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Population ecology and behaviour of European barbel *Barbus barbus*, a recreationally important, translocated fish

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ABSTRACT

The intentional introduction of fish species into new environments for enhancing recreational angling is common. The intentional translocation of European barbel *Barbus barbus* into the middle River Severn in 1956 resulted in their establishment and dispersal throughout the catchment, including its tributary, the River Teme, where they supported high catch rates until the mid-2000s. Anecdotal evidence suggests that since then, there have been large declines in catch rates in the Teme and thus in the size of the adult population. However, data to evidence this were lacking, with minimal ecological data available. The aim of this research was to thus generate new knowledge on the ecology of this invasive *B. barbus* population in the River Teme to provide baseline information that could be used as a basis for fishery and river management. The focus of the research was initially on understanding the reproduction of *B. barbus* in the river, with focus on the quality of the spawning substrate and the temporal and spatial production of 0+ fish in the river via their reproduction. This was followed by investigating the *B. barbus* trophic relationships with other cyprinid species, and in relation to angling. In addition, the tracking of *B. barbus* in the lower river provided insights into the behaviours of individual fish.

The majority of the spawning gravels analysed in the River Teme had generally low fine sediment content and organic matter compared to other lowland rivers, with this potentially important for *B. barbus* larval emergence and survival. The spawning of *B. barbus* involves construction of a nest ('redd') that results in large volumes of sediments being moved and thus they can have a zoo-

geomorphic impact on sediments. Spawning in the Teme *B. barbus* population utilised a protracted spawning strategy, as per their native range, with this strategy also utilised by chub *Squalius cephalus* and minnow *Phoxinus phoxinus*. These results suggest some consistency with the pre-adaptation hypothesis, whereby the non-indigenous *B. barbus* utilised traits in the new range that it utilises in their indigenous range, providing considerable advantages for invasion success.

Investigations into the trophic interactions of the *B. barbus* with other fishes revealed that, in general, there were consistent patterns of partitioning in their trophic and isotopic niches, with little evidence to suggest high inter-specific competitive interactions. Stomach contents analyses revealed that whilst the 0+ fishes were all primarily generalist in their diet, *B. barbus* was the most specialist out of the four analysed fishes, with the trophic niche of invasive *B. barbus* being highly dissimilar to *S. cephalus* and dace *Leuciscus leuciscus*. Stable isotope studies then suggested these patterns of inter-specific niche partitioning remained through the life of these fishes, but with some dietary convergence when larger fishes (generally > 400 mm) had diets composed of high proportions of angler bait based on marine fishmeal. Acoustic tagging of *B. barbus* in the lower river tracked their movements over a 12 month period and revealed that two weirs provided substantial impediments to their movements. There were also considerable differences between the size of the home ranges of individuals, but with this explained more by their method of capture (angling versus electric fishing) than any other variable, suggesting inherent differences in the behaviour of individuals that affect their vulnerability to angler capture.

These results thus provide considerable new knowledge on this invasive *B. barbus* population that can be utilised to better manage populations both in the River Teme and elsewhere in their range. They revealed considerable differences in the behavioural ecology of individuals, but with their invasive population generally having minimal impact on other fishes in the river.

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Author's declaration

I (CGR) confirm that the work presented in this thesis is my own work, with the following exceptions:

Chapter 4, 5 and 5 are based on the following papers that have been submitted or published in collaboration with Robert Britton (JRB), Tea Bašić (TB) and Fatima Amat Trigo (F-AT) as:

Gutmann Roberts C. & Britton JR. Development of feeding specialisms and diet dissimilarities in the 0+ fishes of an invaded riverine cyprinid community. *Ecology of Freshwater Fish* (In revision)
Where CGR and JRB designed the study and collected field samples, CGR collected the data in the laboratory and analysed the data, and led the writing of the manuscript with support from JRB (Chapter 4).

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Where CGR and JRB designed the study and collected field samples, CGR collected the data in the laboratory and analysed the data, and led the writing of the manuscript with support from JRB (Chapter 5).

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Where CGR collected wild fish samples and their food resources, and analysed all data and provided all the results, JRB designed and ran the mesocosm experiments, and designed the pond experiments and collected the data with support from TB, and F.A-T processed scales from wild fish for stable isotope analysis. CGR and JRB designed the methodology for the field data collection, and they wrote the manuscript together.

Chapter 1: INTRODUCTION

1.1 Overview

This research investigates the ecology and behaviour of a population of non-indigenous European barbel *Barbus barbus* and their interactions within the fish community. The study system is the River Teme, a tributary of the River Severn in Western England. *Barbus barbus* was introduced into the River Severn in 1956 and subsequently colonised the Teme where they rapidly became an important species for recreational catch-and-release angling. Whilst there were anecdotal reports of large declines in *B. barbus* catch rates in the River Teme commencing in 2007, there was an absence of data on their populations specifically, and the cyprinid fish community generally, to enable objective assessment. Consequently, this research was designed to overcome this knowledge gap via generating contemporary data on a range of topics associated with *B. barbus* ecology and their interactions with other fishes. The focus of this Introduction chapter is to outline the overarching topics of the Ph.D. and the rationale of the research, before an overview of the individual chapters is presented.

1.2 Anthropogenic impacts on lowland rivers

The land surrounding lowland rivers tends to be highly suitable for agriculture, urbanisation and industry, resulting in the exploitation of a range of provisioning ecosystem services that have multiple environmental and ecological impacts (Middelkoop and Van Haselen 1999). Societies have tended to have close relationships with rivers, given the importance of access to potable water, fish

for food security, and river networks for transport. However, with population increases comes increased exploitation with, for example, levels of groundwater abstraction now becoming unsustainable in many places (Vörösmarty et al. 2010). River engineering programmes that result in channelization and impoundment of river sections to promote navigation and energy generation result in habitat loss and fragmentation, and altered flow regimes and sediment deposition (Kemp 2016). Whilst impoundment includes the construction of large hydropower dams that eliminate river connectivity (Finer and Jenkins 2012), even low-head weirs and culverts can impede connectivity, preventing upstream movements of some fishes (Lucas et al. 2009).

Many lowland rivers have become highly degraded through chemical and nutrient inputs, simplifying biotic communities through reduced species diversity (Riis and Sand-Jansen 2001; Hilton et al. 2006). However, degradation can also result from biotic pollution, with many non-indigenous species being present in freshwaters at the global scale following intentional and accidental introductions, with a small proportion of these developing invasive populations (Gozlan et al. 2010a). Introductions of non-native fishes have mainly been for the purposes of either aquaculture or the enhancement of recreational angling, with species such as largemouth bass *Micropterus salmoides* and common carp *Cyprinus carpio* now invasive around the world (Britton et al. 2010a,b). The net result of such introductions is the biotic homogenization of fish assemblages across different biogeographic regions, resulting in the imperilment of endemism and adaptive capacity (Clavero et al. 2006; Villéger et al. 2011).

1.3 Regulated rivers and consequences for river biota

Lowland river ecology tends to be negatively impacted by increased river regulation resulting from engineering schemes, such as weirs and sluices (Kemp 2016). When a dam, weir or sluice impounds a river, the net effect is to deepen the upstream river section and to regulate the river flow in the downstream section; thus, impoundment can result in a substantial alteration to the hydrology of the system (Petts 1984). Given that the river biota will have evolved within the natural flow regime then these altered conditions generally result in biological degradation, such as reduced species diversity, especially the loss of specialist species that are unable to adapt to the new conditions (Quinn and Kwak 2003; Orsi and Britton 2014). The altered hydrology can also impact the geomorphology, chemistry and physical habitats that further impact biota (Petts 1984; English et al. 1997). Regulated rivers also often have reduced seasonality in flow that then reduces macroinvertebrate diversity (Williams and Winget 1979; Schneider and Petrin 2017).

The combination of altered flow regimes and engineered structures in rivers also results in a loss of longitudinal and lateral river connectivity (Vannote et al. 1980; Junk et al. 2000), with this inhibiting the movements of migratory fishes (Dauble and Geist 2000) and reducing spawning areas for lithophilic fishes (Cadwaller and Lawrence 1990). The net result tends to be a reduction in fish species richness (Joy and Death 2001). Whilst blockages that prevent fish migrations tend to focus on anadromous fishes, even small barriers can impede the movement of many fishes, fragmenting their populations and resulting in reduced gene flow and genetic diversity (Vrijenhoek 1998; Fluker et al. 2014).

The net results of impoundments on fishes thus include changes in fish community composition, with declines in lithophilic species (Penczak and Kruk 2005; Penczak et al. 2012) that are often coincident with increases in phytophilic and eurytopic fishes (Penczak and Kruk 2005; Poulet 2007). Ovidio and Phillipart (2002) revealed that even low head barriers can limit or delay upstream spawning migrations of both salmonid and cyprinid fishes. Concomitantly, the altered flow regimes can also facilitate the establishment and invasion of non-native species (Bunn and Arthington 2002); with this often then further impacting the native species assemblage (Poff and Schmidt 2016).

1.4 Fish introductions and impacts

1.4.1 *Introduced and invasive fish*

Biological introductions have increased worldwide in the last thirty years (Gozlan et al. 2010a, Williamson & Fitter 1998), mainly due to globalised trade and the increased movement of fish by people (Sala et al. 2000, Gozlan 2008). Freshwater ecosystems are particularly vulnerable to biological invasions due to their isolation at the basin level, relatively high levels of endemism and the extant anthropogenic stressors that increase their vulnerability to invasion (Gozlan et al. 2010b, Moorhouse et al. 2015; Section 1.2). The sources of introduced fishes are varied and include aquaculture, ornamental fish and fishery enhancements (Gozlan 2008). The majority of introductions are intentional, having drivers that are economic, commercial and/ or recreational (Gozlan 2008). Despite high rates of fish introductions into freshwaters, not all species are able to adapt and thrive in new environments; of those species that do

establish populations in the wild, a high proportion will only have minor impacts on the native fauna and flora (Gozlan 2008).

Of those introduced, established species that do result in more severe ecological impacts on the receiving communities are described as invasive, their potential consequences include the adverse effects of increased predation pressure, hybridization, competition for refugia, spawning habitats and/ or food resources, and the transmission of non-native parasites (Costedoat et al. 2004; Pinder et al. 2005; Yonekura et al. 2007; Blanchet et al. 2007). Invasive species can also impact the physical habitats and have knock on impacts on an ecosystem scale, often these interactions are hard to measure and may be subtle or indirect (Simberloff 2011). Introduced species can have positive impacts for certain species as they provide a novel food source (Caldow *et al.* 2007; Tablado et al 2010; Wood *et al.* 2017). The effects of an introduced species for native fauna may vary depending on the life stage of the native fauna (Wood *et al.* 2017). For *B. barbus*, detrimental consequences include high levels of genetic introgression through hybridisation with endemic *Barbus* species in rivers in Northern Italy (Meraner et al. 2013), and due to these negative consequences of their interactions they are described herein as invasive. Invasive *B. barbus* also have the potential to cause habitat alterations via their foraging behaviours and whilst these effects are size specific (Pledger et al. 2016), individual *B. barbus* can reduce the stability of sediments and gravels during foraging (Pledger et al. 2014).

Recreational freshwater fishing is one of the key drivers of introductions of non-native fish (Hickley and Chare, 2004; Davis and Darling 2017), a result of the economic and societal benefits that can accrue (McIntosh et al. 2010; Britton and Orsi 2012). Many non-native fishes have been introduced for recreational angling both legally (McIntosh et al. 2010; Weyl et al. 2013; Ellender et al. 2014) and illegally (Hickley and Chare 2004; Benejam et al. 2007). Examples include European catfish *Silurus glanis* (Benejam et al. 2007; Cucherousset et al. 2017), peacock basses of the *Cichla* genus (Britton and Orsi 2012), *M. salmoides* (Ellender et al. 2014) and brown trout *Salmo trutta* (McIntosh et al. 2010; Weyl et al. 2013). Unlike many of fishes utilised and introduced for aquaculture that can occupy lower trophic levels, introduced sport fishes tend to occupy higher trophic levels and thus can have greater negative impacts on native conspecifics and on trophic dynamics via top-down processes (Eby et al. 2006; Britton and Orsi 2012).

The benefits of fish introductions for recreational angling are thus focused on the socio-economic benefits that develop from the enhanced angling opportunities they provide. However, given the potential impacts these species can have on recipient waters and their assemblages (Gozlan et al. 2010a,b) there needs to be a balance between conserving native species and endemism, and promoting new angling opportunities (McIntosh et al. 2009; Weyl et al. 2013). This can now be achieved more easily through the application of risk assessment tools that estimate the ecological risk of species prior to their release (e.g. Copp et al. 2009) and to help managers make decisions about extant invaders (Britton et al. 2011).

1.4.2 *Translocated species*

The focus in invasion biology tends to be on species that have been released into new areas following their transport from other biogeographic regions that often have contrasting climates, faunal communities and habitat structure. By contrast, there has, generally, been less attention on translocated species. The term 'translocation' can refer to the re-introduction of a species into its historic range where is has become extirpated (South et al. 2001), but also to releases of fishes into river basins where the species is non-indigenous but where that species is indigenous to the region or country in question (Copp et al. 2005). For example, roach *Rutilus rutilus* is indigenous to freshwaters in Great Britain, but due to the last glacial period, is non-indigenous to many upland areas in northwest England and Scotland (Winfield et al. 2008). Despite this, non-indigenous populations of *R. rutilus* are present in waters such as the Lake Windermere due to translocations completed by recreational anglers (Winfield et al. 2004, 2008, 2011). From the perspective of the translocated fish, there can be considerable advantages compared to fish from contrasting biogeographic regions, as climatic effects are more likely to match those the fish experienced in their previous range and thus the species might have 'pre-adapted' traits that promote their survival and establishment (Buoro et al. 2016; Section 1.4.3). Thus, providing the physical habitat of the receiving ecosystem meets the requirements of the translocated species, there is a high likelihood of a sustainable population developing (Harig & Fausch, 2002; Buoro et al. 2016).

From here on in the thesis, when the term 'introduced fish' and 'invasive fish' is used, it refers to any fish that has been introduced into a river basin through

anthropogenic means and thus, in contemporary times, would not be naturally found in that river basin. Thus, these terms cover both non-native fishes that have been introduced into a new region and translocated fishes that have been moved from indigenous range into their non-indigenous range within a defined region. The term 'introduced fish' is used where that species has been released into the new river basin but has yet to establish a sustainable population and disperse naturally. By contrast, the term 'invasive fish' refers to species that have been released, have established and have then dispersed more widely, even if that dispersal is only within that river basin.

1.4.3 *Pre-adaptation hypothesis*

It was hypothesised by Darwin (1859) that invaders with similar traits to native conspecifics would be less successful due to the competition they would face, allied with the probability that predators that would be better adapted to preying upon them and parasites would be able to better utilise them as hosts. Contrarily, species with traits that are similar to native species have been hypothesised as being more likely to be invasive, as their traits will facilitate survival in the new environment, with this already demonstrated in some plant species (Duncan and Williams 2002). These hypotheses relate to 'pre-adaptation', whereby the inherent traits of the introduced species facilitate their invasion of their new environment, as they require little or no adaptation to the new conditions (Buoro et al. 2016). For fishes, it was tested by reviewing whether, in invaded waters, the invaders were present with a congeneric native species, with the study being inconclusive in its results (Ricciardi and Mottiar 2006). Buoro et al. (2016) revealed, however, that, the release of hatchery-reared salmonids can represent

a 'native invasion', as the released fishes had similar phenotypic traits to wild con-specifics and thus were 'pre-adapted' to surviving and thriving in the new conditions. Indeed, Carey et al. (2012) suggested that species introduced from biogeographic regions close to the new range were more likely to develop invasive populations than those from more distant regions, a result of conditions in the new environment being similar to their natural range (Carey et al. 2012). The pre-adaptation hypothesis thus has high relevance in the case of determining the outcome of introduced, translocated species.

1.4.4. *Impacts of introduced and invasive fishes*

It was outlined in Section 1.1 that a number of detrimental ecological consequences can result from both fish introductions. Some of these are discussed in more detail in this section.

Increased predation pressure

Introduced fish can increase predation pressure on extant prey communities (Reshetnikov 2003), with many highly invasive fishes having relatively high consumption rates versus trophically analogous native fishes (Alexander et al. 2014; Dick et al. 2014). A potentially major issue with releases of fish is the 'stocking up' of food webs (Eby et al. 2006; Davies & Britton 2015). This is where the fish that are introduced tend to be those in higher trophic positions than the majority of the extant fishes, resulting in high proportions of individuals in high trophic positions, such as apex predators (Eby et al. 2006). The resultant increased predation pressure on prey populations can then incur cascading consequences through the food web via top-down effects. These consequences

can be sufficient to impact the recruitment processes of native fishes and can even result in the extirpation of some prey communities (Reshetnikov 2003). Nevertheless, the ability of those released fishes to incur such deleterious effects will be influenced by the extant fish community structure and habitat characteristics (for example, the availability of prey refugia and the water clarity for predators reliant on visual cues) (Takamura 2007).

Zoo-geomorphic effects

When released into new environments, some introduced fishes can act as 'ecosystem engineers' and consequently are able to dramatically alter the physical habitat. These habitat alterations can alter the biogeochemical properties of the receiving waters and result in increased instability in the hydrological regimes and geomorphological processes (Pledger et al. 2014). In these cases, the fish are acting as zoo-geomorphic agents (Butler, 1995). Freshwater fish can act as geomorphic agents during swimming, burrowing, spawning and feeding activities (Kondolf et al. 1993; Statzner et al. 2003; Shirakawa et al. 2013; Pledger et al. 2014). Many fishes of the Cyprinidae family forage in the benthos and thus increase bedload instability, leading to increased water turbidity. For example, *C. carpio* is now invasive globally and is considered a pest species, primarily through its alteration of habitat quality through their foraging that can transform macrophyte dominated systems to algal dominated (Richardson & Whoriskey 1992; Koehn 2004; Britton et al. 2007). Other cyprinid fishes, such as chub *Squalius cephalus* and sofie *Parachondrostoma toxostoma*, also disturb surface sediments whilst foraging (Canal et al. 2015). Gudgeon *Gobio gobio* is an invasive fish in Italy (Bianco et

al. 2005) and can increase the base-flow transport of sand and gravel through their swimming and foraging behaviours (Statzner et al. 2003).

