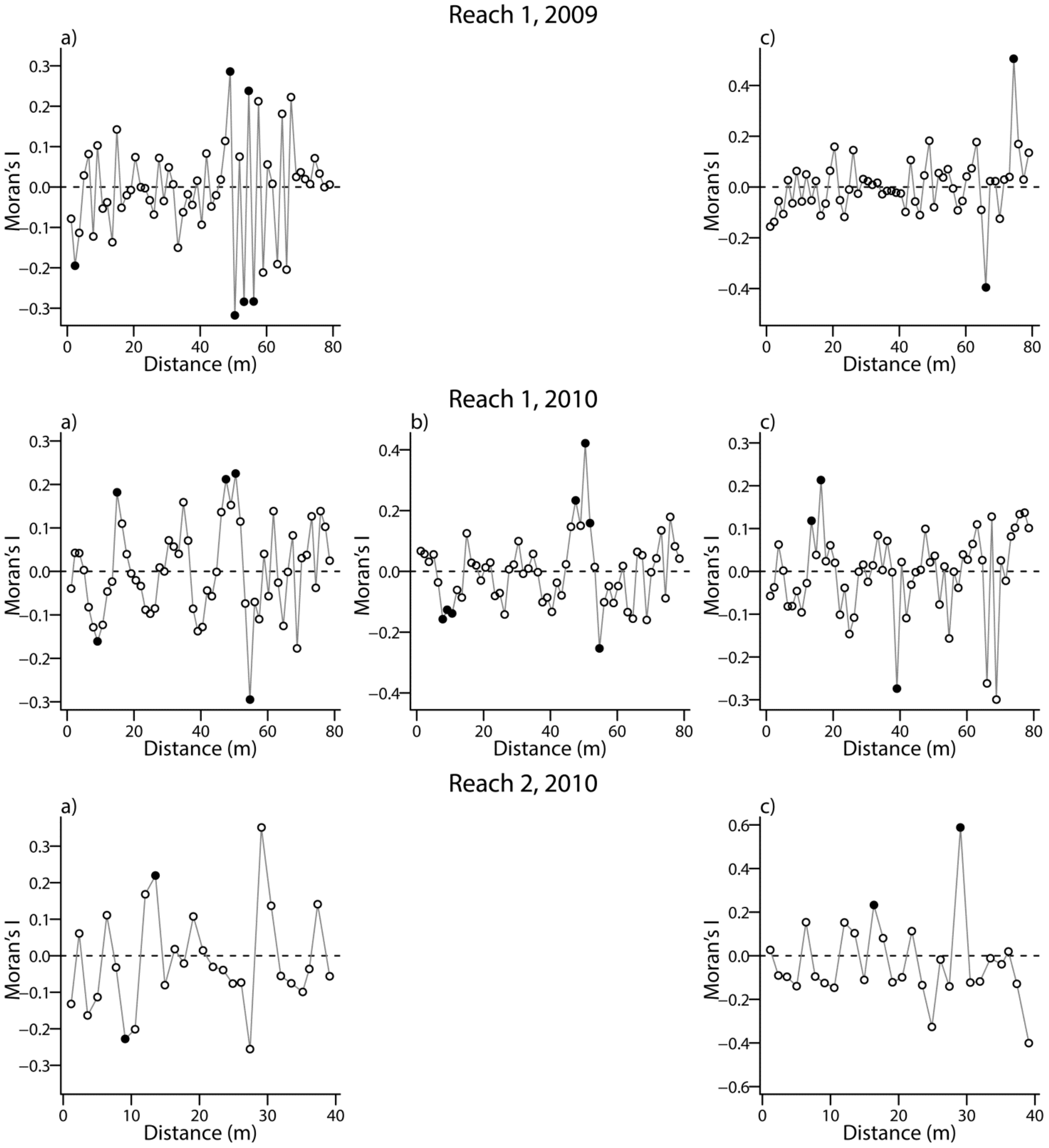
ESM. 2 –Moran's I correlograms for habitat characteristics using pair wise distances along Reach 1 of the Torgal stream in spring 2010. Dark symbols represent Moran's I statistics that are significant after progressive Bonferroni correction ($P < 0.05$). Positive significant values indicate positive autocorrelations, and thus that nearer quadrats are more similar in habitat characteristics than quadrats farther apart. Significant negative values have the opposite meaning.



ESM. 3 –Moran’s I correlograms for habitat characteristics using pair wise distances along Reach 2 of the Torgal stream in spring 2010. Dark symbols represent Moran’s I statistics that are significant after progressive Bonferroni correction ($P < 0.05$). Positive significant values indicate positive autocorrelations, and thus that nearer quadrats are more similar in habitat characteristics than quadrats farther apart. Significant negative values have the opposite meaning.



ESM. 4 - Moran's I correlograms for model residuals of (a) pooled, (b) small and (c) large chub using pair wise quadrat distances along the study reaches of the Torgal in spring 2009 and 2010. Dark symbols represent Moran's I statistics that are significant after progressive Bonferroni correction (P<0.05). Positive significant values indicate positive autocorrelations, and thus that nearer quadrats hold more similar abundances than quadrats farther apart. Significant negative values have the opposite meaning.



Chapter 4.

Linking microhabitat use with models of foraging success in the endangered Mira chub *Squalius torgalensis*

Manuscript

4. Linking microhabitat use with models of foraging success in the endangered Mira chub *Squalius torgalensis*

4.1. Abstract

Identification of essential habitats and the mechanisms affecting habitat selection is critical for Mediterranean cyprinids. Here, we explored the link between focal velocity use and foraging success of the imperilled chub *Squalius torgalensis* in two reaches of the Torgal stream under low flows. Specifically, during spring 2009 and 2010, we quantified habitat selection in relation to velocity, substrata and vegetation conditions prevailing in the field, and tested for variation between reaches, years and length classes. In the laboratory, we quantified variation in the proportion of fish capturing prey in relation to a range of velocity, fish density, group composition and individual length, and then examined whether this variation could reflect differences in individual capture success and reactive distance. Generally, chub displayed random microhabitat use irrespective of size. There was no selection for the low velocities prevailing in the field, though chub occasionally selected areas with boulders, roots and aquatic vegetation. The proportion of fish capturing prey decreased with velocity, and also with density and in groups including large individuals. Similarly, velocity negatively affected capture success and both capture success and reactive distance decreased with density. Results suggest that, under a range of low values, velocity may have little or no effect on microhabitat use by chub because capture success was high, but that increases in velocity may result in less prey captures, even though chub may still have the potential to capture prey at high flows. Our findings provide direct observation data on habitat and foraging success that are crucial for understanding the process of habitat selection by chub, and clearly point to the

importance of quantifying habitat choice in Mediterranean cyprinids using mechanistic, fitness-based criteria.

4.2. Introduction

Streams are highly heterogeneous in their physical characteristics, in both space and time, and over a broad range of scales (Palmer et al. 1997; Ward et al. 2002; Winemiller et al. 2010). This variability may manifest itself in habitat selection by stream fishes, which often prefer certain habitats and avoid others (e.g., Santos & Ferreira 2008; Martínez-Capel et al. 2009; Kanno et al. 2012; Anglin & Grossman 2013). However, there is still a limited biologically realistic understanding of the factors determining habitat selection in stream fishes, although it has been considered essential for both basic scientific and management/conservation perspectives (Grossman 2014).

Microhabitat habitat selection by stream fishes typically is quantified by comparing habitat availability to habitat use, either using fish-habitat relationships (Santos et al. 2010; Compton & Taylor 2013; Martelo et al. 2014; McEwan & Joy 2014) or via direct observation of individual fishes (Martínez-Capel et al. 2009; Kanno et al. 2012; Anglin & Grossman 2013). Water velocity frequently has a strong effect on microhabitat selection (e.g., Hill & Grossman 1993; Santos & Ferreira 2008; Donaldson et al. 2013), though it may be most influential for drift-feeding fishes because it controls both the energy expended to maintain position and access to prey (Facey & Grossman 1990; Hughes & Dill 1990; Hill & Grossman 1993). However, relatively few studies have attempted to identify the mechanisms underlying selection for microhabitats with particular velocities. For example, it is still unclear whether individual fishes select these microhabitats because they have higher net energy gain, greater protection from predators, or greater swimming efficiency.

Mechanistic habitat selection models generally are based on optimal foraging theory, and assume that individuals will occupy microhabitats that yield the highest rates of net energy intake, and consequently increase their fitness (see Grossman 2014 and references therein). The majority of foraging models have been developed for salmonids (Hughes & Dill 1990; Nislow et al. 1999; Piccolo et al. 2014) and far fewer are available for cyprinids (but see Hill & Grossman 1993; Grossman et al. 2002; Hazelton & Grossman 2009), despite the fact that they are among the most diverse and imperilled fishes worldwide (Jelks et al. 2008). In general, foraging models demonstrate that prey encounter rates and energy expenditures increase with velocity whereas capture success decreases, with maximum energy intake typically occurring at intermediate velocities. The accuracy of predictions of foraging models frequently are more sensitive to changes in benefits than they are to changes in costs (Hughes & Dill 1990; Hill & Grossman 1993, but see Hughes et al. 2003), and models based solely on prey capture success have successfully predicted focal point velocities occupied by cyprinids (Grossman et al. 2002).

Studies on habitat use by Mediterranean cyprinids have increased significantly in recent years (Santos et al. 2004; Santos & Ferreira 2008; Martínez-Capel et al. 2009; Martelo et al. 2014), but there is still a dearth of information regarding the mechanisms underlying habitat choice. In addition, there is considerable controversy about the factors influencing habitat use, with substratum coarseness, roots, aquatic vegetation and depth emerging as prevalent for some species (Grossman & de Sostoa 1994a, b; Martelo et al. 2014) whereas velocity appears the most influential factor for others (Santos et al. 2004; Santos & Ferreira 2008; Martínez-Capel et al. 2009; Santos et al. 2010).

Clarifying microhabitat use, especially velocity selection, and understanding how it may be related to foraging success, is therefore important for Mediterranean cyprinids, which face increasing alteration and loss of aquatic habitats (Smith & Darwall 2006).

In this study, we combined field observations on microhabitat selection with laboratory experiments on foraging success, in an attempt to identify the functional mechanism that may determine microhabitat selection by Mira chub *Squalius torgalensis* in the Torgal stream. Specifically, laboratory studies on foraging success were conducted over a range of velocities typically occurring during low and high flows, and the former was compared with field observations of focal velocity use during low flows. Our objectives were to: 1) quantify microhabitat use by chub and analyse differences among reaches, years and length classes, 2) quantify the effects of velocity, fish density, group composition, and individual length on foraging success, and 3) compare patterns of foraging success at a given focal point velocity to focal points occupied by chub in the Torgal stream.

4.3. Methods

Study reaches and species

The study was conducted in two reaches of the Torgal, a small (28 km) Mediterranean stream situated in the SW Portugal (37°38’N, 8°39’W). In brief, the Torgal basin drains siliceous igneous rocks, slates and greywackes. The landscape comprises cork oak *Quercus suber* woodlands and eucalyptus *Eucaliptus globulus* plantations interspersed with pasture and dry grain fields. Riparian vegetation is dominated by well developed galleries of alder *Alnus glutinosa* and ash *Fraxinus angustifolia*, with an understory of Mediterranean scrub. Human settlement is sparse and there are no major anthropogenic impacts such as urban pollution, impoundments, angling or other recreational activities. Because of the Mediterranean climate, the stream experiences wet and dry periods that are seasonally predictable but show variable intensity from year-to-year. Headwaters are ephemeral and downstream reaches typically consist of dry to isolated pools and runs in summer. In dry years, large floods are lacking, the drying period is extended, and surface water is restricted to the deepest pools. Conversely, in wet years there are major floods, and flows may persist through summer in downstream reaches.

The study reaches were located in the downstream portion of the Torgal stream and consisted of relatively shallow runs (< 80 cm deep in spring), with clear water, intact riparian vegetation and persistent flows through summer. The upstream reach (Reach 1) was 80 m in length, and bounded by deep pools and shallow rapids with boulders whereas the lower reach (Reach 2) was 40 m in length and bounded by deep pools and gravel bars. The fish assemblages of study reaches included six native species although chub dominated assemblages in abundance (72 - 87%) and biomass (Martelo et al. 2014). Southwestern arched-mouth nase *Iberochondrostoma almacai* (4 - 12%) was the next most abundant species occurring in both reaches and years. Western three-spine stickleback *Gasterosteus gymnurus* (7%), European eel *Anguilla Anguilla* (0.3%) and Southern Iberian spined-loach *Cobitis paludica* (0.3%) also were present but only in Reach 1 in 2009 whereas, Southern Iberian barbel *Luciobarbus sclateri* (23%) was present only in Reach 2 in 2010. The exotic pumpkinseed *Lepomis gibbosus* occurred throughout the study, but always in low numbers (0.3-1%).

Chub are endemic to SW Portugal, and currently listed as critically endangered according to the Portuguese Red Data Book (Rogado et al. 2005). Chub are small (maximum 16 cm, total length), short-lived (maximum age 5+ years), early maturing (all individuals are sexually mature at age 2+ years) (Magalhães et al. 2003), and occupy mid-water column microhabitats (Martelo et al. 2013). Individuals show some flexibility in foraging behaviour, shifting between drifting and benthic feeding depending on prey availability (Martelo et al. 2013). Generally, chub spawn in spring, between March and June, and have low reproductive allocation (Magalhães et al. 2003). Annual recruitment tends to be severely reduced during dry years (Magalhães et al. 2003; Magalhães et al. 2007). Mobility may vary both among individuals and over time, tending to be greater among chubs leaving dry season pools (Pires et al. 2014).

Microhabitat availability and use

Microhabitat availability and use were quantified in Reach 1 in late spring 2009 and 2010 and in Reach 2 in late spring 2010. We sampled in late spring to avoid biases produced by reproductive behaviour and we observed no reproductive behaviour during sampling. Rainfall records from a gauging station located near the study sites (37°42′N, 8°32′W), indicate that October 2008 to September 2009 was the driest 12 month period on record in the Torgal stream since 1931 (129 mm). In addition, rainfall in the following year (October 2009 to September 2010), also was close to the long-term annual median (690 versus 658 mm).

We quantified microhabitat availability and use following the methods described in Grossman & de Sostoa (1994a, b) and Inoue & Nunokawa (2002). In brief, we established a 1 x 1 m grid over the wetted channel of the study reach using transect ropes and numbered spikes. Spikes were driven into the banks at the ends of transect lines, and marked with coloured tape. Microhabitat availability was quantified using a stratified random sampling design, selecting 80% of quadrats in the mid channel and 10% along each margin. Depth (meter stick, ± 1 cm) and average current velocity (Global Water FP101 electronic velocity meter, ± 3 $cm.s^{-1}$) were measured at the centre of each quadrat. Average current velocity was measured at 60% depth in quadrats ≤ 75 cm deep, and at 20% and 80% depth in quadrats > 75 cm deep. In addition, we visually estimated the proportions of nine habitat variables in a 20 x 20 cm quadrat in the centre of the 1 x 1m quadrats. We categorized substrata based on maximum linear dimension, as: mud (≤ 0.2 cm), gravel (0.3 – 2.5 cm), small cobble (2.6 - 15 cm), large cobble (16 – 30 cm), and boulders (> 30 cm). The remaining habitat variables were the proportions of roots, debris, aquatic vegetation, and overhanging vegetation (< 50 cm above water surface).

We quantified microhabitat use via snorkelling by entering the reach from downstream and slowly moving upstream to minimize disturbance. Upon sighting an undisturbed fish, its exact position was marked using a numbered weight, its species identified and its standard length (SL, cm, ± 1 cm) visually estimated. Fish less than 5 cm, SL, were excluded because they were not reliably identifiable to species underwater. Habitat variables were then measured at each fish location as described above.

Foraging success experiments

Although our primary objective was to quantify the effects of water velocity on foraging success of chub, experiments also included fish length, fish density and group composition as treatments, because these factors also may affect foraging success (Hazelton & Grossman 2009; Kaspersson et al. 2010).

Following the methods of Zamor & Grossman (2007) and Hazelton & Grossman (2009), an 2.0 x 1.0 x 0.6 m (length x width x height) fiberglass tank was used, in which a 25 Kg thrust electric trolling motor generated flow. The experimental chamber (1.0 x 0.6 x 0.5 m), was enclosed at each end with mesh screens that acted as collimators to produce semilaminar flow, and had a 2 cm layer of small cobble. We marked the experimental chamber at 1 cm intervals on the bottom and sides of the front wall, and placed 1 cm^2 plastic quadrats across its bottom, at 10 cm intervals, to facilitate location of individuals. Temperature was maintained at 15 - 17 ^{0}C, pH near 7, and 12h:12h photoperiod, which represent spring average conditions at the Torgal stream in the study years.