Loss of genetic integrity

Where fishes are introduced that are taxonomically similar to extant fishes then there is risk of genetic introgression between these species via hybridisation. Hybridisation can alter the gene pool for the native species and potentially lead to negative consequences for their population (Rubidge & Taylor 2005). Whilst the outcomes of hybridization can be difficult to generalize, hybrids can be fertile and lead to introgression via reproduction with native species (Schribner et al. 2001). In the case of the invasive goldfish *Carassius auratus*, their reproduction with both crucian carp *Carassius carassius* and *C. carpio* results in fertile hybrids that are then able to back-cross with the species, as well as reproduce with other hybrids; this results in very high levels of introgression within a small number of generations (Hänfling et al. 2005; Tóth et al. 2005). The anthropogenic movement of fish between river basins can also result in genetic introgression between distinct genetic units. For example, in *B. barbus* populations in England, genetic analyses confirmed the single release of *B. barbus* into the River Severn basin from the River Thames basin (Antognazza et al. 2016). These analyses also revealed genetic disruption to the indigenous *B. barbus* population of the Yorkshire Ouse catchment that had unique haplotypes compared with other English populations, with these now impacted by introgression that resulted from stocking of hatchery reared fish from other basins (Antognazza et al. 2016).

Introduced pathogens

The release of fishes into new ranges potentially also results in the release of their parasitic fauna (Britton 2013). Whilst the process of introduction can result in 'enemy release' that minimises the numbers of released parasites, some are still released into the new system (Sheath et al. 2015). This is potentially problematic, as these parasites have the potential to 'spill over' into native fishes (Peeler et al. 2011; Britton 2013). When this occurs then the naïve hosts can incur substantial pathological and ecological consequences due to their lack of co-evolution with the pathogen that results in poor immune responses and anti-parasite behaviours (Kirk 2003, Gozlan et al. 2010a; Britton et al. 2011b). The transmission of introduced pathogens to native fishes can result in substantial consequences, such as for European eel *Anguilla anguilla* infected with the nematode parasite *Anguillicolloides crassus* from the Japanese eel (Kirk 2003). This can damage the swim-bladder of adult *A. anguilla*, potentially inhibiting their ability to return to their spawning grounds in South Atlantic Ocean (Pegg et al. 2015).

Increased competition in native fish community

Many fishes, and especially cyprinid fishes, often utilise generalist feeding strategies, with the selection of prey items often being limited by gape size. Therefore, overlap in the exploitation of food resources is often common between introduced and native fishes, and can lead to interspecific competition where resources are limited (Gozlan et al. 2010). Mosquitofish (*Gambusia spp.*) have been introduced in many countries to control mosquito but have led to declines in native fishes partly due to competition for food resources (Rincon et

al. 2002). Abiotic conditions can play a role in the competitive ability of introduced species, hence an introduced species might differ in their inter-specific interactions with native fish depending on the environment (Alcaraz et al. 2008). Nevertheless, niche partitioning is also often observed, whereby rather than sharing limiting food resources, populations become more specialised in their diet under situations of increased competition for resources following an introduction, with this aligned to the 'niche variation hypothesis' (Tran et al. 2015).

Socio-economic benefits

Whilst many fish introductions have been associated with reductions in biodiversity (Gozlan et al. 2005), introductions have continued to be completed due to the societal benefits they potentially deliver. The use of non-native fishes in aquaculture has provided substantial economic benefits (Gozlan et al. 2008), but the escape of these fish into the wild has resulted in numerous invasions and ecological impacts (e.g. Savini et al. 2010; Copp et al. 2016). Some fish introductions have also benefitted recreational angling, with the North American Rainbow trout *Onkorynchus myskiss* having been introduced into many countries for sport angling, including in Africa, Europe and South America (Fausch et al. 2001). Such is the economic and recreational importance of many fishes, their releases have historically been given precedence over conserving native fish communities (Cambray 2003).

1.5 European barbel, *Barbus barbus*: An introduced, translocated and invasive fish

In their native range, *B. barbus* is often used as a flagship species to indicate 'healthy' lowland rivers through their requirement for high levels of dissolved oxygen, lithophilous spawning and affinities for rivers of low anthropogenic disturbance (Freyhof 2013). *Barbus barbus* is of 'Least Concern' on the IUCN Red List, but is locally threatened in many areas (Britton and Pegg 2011), with minimal data available on many populations due to the large, deep, fast-flowing rivers they usually inhabit (Freyhof 2013). Barbel are present in many European rivers, but their Western extent is the British Isles, where they are only indigenous to eastern flowing rivers, such as the River Great Ouse and River Trent (Freyhof 2013; Antognazza et al. 2016). This is a result of connectivity with continental Europe at the end of the last glacial period that enabled re-colonization of these rivers via the Danube and Rhine (Wheeler and Jordan 1990; Section 1.8). In some Eastern European countries, *B. barbus* are caught by anglers for consumption. However, in general, they are used primarily for catch-and-release angling where they are now a very popular sporting species due to their relatively large body sizes and hard fighting abilities (Britton & Pegg 2011). Their popularity with anglers is such that they have been introduced into many rivers outside of their native and indigenous ranges, in countries including Italy, England and Wales (Wheeler & Jordan 1990; Antognazza et al. 2016).

A rheophilic cyprinid fish, *B. barbus* prefers running waters in the middle and lower reaches of rivers, although they can survive in lentic conditions as well (Taylor et al. 2004). Individuals are capable of moving relatively large distances

for spawning and foraging, with individual behavioural differences related to movement (Baras and Cherry 1990; Baras 1995; Twine 2013). Upstream movements tend to occur in spring in preparation for spawning on shallow riffle gravels (Lucas & Batley 1996, Vilizzi et al. 2006). Water temperature is the key initiator of spawning (Baras 1995), whilst feeding dynamics are largely affected by light intensity and water temperature (Britton et al. 2011). The 'barbel zone' tends to have other cyprinid species also present such as *S. cephalus*, dace *Leuciscus leuciscus* and roach *Rutilus rutilus* (Huet 1954).

Introductions of non-native *B. barbus* have occurred across Europe, where impacts have mainly focused on genetic introgression with endemic *Barbus* species, with less attention on ecological impacts (Meraner et al. 2013). An exception is Carosi et al. (2017), who revealed that invasive *B. barbus* had negative effects on the endemic *B. tyberinus* and, in some places, resulted in their extirpation. This study was, however, limited to population level assessments, with no data provided on ecological interactions, such as tropic relationships. Nevertheless, it revealed that in the Tiber region where invasive *B. barbus* have been present since the 1998, there were large overlaps in their habitat ranges, with the range and density of invasive *B. barbus* increased between 2000 and 2015, with their upstream dispersal continuing (Carosi et al. 2017). When inhabiting the same area as *B. barbus*, *B. tyberinus* had lower body condition, potentially suggesting a negative effect of interspecific competition, although this was not quantified further (Carosi et al. 2017). The ecological impacts of invasive and translocated *B. barbus* are largely unknown in areas outside of their indigenous range in England, despite populations being stocked regularly in their

native range with hatchery reared fishes and invasive populations being present in many western flowing rivers (Bašić and Britton 2016).

1.6 Influence of engineered structures on the movements of *Barbus barbus*

Engineered structures, such as weirs and dams, alter habitats and often make them unfavourable to *B. barbus*, especially in upstream areas where habitats become lacustrine and homogenised (Penczak 2006). Whilst the majority of *B. barbus* have relatively small home ranges (< 1 km; Hunt and Jones 1974; Britton and Pegg 2011), a small proportion have much larger home ranges (> 8 km; Britton and Pegg 2011), with large upstream movements often occurring during the spawning period where movements in excess of 20 km have been recorded (Lucas and Batley 1996). Weirs can thus impede these upstream movements (Lucas and Batley 1996; Ovidio and Philippart 2002), resulting in delayed spawning or spawning in less favourable areas (Lucas and Frear 1997). Delayed migrations can increase energy expenditure, with fish dropping back downstream as they await the right river conditions to traverse the blockage (Lucas and Frear 1997). The ability to traverse migration blockages varies between individuals, but does not appear to relate to body size (Lucas and Frear 1997; Slavik et al. 2009). Despite ecological enhancements of weirs including fish passes, these have largely been designed for salmonid fishes and are rarely utilised by *B. barbus* (Baras et al. 1994). When they do utilise these passes, it is usually only under specific environmental conditions (Baras et al. 1994; Slavik et al. 2009), such as high flow events when successful passage over weirs also increases (Baras et al. 1994; Slavik et al. 2009). When upstream passage is not possible, these blockages can result in high levels of spawning activities in

downstream areas, potentially increasing competition for suitable spawning substrate (Melcher and Schmutz 2010). Despite the evidence of *B. barbus* in their native range being unable to pass or being delayed by in-stream barriers, their movement in their non-native range may differ via adaptive behaviours; thus any increase in their passage might impact fish assemblages upstream, as these areas often act as refuges for native and endemic species from invasive fishes (Carosi et al. 2017). Moreover, *B. barbus* movement is often river-specific and can relate to the experience of the individual fish (Benitez et al. 2017).

1.7 *Barbus barbus* as zoo-geomorphological agents

Barbus barbus foraging and swimming behaviours also disturbs surface sediments by affecting grain-size distributions and bed material structure from the micro-topographical scale up to the riffle and reach scale (Statzner et al. 2003; Statzner and Sagnes 2008; Pledger et al. 2014, 2016, 2017). By altering grain-size distributions and bed material structure, *B. barbus* can affect bed mobility and bedload transport (Pledger et al. 2014, 2016). Lithophilic freshwater fishes also interact with sediments during spawning when, for example, they create nests or 'redds' in the riverbed. In this regard, the effects of salmonid spawning have been well documented, with spawning Atlantic salmon *Salmo salar* affecting bed surface coarsening, sorting, grain mobility and scour (Montgomery et al. 1996; Hassan et al 2015). Spawning salmon can also reduce surface gravel size (Peterson and Foote 2000), alter the amount of fine sediment and organic matter within the spawning gravel (Moore et al. 2007). These changes can also alter the morphology of the riparian zone (DeVries 2012). The vertical mixing of sediment by spawning salmon can have the same

effect as flood events (Gottesfeld et al. 2004), with habitat changes from spawning salmon recently being linked to landscape scale processes (Fremier et al. 2017).

Although Hancock et al. (1976) described *B. barbus* as shedding eggs onto the gravels, there has been limited descriptions of the physical impacts of their spawning on the sediment characteristics. Anecdotal evidence suggests that *B. barbus* do cut a spawning redd, similar to *S. salar* (Spenature, 2013). Despite this, *B. barbus* redds and the sediment composition within them has not been quantitatively measured, despite being potentially important for egg survival and larval emergence (Kemp et al. 2011; Bašić et al. 2017).

1.8 Translocated *Barbus barbus* in the River Severn catchment

Biogeographically, the influence of the last glacial period had a strong influence on the distribution of some fish species in Great Britain, with a land bridge between Eastern England and mainland Europe providing connectivity for some English rivers with catchments such as the Rhine and Danube that was lost as sea levels rose (Wheeler and Jordan 1990; Section 1.5). This land bridge enabled fishes to recolonize these rivers in England as they were able to disperse from their glacial refugia and thus for some species, such as *B. barbus*, they are only indigenous to eastern flowing rivers in England and not those in the west. Consequently, in England, the species is naturally occurring in the Yorkshire Ouse, Trent and Thames river basins, plus some smaller basins along the east coast (Wheeler and Jordan 1990; Antognazza et al. 2016). For species such as *S. cephalus*, their status in some western flowing rivers, such as the River Severn,

is less clear and thus for the purposes of this research, they are considered as indigenous to these catchments but with the caveat that there remains some uncertainty over their status.

The re-distribution of *B. barbus* in England commenced in the 1890s, when the Hampshire Avon in Southern England had fish introduced from the Thames catchment, with subsequent releases into this river using fish from the Rivers Kennet and Lea (Antognazza et al. 2016). The River Severn had *B. barbus* introduced in 1956 with a release of 509 adult fish from the River Kennet and remains the only known release of fish in either the Severn or its tributary, the River Teme (Wheeler and Jordan 1990), with this also now confirmed genetically (Antognazza et al. 2016). This introduction was very successful, and the Severn and Teme have been important recreational fisheries for *B. barbus* since the 1970s.

However, since 2007, the *B. barbus* fisheries of the River Severn and Teme have been reported as being in decline, raising considerable concern in the region (e.g. Angling Trust 2013). An issue associated with this apparent decline from this period to the present is a lack of data on the cyprinid fish communities of the River Teme from any period. This results from the physical habitat of the middle and lower river comprising of pool-riffle sequences with relatively fast flows, widths to 20 m and depths to over 3 m. In combination, these make quantitative fish community sampling extremely challenging both logistically and in capturing representative samples. Consequently, it has been highly difficult for angling and fishery regulators to respond to challenges by angling groups on this

reported decline in both fish stocks and angling performance, as there are no data which comparisons can be made from. It also means that there is little or no knowledge on many aspects of *B. barbus* ecology, including their spawning strategies, juvenile fish dynamics, life history traits, movements and trophic ecology. These are considerable knowledge gaps that require filling before informed decisions can be made on how the population might be better managed.

1.9 Research aim and objectives

The aim of the PhD research is to thus quantify aspects of the zoo-geomorphology, ecology and behaviour of the translocated *B. barbus* in the River Teme, Worcestershire, England. From a research perspective, *B. barbus* is being used as a model translocated species that has developed a highly invasive population in the River Severn basin, with the Teme used as the model study system ('model river'). Through comparisons of ecological data between invasive *B. barbus* and other fishes in the community, their interactions can be quantified and ecological impacts assessed (from an invasion perspective). From an applied perspective, the research is the starting point of investigations into the *B. barbus* population of the River Severn basin in order to inform debate on the next steps in their management and in relation to the apparent catch rate declines. In the research, whilst the focus is on *B. barbus*, a cyprinid fish community perspective is provided wherever feasible. Salmonid fishes are, however, rarely utilised in the research, as they tend to be more dominant in the upper River Teme where cyprinid fish are present in much lower abundance. The research objectives (O) were thus to:

O1. Quantify the spawning habitat of *B. barbus* and redd characteristics in the River Teme at baseline flows (Chapter 2);

O2. Evaluate, using larval and juvenile fish, the spawning strategies of *B. barbus* in the River Teme and in relation to the pre-adaptation hypothesis (Chapter 3);

O3. Quantify the spatial, temporal and ontogenetic diet composition and feeding relationships of the juvenile cyprinid fishes of the River Teme (Chapter 4);

O4. Assess the trophic relationships of juvenile and adult *B. barbus* and native cyprinid fishes in the River Teme (Chapter 5), and in relation to trophic subsidies from angling baits (Chapter 6); and

O5. Quantify the intra- and inter-individual variability in the movements of adult *B. barbus* in the River Teme according to season, river flow and temperature (Chapter 7).

1.10 River Teme and the study reaches

The River Teme begins in Powys, Wales and runs 134 km until its confluence with the River Severn at Worcester, England (Fig. 1). The River Teme catchment is 1,648 km^2 in area and runs through predominantly rural areas over Silurian and Devonian rocks (Natural England 1996). The channel maintains a pool-riffle morphology and is underlain by sandstones and mudstones, which transitions to clays and silts when the river nears Worcester. The river has SSSI classification, partly due to the river's characteristic geology (Natural England 1996). There are some small urban areas located along this stretch of river, but land use is primarily for medium sized livestock and arable agriculture (Environment Agency 2017), where in some locations poaching is visible along the banks (Fig. 2). The upper reaches of the river regularly dry up during the summer months,

leaving isolated pools. The study reach is within a lowland river on soft deposits and is largely unmodified but with poor habitat, due to intensive agriculture which extends to the river bankside (Severn Rivers Trust 2012). The lower reaches are a stream order 4 river (Maddock & Hill 2007) that maintains a mean width of 9 m and has a low gradient channel slope (Pinder 2016a).

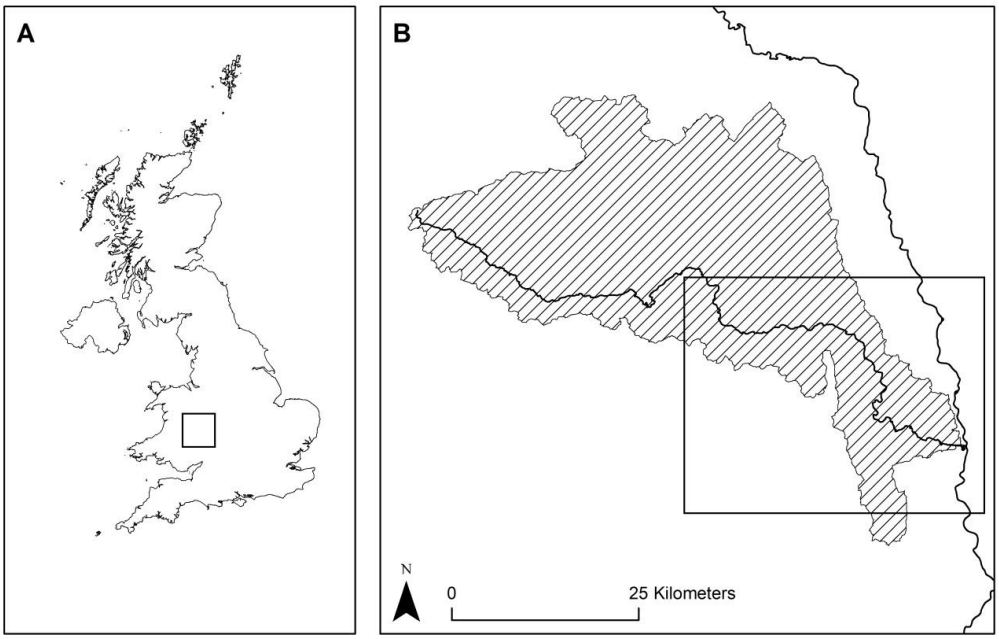

Figure 1. A) Location of the River Teme catchment within the UK; and B) River Teme study reach (within box) within the catchment of the River Teme (striped area). Exact sampling locations vary between chapters.