We collected 432 chub, 216 small chub measuring 6.0 - 7.0 cm fork length (FL ± SD = 6.4 ± 0.3 cm; mass ± SD = 3.4 ± 0.6 g) and 216 large chub measuring 8.0 - 9.0 cm (FL ± SD = 8.3 ± 0.4, mass ± SD = 7.6 ± 1.2 g), in March and April 2009 and between November 2009 and July 2010 in the Torgal stream, using minnow traps. None of these fish showed evidence of physiological stress or injuries. For acclimation to the laboratory, fish were held in a 900 L fiberglass recirculating tank with water conditions similar to those in the experimental tank for three days. This period allowed fish to recover from collection stress and exhibit normal feeding. Fish were fed red chironomid larva *ad libitum* once daily. We used red chironomid larva because they are comparable to natural chironomid in chubs' diet (Magalhães 1993a, b), and were readily visible and consumed by chub. After acclimation, individuals were anaesthetised with clove oil, measured (± 1 mm, FL), and tagged with coloured plastic discs posterior to the dorsal fin (Hazelton & Grossman 2009). To prevent infections from tagging, fish were treated with antibiotic (MyOxin). After tagging, we kept fish for three additional days in the holding tank, to assure they recovered and regained balance and swimming. Prior to experiments, fish were moved into a separated chamber of the holding tank for a 48 hour non-feeding period, to ensure all individuals were motivated to feed. Because of the imperilled status of chub, after experiments chub in good condition (i.e., feeding normally and with no external parasites) were returned to the Torgal stream. Laboratory procedures conformed to the Portuguese legislation regarding animal capture (ICNB), manipulation and experimentation for scientific purposes, and IAUCUC guidelines of the University of Georgia.

To test for the effects of fish density, fish length, and group composition, trials were conducted with groups of two, four and six individuals belonging to either one or two length classes (i.e., small and large chub). These trials included: 1) trials with varying densities of a single length group (either small or large), and 2) trials with varying length groups and varying densities (one large and small chub, two large and small chub, and finally, three large and small chub). Each combination was tested at four average velocities, two (0.2 and 8 cm.s^{-1}) encompassing the range of velocities occupied by chub during the study, and two (12 and 26 cm.s^{-1}) representing velocities prevailing in the Torgal stream during moderate to high flows (Pires 2012). These resulted in 36 treatment combinations, which were replicated three times, thus totalizing 108 independent trials. Each fish was used in only one trial.

Before trials began, fish were allowed three hours to acclimate to the experimental tank and return to active feeding. During acclimation, velocity was increased gradually to the treatment level. Velocities were measured using an electronic velocity meter (Global Water FP101 ± 3 cm.s^{-1}) at 60% depth, and also mapped in 10 × 10 cm grids at three cross-sections of the tank (length x width x height). Velocity across the tank varied less than 2 cm.s^{-1} in all treatments except 26 cm.s^{-1}, for which variation was between 4 and 6 cm.s^{-1}.

During each trial fish behaviour was observed and videotaped from behind a black plastic blind. A trial consisted of the 20 releases of individual prey, at approximately two minute intervals, through one of three randomly selected (random number generator) silicone tubes at the front of the experimental chamber. Fish were classified as reacting to prey if they oriented directly towards the prey. A capture represented a fish grasping a prey independently of whether or not the prey was consumed. If a fish did not react after presentation of five consecutive prey, we removed it from the tank and did not include the data in analyses. Reactive distance was measured by recording the locations of both fish and prey at the time the fish oriented to the prey (Hazelton & Grossman 2009).

4.4. Data analyses

4.4.1. Microhabitat availability and use

Prior to statistical analyses, we transformed skewed habitat variables to approach normality and reduce the influence of peak values, using the angular transformation for proportional data and the log10 transform for continuous variables.

Because some habitat variables were strongly intercorrelated (Pearson's r, $| r | \geq 0.50$), we used Principal Components Analysis (PCA) to describe the main gradients of variation in microhabitat use and availability (Legendre & Legendre 1998). To avoid bias in the ordination, overhanging vegetation was excluded from analysis because it included a large number of zeros (95%). We tested the null hypothesis of randomness in microhabitat use using non-parametric Mann-Whitney U-tests (Zar 1996), with significance set at 0.05, and adjusted for multiple testing using the Bonferroni sequential method (Legendre & Legendre 1998). Additionally, for clarity of presentation we plotted the 95% confidence ellipses of mean scores for microhabitat availability and use in the ordination diagram (Anglin & Grossman 2013). For consistency between field and laboratory data, fish SL was converted into FL (fork length) using linear regression ($FL = 0.1387 + 1.1023SL$, $R^2 = 0.99$, $F = 8361.8$, $P < 0.0001$, $n = 93$). We compared scores for small (6.0 - 7.0 cm, FL) and large (8.0 - 9.0 cm, FL) chub using Mann-Whitney U-tests (Zar 1996), because previous studies have demonstrated that length-related variations in microhabitat use by stream fishes are common (Grossman & de Sostoa 1994a, b; Santos & Ferreira 2008). Analyses were conducted with the STATISTICA vs.10.0 software (StatSoft 2011).

4.4.2. Foraging success

Analysis of foraging success involved a two-stage procedure based on information theoretic and model averaging approaches (Burnham & Anderson 2002). We started by quantifying variation in the proportion of fish capturing prey among trials and identifying which variables could explain it, and then examined whether this variation could reflect individual differences in capture success and reactive distance. Because fish density in experiments varied from 2 to 6, but the number of prey released was constant among trials, the probability that an individual will capture a prey will inevitably decrease with density. To account for this trivial density effect, we included the number of prey per fish per trial in all candidate models as a fixed effect explanatory variable.

Proportion of fish capturing prey

We quantified relationships between the proportion of fish capturing prey among trials and explanatory variables with generalized linear models using the binomial distribution with logit link (Crawley 2007). Explanatory variables included velocity, fish density, and group composition. Group composition was defined using two binary variables describing the presence of small and large fish in the group (i.e., groups containing small individuals were coded as 1/0, groups containing large individuals were coded 0/1, and groups containing both small and large individuals were coded as 1/1). Explanatory variables were standardized to a mean of 0 and a standard deviation of 1 to facilitate interpretation of relative effect sizes (Crawley 2007; Hazelton & Grossman 2009). Except small and large, which were used together for analyses, there were no significant correlations between explanatory variables (Pearson's r, $|r| < 0.001$).

Capture success and reactive distance

To identify factors affecting individual capture success and reactive distance, we used generalized linear mixed models (GLMM, Zuur et al. 2009). Capture success in each fish's response to individual prey was a binary variable (0 = no capture, 1 = capture), and thus we used the binomial distribution with logit link for capture success models (Crawley 2007). Reactive distance was only determined for individuals that reacted to prey; it corresponded to the distance between the coordinates of the fish's snout (i.e., x_1, y_1, z_1) and the prey (i.e., x_2, y_2, z_2), at the moment fish oriented towards the prey, calculated using the Pythagorean theorem as follows: distance = $\sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2 + (z_2 - z_1)^2}$ (Zamor & Grossman 2007). The normal distribution with identity link was used for reactive distance models (Crawley 2007).

Explanatory fixed variables included velocity, fish density, fish length (FL), and group composition as a binary variable (0 = homogeneous length classes, 1 = heterogeneous length classes). These variables also were standardized to a mean of 0 and a standard deviation of 1 (Crawley 2007; Hazelton & Grossman 2009), and all variables were used in analyses because they were not correlated (Pearson's r, $| r | \leq 0.05$).We investigated two random effects structures because observations likely were not independent both within and among individuals (Zuur et al. 2009). The first included a random effect of fish identity to account for correlation in capture success and reactive distance within individuals. The second allowed the relationship between capture success and reactive distance and fish length to vary among individuals. We selected the best random effects structure using Akaike's Information Criterion (AIC, Burnham & Anderson 2002), from models including the same fixed effects structure and all explanatory variables.

4.4.3. Model selection and averaging

We constructed two sets of mechanistic, a priori candidate models of increasing complexity (Table 1), nine for the proportion of fish capturing prey per trial and 16 for individual capture success and reactive distance. The simplest models considered only the intercept, whereas the most complex models included all explanatory variables (i.e., global models). Global models were fitted and checked for normally distributed errors by examining residual plots (Crawley 2007). Reactive distance was then log transformed to ensure normality of errors.

Table 1 – Candidate models used to evaluate relationships between the proportion of chub capturing prey per trial, and the individual capture success and reactive distance and explanatory variables. All models included prey.fish^{-1} to correct for the eventual trivial effects of density.

No.	Model description
	Proportion of fish capturing prey
1	Velocity
2	Density
3	Small + large
4	Velocity + density
5	Velocity + small + large
6	Density + small + large
7	Velocity + density + velocity x density
8	Global
9	Intercept
	Capture success and reactive distance
1	Velocity
2	Density
3	Group composition
4	Length
5	Velocity + density
6	Velocity + group composition
7	Velocity + length
8	Velocity + density + group composition
9	Density + group composition
10	Velocity + density + velocity x density
11	Velocity + group composition + velocity x group composition
12	Velocity + length + velocity x length
13	Density + group composition + density x group composition
14	Velocity + density + group composition + velocity x density x group composition
15	Global
16	Intercept

The relative support for each of the candidate models was assessed via ΔAIC with models for which $\Delta AIC < 2$ having substantial support (Burnham & Anderson 2002). Akaike weights (w_i) of the models were then calculated and only models with Akaike weights > 10% of the model with the best fit were interpreted (Burnham & Anderson 2002; Hazelton & Grossman 2009). For the proportion of fish capturing prey, we quantified model selection uncertainty by calculating model-averaged coefficient estimates (β) and evaluating the precision of these estimates using 85% confidence intervals (Arnold 2010). Estimates whose confidence intervals included zero were not interpreted. Additionally, we estimated the relative importance of explanatory variables (w_+) by summing their Akaike weights over all interpretable models where the variable was included (Burnham & Anderson 2002). For capture success and reactive distance, coefficient estimates and 85% confidence intervals were not model-averaged as this is inappropriate for models containing both random and fixed effects (Kenneth Burnham, personal communication).

Finally, we calculated goodness of fit for the interpretable models (R^2). For capture success and reactive distance models we produced two statistics for each interpretable model – R^2 marginal (R^2_m) that indicates the variability explained by fixed effects, and R^2 conditional (R^2_c) that indicates the variability explained by random and fixed effects (Nakagawa & Schielzeth 2013). Analyses were conducted using R 2.15.2 (package MuMIn, Barton 2011 and package lme4, Bates et al. 2012) and the STATISTICA vs.10.0 software (StatSoft 2011).

4.5. Results

4.5.1. Microhabitat use

Habitat characteristics were identical in 2009 and 2010 in Reach 1, except for velocity and depth, which increased in 2010. Reach 2 differed from Reach 1, being characterized by substantially less boulder and large cobble (Table 2).

Table 2 – Mean ± standard error of habitat characteristics available and used by small and large chub in each Reach and year. Abbreviations are as follows: V –velocity, D – depth, B – boulder, LC – large cobble, SC – small cobble, G – gravel, M – mud, De – debris, R – roots, AV – aquatic vegetation, and OV – overhanging vegetation.

	Reach 1, 2009			Reach 1, 2010			Reach 2, 2010		
	Available	Small	Large	Available	Small	Large	Available	Small	Large
HC	n = 50	n = 49	n = 18	n = 57	n = 73	n = 11	n = 22	n = 20	n = 16
V, cm.s^{-1}	1.4 ± 0.6	0.1 ± 0.1	0.8 ± 0.4	7.7 ± 0.9	6.6 ± 0.8	8.3 ± 2.4	4.1 ± 1.3	2.6 ± 0.8	4.6 ± 1.4
D, cm	35.7 ± 2.2	46.7 ± 1.2	51.7 ± 2.3	46.1 ± 2.3	52.3 ± 1.6	61.1 ± 2.3	39.5 ± 2.9	48.0 ± 2.2	55.3 ± 2.2
B, %	42 ± 6	63 ± 5	68 ± 7	27 ± 5	26 ± 4	24 ± 9	1 ± 1	4 ± 3	
LC, %	13 ± 3	5 ± 2	4 ± 2	13 ± 3	28 ± 4	30 ± 11	1 ± 1	5 ± 4	2 ± 1
SC, %	16 ± 3	12 ± 2	9 ± 3	8 ± 2	9 ± 2	17 ± 6	45 ± 8	45 ± 8	63 ± 8
G, %	20 ± 4	9 ± 2	9 ± 5	34 ± 5	18 ± 3	10 ± 5	32 ± 6	30 ± 7	24 ± 7
M, %	9 ± 4	11 ± 3	10 ± 4	18 ± 4	19 ± 3	19 ± 10	20 ± 5	17 ± 5	12 ± 3
De, %	4 ± 2	11 ± 3	5 ± 2	11 ± 3	9 ± 3	3 ± 1	18 ± 5	12 ± 3	27 ± 9
R, %	3 ± 2	21 ± 4	23 ± 6	4 ± 2	15 ± 4	12 ± 5		4 ± 4	1 ± 1
AV, %	1 ± 0	8 ± 2	10 ± 4	4 ± 2	12 ± 3	5 ± 4			
OV, %		2 ± 1	1 ± 1	2 ± 1			6 ± 4		1 ± 1

We observed a total of 142 small and 45 large chub throughout the study (Table 2). Chub displayed random microhabitat use except in Reach 1 (Fig. 1). The first two components of the PCA explained 41% of the variation in the habitat data (Fig. 1). The first component identified a gradient of boulder, roots and aquatic vegetation, and the second component contrasted areas with high depth and velocity, with areas with large quantities of mud and debris. Microhabitat use differed significantly from random on PC1 in Reach 1 in 2009 for both small (Mann-Whitney U-test, $U = 637$, $p < 0.0001$) and large chub ($U = 160$, $p < 0.0001$), which tended to be over-represented in microhabitats with boulders and large quantities of roots and aquatic vegetation.

Ontogenetic variation was low, being observed only in Reach 2 in 2010 ($U = 84$, $p < 0.016$), with small chub using areas with less gravel and small cobble than large chub.

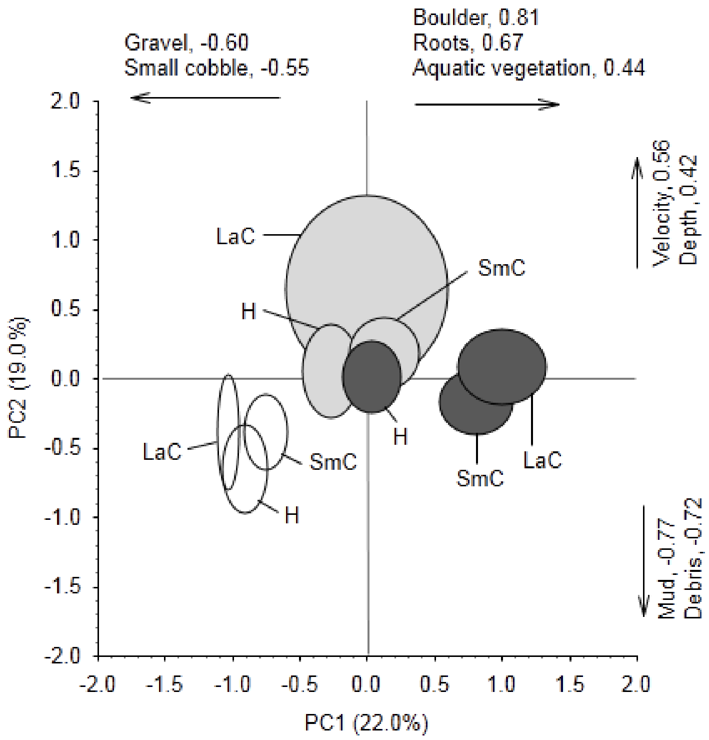


Figure 1 – Ordination diagram of Principal Component Analysis (PCA) of microhabitat use for small (SmC) and large chub (LaC) and microhabitat availability (H) in the in the Torgal stream. Ellipses represent the 95% confidence limits of mean scores for Reach 1 in 2009 (dark grey), Reach 1 in 2010 (light grey), and Reach 2 in 2010 (blank).

4.5.2. Foraging success

Proportion of fish capturing prey

A total of 319 in 432 fish (74%) captured prey, with the proportion of fish capturing prey per trial varying between 0.17 and 1.00. We identified three interpretable models for the proportion of fish capturing prey that explained between 0.41 and 0.46 of the variation in the data (Table 3). The average model indicated that the proportion of fish capturing prey decreased with velocity and in groups containing large individuals, and also with density (Table 4).

Table 3 – Summary results for model selection analyses for the relationships between the proportion of chub capturing prey, and capture success and reactive distance and explanatory variables. Values are the Akaike's Information Criterion (AIC), the change in AIC between subsequent models (ΔAIC) and the Akaike weights (w_i) for each interpretable model (models with Akaike weights > 10% of the model with the best fit).