The 46-km study reach is within the middle and lower reaches of the river, from Ludlow (upstream) to Worcester (downstream) (Fig 1B). This reach as selected as it is representative of the 'Barbel Zone' (Huet, 1959), allied with anecdotal evidence suggesting the weir at Ashford Carbonel (close to Ludlow) is the upstream limit of *B. barbus* in the river. The Teme is a spate river, with very high winter flows and relatively incised channels (Fig. 2). These conditions have made regular monitoring of fish species difficult, with minimal historical data

on non-salmonid fishes. Despite the lack of data on the cyprinid fishes, there are many specialist barbel anglers that had fished the River Teme that reported a decline in their catch rates (Angling Trust 2013). Of the potential suggested causes of the decline in *B. barbus*, large floods in June 2007 have been mentioned, in conjunction with lack of access to high quality spawning gravels and the low availability of refuge habitat during high flow events (Angling Trust 2013). There has also been discussion about the upstream permeability of Powick Weir (the weir close to the River Severn confluence; Fig. 2) for a range of non-salmonid fishes, including *B. barbus*. Despite the long-term presence of non-indigenous *B. barbus* in the River Teme, there have been no studies undertaken to address the ecological implications of their invasion.

Figure 2. Top: Flooding on the River Teme at Powick Bridge, Worcester; Middle left: Powick weir, the weir furthest downstream on the river and approximately 3 km from the Severn confluence; Middle right: Poaching caused by cattle downstream of Powick Bridge; and Bottom: Incised banks downstream of Powick Bridge.

Chapter 2: Spawning habitat and redd characteristics of *Barbus barbus*

2.1 Abstract

Lithophilic fishes can have considerable impacts on river sediments during activities such as foraging and spawning, but in non-salmonid species, these have received minimal attention. Here, the spawning habitats of *B. barbus* were quantified to identify their sediment preferences and zoo-geomorphic capabilities, with the River Teme used as the study area. The riffles where *B. barbus* spawned in the river were generally shallow (< 0.5 m depth), with fast flowing and turbulent water, and with relatively stable bed sediments. Sediment size distributions revealed the surface and sub-surface sediments were generally coarse, moderately well sorted and leptokurtic, but with more fine sediment within the subsurface sediment than at the surface. The mean area of individual spawning redds was 3.47 ± 0.42 m^2, with quantification of their morphology revealing the tailspill of the redd tended to be longer and wider than the pit, and tailspill height being typically twice the depth of the pit. Sediments within the pit and tailspill were coarse and moderately well sorted, with the level of fine sediment in the redd surface being generally low. There were no significant differences between the surface characteristics of redd compared with their surrounding riffle. A typical spawning *B. barbus* female was estimated to be able to move a surface particle of maximum size 121 mm, resulting in there being few particles that these fish could not move on a spawning riffle. The reproductive capacity of each spawning riffle was estimated as 73,113 eggs/m^2. These results suggested that the River Teme provided adequate spawning

substrates for *B. barbus*, with relatively low fine sediment content compared to other rivers where *B. barbus* are present. The results also indicated that *B. barbus* can act as important zoo-geomorphic agents during their spawning activities through their ability to move large amounts of relatively large sediment particles.

2.2 Introduction

River habitats are characterised by a range of geological, morphological and hydrological processes that in turn define the characteristics of the riparian vegetation, river channel and floodplain (Cowx and Welcomme 1998). These characteristics are important, given that fishes rely on high-quality spawning and nursery habitats to facilitate their recruitment success (King et al. 2003; Zeug and Winemiller, 2008). The availability of these habitats, as dictated by hydro-geomorphological processes, is thus critical for the sustainability of fish populations (Freeman et al. 2001; Aarts et al. 2004). The habitat selectivity of many riverine fishes varies throughout their lifecycles and is particularly strong during the spawning period (Grossman et al. 1987; Labbe and Fausch 2000). The spawning habitat requirements for many salmonid fishes are now relatively well understood (e.g. Kondolf and Wolman 1993; Kondolf 2000; Buffington et al. 2004). For example, whilst Atlantic salmon *Salmo salar* reproduce in gravels across a wide range of sediment characteristics, they tend to prefer grain sizes of 2 to 64 mm (Moir et al. 2002). For lithophilic fishes of the Cyprinidae family, the importance of sediment characteristics for their spawning is comparatively poorly understood (Bašić et al. 2017), despite their high ecological and recreational importance in many rivers (Winfield and Nelson 1991).

River flows play an important role in determining where and how sediment is transported and deposited (Bridge 1993), and thus influences the composition and quality of fish spawning substrates (Bunn and Arthington 2002). Furthermore, hydraulic conditions and their impact on substrate composition can

influence the hyporheic physio-chemical water conditions (Greig et al. 2007). For gravel spawning fishes, this can have implications for the oxygen supply and interstitial flow to eggs and pre-emerged larvae (Lapointe et al. 2004), and so emphasises the importance of flows in regulating spawning habitat quality (Goode et al. 2012). In addition, high levels of fine sediment (< 2 mm diameter; 'fines') can impact the recruitment success of salmonid fishes via sediment compaction that results in shallower nest ('redd') construction (Kemp et al. 2011). High levels of fines also result in reduced permeability of the redd that inhibits oxygen delivery to eggs and larvae, potentially causing egg death and/ or delayed larval emergence (Kemp et al. 2011). Similar to studies of riverine fish spawning habitat characteristics, most studies on spawning habitat quality have also focused on salmonids (e.g. Montgomery et al. 1996; Youngson et al. 2004; Zimmerman and Lapointe 2005; Sear et al. 2016). Whilst these studies might have some transferability to non-salmonids, there are also likely to be many key differences between salmonids and other fish families. For example, as salmonid fishes tend to reproduce in the winter period then their egg incubation period tends to be considerably longer than riverine cyprinid fishes that reproduce in spring/ early summer, and so the importance of fines in spawning substrates might be lower in cyprinid versus salmonid fishes (Bašić et al. 2017).

The construction of spawning redds by salmonid fishes has been well documented (e.g. Montgomery et al. 1996, Peterson and Foote, 2000, Gottesfeld et al. 2004, Moore et al. 2007, Hassan et al. 2015). During spawning, the female excavates a depression in the riverbed that results in localised coarsening and

fining of the bed surface within and downstream of the depression respectively (Kondolf et al. 1993). These zoo-geomorphological actions affect stream bed mobility (Montgomery et al. 1996), with the extent of disturbance equivalent to the displacement of gravels caused by flood events (Gottesfeld et al. 2004). There are biophysical limits to redd building, which alter inter- and intra-specific effects of this zoo-geomorphological process, and thus this influences the size of grains that are moved (Riebe et al. 2014).

Recent studies on *B. barbus*, a lithophilic cyprinid fish, have revealed they can act as strong zoo-geomorphological agents through their foraging behaviours altering stream bed characteristics (Pledger et al. 2014, 2017). Studies on their reproduction have tended to focus only on their pre-spawning movements (Baras and Cherry 1990; Baras et al. 1994; Baras 1997). Studies on their habitat spawning requirements have been very limited, with Melcher and Schmutz (2010) revealing that they preferred spawning in fast flowing, shallow water (~37 cm) and in loose gravel close to overhanging vegetation. In this study, however, only the dominant substrate was recorded, with no quantitative assessment of sediment composition at the surface or subsurface, or the fines content (Melcher and Schmutz, 2010). Thus, the substrate characteristics of the gravels used by spawning *B. barbus* and their zoo-geomorphological impacts have not been quantified.

Given this paucity of knowledge on the spawning substrate characteristics and quality of non-salmonid river fishes, this chapter investigates the spawning habitats of a lithophilic model fish in a model river study system. *Barbus barbus*

was selected as the model species, as they are known to reproduce on gravel riffles and generally construct a spawning redd (Balon 1975, Hancock et al.1976). The model river was the River Teme, Worcestershire. Although *B. barbus* are non-indigenous in this river, their population has been present since the 1970s (Antognazza et al. 2016), with subsequent work in Chapters 3 and 4 revealing the consistent production of relatively high numbers of 0+ fish over three successive spawning years. This reproductive success is a contrast to areas in their native range in Britain, where *B. barbus* are either present in very small numbers and proportions in 0+ fish samples (e.g. River Trent; Nunn et al. 2007a, b) or their spawning success is negligible, with their populations supported primarily by hatchery-reared stocked fish (e.g. River Great Ouse; Antognazza et al. 2016; Bašić et al. 2017). Using field studies, the aim here was to quantify their spawning habitat conditions and redd characteristics through completion of the following objectives (O): (O1) assess the sediment size distribution and organic content of the river bed sediments utilised by spawning *B. barbus*; (O2) quantify the hydraulic conditions (depth and velocity profiles), physicochemical properties and bed mobility of spawning sites; (O3) characterise the morphology and properties of *B. barbus* spawning redds; and (O4) determine the reproductive capacity of their spawning sites.

2.3 Materials, methods and data analysis

2.3.1 *Study site*

The study reach used in the model river was selected as it is representative of the 'Barbel Zone' (Huet, 1959; Fig. 3b) and hosts a translocated population of *B. barbus*, the model species. Additionally, several other lithophilic species, such as *S. cephalus, L. leuciscus* and *T. thymallus*, occupy the reach, utilising and so relying on the same gravels during spawning. Seasonally, the anadromous *Alosa* spp. and *Petromyzon marinus* also enter the study reach to spawn on and amongst the shallow gravel riffles (Pinder 2016a).

Sampling for sediment size distributions and hydraulic conditions was completed between May and July of 2015 to 2017, and utilised a total of 13 sites (Fig. 3c). The *B. barbus* spawning sites that were sampled covered the following areas (upstream to downstream): Ashford Carbonel (2 sites), Tenbury Wells, Stanford Bridge (2 sites), Knightwick, Branford (2 sites), and Powick (5 sites) (Fig 3C). Each site was selected by identifying riffle areas typically used by *B. barbus* for spawning, with this confirmed from either observing spawning or from their progeny being sampled in downstream nursery habitats (Table 1). All 13 sites were included for surface sediment sampling but due to limited access and permission constraints, only 10 sites were sampled for subsurface sediments. Historical water quality data (Environment Agency) were used to investigate water physico-chemistry at 5 sites across the sample reach. Redd nest measurements for characterising the morphology and properties of *B. barbus* redds were made at 1 site at Stanford Bridge and 3 sites at Powick, with only

four sites being used due to limited sightings of visible and accessible *B. barbus* redds.

2.3.2 *Sediment size distributions*

Sediment size distribution was initially assessed by measuring the surface grain size distribution by Wolman sampling using a gravelometer (Wolman 1954; Bunte et al. 2001). To gain representative samples (Rice and Church 1996), 400 surface grains were measured (to 2mm) in the field at each of the 13 sites (Table 1) and to negate sampling bias, grain selection was achieved by random selection. To avoid reselecting grains, step-pacing between sampling points was always greater than the maximum grain size (180 mm) and measured grains were placed downstream of the sampling area. Surface sediment samples were taken between May and July 2015 under base flow conditions (Table 1).

Sub-surface sediments are also important to characterise, as these partly determine quality of spawning substrate by influencing egg mortality, and larval survival and emergence (Lapointe et al. 2000; Bryce et al. 2010; Franssen et al. 2014; Sear et al. 2016). The sediment size distributions of the sub-surface sediments were thus collected randomly from 10 sites (Table 1) using a McNeil sampler and Koski plunger (coring tube dimensions: 16 x 26 cm).

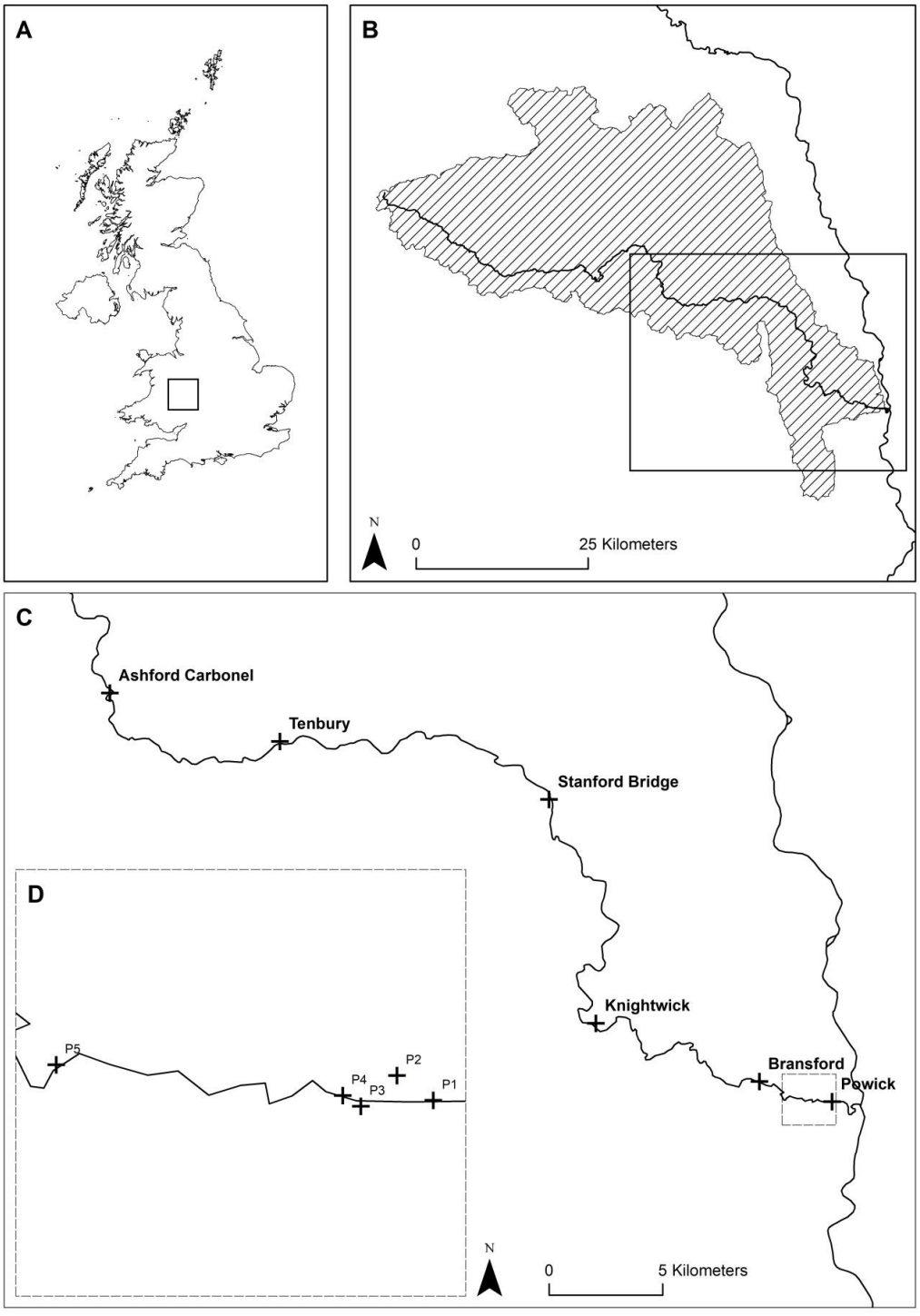

Figure 3. A) Location and B) catchment of the River Teme with the study reach within the box and C) study sites (marked as crosses) between Ashford Carbonel and Powick, at which sampling took place. D) Detailed locations of study sites at Powick with P4 just downstream of Powick weir.

Table 1. Site locations and dates for surface and subsurface sampling. '-' denotes the three sites where subsurface sampling was not completed. The 'Spawning confirmed' column states whether adults were observed mating ('Adults') and/ or whether *B. barbus* eggs were found and identified ('Eggs'), or where juvenile *B. barbus* were subsequently captured downstream ('Juveniles').

Site	Surface sampling dates	Subsurface sampling dates	Spawning confirmed
Ashford Carbonel U/S	01/07/15	21/07/15	Juveniles
Ashford Carbonel D/S	01/07/15	27/07/15	Juveniles
Tenbury	01/07/15	27/07/15	Juveniles
Stanford Bridge U/S	30/06/15	-	Juveniles
Stanford Bridge D/S	30/06/15	-	Juveniles
Knightwick	02/07/15	28/07/15	Juveniles
Bransford U/S	30/06/15	20/07/15	Adults
Bransford D/S	20/07/15	20/07/15	Juveniles
Powick 5 (U/S)	03/07/15	-	Juveniles
Powick 4	29/06/15	30/07/15	Eggs
Powick 3	27/05/15	30/07/15	Adults & Eggs
Powick 2	24/06/15	21/07/15	Eggs
Powick 1 (D/S)	24/06/15	31/07/15	Adults

Following sampling, the bulk sub-surface samples were processed whereby these subsurface sediments were oven-dried at 100°C and sieved into size fractions (0.032, 0.064, 0.125, 0.25, 0.5, 1, 2, 2.8, 4, 5.6, 8, 11.2, 16, 22.4, 31.5 and 45 mm) using a sieve shaker and sieve stacks. The mass of each size fraction was weighed (nearest 0.1 g). A 10 g subsample of fines (< 2 mm) was then collected from each of the bulk samples from each site and used to measure organic content using 'Loss On Ignition' (LOI, %) (Heiri et al. 2001). For this, samples were dried at 100 °C for a further 24 hours and then transferred into crucibles of known-weight to calculate pre-ignition mass (mpre, Equation 1a; to 0.0001 g) before being transferred to a Carbolite furnace at 550 °C for three hours. Once cooled, the crucibles were weighed once more to calculate the post-ignition mass (mpost, Equation 1b). Percentage of organic content (i.e. LOI) in the subsurface fines sediment was then calculated using the LOI formula (Equation 1c).

Mpre = weight of crucible and sediment – weight of crucible (Equation 1a)

Mpost = weight of crucible and sediment – weight of crucible (Equation 1b)

Organic matter (%) = ((Mpre-Mpost)/Mpre) * 100 (Equation 1c)

Surface and subsurface sediment percentiles ('D'; D5, D10, D25, D50, D75, D84, D90 and D95, mm) were extracted from cumulative distributions, with mean grain size, sorting, skewness and kurtosis (Trask 1932) values calculated using Equations 2 to 5. Percentages of fine sediment (> 2.001 mm), sand (0.064 mm to ≤ 2.000 mm diameter) and silt (diameter ≤ 0.063 mm) in the substrates

were determined. Summary statistics were used to calculate site and reach means.

$$Mean = \frac{D25 + D75}{2} \quad \text{(Equation 2)}$$

$$Sorting = \sqrt{\frac{D25}{D75}} \quad \text{(Equation 3)}$$

$$Skewness = \frac{D25 * D75}{D50^2} \quad \text{(Equation 4)}$$

$$Kurtosis = \frac{D75 - D25}{2 * (D90 - D10)} \quad \text{(Equation 5)}$$

2.3.3 *Characterising and analysing hydraulic conditions*

The following measurements were taken to determine the hydraulic conditions (as flow/ velocity and depth profiles) that characterised the *B. barbus* spawning riffles. A Valeport Open Channel Flow Meter (Model 801) measured mean water flow at 10 sites (Table 2). On each sampling occasion, flow measurements were taken at 12 locations across the spawning riffle, in four rows that were equally spaced along the channel, with a measurement on the and right of the channel, plus in the mid-channel, of each row. Each measurement lasted 60 s. At each location, measurements of flow depth and velocity (near-bed and 0.6 depth; cm s^{-1}) were made. Velocity profiles were collected from the same sites (Table 2) using the same flow meter at 3 locations per site, with point measurements from 3 cm above the bed and every 0.5 cm throughout the bottom 30 % of the flow, and at 5 cm increments above. Measurements of riffle width (wetted channel), depth and length were taken per site using a tape measure. Between one and four

measurements of width were taken, depending on the regularity of channel width, with one length measurement was taken per riffle and with three depth measurements always being taken. A Leica dumpy level was used for measuring surface-water and bed slope at each site, with three measurements (near bank, middle, far bank) taken at the upstream and downstream end of each site. All water and bed slope measurements were taken on 07/10/15 at the same 10 sites as the flow measurements.

Table. 2. Site locations and dates for velocity profiles and flow depths

Site	Date of hydraulic sampling
Ashford Carbonel U/S	15/10/15
Ashford Carbonel D/S	22/08/15
Tenbury	19/08/15
Stanford Bridge U/S	-
Stanford Bridge D/S	-
Knightwick	15/09/15
Bransford U/S	17/08/15
Bransford D/S	15/09/15
Powick 5 (U/S)	-
Powick 4	17/08/15
Powick 3	18/08/15
Powick 2	17/08/15
Powick 1 (D/S)	18/08/15

Hydraulic radius (R) was calculated using channel dimensions (cross-sectional area A and wetted perimeter P) during base flow (Equation 6). Water surface slope (S) was calculated from surface dumpy measurements (upstream to downstream), divided by riffle length. Triplicate measurements per site were used to calculate mean slope for each site. Mean bed shear stress for each site (τo; Nm^{-2}) was calculated to determine the amount of energy in the flowing water at the sites (Equation 7). Standard values for water density (ρw = 998.2 kg/m^3) and gravity (g = 9.81 m/s^2) were used in Equation 7.

$$R = \frac{A}{P} \qquad \text{(Equation 6)}$$

$$\tau o = \rho w g R S \qquad \text{(Equation 7)}$$

The critical shear stress (τc; Nm^{-2}) was calculated to estimate the amount of energy required to move sediment at each of the sites (Equation 8), using the previously calculated D_{50} (mm) from the sediment characteristics and standard sediment density (ρs = 2650 kg/m^3). The value 0.035 used to calculate critical shear stress is the $\tau c50$ value from Parker and Klingemans's (1982) calculation for a typical gravel stream with mixed grain sizes, which is also used in Montgomery et al. (1996). The ratio of bed shear stress (τo) to critical shear stress (τc) was calculated, and represents the bed mobility index (Lapointe et al. 2000). Reynolds number was used to quantify whether the flow type was turbulent or laminar (Equation 9), where velocity (v) was calculated from the mean 0.6 m depth velocities, with standard water viscosity υ used (10^{-6} m^2/s). Mean water discharge (m^3s^{-1}) was calculated from time series data downloaded from the Environment Agency flow gauging station at Knightsford Bridge between May and July 2015 (Environment Agency 2017).

$$\tau c = 0.035(\rho s - \rho w)gD50) \quad \text{(Equation 8)}$$

$$Re = \frac{(VR)}{\upsilon} \quad \text{(Equation 9)}$$

2.3.4 *Characterising and analysing the physico-chemical properties of spawning sites*

Water temperature (to 0.1 °C), dissolved oxygen concentration and saturation (mg l^{-1} and %), Ammonia (N, mg l^{-1}), Nitrite (NH_3, mg l^{-1}), pH and conductivity were collated for 5 sites from Environment Agency records (Environment Agency 2017; Table 3). Where available, 2015 records were used, with 2013/ 2014 records used when these were not available. Their mean values were calculated for sampling dates May and August, for each site and then these site values were used to calculate a reach-mean.

Table 3. Dates of sample collection from the Environment Agency Data that was used to calculate means for the 5 sites across the River Teme study reach, pH was not always recorded at the same time as the other water quality parameters, where it was sampled at the same time that column is marked with '-'

Site name	pH sampled	Water quality sampled
Ashford Carbonel	-	26/05/15
		11/08/15
Tenbury	-	22/05/15
		19/08/15
Stanford Bridge	03/10/13	08/05/14
	07/11/13	07/07/14
	20/11/13	
Knightsford	29/10/13	13/05/14
	11/11/13	15/07/14
	20/11/13	
Powick	-	29/05/15
		23/06/15
		03/08/15
		04/08/15

2.3.5 *Morphology and properties of B. barbus spawning redds*

The dimensions of six *B. barbus* redds were measured at 2 sites between May 2015 and June 2017 (Table 4). The redds measured in 2015 and 2016 were in areas that spawning *B. barbus* had been observed and so were assumed to be from *B. barbus*. This was justified as the only other redd building species spawning in the river at that time of year is *P. marinus*, which has a much more circular redd with excavated stones around a crater (Pinder et al. 2016b). Redds measured in the 2017 spawning period were assessed to also determine the species of fish eggs present, whereby the top 50 mm of sediment was disturbed

with a pencil in a circular motion, with a hand-held aquaria net placed behind the redd to collect the eggs. Eggs were identified to species level by their colouration and morphology (APEM 2009), where previous visual ID has been > 99 % accurate (Pinder et al. 2016a). Low redd sample size was due to constraints of access and sporadic spawning activity, and to limit disturbance of spawning fish. Measurements of the two main areas of the redd were taken; the pit, which is an excavated hole or depression in the bed, and the tailspill, an area where grains, mobilised from the pit during spawning, are deposited (Fig. 4).

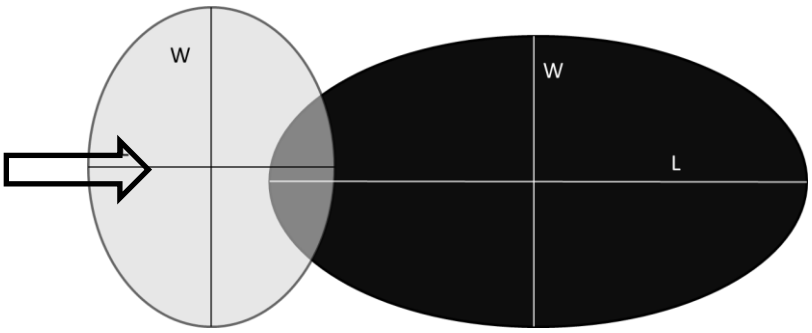

Figure 4. Schematic diagram of the lengths (L) and widths (W) measured from the pit (light grey) and the tailspill (black), the overlapping area between the two is shown in grey. Arrow indicates direction of flow.

In each case, the depth of water above the pit bottom, tailspill top and surrounding river bed were measured using a metre rule. The aerial extents of nests (m^2; Equation (10)) were calculated using the pit and tailspill length, and width data (Fig. 4). The total area of the redd (A), including pit (A_{PIT}) and tailspill ($A_{TAILSPILL}$) areas, was also calculated by combining the two areas for each of the measured redds. Note, however, this method overestimates the area, as each of these areas slightly overlap (Fig. 4). The volume (V) of the redd pit and

tailspill were then calculated (Equation 11), based on the assumption that it was a half-ellipsoid (McCart 1969) and where the pit depth and tailspill height were used as depth.

$$A = length * width * \pi \quad \text{(Equation 10)}$$

$$V = \left(\frac{4}{3} * \pi * depth * \frac{length}{2} * \frac{width}{2}\right) \div 2 \quad \text{(Equation 11)}$$

Surface sediment sampling was then carried out to identify size distribution characteristics of sediments within the pit and tailspill of nests, which would then allow for comparisons with the rest of the riffle. A random sample of 30 pebbles was collected from both the pit and tailspill of nests. These grains were then measured using a gravelometer, which were discarded once measured to avoid duplicate measures. Only 30 pebbles were measured due to the small nest areas (A_{PIT} = 1.2 ± 0.2 m^2, $A_{TAILSPILL}$ = 2.3 ± 0.3 m^2) and to minimise the impact of nest disturbance, which could have implications for egg development and incubation success. Additionally, three axes were measured (a, b and c) of five of the largest grains on the surface of each tailspill to investigate the maximum particle size moved during spawning, which allowed calculation of volume (m^3), assuming that grains were ellipsoid (Dorr 1994). Pairwise t-tests in R (R Core Team 2017) were carried out to compare surface characteristics (D5, D50, D95, mean, sorting, skewness, kurtosis and fines) between the pit and tail of the redds, and the redd pit and tail with the adjacent riffle area.

Table 4. Site, date and given code for the six redds that were measured and whether *B. barbus* eggs were recorded; '-' denotes where eggs were not recorded

Site	Date	Code	Eggs
Stanford Bridge US	30/06/15	SB_1	-
Stanford Bridge US	30/06/15	SB_2	-
Powick, Site 2	17/05/16	P_1	-
Powick, Site 2	16/05/17	P_2	✓
Powick, Site 3	16/05/17	P_3	✓
Powick, Site 4	16/05/17	P_4	✓

2.3.6 *Reproductive capacity of B. barbus spawning sites*

In salmonid fishes, body length (L) is positively correlated to redd area and the maximum size of particles that can be moved (Crisp and Carling 1989; Riebe et al. 2014). There have been no studies completed on the size and redd dimensions of cyprinid fish redds. Consequently, the relationships between redd morphology and *B. barbus* length used methods that are used to describe salmonid redd relationships, principally the redd area (as 'A_{REDD}', Equation 12).

$$A_{REDD} = 3.3[\frac{L}{600}]^{2.3} \quad \text{(Equation 12)}$$

To determine *B. barbus*-specific relationships in Equation 12, post-spawning fish would have needed to be sampled and measured, with this unable to be completed logistically and ethically. Therefore, salmonid-relationships were used as a proxy for *B. barbus*. Threshold particle size (D_T, mm) is a measure of

the grains a fish of a given length can move during redd construction, as was calculated using Equation 13 (Riebe et al. 2014).

$$D_T = 115\left(\frac{L}{600}\right)^{0.62} \quad \text{(Equation 13)}$$

The mean length (L) of spawning *B. barbus* females was calculated from eight *B. barbus* that could relatively positively be identified as females that were sampled in September 2015 downstream of Powick weir, with their mean length being 651 ± 37 mm (95% CI) (Chapter 7). Then, using the D_T calculated for the mean length of *B. barbus* at this site (Equation 13), the fraction of moveable particles, F_m was calculated using Equation 14 (Riebe et al. 2014). To calculate the difference between threshold size and mean grain size (z) in Equation 14, Equation 15 (Bowling et al. 2009) was used, based on the previously calculated threshold size (D_T) and surface sediment percentiles (D_{50} and D_{85}).

Next, the potential number of redds for a given area (N_{REDDS}; redds/ m^2; 16) was calculated, using the calculated area of the redd (A_{REDD}) and the area of the riffle covered in moveable particles (F_M). The value calculated as N_{REDDS} represented the spawning capacity of the site. Finally, the reproductive capacity of a site, N_{EGGS} (eggs/m^2, Equation 17), was calculated using fecundity estimates (F). As *B. barbus* fecundity-length relationships were not available then substitute data were used from *B. sclateri* in Equation 18 (F(B); Herrera et al. 1988)). Equation 18 was then tested on two *B. barbus* studies to determine the accuracy of reproduction estimates, where in both studies, the fish lengths and total fecundity estimates were provided (Appendix 1). This suggested that the reproductive capacity estimates in Equation 18 could be an overestimation,

but only by up to 7,082 eggs per fish (Appendix 1). Consequently, the egg estimates for *B. barbus* from Equation 18 are approximate, but due to the inherent variation in fecundity between studies, the potential for over-estimation was not accounted for in the final estimates.

$$F_M = [1 + e^{-1.702z}]^{-1} \quad \text{(Equation 14)}$$

$$z = \left[\frac{\log(\frac{DT}{D50})}{\log(\frac{D84}{D50})}\right] \quad \text{(Equation 15)}$$

$$N_{REDDS} = \frac{F_M}{A_{REDD}} \quad \text{(Equation 16)}$$

$$N_{EGGS} = \frac{F_M E}{A_{REDD}} \quad \text{(Equation 17)}$$

$$F(B) = 6.07 * 10^{-4} * \mathrm{L}^{3.0667} \quad \text{(Equation 18)}$$

The reproductive capacity of the sites was then also calculated using the *B. barbus* specific redd measurements from this study, with the mean redd area (A; Equation 10) used instead of A_{REDD} (Equation 12).

2.4 Results

2.4.1 *Sediment size distributions*

The reach-averaged surface sediments were coarse (median grain size D_{50} = of 27.9 ± 5.1 mm), moderately well sorted (sorting = 0.64 ± 0.03), positively skewed (skewness = 0.92 ± 0.04) and leptokurtic (kurtosis = 0.24 ± 0.01) (Table 5). Reach-averaged subsurface sediments were also relatively coarse (D_{50} = 12.8 ± 2.6 mm and D_{95} = 50.1± 6.4 mm), well sorted (sorting = 0.38 ± 0.03), positively skewed (skewness = 0.62 ± 0.05) and leptokurtic (kurtosis = 0.29 ± 0.01) (Table 5).

There was more fine sediment within the subsurface sediment (19 ± 4 %) that at the surface (2 ± 1%) (Table 5). Surface fine sediment ranged from 0 to 6.5 % (Fig. 5a), whilst subsurface fine sediment ranged from 10 to 43 % (Fig. 5b). The subsurface fine sediment was mostly made up of sand (Table 5, Fig. 5b). Subsurface organic content ranged from 1.5 % (LOI) at Bransford 1 to 2.5 % (LOI) at Ashford Carbonel 1 (Fig. 6).

Table 5. Reach-averaged values for surface and subsurface sediment parameters showing mean ± 95% confidence interval, River Teme 2015

Parameter	**Surface**	**Subsurface**
	Mean ± CI	**Mean ± CI**
D_5 (mm)	6.7 ± 1.7	0.5 ± 0.1
D_{50} (mm)	27.9 ± 5.1	12.8 ± 2.6
D_{84} (mm)	50.9 ± 7.5	33.7 ± 4.9
D_{95} (mm)	76.6 ± 10.9	50.1 ± 6.4
Mean (mm)	29.2 ± 5.0	15.2 ± 2.6
Sorting	0.64 ± 0.03	0.38 ± 0.03
Skewness (mm)	0.92 ± 0.04	0.62 ± 0.05
Kurtosis	0.24 ± 0.01	0.29 ± 0.01
Fine sediment (%)	1.7 ± 1.0	18.8 ± 4.2
Sand (%)	NA	18.4 ± 4.2
Silt (%)	NA	0.5 ± 0.1
Organic content (%)	NA	1.9 ± 0.2