Models	AIC	ΔAIC	w_i
Proportion of fish capturing prey			
Velocity + small + large	209.47	0.00	0.53
Global	210.38	0.91	0.33
Velocity + density	213.69	4.22	0.06
Capture success			
Velocity + density	7345.77	0.00	0.39
Velocity + density + group composition	7347.75	1.98	0.15
Velocity + density + velocity x density	7347.75	1.99	0.14
Velocity	7347.87	2.11	0.14
Velocity + length	7349.29	3.52	0.07
Velocity + group composition	7349.86	4.09	0.05
Reactive distance			
Density	2824.70	0.00	0.90

Table 4 – Model-averaged coefficient estimates (β), 85% confidence intervals (CI) and Akaike weights (w_+) for each parameter in interpretable models (models with Akaike weights > 10% of the model with the best fit) of the proportion of chub capturing prey. † Regression coefficients with 85% confidence intervals including zero.

		85% CI		
Parameter	β	Lower	Upper	w_+
Velocity	-0.43	-0.63	-0.24	1.00
Density	-0.74	-1.41	-0.07	0.38
Large	-0.31	-0.52	-0.10	0.92
Small	0.09	-0.11	0.28†	0.92
Velocity x density	-0.08	-0.31	0.15†	0.30

Capture success and reactive distance

A total of 1771 prey captures was recorded, with mean proportion of captures per individual being 0.2 (SD = 0.2, range = 0.0-0.9). Reactions to prey by chub summed 2520, with mean reactive distance being 28.4 cm (SD = 11.7, range = 2.8-93.4 cm).

Calculation of AIC for the two random effects models indicated no differences in their relative support (ΔAIC ≤ 4), and consequently the simplest random effect structure including fish identity only was used in further analyses.

Both capture success and reactive distance were affected by fish density, but only the former was influenced by velocity (Tables 3 and 5). Six interpretable models were identified for capture success (R^2_c = 0.43, R^2_m = 0.09, Table 3). These models elucidated negative relationships between capture success and both density and velocity, although the latter had a weaker effect (Table 5 and Figure 2). Group composition, velocity x density and fish length also were included in interpretable models (Table 3), however, the confidence intervals of these terms included zero, and thus were not interpreted (Table 5). For reactive distance, there was a single interpretable model (R^2_c = 0.30, R^2_m = 0.03, Table 3), identifying a decrease in the distance of reaction with fish density (Table 5).

Table 5 – Coefficient estimates (β) and 85% confidence intervals (CI) for each parameter in interpretable models (models with Akaike weights > 10% of the model with the best fit) of chub capture success and reactive distance. † Regression coefficients with 85% confidence intervals including zero.

	Capture success			Reactive distance		
		85% CI			85% CI	
Models and parameters	β	Lower	Upper	β	Lower	Upper
Velocity + density						
Velocity	-0.30	-0.42	-0.18			
Density	-0.46	-0.80	-0.12			
Velocity + density + group composition						
Velocity	-0.30	-0.42	-0.18			
Density	-0.46	-0.80	-0.12			
Group composition	-0.01	-0.12	0.10†			
Velocity + density + velocity x density						
Velocity	-0.30	-0.42	-0.18			
Density	-0.46	-0.80	-0.13			
Velocity x density	-0.01	-0.12	0.10†			
Velocity						
Velocity	-0.31	-0.43	-0.19			
Velocity + size						
Velocity	-0.31	-0.42	-0.19			
Length	0.06	-0.06	0.17†			
Velocity + group composition						
Velocity	-0.31	-0.43	-0.19			
Group composition	-0.01	-0.12	0.11†			
Density						
Density				-0.12	-0.20	-0.05

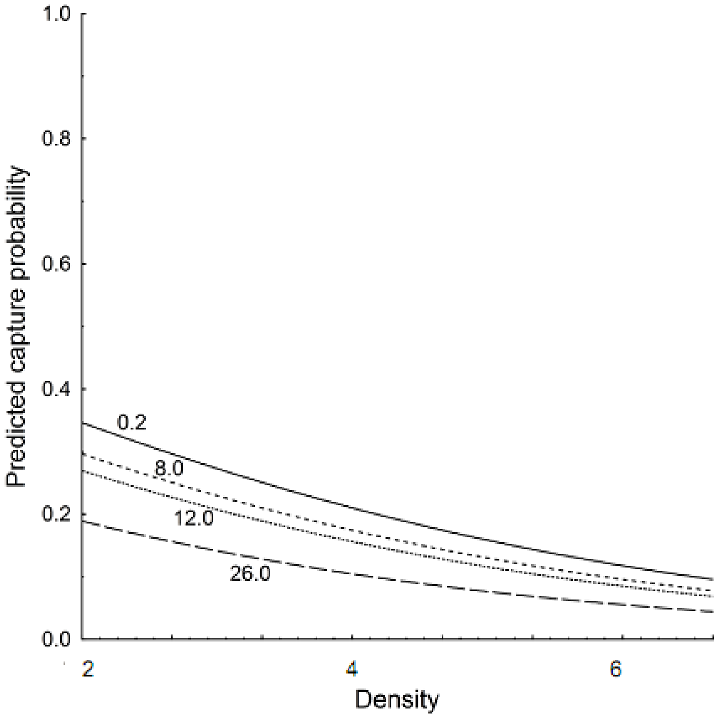


Figure 2 – Predicted variation in capture probability by chub with fish density at velocities of 0.2, 8, 12 and 26 (cm.s^{-1})

4.6. Discussion

Chub generally showed random microhabitat use during low flows, only occasionally, favouring areas with boulders, roots and aquatic vegetation. In addition, length-related differences in microhabitat use were low, albeit sometimes small chub used areas with less gravel and small cobble than large chub. Although velocity was not an important microhabitat characteristic for chub in study reaches, foraging success experiments indicated that the proportion of fish capturing prey and individual capture success may decrease with velocity. Results suggest that velocity may have little or no effect on microhabitat use by chub under low flows, because capture success was still high at the range of low velocities prevailing during the study.

However, increases in velocity will likely result in less prey captures, although chub may still have the potential to capture prey at high flow conditions.

Our research may be one of the first attempts to apply formal foraging theory to tests of habitat selection in Mediterranean fishes. It also is one of few European studies to document focal microhabitat use using underwater observations, which facilitate precise *in situ* habitat observations of individual fish (Heggenes et al. 1990; Almeida & Grossman 2012).Specifically, our findings provide direct habitat and foraging success data that should help in understanding the process of habitat selection and in quantifying optimal habitat for chub, suggesting that this approach also may be adequate for other Mediterranean cyprinids. Nevertheless, our study has several limitations that should be addressed in future studies. First, microhabitat use was quantified over a short time period in two years with similar low flows, which does not cover the full range of velocity that may be experienced by chub. Additionally, the variance explained by our models was relatively moderate indicating that other unmeasured factors may play a role in foraging success. Finally, our experiments were based exclusively on drift foraging, and thus the relation between benthic foraging success and chub microhabitat use remains unknown.

In general, chub showed random microhabitat use in relation to velocity, depth, mud and debris, only occasionally, selecting areas with boulders and large amounts of roots and aquatic vegetation. This pattern suggests that, during low flows, chub probably tolerate well a broad range of microhabitat conditions, which apparently are used accordingly to their availability. Nevertheless, chub may sometimes be selective and show ontogenetic variation in habitat use as has already been noted for other chub species (e.g., *S. aradensis* and *S. carolitertii*) in Mediterranean streams (Grossman & de Sostoa 1994a, b; Santos et al. 2004; Santos & Ferreira 2008; Martínez-Capel et al. 2009). Overall, it seems that chub may be extremely flexible in their microhabitat use patterns, a strategy that typically is favoured in environmentally unstable lotic ecosystems (Poff & Allan 1995), such as Mediterranean streams (Magalhães et al. 2002).

The proportion of fish capturing prey varied with velocity probably reflecting individual variation in capture success and reactive distance of individual fish. Moreover, the proportion of fish capturing prey decreased in groups with large individuals, suggesting that these individuals may be particularly sensitive to changes in prey supply compared to small conspecifics. Nevertheless, neither group composition nor fish length affected individual capture success or reactive distance, making it difficult to infer which mechanism may be at play here.

The proportion of fish capturing prey and both capture success and reactive distance decreased with fish density, but this result should be interpreted with careful. Although we tried to account for the negative effect of density on chub foraging success associated to our experimental design, this may not have been completely eliminated. Nonetheless, the importance of density to foraging success of chub was noted by Martelo et al. (2013), who found that chub in small shoals foraged and searched for prey with greater frequency than fish in large shoals. Consequently, we considered that density likely influences the behaviour of chub, but its effect on foraging success and microhabitat selection needs further clarification.