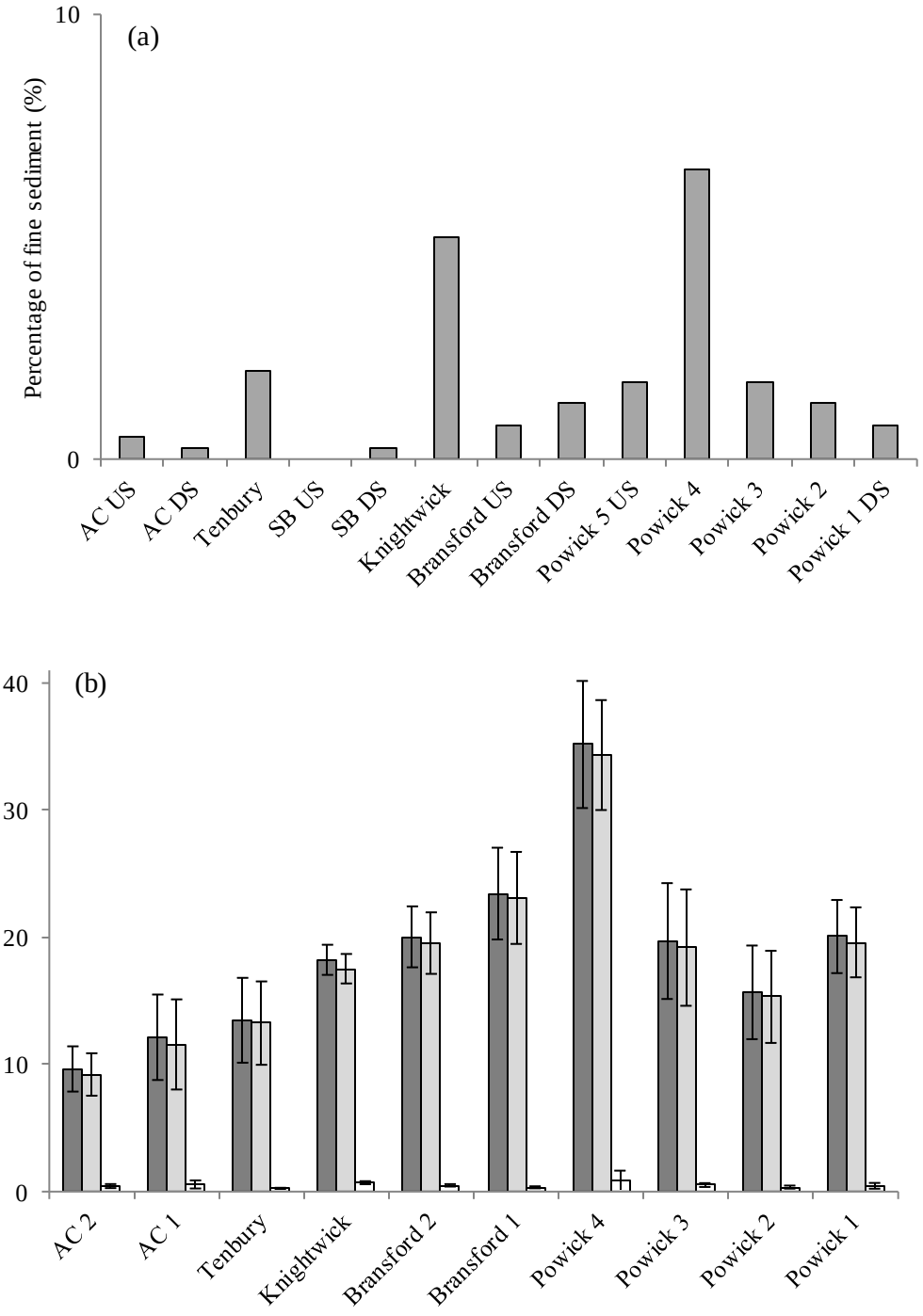

Figure 5. Upstream (left) to downstream (right) sites in the River Teme with the percentage (%) of (a) surface fine sediment (< 2mm) and (b) subsurface fine sediment (dark grey), sand (light grey; 0.063 mm < diameter ≤ 2 mm) and silt (white; ≤ 0.063 mm) with 95% CI error bars. Ashford Carbonel (AC) and Stanford Bridge (SB) have abbreviated site names

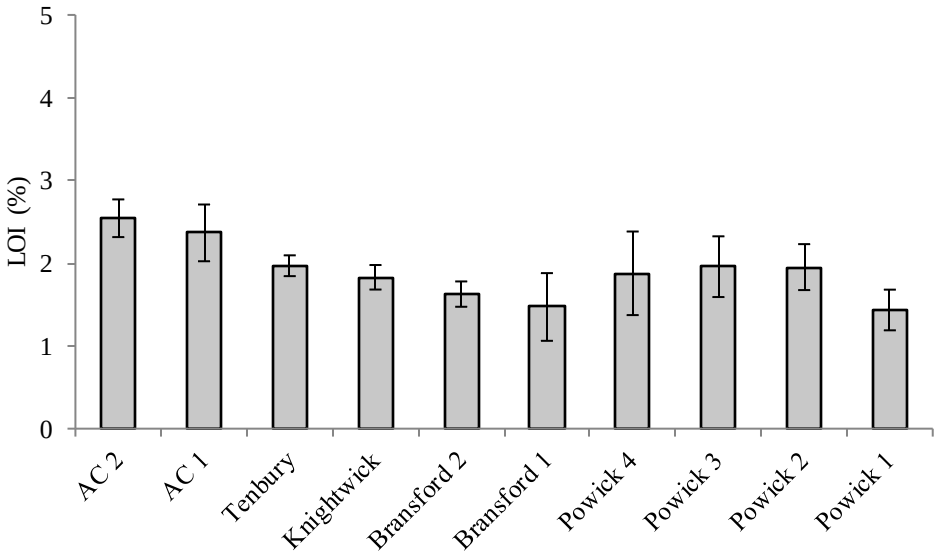

Figure 6. Upstream (left) to downstream (right) sites in the River Teme with the percentage of organic matter (LOI) within fine sediments (%)

2.4.2 *Hydraulic conditions and bed mobility*

A total of 5,930 m^2 of potential *B. barbus* spawning riffles were measured. The area of riffle varied between sites (range = 85 to 1,284 m^2 for Ashford Carbonel US and Tenbury, respectively), with a mean riffle area of 500 ± 249 m^2 (Table 6). The mean surface water slope and mean bed slope were similar across the riffles (Table 6), although there was variation between sites in surface water and mean bed slopes. Reach mean shear stress was 2.17 ± 1.23 Nm^{-2} with large variation between sites (Table 6), which was low compared with the critical shear stress of 15.27 ± 3.41 Nm^{-2} (Table 6). Therefore, mobility ratio was low at 0.12 ± 0.05 (Table 6), indicating relatively stable bed sediments. In general, spawning riffles were relatively shallow (mean = 35.7 ± 3.6 cm), fast (0.38 ± 0.02 and 0.59 ± 0.02 m s^{-1} for near bed and 0.6 depth velocities, respectively; Table 6) and turbulent (Reynolds number = 113,284 ± 17,611).

Table. 6. Reach-mean values and 95% confidence interval for riffle dimensions and hydraulic characteristics in the *Barbus barbus* spawning riffles measured at 10 sites in the River Teme (Ashford Carbonel to Powick), and the mean discharge from Knightsford gauging station (Environment Agency, 2017)

Parameter	**Mean (± 95 % CI)**
Riffle area (m^3)	500.3 ± 249.0
Riffle length (m)	29.3 ± 12.0
Riffle width (m)	15.0 ± 4.5
Site length (m)	30.8 ± 12.0
Site width (m)	18.0 ± 3.7
Depth (cm)	35.7 ± 3.6
Wetted width (m)	89.5 ± 5.9
Bed slope (%)	0.33 ± 0.38
Water surface slope (%)	0.34 ± 0.17
Flow depth (m)	0.35 ± 0.01
Near-bed velocity ($m\ s^{-1}$)	0.38 ± 0.02
0.6 depth velocity ($m\ s^{-1}$)	0.59 ± 0.02
Reach mean shear stress (to Nm^{-2})	2.17 ± 1.23
Critical shear stress (tc $N\ m^{-2}$)	15.27 ± 3.41
Mobility ratio	0.12 ± 0.05
Reynolds	113,284 ± 17,611
Mean discharge ($m^3\ s^{-1}$)	5.43 ± 0.06

2.4.3 *Physico-chemical properties*

Reach-averaged pH was 8.2 ± 0.1 and mean temperature between sites was 15.11 ± 0.76 °C (Table 7). Conductivity increased from upstream to downstream, with Ashford Carbonel having lower conductivity compared to Powick. Concentrations of ammonia in both N and NH_3 form (Table 7) were low. Dissolved oxygen was relatively high at 9.51 ± 0.51 mg l^{-1}.

Table 7. A list of reach-averaged mean values and 95% confidence interval for water quality parameters across the River Teme including Ashford Carbonel, Tenbury, Stanford Bridge, Knightsford and Powick. Source: Environment Agency Data 2013 to 2015 (Environment Agency 2017).

Parameters	**Mean (± 95 % CI)**
pH	8.15 ± 0.09
Temperature (°C)	15.11 ± 0.76
Conductivity (uS/cm)	433.6 ± 34.5
Ammonia (N), mg/l	0.04 ± 0.02
NH_3 un-ion, µg/l	1.05 ± 0.35
Dissolved oxygen saturation (%)	94.71 ± 4.79
Dissolved oxygen (mg/l)	9.51 ± 0.51

2.4.4 *Morphology and properties of redds*

Six redds were measured over a three-year period, with *B. barbus* eggs all recorded in the three measured redds in 2017. However, they also contained eggs

of shad (*Alosa* spp.), with one redd also containing eggs of *P. phoxinus* and *S. cephalus*.

In each redd, the tailspill tended to be longer and wider than the pit, a mean difference of 29 ± 22 cm and 19 ± 17 cm respectively (Table 8). Tailspill height ranged between 0 and 29 cm, and was typically twice the depth of the pit. Pit depth ranged between 4 and 11 cm. Total redd area ranged from 1.37 to 9.11 m^2 at the Powick sites, and 2.23 to 2.58 m^2 at Stanford Bridge. The mean redd area (A) was 3.47 ± 0.42 m^2. The area of the tailspill was larger than that of the pit for 5 of the 6 redds. Pit volume ranged from 1,900 to 34,819 cm^3 (mean ± 95% CI; 14,390 ± 5,338 cm^3) and the tailspill volume ranged from 0 to 234,834 cm^3 (mean ± 95% CI; 74,754 ± 43,284 cm^2). Pit volume was larger than the tailspill volume for 2 redds, and for four redds the converse relationship was found. The volume of the largest pebble found at the tailspill surface (n = 3) was 52 cm^3, with the b axis of that grain being 2.5 cm. The largest grain in the other two redds were 14 and 43 cm^3.

The grain size distributions between the pit and tailspill materials did not vary significantly (t-tests, $P > 0.05$ in all cases; Table 9). Sediments within the pit and tailspill were coarse (mean = 22.2 ± 9.5 and 28.2 ± 3.7 respectively) and moderately well sorted (sorting = 0.58 ± 0.07 and 0.62 ± 0.05 respectively) (Table 8). Whilst fine sediments appeared more prevalent at the surface of the pit than the surface of the tailspill (Table 8), the difference was not significant ($P > 0.05$; Table 9). Generally, the level of fine sediment found on the redd surfaces were low, but the pit at Powick 4 was 27 % fines content (Fig. 7d),

which was higher than the surrounding bed (6.4%; Fig. 5a). There were no significant differences between the surface characteristics of the pit and tailspill areas of the nests compared with their surrounding riffle for D5, D50, D95, mean, sort, skewness, kurtotsis and percentage of fine sediment (Table 9, Fig. 8).

2.4.5 *Reproductive capacity of B. barbus spawning sites*

The mean length of a female *B. barbus* length in the Powick area of the river was estimated to be able to move a surface particle (D_T) of 121 mm. This meant that the proportion of coverage of moveable particles (F_M) within riffles from the study reach was 0.98 ± 0.01, suggesting there are very few particles that mature female fish cannot move on a spawning riffle. When the mean calculated redd area (A) of 3.47 m^2 was used, then the spawning potential of the study reach riffles was recalculated. This changed the N_{REDDS} value to 0.29 ± 0.00 redds m^{-2}. Calculated fecundity (F) for mean female lengths in this study was 257,683 eggs. When this value for fecundity (F) was used with the calculated *B. barbus* redd area, the reproductive potential of the sites was estimated as 73,113 eggs m^{-2}.

Table 8. Reach-averaged values and 95% confidence interval for redd pit and tailspill dimensions and surface sediment parameters (mean ± 95% CI) at Powick (n = 4) and Stanford Bridge (n = 2)

Parameter	Redd pit	Redd tailspill
Length (cm)	60 ± 20	89 ± 28
Width (cm)	63 ± 20	82 ± 24
Depth/ height (cm)	7 ± 1	14 ± 5
Area (m^2)	1.19 ± 0.15	2.28 ± 0.26
Volume (cm^3)	14,390 ± 5,338	74,754 ± 43,284
D_5 (mm)	4.4 ± 3.3	4.4 ± 1.5
D_{50} (mm)	21.6 ± 14.3	25.1 ± 5.4
D_{84} (mm)	36.4 ± 18.6	51.1 ± 11.2
D_{95} (mm)	54.5 ± 29.7	71.5 ± 16.7
Mean (mm)	22.2 ± 13.1	28.2 ± 5.1
Sorting	0.58 ± 0.01	0.62 ± 0.01
Skewness	0.90 ± 0.01	1.01 ± 0.01
Kurtosis	0.25 ± 0.01	0.25 ± 0.01
Fine sediment (%)	3.17 ± 1.38	0.67 ± 0.56

Table 9. Paired t-test comparison results between nest (Pit and Tailspill) surface characteristics of *Barbus barbus* redds and the adjacent riffle surface characteristics. Degrees of freedom for all tests were 5.

	Pit v Tailspill		Pit v Riffle		Tailspill v Riffle	
	t	*P*	t	*P*	t	*P*
D5	-0.01	0.99	-0.48	0.65	-0.88	0.42
D50	-0.55	0.61	-0.37	0.73	0.08	0.94
D95	-1.63	0.16	-1.90	0.12	-1.27	0.26
Mean	-0.99	0.37	-0.67	0.53	0.28	0.79
Sorting	-0.59	0.58	-0.34	0.75	0.35	0.74
Skewness	-1.28	0.26	-0.92	0.40	0.58	0.59
Kurtosis	-0.21	0.84	0.12	0.91	0.47	0.66
Fines	1.78	0.14	2.48	0.06	0.18	0.87

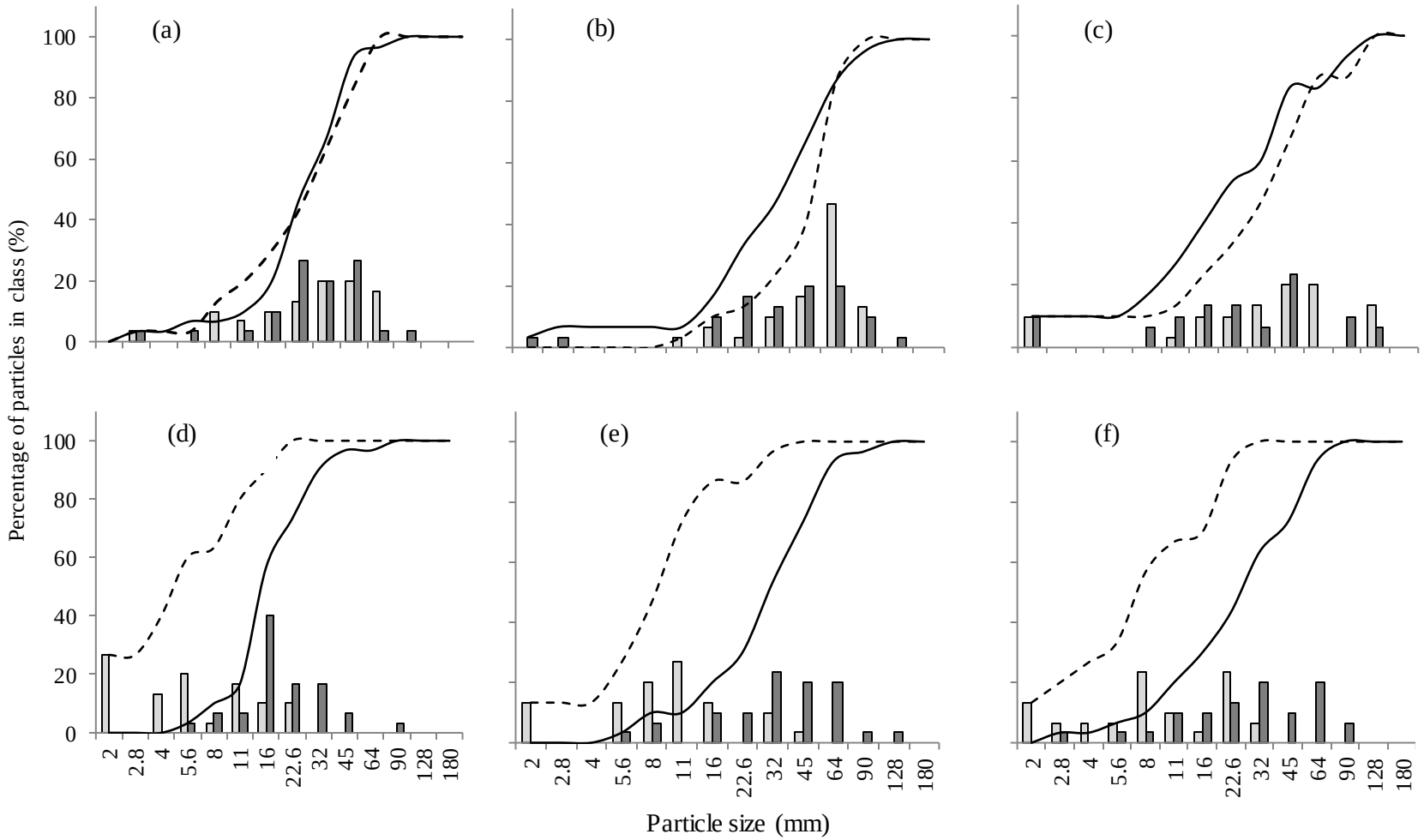

Figure 7. Grain size distributions of *B. barbus* spawning pit (light grey, dashed lines) and tailspill (dark grey, solid lines) materials from 6 redds across two sites (Stanford Bridge and Powick). Bars represent percentage of sediment in each size class and lines represent cumulative percentage. a) Powick 2 2016, b) Powick 2 2017, c) Powick 3 2017, d) Powick 4 2017, e & f) Stanford Bridge 2 2015

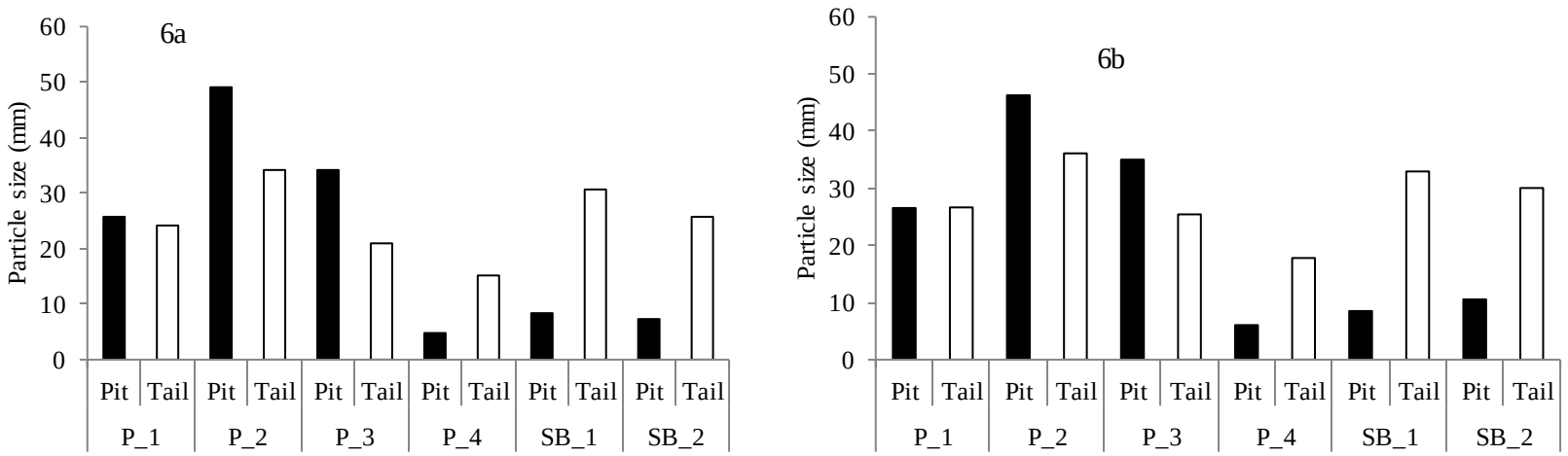

Figure 8. Surface characteristics; (a) mean particle size (mm) and (b) D_{50} of particles, at six redds from the pit (black) and tailspill (clear).

2.5 Discussion

The results of this Chapter identified the sediment and water quality conditions of *B. barbus* spawning sites, with the results consistent with those of other studies describing *B. barbus* spawning habitat (Bašić 2016). Whilst there has been limited work on quantifying the sediment characteristics of *B. barbus* spawning habitat, it has been studied using similar methods in the River Great Ouse, part of the *B. barbus* native range in England (Bašić 2016). There were some differences between the reach means between the two rivers, with both the surface and subsurface sediment sizes being larger at the River Teme across the D_5 to D_{95} and mean sediment sizes. The River Great Ouse also had higher fine sediment content at both the surface and subsurface, by a mean of +0.3 % at the surface and +3.0 % for the subsurface. Recruitment of *B. barbus* is negligible in the River Great Ouse (Antognazza et al. 2016), despite observations of spawning adults (Twine 2013), whereas there are relatively high levels of 0+ fish production in the River Teme (Chapter 3). It is thus reasonable to assume that spawning substrates in the River Teme are of better quality than the Great Ouse and this could at least partially relate to this difference in sub-surface fine content.

The surveys of riffles were not exhaustive and thus there were riffles within the study reach that were not measured. Nevertheless, this study measured a total area of 5,930 m^2 of potential *B. barbus* spawning riffles. There was an overall pattern of decreasing median (D50) surface sediment size from upstream to downstream. There was also a pattern of increasing fine subsurface sediment

from upstream to downstream, although this appeared to decrease again from Powick 3 to 1. There was little site variation in levels of organic matter, but overall it decreased from upstream to downstream. Fine sediment content was much greater within the sediment than at the surface, which was expected, as fine sediments often either ingress into the bed causing subsurface fining, or are entrained from the surface layer, resulting in surface substrate coarsening (Reid et al. 1997). The subsurface fine sediment percentage was very similar to the mean value across other British rivers, where it was estimated at approximately 19 % across four rivers by Greig et al. (2005). In the Teme, the fine sediment was primarily sand, with very little silt present. Organic content was relatively low at 1.9 % when compared to the four rivers of Greig et al. (2005), where the lowest recorded level was 3 %.

The spawning riffles in this study were slightly deeper with higher flows than those reported in Baras (1992), where depths of 10 to 26 cm deep were reported. The flows (10 cm from bed) in Baras (1992) were 28 to 43 cm s^{-1} and 54 cm s^{-1} in Bašić (2016), whereas in this study they were 59 cm s^{-1} at depths of 60 cm. Flow levels are an important indicator of fish spawning intensity, as well as temperature, with several lithophilic fishes having a positive relationship between flow (discharge) and spawning intensity (King et al. 2016). Water quality conditions in the Teme also appeared favourable compared with European standards outlined in the Water Framework Directive (Directive 2014), and so were not thought to be impeding the reproduction of *B. barbus* in the river.

Fine sediment and organic content are both important factors in egg and larval survival and development, largely due to their influence on the permeability of the gravels for water flow that removes waste products and maintains oxygen levels (Greig et al. 2005). Organic content can reduce the oxygen supply when it decomposes within the hyporheic layer. Whilst there is limited work on the impacts on cyprinid fishes by fine sediment and organic matter in spawning gravels, in *S. salar*, there was very high survival of salmonid eggs in an artificial redd placed into a chalk stream in Southern England, when the fine content was 12 % fine sediment and 3 % organic matter at the surface and 19 % and 2 % respectively at the subsurface (Greig et al. 2005). Survival from egg to emergence for *B. barbus* is not affected by sand content up to 40 % although it does affect the timing of larval emergence when levels are above 20 % (Bašić 2016). These values suggest that fines content in the Teme should not impact either egg survival or the timing of emergence, given fine contents were generally below 20 %.

The process of redd building can remove fine sediment from the bed before the eggs are deposited (Hassan et al. 2008), so whilst fine content can be measured in spawning areas, these may not be the conditions that the eggs and larvae will experience within the redd. Thus, it is important that these are measured both in- and outside of redds, Where fine contents in both redds and riffles are flagged as a concern then management techniques to reduce fine sediment can involve catchment scale management, such as reducing diffuse inputs (Pulg et al. 2013), but where there is just a localised issue then the method of gravel jetting can reduce fines from a localised area but only over short periods (Bašić et al. 2017).

In the study area, the areas downstream of Powick Weir had the highest levels of fine content in redds. This weir is due for removal in summer 2018 (Sharpe 2017) and so the removal of this impoundment could alter the levels of fine sediment in downstream areas in future, which could benefit *B. barbus* spawning success.

This study confirmed *B. barbus* redds can also contain eggs from three other species, with eggs of *S. cephalus*, *P. phoxinus,* and *Alosa* spp. all identified as present. Previous studies in the Teme have shown these species all using the same spawning riffles (Pinder et al. 2016a), but not necessarily sharing the same spawning microhabitats. Therefore, whilst it is important to collect species-specific information on spawning substrates, these results suggest that improving spawning gravels for one species (e.g. via reductions in fine content to improve *B. barbus* reproductive success) should have an umbrella effect for other species. None of the sediment characteristics within *B. barbus* redds were significantly different to the rest of the surrounding riffle, which suggests that their spawning may not affect the sediment structure. However, the pits that were excavated had moved a volume of 14,390 cm^3 of sediment and the tailspill had a volume of 74,754 cm^3, which suggests that the topography was significantly different to the "flat" riffle surface, with increasingly varied topography potentially affecting bed mobility and bed transport amounts (Mongomery et al. 1996). As there was a large variation in the redd volume values that could have been related to the body size of the fish that excavated them (Crisp and Carling 1989; Riebe et al. 2014), then further work could focus on the relationship between body size of

spawning *B. barbus* and the extent of the changes in the sediments that results from their redd building activities.

In summary, this Chapter has extended the knowledge of the spawning habitat used by *B. barbus* and, specifically, has helped increase understandings of their utilisation of spawning habitats in their non-indigenous range. Understanding the dimensions and structure of redds should also assist fishery and river managers to identify suitable spawning areas for *B. barbus*, and where absent, how they can be created through habitat works.

3. Do protracted spawning strategies pre-adapt introduced fishes to be successful invaders? Evidence from non-indigenous European barbel *Barbus barbus*

3.1 Abstract

In temperate, lowland rivers, single spawning strategies usually result in reproduction early in the year, providing 0+ fish with an extended growth season in a trade-off with increased mortality risks from early summer floods. Protracted spawning strategies reduce this risk by producing 0+ fish over extended periods, but in a trade-off with limited growth seasons for some individuals. Fish spawning strategies in cyprinid fish community of the River Teme were investigated here for the presence of single versus protracted spawning strategies, using 0+ fish samples collected over three consecutive reproductive seasons. Temporal analyses of 0+ fish lengths during the first growth season revealed that in non-indigenous *B. barbus* and indigenous *S. cephalus* and *P. phoxinus*, protracted spawning events were evident each year, with 0+ fish of < 20 mm regularly appearing in samples collected between June and August. Fish of < 20 mm appearing in samples in August were still relatively small at the end of the growth season. Only indigenous *L. leuciscus* utilised a single spawning strategy. As *B. barbus* also use protracted spawning strategies in their native range, these results suggest rather than being mediated by reproductive plasticity in their invasive range, this spawning behaviour aligns to the pre-adaption hypothesis of invasion biology, with this potentially conferring considerable invasion advantages via enhanced 0+ fish survival and recruitment.

3.2 Introduction

A range of factors influence the probability of introduced species establishing self-sustaining populations, with relevant hypotheses including propagule pressure, biotic resistance and enemy release (Lockwood et al. 2005; Britton 2012; Sheath et al. 2015). Hypotheses also include the 'pre-adaptation hypothesis' that suggests where introduced species share similar ecological traits and behaviours with native species then they should benefit through, for example, a similar ability to acquire resources (Duncan & Williams 2002; Ricciardi & Mottiar 2006; Buoro et al. 2016). Following their introduction, the utilisation by an non-indigenous species of spawning strategies that are similar to their native range, and that are also used by native species in the new range, should thus also increase their establishment probability, as there is little requirement for their reproductive traits to adapt to the new conditions (Schlaepfer et al. 2010; van Kleunen et al. 2011).

In temperate lowland rivers, the new environmental conditions faced by introduced fishes include episodic flood events that can be deleterious to cohorts of larval and juvenile fish in their first year of life ('0+ fish'), especially when these events occur in early summer when individuals are still in early developmental stages (Nunn et al. 2002, 2007a,b). The probability of over-winter survival and recruitment of 0+ fishes in these rivers also tends to be positively correlated to their body lengths at the end of their first growth season (Kirjasniemi & Valtonen 1997; Mills & Mann 1985; Nunn et al. 2003). Whilst spawning strategies utilised by temperate riverine cyprinid fishes vary between

species, each strategy is assumed to enhance the numbers of 0+ fish that survive their first year of life and subsequently recruit (Beardsley & Britton 2012a). For species such as dace *Leuciscus leuciscus*, spawning tends to be a single event in early spring when no other cyprinids are reproducing, maximising their access to spawning substrates whilst providing their progeny with a prolonged growth season that enables individuals to attain relatively large body lengths (e.g. > 50 mm) (Mann 1974; Nunn et al. 2002; Beardsley & Britton 2012b). This strategy, however, also means that a flood event in early summer could result in high rates of mortality and/ or downstream displacement (Nunn et al. 2003, 2007a). An alternative strategy is the use of fractional or batch spawning events (hereafter referred to as 'protracted spawning'). Utilised by species such as *S. cephalus*, these events involve a trade-off between prolonged spawning efforts in adults (potentially in excess of two months) versus the reduced likelihood of the entire cohort being exposed to the same level of mortality risk from stochastic events (Nunn et al. 2002, 2007a). There is also then a potential trade-off in the 0+ fish between elevated survival rates during the growth season versus achieving lower body sizes at the end of that season, a result of a relatively short first growth season (Bolland et al. 2007). This then potentially limits the over-winter survival of the individuals produced later in the season (Nunn et al. 2007a,b).

The fish communities of rivers in eastern England tend to be relatively diverse, a legacy of their previous connectivity with the Rhine-Danube systems after the last glacial period (Wheeler and Jordan 1990). In comparison, western flowing British rivers tend to have lower fish diversity and has resulted in non-indigenous species, such as *B. barbus*, being introduced to diversify angling (Wheeler and

Jordan 1990; Antognazza et al. 2016). In river basins such as the Severn and Wye, these non-indigenous *B. barbus* have been very successful, establishing abundant populations (Amat Trigo et al. 2017), despite the propensity of these rivers to flood regularly (Marriot 1992). Moreover, *B. barbus* is now invasive in many other European rivers (Meraner et al. 2013; Zaccara et al. 2014). Despite this, there remains minimal knowledge on how their spawning strategies in their non-indigenous range influence their 0+ fish cohorts and how these potentially influence their invasion success.

Consequently, the aim of this Chapter was to test the pre-adaptation hypothesis of invasion biology via the early life dynamics of 0+ fish cohorts in a lowland river, using non-indigenous *B. barbus* as the model invasive species. The River Teme, western England (a tributary of the River Severn), was the study river, with samples of 0+ cyprinid fishes collected from three locations and over three successive growth seasons. The pre-adaptation hypothesis was tested by assessing whether the non-indigenous *B. barbus* population utilised a single or protracted spawning strategy in the river and how this compared to three indigenous fish populations. Comparisons were also made with literature on native *B. barbus* spawning strategies. In combination, these results enabled the testing of the pre-adaptation hypothesis. The progression of the 0+ fishes through their first summer of life in the study river was then analysed to identify potential implications of single versus protracted spawning events. Realised body lengths at the end of their first growth season were also assessed and, correspondingly, the potential of these 0+ fish to over-winter successfully.

3.3 Materials and Methods

3.3.1 Sample sites

Three sampling sites were used in the study that covered most of the non-indigenous range of *B. barbus* in the River Teme (Fig. 9). Due to negligible off-channel habitat throughout the river, each sampling site consisted of areas of reduced flow rates within the river channel. Site 1 was the furthest upstream, located at Tenbury Wells (52°19'N, -2°24'W) (Fig. 9). The sampled areas was located immediately downstream of a road bridge at the downstream end of a large gravel island, near to the right-hand bank. This site is in an urbanised area with little agriculture directly nearby, with a public footpath running along the left-hand bank. Riparian vegetation included overhanging trees (*Salix* spp.). Within the river, there was minimal instream vegetation, with the river generally running over gravel at depths of < 1m). The sampling area comprised of an area of minimal/ negligible flow, close to the right-hand bank. Site 2 was located at Knightwick (52°12'N, -2°23'W) (Fig. 9). Sampling was from the right-hand bank, with samples taken either at the downstream end of an exposed gravel beach, or upstream of the gravel beach, in shallow water of a maximum depth of 1 m. Again, instream vegetation was minimal, with the sampling area comprising of relatively slack water over a gravel substrate that was contiguous with a gravel riffle. Site 3 is the most downstream site at Powick (52°10'N, -2°14'W) (Fig. 9), with the sampling area located at the downstream end of a gravel riffle used by spawning *B. barbus* (Pinder et al. 2016a). Sampling was conducted from the left-hand bank in an area of relatively shallow water. The right-hand bank was steep,

incised and suffering erosion with sheep grazing pasture extending up to the river.

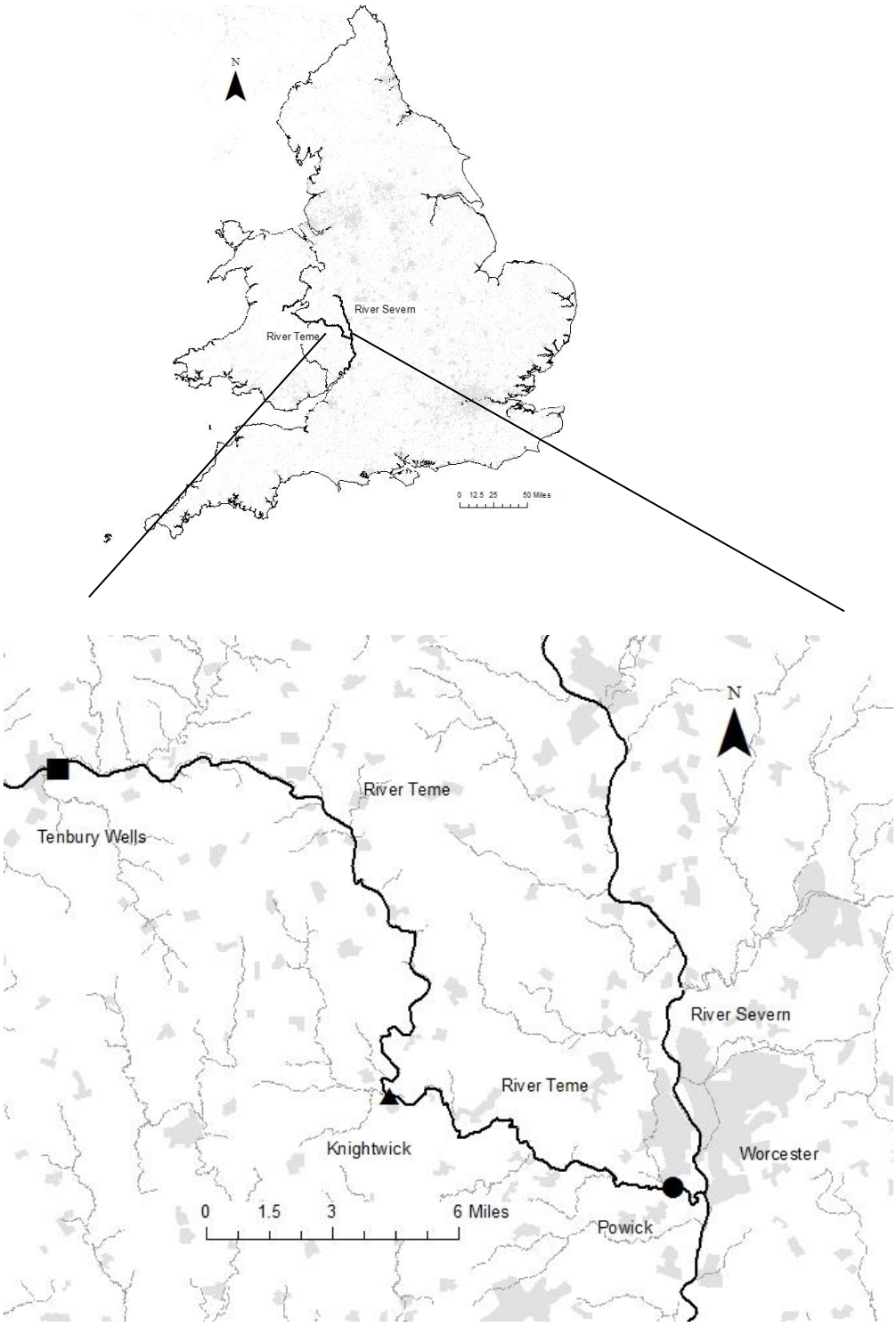

Figure 9. Map of the three survey sites – Site 1Tenbury (■), Site 2 Knightwick (▲) and Site 3 Powick (●) on the River Teme (full black line) and River Severn (full black line), all other rivers as grey dashed lines. Urban areas are shaded grey.