Capture success and reactive distance were independent of fish length and group composition which is consistent with the generally high overlap between small and large chub in microhabitat use. Similarly, Martelo et al. (2013) found that time spent foraging by chub was not influenced by fish length in the same years and reaches. These findings suggest that individual processes such as energy requirements and the ability to find and capture food may not be strongly related to chub length. Nevertheless, exclusion of young chub from our study due to sampling constraints may have weakened ontogenetic variation in habitat use, typically found for other Mediterranean fish species (Grossman & de Sostoa 1994a, b; Santos & Ferreira 2008; Santos et al. 2010). Further studies should thus attempt to include smaller individuals.

Capture success by chub decreased with velocity, but individuals seemed to have the potential to capture prey at much higher velocities than they occurred in the Torgal stream during our study. Negative effects of velocity on capture success have been reported for other cyprinids (Grossman et al. 2002; Hazelton & Grossman 2009) and salmonids (Hill & Grossman 1993; Piccolo et al. 2008), with high velocities probably resulting in an increase in energy expended per prey, and consequently reduced individual fitness (Facey & Grossman 1990). In the case of chub, however, capture success was high at the low velocities (0.2-8 m.s^{-1}) prevailing in the Torgal stream (mean = 4, SD = 6, range = 0-29 m.s^{-1}), which may have contributed to the random use of this variable. Future field studies encompassing periods of higher velocities in wet years in the Torgal stream are thus needed to fully uncover mechanisms behind microhabitat use by chub.

Our study provides direct habitat and foraging success data that help in understanding the process of habitat selection and in quantifying optimal habitat for chub. Although further testing is required before general conclusions regarding the mechanics of chub microhabitat use can be drawn, our results suggest this may at least be partially related to some aspect of fish fitness, as foraging success likely determined the relationship between chub and velocity. . Moreover, our study highlights that for both basic scientific and management/conservation perspectives, it is important to quantify habitat choice in Mediterranean cyprinids using mechanistic, fitness-based criteria.

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Chapter 5.

General discussion

5. General discussion

5.1. Overall patterns

The main objectives of this thesis were to identify the physical factors influencing activity, distribution and microhabitat use of fish in Mediterranean streams, and explore the mechanisms that may underlie these patterns, using as a case study the Mira chub *Squalius torgalensis*, an imperilled cyprinid endemic to SW Portugal. Given the complexity and dynamism of distribution and microhabitat use by stream fishes, addressing these issues required innovative mechanistic models that link physical characteristics to individual fitness, and account for behaviour traits.

Chub activity during the day was highly variable among individuals, with foraging changing from drift to benthic on an annual-basis and with shoal size whereas time spent cruising vs. searching was influenced by temperature and body size. Spatial distribution was aggregated, probably as a result of spatial patchiness and autocorrelation in physical habitat resources, though social attraction also may have been influential for small individuals. Microhabitat use was generally unrelated to velocity, and little variable throughout ontogeny. Substratum coarseness, amount of debris, roots and aquatic vegetation and depth were generally influential, though varied in importance across reaches. Focal microhabitat use generally was random, irrespective of fish size. Experiments indicated that the proportion of fish capturing prey decreased with velocity, and also with density and in groups including large individuals. Capture success was negatively influenced by velocity, and density affected both capture success and reactive distance.

Taken together, results indicate that the activity of chub, and possibly that of other Mediterranean cyprinids, may be highly variable at the individual level and affected by multiple interacting extrinsic and intrinsic factors. Aggregated distributions apparently are a result of patchiness and autocorrelation in physical resources, and social interactions. Random use of velocity may be associated with capture success, tending to be high at the low velocities prevailing during low flows. Moreover, individuals tend to capture less prey at high velocity, but may still be effective at capturing prey during high flows. Overall, this high flexibility both in behaviour and microhabitat use may be important to cope with highly variable physical conditions in Mediterranean streams, with maintenance of habitat patches with variable substrata, cover and depth likely favouring local persistence. This thesis highlights the importance of using mechanistic, fitness-based models for understanding the process of microhabitat selection.

5.2. Aggregation by chub – resource patchiness or social attraction?

The majority of fishes form aggregations at one stage or another during their life history (Pitcher & Parrish 1993). Aggregations may reflect the way individuals respond to resource patchiness, with individuals assorting uniformly relative to resource availability and quality (Freeman & Grossman 1993; Breau et al. 2007; Einum et al. 2008; Argentina et al. 2010). Ecological examples are abundant and include fishes responses to prey patchiness (Thompson et al. 2001), macrophytes (Argentina et al. 2010) and breeding nests (Einum et al. 2008), or rocks used as oviposition sites by aquatic insects (Lancaster et al. 2003). This view also applies to chub distribution which mainly associated with physical resources that were patchily distributed and spatially autocorrelated (e.g., debris, roots and aquatic vegetation, Chapter 3). In addition, abundance of large chub was spatially autocorrelated, further corroborating this view. The abundance of chub, particularly of large individuals, may therefore be closely coupled with the presence of essential physical habitat characteristics, and hence, their distribution may be contingent on the distribution of habitat resources.

Nonetheless, aggregation may not only be a simple pattern but also have a functional purpose (Freeman & Grossman 1992; Parrish & Edelstein-Keshet 1999). Many fish species aggregate as a result of social attraction among a number of individuals moving within a given area, with individuals joining the group increasing in survivorship or reproductive success due, for example, to enhanced foraging success and lower predation risk (Ryer & Olla 1992; Bednekoff & Lima 1998; Allouche & Gaudin 2001; White & Warner 2007). In the case of chub, social attraction may have influenced the distribution of small individuals because they were not always spatially autocorrelated and the strength of aggregation increased when their abundance was highest (Chapter 3). Nevertheless, foraging success did not increase within the range of group sizes used in experiments (Chapter 4), and predation risk probably is low in the Torgal stream (Beja 1996; Magalhães et al. 2002). Consequently, the functional role of social attraction in driving small chub aggregation is still unclear.

In conclusion, the mechanisms underlying aggregation may integrate the distribution of habitat resources, as well as social interactions among individuals. The importance of both processes, however, presumably vary between size classes, with non-social aggregation being superimposed on social aggregation for large fish, but the later increasing in importance for small fish.

5.3. Chub behavioural flexibility

Fishes inhabiting unpredictable and unstable streams often show great flexibility in behaviour (Grossman & de Sostoa 1994a, b; Grossman & Ratajczak 1998; Ayllón et al. 2010; Kanno et al.

2012), and this also appeared to be the case of chub. Indeed, chub showed 1) variable activity patterns, 2) use of a broad range of microhabitats, and 3) effective foraging over a wide range of velocities. Behavioural flexibility may be important for fishes to cope with strong spatial and temporal environmental variability in Mediterranean streams, and hence, to enhance their local persistence.

Activity patterns varied substantially on an individual and annual basis, being affected by multiple factors including temperature (Chapter 2). Chub spent more time searching for prey and less time cruising when temperature increased, an effect already documented in previous studies and often related to the positive effect of temperature on metabolic rates (Fraser et al. 1993; Greenwood & Metcalfe 1998; Linløkken et al. 2010). Moreover, chub foraging mode shifted from drift to benthic foraging, likely as a result of annual differences in prey availability (Chapter 2, Appendix). Variations in prey availability have been reported to produce changes in foraging mode for several fish species (e.g., Nakano et al. 1999; Katano 2011), and also to influence the diet of many Mediterranean cyprinids (Magalhães 1993; Gomes-Ferreira et al. 2005).

Chub favoured different habitat characteristics between reaches, namely related to substratum composition, cover and depth (Chapter 3). Although a number of factors may contribute to spatial variation in microhabitat use (e.g., foraging tactics, competitive interactions), here, this was apparently associated with variation in microhabitat availability. The influence of microhabitat availability on variation in microhabitat use patterns was previously noted for fishes in Mediterranean streams (Grossman & de Sostoa 1994a, b; Santos et al. 2004; Santos & Ferreira 2008) and elsewhere (Grossman & Ratajczak 1998; Henry & Grossman 2007; Kanno et al. 2012). Focal analyses of microhabitat use (Chapter 4), a novel approach in Mediterranean streams, revealed that individuals may exhibit random microhabitat use, providing additional evidence for behavioural flexibility. Flexible microhabitat use may be an important strategy for chub to cope with changes in microhabitat availability across reaches and over seasons and years, that typically involve variation in water velocity and depth, substratum composition and amount of debris and aquatic vegetation (Grossman & de Sostoa 1994a, b; Santos et al. 2004; Santos & Ferreira 2008).