3.3.2 Sampling methodology

There are two primary methods to sample 0+ fishes: micro-mesh seine netting (Nunn et al. 2002, 2003, 2007a,b) and point abundance sampling by electric fishing (PASE; Copp 2010). Comparison of the two methods by Cowx et al. (2001) revealed that although PASE provides better sampling resolution for larval fishes (generally < 15 mm), thereafter micro-mesh seine netting captures more representative samples of the larger, juvenile 0+ fishes. Moreover, PASE is only appropriate for use where access to long stretches of river enables the use of randomised point sampling, whereas the use of a micromesh seine net is more suitable when only limited 0+ fish habitat is available, as per the River Teme. Consequently, the primary method used was micro-mesh seine netting in appropriate larval and juvenile fish habitats at each site. These nursery habitats were identified by areas of habitat off the main flow of the river, where there was sufficient depth (up to 1 m) and cover (including over-hanging trees and large stones) to provide refuge for the 0+ fishes (Fig. 10).

In 2015, sampling commenced in early July and concluded in October (Table 10). In 2016, sampling commenced earlier to determine the arrival of larvae in nursery habitats, hence sampling commenced in late May and concluded in October (Table 10). In 2017, sampling commenced in May and continued through to September. The rationale for concluding sampling in September/ October was a series of low catches of cyprinids in the final samples as the 0+ fished utilised alternative, non-accessible habitats, in combination with rising water levels that severely limited safe access to the sampling sites. Indeed, by October the few *B. barbus* and *S. cephalus* that were caught were all juveniles

in the final stages of development (scales all over body and all fins fully formed) and thus were likely to be starting to utilise deeper water that was not accessible for sampling by micro-mesh seine netting (Copp 1992, Bischoff & Freyhof 1999).

Sampling thus utilised a micro-mesh seine net of 25 m length, 3m depth and 2.5 mm mesh size. At each site, between 1 and 3 hauls of the net were completed, with this number of hauls dependent upon the number of fish captured. For example, where a relatively large sample was captured in the first haul (e.g. > 200), no further sampling was required. All of the fish were removed from the net and, where catches were sufficiently high at the species level (e.g. > 100 per species) then sub-samples were taken for subsequent analysis. Sub-samples were taken randomly from the main sample with a small hand net, euthanized (anaesthetic overdose, MS-222) and then preserved in 70 % IMS. They were then kept in chilled conditions (approximately 5 ^{o}C) until their processing in the laboratory.

Table 10. Micromesh seine net sampling dates at sites on the River Teme. * Micromesh drift net at Site 3 only.

Date	Sites sampled
07/07/15	S1
08/07/15	S2, S3
23/07/15	All sites
04/08/15	All sites
20/08/15	All sites
08/09/15	All sites
22/09/15	S3
05/10/15	All sites
24/05/16	All sites
06/06/16	All sites
29/06/16	All sites
08/07/16	S1, S2
13/07/16	S3
25/07/16	S3
28/07/16	S1, S2
09/08/16	All sites
25/08/16	All sites
30/08/16	S3
12/09/16	All sites
01/10/16	S2
15/05/17	All sites
24/05/17	S3
05/06/17	All sites
19/06/17	All sites
02/07/17	All sites*
26/07/17	All sites
08/08/17	All sites
22/08/17	All sites
06/09/17	All sites

Figure 10. Sample sites on the River Teme: (top) Site 1 (looking downstream), (middle) Site 2 (looking upstream), and (bottom) Site 3 (Left - looking upstream, Right – looking downstream), photos taken in July 2015, except bottom right taken 2017.

3.3.3 Data collection and analysis

In the laboratory, for each sampling date and site, the fish were identified to species level (Pinder 2001) and measured using digital calipers (standard length (L_S), nearest 0.1 mm). Assessment of these data involved calculating their length distributions in 1 mm class intervals for *B. barbus*, *S. cephalus*, *P. phoxinus* and *L. leuciscus*. These length distributions were plotted temporally by species and site to identify whether there was the appearance of 'new' fish into the cohort throughout the summer, i.e. whether fish of < 20 mm were regularly appearing in samples collected in July and August that would suggest protracted spawning activities, as per Nunn et al. (2002, 2007a).

These length distributions were then used to identify the presence of length modes in the samples per site and to assess their growth through each growth season using modal progression analysis (MPA). This method was used here on the assumption that each mode identified by MPA represented a discrete spawning event and that each mode could be tracked through subsequent samples. For each species, site and sampling date, the length distributions were analysed for MPA by decomposition assessment using Bhattacharya's method in FiSAT (Bhattacharya 1967; Bolland et al. 2007; Hamidan & Britton 2015). This analysis identifies the presence of modes in each length distribution by separating them into a series of normal distributions (King 2007) (Fig. 11). For each mode, the output was the number of individuals, their mean length and standard deviation (SD) (Bolland et al. 2007), with modes separated by application of a separation index (SI), calculated as the ratio of the difference between successive means and the difference between the SD of their modes.

Values of the SI > 2.0 indicate significant length differences with the other identified modes (Bhattacharya 1967; Bolland et al. 2007). The overall output of MPA for each site per sample and species was thus the number of modes in the cohort and their mean length (± SD), plus their SI with adjacent modes. These outputs were then plotted for mean length (+/- 95% confidence interval) per mode and per sample for each species and site to identify and track the modes over time. This enabled visual assessment of the different modes in the cohort over time.

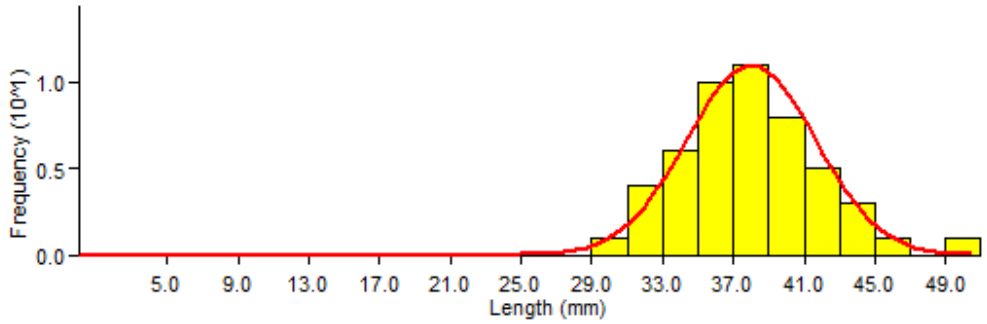

Figure 11. An example of a graphical output from modal progression analysis completed in FiSAT using Bhattacharya's method. Here, only a single mode has been identified by the analysis, with its normal distribution shown by the line. Allied to the graphical output, a mean length and standard deviation is calculated; where more than one mode is identified, the separation index (SI) between successive modes and the number of fish per mode is calculated.

Due to low sample sizes of *B. barbus* and *S. cephalus* being caught at Site 3 in 2016, these data were omitted from analyses. Site 1 also had a low sample size of *B. barbus* in 2016 and was not included for analysis of length modes.

3.4 Results

3.4.1 Data overview

The main fish species sampled at Sites 1 and 2 were *P. phoxinus*, *S. cephalus* and *B. barbus* (Table 11). Other species occasionally present in samples but at numbers that were insufficient for further analyses included gudgeon *Gobio gobio*, stone loach *Barbatula barbatula*, bullhead *Cottus gobio* and three-spined stickleback *Gasterosteus aculeatus*. Whilst the composition of catches was similar at Site 3, catches also included *L. leuciscus*, with this the only site where these were recorded regularly (Table 11).

Table 11. Number of larval and juvenile 0+ cyprinid fish analysed for length progression from three different sites on the River Teme between 2015 and 2017. Values in bold were excluded from analysis due to low sample size. NA where no samples were taken on that occasion.

2015	Date						
	07/07 08/07	23/07	04/08	20/08	08/09	22/09	05/10
B. barbus							
S1	19	72	153	67	49	NA	**0**
S2	94	59	65	67	44	NA	20
S3	32	67	37	31	15	**9**	**6**
S. cephalus							
S1	11	23	35	51	30	NA	**11**
S2	23	39	15	28	18	NA	16
S3	**4**	160	114	64	91	93	60
P. phoxinus							
S1	30	30	40	30	30	NA	93
S2	30	30	30	30	208	NA	183
S3	11	17	30	31	148	30	30
L. leuciscus							
S3	45	26	50	76	53	54	47

2016	Date								
	24/05	06/06	29/06	08/07 13/07	25/07 28/07	09/08	25/08 30/08	12/09	01/10
B. barbus									
S1	**0**	**0**	5	16	20	12	13	22	NA
S2	**0**	**0**	70	39	134	44	27	122	30
S3	**0**	**0**	**0**	**0**	**4**	**8**	**20**	**18**	**NA**
S. cephalus									
S1	**0**	34	48	30	55	**2**	**5**	**4**	**NA**
S2	**1**	100	100	23	24	**11**	**19**	**20**	**17**
S3	**17**	**0**	**16**	**6**	**4**	**7**	**12**	**6**	**NA**
P. phoxinus									
S1	1	111	88	105	96	80	96	25	NA
S2	95	100	142	55	125	118	88	104	101
S3	100	50	100	69	11	100	70	56	NA
L. leuciscus									
S3	**0**	18	100	43	39	62	**2**	**18**	NA

2017	Date						
	05/06	19/06	02/07	26/07	08/08	22/08	06/09
B. barbus							
S1	1	25	76	48	36	40	28
S2	38	31	60	34	45	36	34
S3	15	35	62	46	34	34	33
S. cephalus							
S1	22	25	57	23	41	16	39
S2	10	4	37	51	48	44	50
S3	3	13	35	39	12	8	26
P. phoxinus							
S1	28	95	100	50	50	52	50
S2	46	34	60	50	50	50	50
S3	**0**	16	77	50	38	50	50
L. leuciscus							
S3	4	8	50	52	33	33	35

3.4.2 Barbus barbus

Across the 0+ *B. barbus* samples collected in 2015, lengths ranged between 13 and 37 mm across the three sites, with the fish of smallest SL being recorded in July, with fish present > 30 mm at all sites from samples collected in August (Fig. 12). Similarly, the length range of 0+ *B. barbus* in 2016 was 12 to 37 mm (Fig. 12). The earlier start date of sampling in 2016 revealed that although no *B. barbus* were present in samples collected on 24/05/16 and 06/06/16, they were from 29/06/16, when fish were present in samples at Sites 1 and 2 at 12 and 13 mm respectively (Fig. 13). In 2017, when sampling commenced in May, *B. barbus* were first detected in samples on 05/06/17 (Fig. 14).

A relatively large size range of 0+ *B. barbus* was present in each 2015 sample, with this peaking in samples collected on 20/08/15 when the differences in lengths between the smallest and largest fish in samples was up to 18 mm (17 to 35 mm; Fig. 12). Length frequency distributions revealed the appearance of fish of < 15 mm in samples collected into August, with MPA able to consistently identify three to four modes in the cohorts over the sampling period and across the sites where the SI was > 2.0 (Fig. 12, 15).

In 2016, *B. barbus* of 11 to 12 mm were present in samples collected on 28/07/16, with fish of 9 to 12 mm then present in samples collected on 09/08/16 when other 0+ *B. barbus* were present to over 30 mm (Fig. 16).. Due to low sample sizes of 0+ *B. barbus* caught at Sites 1 and 3 in 2016, they could not be included in model progression analysis. Modal progression analysis on the 2016 samples for Site 2 revealed three modes in the cohorts over the sampling period,

with all three modes present in samples collected on 28/07/16 and 09/08/16 (Fig. 13). In the samples collected in 2017, five modes in the cohorts were detected at all three sites and most could be tracked through the sampling period (Fig. 17).

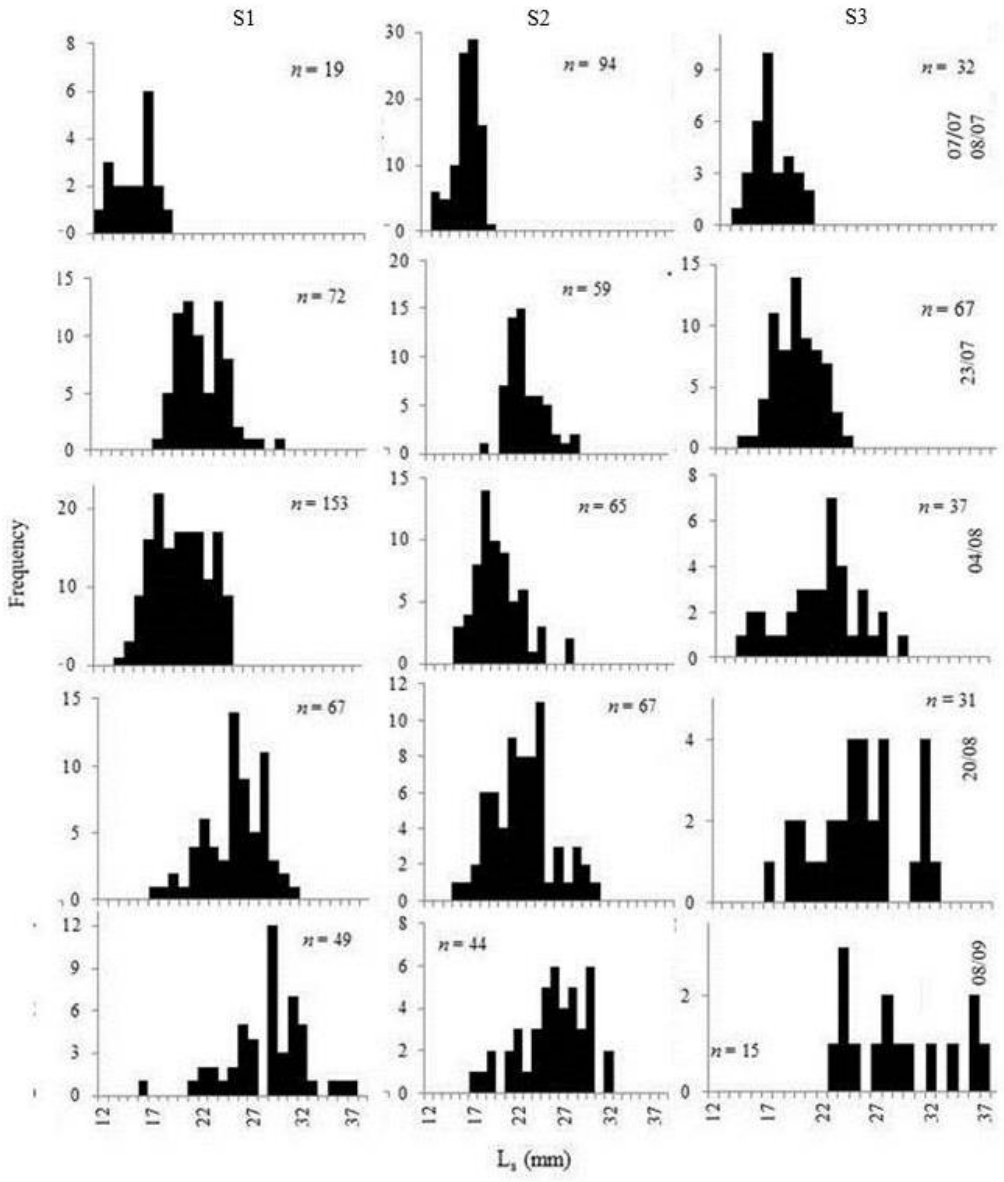

Figure 12. Standard length (L_S mm) distributions of *Barbus barbus* at S1, S2 and S3, River Teme from July to September 2015. Note differences in values on the Y axis for comparative purposes.

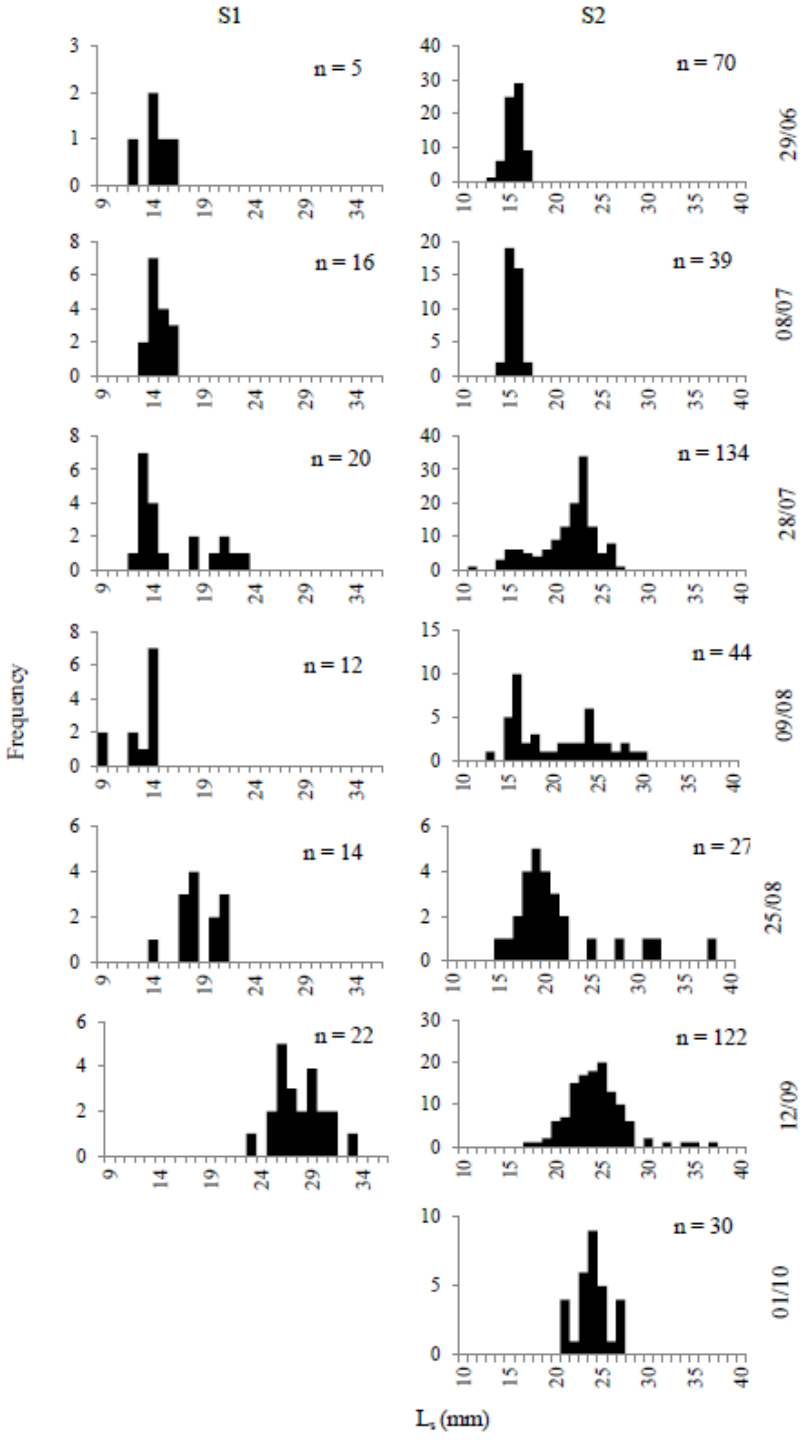

Figure 13. Standard length (L_S mm) distributions of 0+ *Barbus barbus* at S1 and S2, River Teme 2016. Note differences in values on the Y axis for comparative purposes.