Finally, foraging success experiments suggest that chub may have the potential to capture prey over a large range of velocities, though capture success may decrease with velocity (Chapter 4). This may be especially important for chub to cope with high flows prevailing in winter and early spring. Other cyprinids (e.g., rosyside dace *Clinostomus funduloides* Girard) inhabiting different environments, also seemed to be effective in capturing prey at high velocities (Grossman et al. 2002).

As suggested by these findings, chub may be flexible to changes in temperature and prey availability, and able to tolerate a broad range of microhabitat conditions. Such flexibility may be an important behavioural attribute of chub to persist in Mediterranean streams, where these factors may change substantially over time and space (Gasith & Resh 1999). It is expected that species inhabiting harsh and highly variable systems, such as Mediterranean streams, would be adapted to their variability because selective, filtering pressures on species attributes appropriate for local persistence might have been at work for long periods of time (Poff 1997; Magalhães et al. 2007). Behavioural flexibility may be such an attribute, which may have "passed" through environmental "filters" occurring at multiple scales, contributing to the success of chub in coping with prevailing harsh environmental conditions.

5.4. Chub microhabitat use

When velocity is unimportant

Water velocity is typically one of the most important microhabitat use components for drift and epibenthic feeding fishes (Santos et al. 2004; Santos & Ferreira 2008; Martínez-Capel et al. 2009; Donaldson et al. 2013), because it affects the cost of holding position, the supply of drifting prey and prey capture success (Hughes & Dill 1990; Piccolo et al. 2008; Hazelton & Grossman 2009). However, in the case of chub no strong association between velocity and microhabitat use was found (Chapters 3 and 4). Instead, chub were strongly associated with coarse substrata, roots, aquatic vegetation and depth (Chapter 3) or used microhabitat randomly (Chapter 4). A similar result was obtained by Grossman & de Sostoa (1994a, b), who found that substratum composition and depth were the most important microhabitat characteristics for cyprinids in the River Matarrana. Nevertheless, all these studies were conducted under low flows, and consequently, it is possible that velocity plays a more important role in microhabitat use by cyprinids in Mediterranean streams during high flows.

Microhabitat selection by chub likely is linked with foraging success, which is increasingly recognized as an important mechanism underlying habitat selection by stream fishes given their direct association with fitness (Grossman 2014). First, capture success was high at the range of low velocities prevailing during the study (Chapter 4), which likely explains why chub used this physical characteristic randomly. Second, chub were observed foraging on coarse substrata (e.g., boulder and cobbles) and roots, two of the most consistently used physical resources (Chapter 3 and 4). Third, chub spent most of the day foraging or searching for prey (Chapter 2), and hence microhabitat use likely also was related to these activities rather than to physical habitat characteristics alone. Overall, this agrees with previous studies pointing the importance of foraging success and prey availability, as drivers of microhabitat selection by stream fishes in systems in which predation is not substantial (review in Grossman 2014).

Taken together results indicate that microhabitat selection by chub may be linked to physical habitat characteristics that affect individual foraging success, though the effect of other factors, such as fish density, needs clarification. Clearly, a deeper understanding of the mechanics of chub microhabitat selection will require field observation under high flows, and testing of the link between physical characteristics actually used by chub during low flows (e.g. coarse substrata, roots) and foraging success. Nonetheless, this thesis highlights the importance of quantifying microhabitat selection by Mediterranean cyprinids using mechanistic, fitness-based criteria.

Size-related differences

Ontogenetic differences in habitat use typically have been related to differences among size classes in the ability to avoid predators, use resources, and energetic demands (Harvey & Stewart 1991; Rosenberger & Angermeier 2003; Ayllón et al. 2010; Henderson & Johnston 2010). Unlike many stream fishes chub did not show substantial differences in microhabitat use between size classes (Chapter 3 and 4). The low ontogenetic variation in microhabitat use were accompanied by no effect of either fish length or group composition on foraging success (Chapter 4), suggesting that energy requirements and the ability to find and capture prey may not be strongly related to individual length under the range of velocities prevailing in study reaches and used in experiments. Nonetheless, body size has been directly related to the ability of fishes to maintain position under high velocities, with larger individuals having greater swimming abilities than small individuals (Hill & Grossman 1993; Rosenberger & Angermeier 2003). This phenomenon, thus suggests, that higher velocities may be needed to produce size-related differences in both microhabitat use and foraging success by chub.

Although size-related differences in microhabitat use are common among Mediterranean stream fishes, these have typically been documented under normal to high flow conditions, in which velocity likely is more variable and reach higher values than in this study (Santos et al. 2004; Santos & Ferreira 2008; Martínez-Capel et al. 2009; Santos et al. 2010). Competition with conspecifics also is usually an explanation for microhabitat use shifts over ontogeny, but unlikely to have been important for chub because 1) in spring , food supply is generally high in Mediterranean streams (Gasith & Resh 1999), 2) there were no yearly shifts in microhabitat use despite fish abundance variation (Chapter 3), 3) behavioural aggression was never observed between small and large chub, and 4) chub spent more time foraging and searching for prey in small than in large shoals (Chapter 2). Improve the understanding of size-related differences in microhabitat use by chub will thus involve addressing ontogenetic variation over a wider range of physical and biological conditions.

Complementarity in scales of microhabitat use

Studies conducted at only one spatial scale may be limited because different factors can influence habitat use at different scales (Wiens 1989; Mayor et al. 2009), hence, a more useful approach may be to investigate habitat use at multiple spatial scales (Kotliar & Wiens 1990; Altmoos & Henle 2010). Here, microhabitat use was quantified using two methodologies differing in the scale of observation, which produced different results. In Chapter 3 habitat characteristics and fish were sampled in 1x1m quadrats and chub showed strong associations with microhabitat characteristics, whereas in Chapter 4 they were sampled at the focal level (0.20x0.20m quadrats) and chub generally showed random microhabitat use. These differences in results suggest that a multi-scale hierarchical approach should be adopted in microhabitat analysis, to provide a complementary view of patterns and processes. The influence of observation scale have previously been reported in other studies, albeit these typically use more disparate spatial scales (Torgersen & Close 2004; Santos et al. 2010; Compton & Taylor 2013; McEwan & Joy 2013).

Two hypotheses may be advanced to explain differences in microhabitat use with observation scale. First, physical habitat characteristics frequently are patchily distributed on several hierarchical levels (Pringle et al. 1988; Winemiller et al. 2010), and patchiness of this type has been documented to affect the abundance and distribution of stream fishes (Freeman & Grossman 1993; Thompson et al. 2001; Argentina et al. 2010). Therefore, although habitat characteristics were patchily distributed at 1m distances (Chapter 3), it is possible that there was less or no variation at smaller scales, thus leading to random focal microhabitat use. Second, fishes may not be able to differentiate among habitat characteristics at all spatial scales (Kotliar & Wiens 1990; Thompson et al. 2001), consequently, individual chub may be unable to perceive patchiness in habitat characteristics at the focal level. For example, Thompson et al. (2001) found that longnose dace *Rhinichthys cataractae* Valenciennes responded to patchiness of macroinvertebrate prey at the secondary (foraging patches < 5 m in diameter) and tertiary levels (stream reaches) but not at the primary level (individual cobbles separated by < 1 m). Testing patchiness in physical characteristics at the focal level is thus needed, as well as assessing the ability of chub to differentiate patches at various spatial scales.

The approach adopted in this thesis acknowledges the influence of scale on chub habitat use and recognises the importance of using multiple scale analyses in assessing distribution and habitat selection. There have been few underwater observational studies using such an approach to quantify microhabitat use by fishes. However, this appears highly advantageous because it provides complementary perspectives about microhabitat use and allows the integration of both field and laboratorial results, through the comparison of focal observations of microhabitat use with individual fitness criteria (i.e., prey capture success).

5.5. Final remarks and future prospects

The recent acceleration in research on Mediterranean stream fishes is promising (Magalhães et al. 2007; Filipe et al. 2010; Pires et al. 2010; Ribeiro et al. 2013), and represents an opportunity for rapid advances in the development of effective conservation and management plans. Nevertheless, there is still a shortage of information regarding various small scale ecological aspects, as well as, a lack of adequate and biological realistic quantitative approaches to study them. The majority of studies analysing microhabitat use by fishes are not based on direct observations, which facilitate precise *in situ* habitat use observations of individual fish (Heggenes et al. 1990; Almeida & Grossman 2012). In addition, studies are mostly descriptive with no attempts to determine the causations of diagnosed patterns or to include spatial information (Santos et al. 2004; Santos & Ferreira 2008; Martínez-Capel et al. 2009) that often has substantial influence on distribution patterns and species-habitat relationships (Keitt et al. 2002; Lancaster et al. 2003). Further, studies often ignore the type of behaviour fishes performed while using a certain microhabitat, which may limit the ability to understand relationships between activity and habitat use (David & Closs 2003; Clavero et al. 2005). In the view of the high number of endemic and imperilled fish species in Mediterranean streams (Smith & Darwall 2006), this lack of ecological information is concerning.

Our study, despite its limitations, tried to overcome the shortcomings mentioned above: 1) it is based on underwater and video observations that enable the exact location of individuals and the collection of behavioural information without disturbing the fish (Heggenes et al. 1990), 2) it incorporates resource patchiness and spatial explicit information into analyses of chub distribution and models of microhabitat use, 3) it follows a mechanistic approach in an attempt to determine whether foraging success underlies chub microhabitat use (i.e., focal-point velocity), and 4) it analyses activity patterns, which may be intimately related to habitat use.

By taking an innovative approach to address fundamental ecological questions, this study has therefore contributed to increase the understanding of behaviour, spatial distribution and habitat use by stream fishes, and shed light on the mechanisms underlying them. Analysis of activity patterns provided evidence of how cyprinids allocated their time to specific activities during the day, and of their high individual behavioural flexibility, important for persistence in highly variable and unstable streams. Examination of distribution improved the understanding of how spatial variation in physical resources and social attraction may shape fish aggregation, and showed the importance of using spatial explicit information. Investigation of microhabitat use produced unexpected results regarding the influence of velocity on fish occurrence and abundance, but allowed the identification of physical resources that should be maintained for local persistence under low flows. Finally, the combination of focal microhabitat use with experimental essays on prey capture provided the first evidence that foraging success may be implicated in microhabitat selection by Mediterranean cyprinids, and corroborates other studies arguing that models of habitat selection must be based on criteria directly linked with some aspect of individual fitness. This information is important for purposes ranging from the elaboration of ecological theory to the conservation and management of Mediterranean cyprinids.

Nevertheless, future studies can be conducted to clarify and strengthen the results of this thesis. Examples of possible improvements are as follows:

- Extend the taxonomic, spatial and temporal scope of the habitat analyses by conducting multiple fish and physical microhabitat observations in different habitat contexts, and over low to high flows. This information will clarify how microhabitat use may be influenced by a wider range of physical habitat characteristics, especially, water velocity and the extent to which this may be contingent on species and geographic context;
- Testing for effects of inter and intraspecific competition (Almeida & Grossman 2012) in habitat use. This can be achieved by conducting field removal experiences consisting in quantifying and comparing microhabitat use before and after fish removal. This has successfully been done with other cyprinids (Petty & Grossman 2010), but has never been tested in Mediterranean fishes, for which there is a dearth of empirical data regarding biotic interactions;
- Use neutral landscape models for understanding the relationship between aggregation and resource patchiness. Point pattern analysis quantifies the distribution of resource patches and combined with neutral landscape models allows to test whether the spatial pattern of used resources is independent of the background distribution of available resources (Lancaster et al. 2003). This could be applied not only with physical habitat but also with prey resources;

- Address the interaction between activity and microhabitat use by explicitly examining how activity is related to microhabitat (Schrandt et al. 2012). This may allow the identification of feeding, spawning or resting microhabitats, important for understanding population dynamics and planning more effective restoration of stream habitat (Rosenfeld 2003; Pander & Geist 2010);
- Improve foraging success-based models by approaching energy gain not only using prey capture success but also the energetic value of prey items, and estimate energetic costs of occupying a certain velocity using respiration rates at varying velocities (Hill & Grossman, 1993). Finally, estimate intra and interspecific competition parameters (Hazelton & Grossman, 2009) and validate the model in wet years.

As a general conclusion of this thesis, it arises that traditional correlative approaches may be insufficient to understand the distribution and microhabitat use of Mediterranean stream fishes, and that approaches based on a mechanistic understanding of the processes involved using criteria directly linked with some aspect of individual fitness, such as reproductive output, growth, or survivorship are needed. Future studies should thus attempt to scale up mechanistic habitat use models spatially and temporally (Dormann et al. 2012) to better represent the hierarchical nature and the temporal dynamism of habitat selection and use by Mediterranean stream fish.

5.6. References

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Appendix

Abundance of benthic and drift macroinvertebrates

Appendix

Table A1 – Abundance of benthic macroinvertebrates in the study reaches of the Torgal stream, in spring 2009 and 2010. Values are the mean number (N), standard deviation (SD) and percentage (%) of each taxa in 23 and 24 samples collected in Reach 1 in 2009 and 2010, respectively, and 23 samples collected in Reach 2. Macroinvertebrates were sampled from small cobbles, gravel and silt using a Hess sampler, and scraped from boulders, large cobbles, tree trunks and roots, collected in a 40x40cm quadrat. Taxa are listed by order of abundance in Reach 1 in 2009, and those contributing to more than 10% of total numbers in each reach and year are highlighted in grey.

Taxa	Reach 1						Reach 2		
	2009			2010			2010		
	N	SD	%	N	SD	%	N	SD	%
Gastropoda	65.4	122.7	47.6	20.3	33.1	27	0.2	0.6	0.6
Chironomidae (pupae included)	35.0	49.6	25.5	32.8	66.7	43.6	18.2	31.1	52.5
Coleoptera	12.7	33.5	9.2	9.0	23.4	11.9	1.0	2.9	2.8
Crustacea	11.0	41.6	8.1				6.4	23.6	18.6
Tricoptera (pupae included)	6.3	5.0	4.6	3.8	4.4	5.1	2.2	3.4	6.3
Ephemeroptera	3.0	4.3	2.2	3.5	5.5	4.7	2.2	3.9	6.4
Ortophera	0.6	1.1	0.4						
Turbellaria	0.6	1.0	0.4	1.8	3.7	2.4			
Megaloptera	0.6	1.1	0.4	1.1	3.1	1.4	0.3	1.1	0.8
Plecoptera	0.6	1.4	0.4	1.1	1.8	1.5	3.5	10.5	10.1
Arachnidae	0.4	0.9	0.3						
Bivalvia	0.4	1.4	0.3						
Odonata	0.2	0.7	0.2	0.7	1.0	0.9	0.1	0.3	0.3
Coleoptera (adults)	0.1	0.3	0.1	0.1	0.6	0.2	0.0	0.2	0.1
Clitellata	0.1	0.3	0.1	0.2	0.7	0.3	0.1	0.5	0.4
Diptera (adults)	0.1	0.3	0.1						
Hymenoptera		0.2							
Diptera (non Chironomidae)				0.8	1.1	1	0.4	0.9	1.3

Table A2 – Abundance of drift macroinvertebrates in the study reaches of the Torgal stream, in spring 2009 and 2010. Values are the mean number (N), standard deviation (SD) and percentage (%) of each taxa in 4 and 11 samples collected in Reach 1 in 2009 and 2010, respectively, and 4 samples collected in Reach 2. Samples were collected using a drift net placed approximately 15cm from the bottom, during 30min. Velocity was measured both at the beginning and end of this period, at the mouth of the net and a mean was calculated. Taxa are listed by order of abundance in Reach 1 in 2009, and those contributing to more than 10% of total numbers in each reach and year are highlighted in grey.

Taxa	Reach 1						Reach 2		
	2009			2010			2010		
	N	SD	%	N	SD	%	N	SD	%
Gastropoda	19.0	9.0	65.5	0.5	1.2	29.4	0.0	0.0	
Chironomidae (pupae included)	6.8	1.7	23.3	0.3	0.5	17.6	0.5	1.0	33.3
Odonata	2.3	3.9	7.8	0.0	0.0		0.0	0.0	
Tricoptera (pupae included)	0.8	1.0	2.6	0.1	0.3	5.9	0.3	0.5	16.7
Diptera (adults)	0.3	0.5	0.9						
Coleoptera (adults)				0.2	0.6	11.8			
Arachnidae				0.2	0.6	11.8			
Hymenoptera				0.2	0.6	11.8			
Ephemeroptera				0.1	0.3	5.9			
Plecoptera				0.1	0.3	5.9			
Crustacea							0.3	0.5	16.7
Diptera (non Chironomidae)							0.5	1.0	33.3