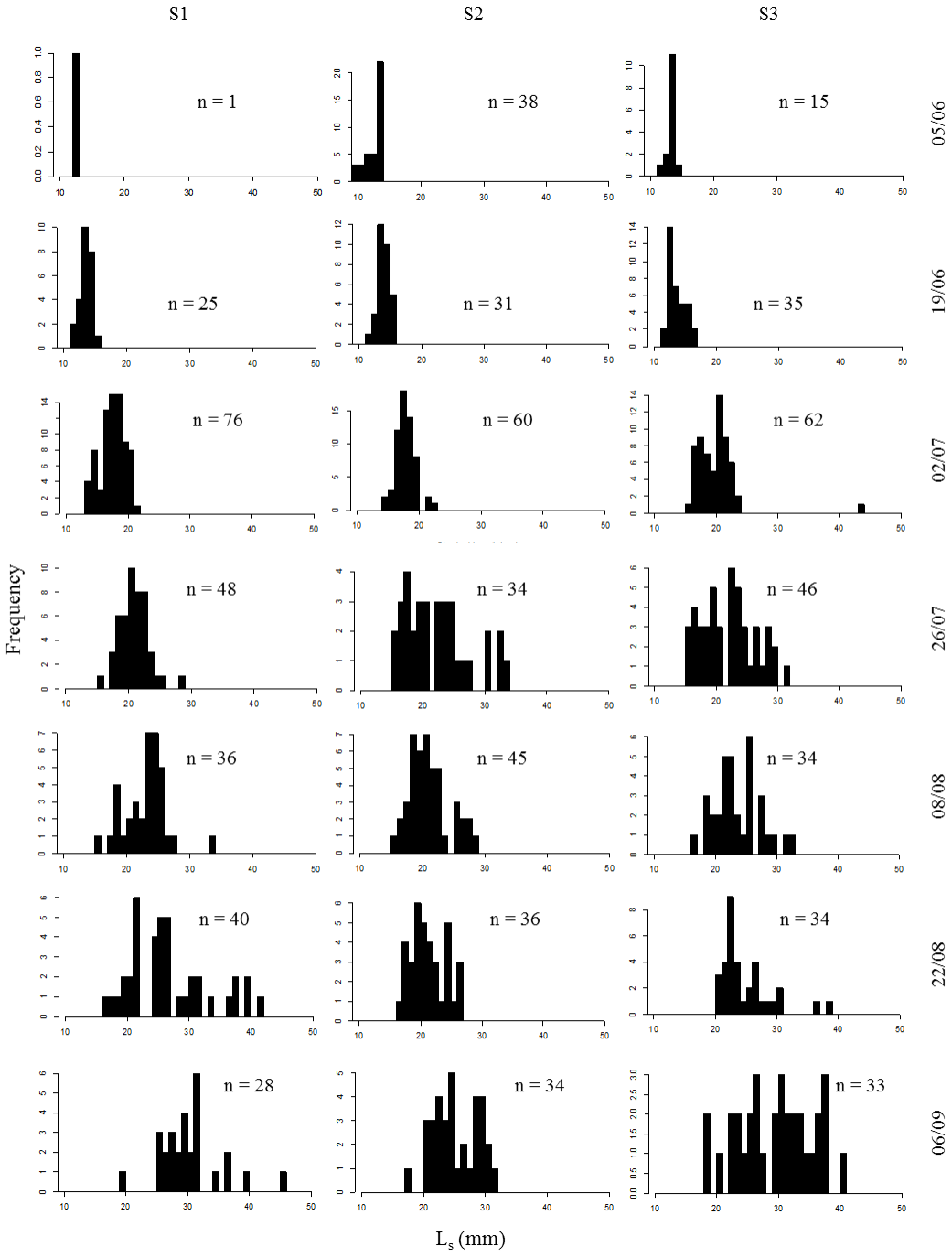

Figure 14. Standard length (L_S mm) distributions of 0+ *Barbus barbus* at Site 1, 2 and 3, River Teme 2017. Note differences in values on the Y axis for comparative purposes.

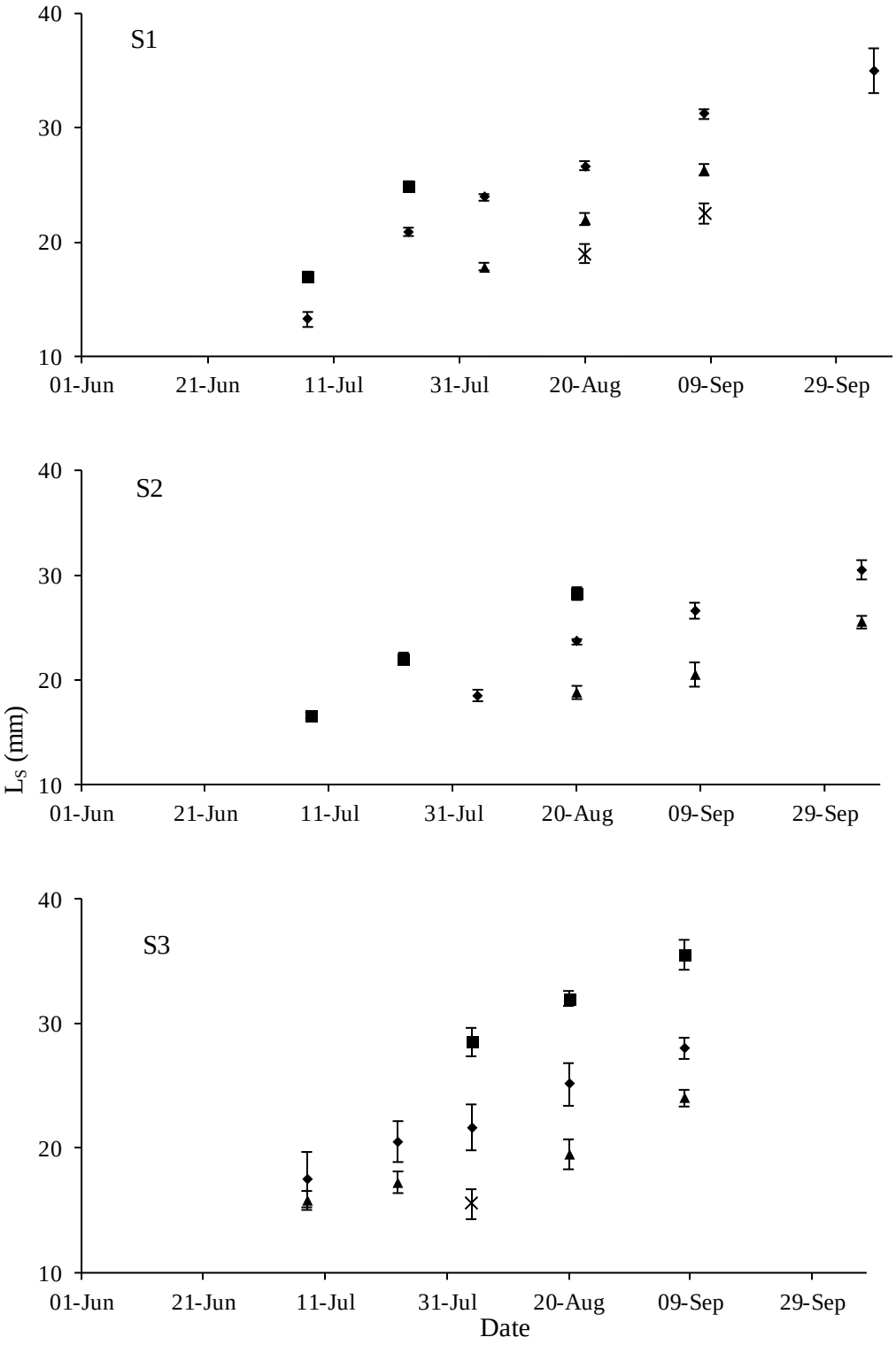

Figure 15. Mean length (mm, ± 95% CI) per mode of *Barbus barbus* from Site 1 - 3 River Teme in 2015, as identified by Modal Progression Analysis. The symbols represent each mode.

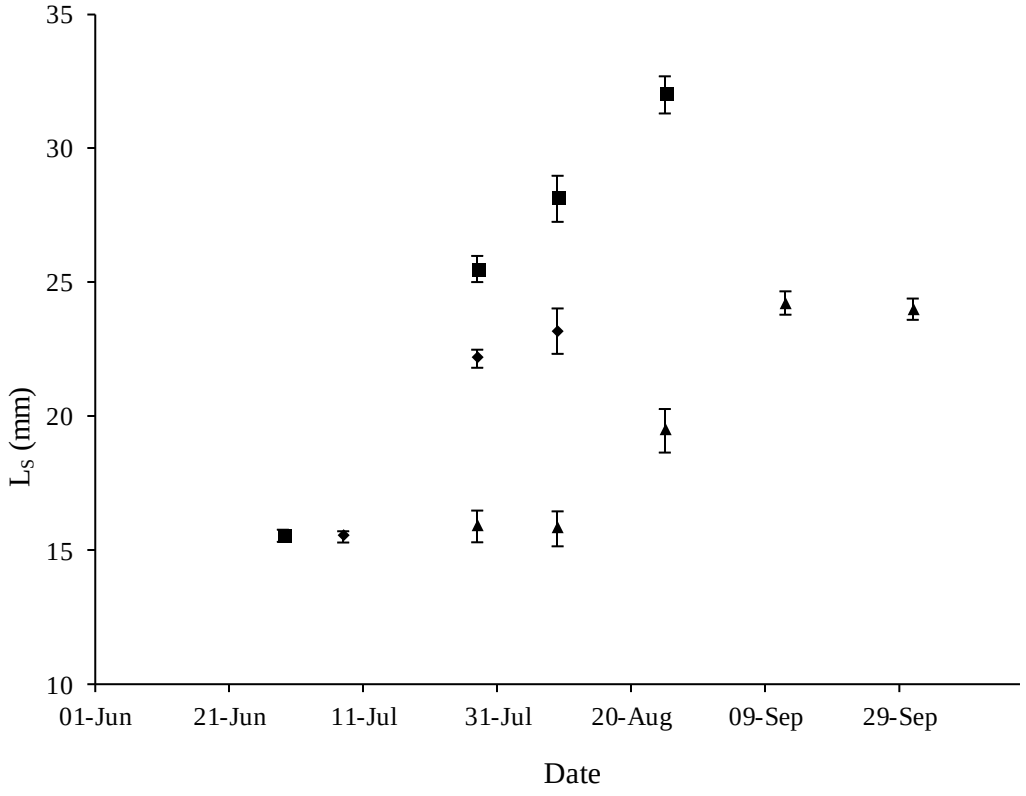

Figure 16. Mean length (mm, ± 95% CI) per mode of *Barbus barbus* from Site 2, River Teme from 2016, as identified by Modal Progression Analysis. The symbols represent each mode.

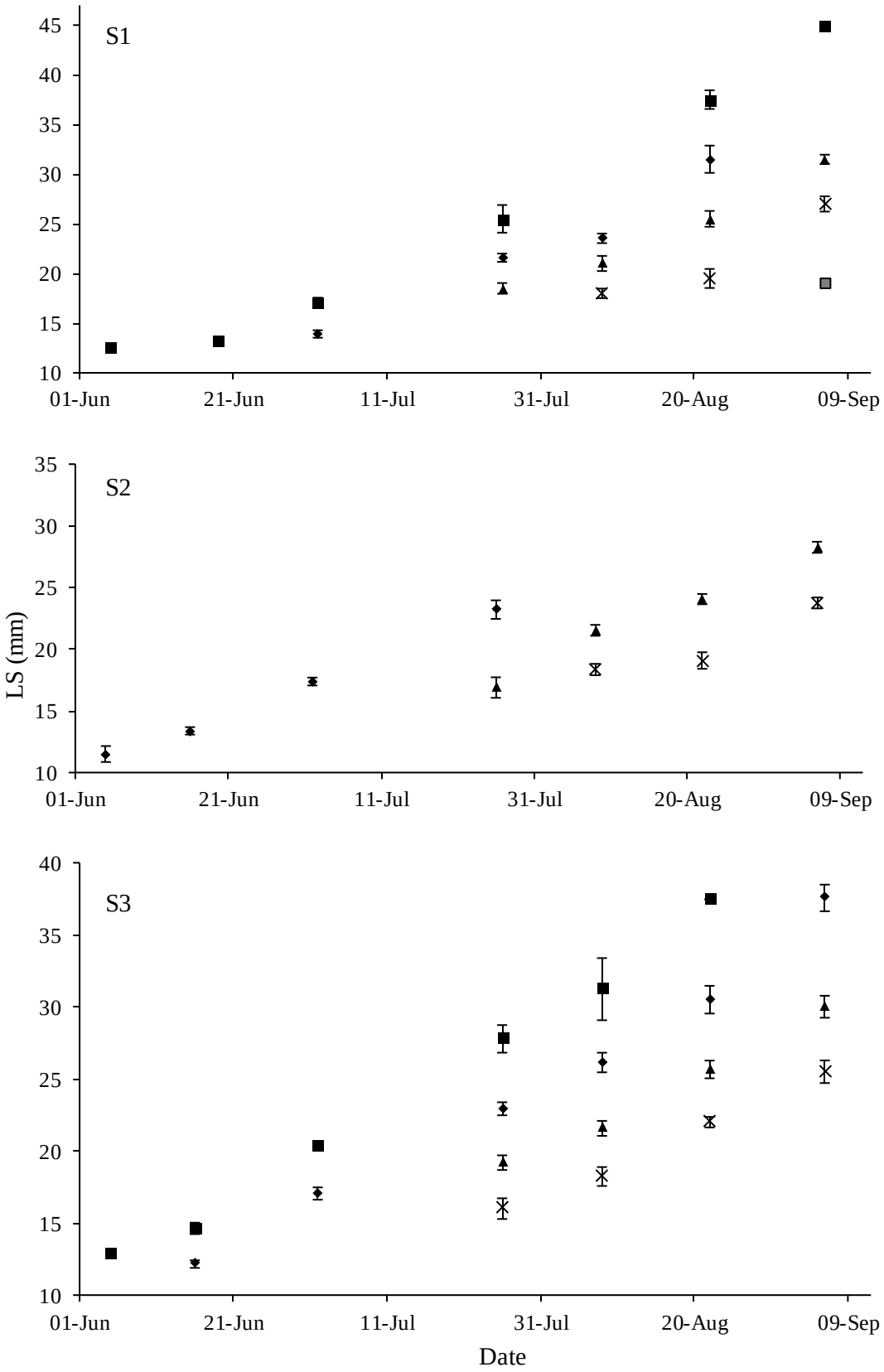

Figure 17. Mean length (mm, ± 95% CI) per mode of *Barbus barbus* from Site 1 - 3, River Teme from 2017, as identified by Modal Progression Analysis. The symbols represent each mode.

3.4.3 Squalius cephalus

From the initial sample collection in early July 2015, 0+ *S. cephalus* were present in samples at all sites, with fish present of lengths of 13 to 37 mm (Fig. 18). Individual fish of < 20 mm were always present in these samples until late August, when fish > 30 mm were also present (Fig. 18). In the 2016 samples, 0+ *S. cephalus* appeared in samples from 06/06/16, earlier than *B. barbus*, with individuals present between 7 and 44 mm in all samples (Fig. 19). In 2017, samples appeared at the beginning of June again (05/06/17), with samples less than 10mm also being found in mid-June (Fig. 20). The *S. cephalus* caught on the 02/07/17 that were larger than 35 mm were significantly separated from previous modes and expected to be 1+ fish hence were not included in the modal progression analysis (Fig. 20).

As with *B. barbus*, there was considerable variability in the size ranges of *S. cephalus* in the samples collected on each occasion (Fig. 18 to 20). Evidence for extended spawning periods in each year were apparent in the length frequency distributions (Fig. 18 to 20), with MPA able to consistently identify and track the progression of up to five modes through samples (Fig. 21 to 23). For example, in S1 in 2015, following the initial sample on 07/07/16, fish of < 15 mm continued to be present in samples collected on 23/07/15 and 20/08/15 (Fig. 18 to 20). There was variability in the numbers of modes identified in cohorts, with Site 1 having up to three modes and Site 2 having up to five modes in 2017 (Fig. 18 to 20).

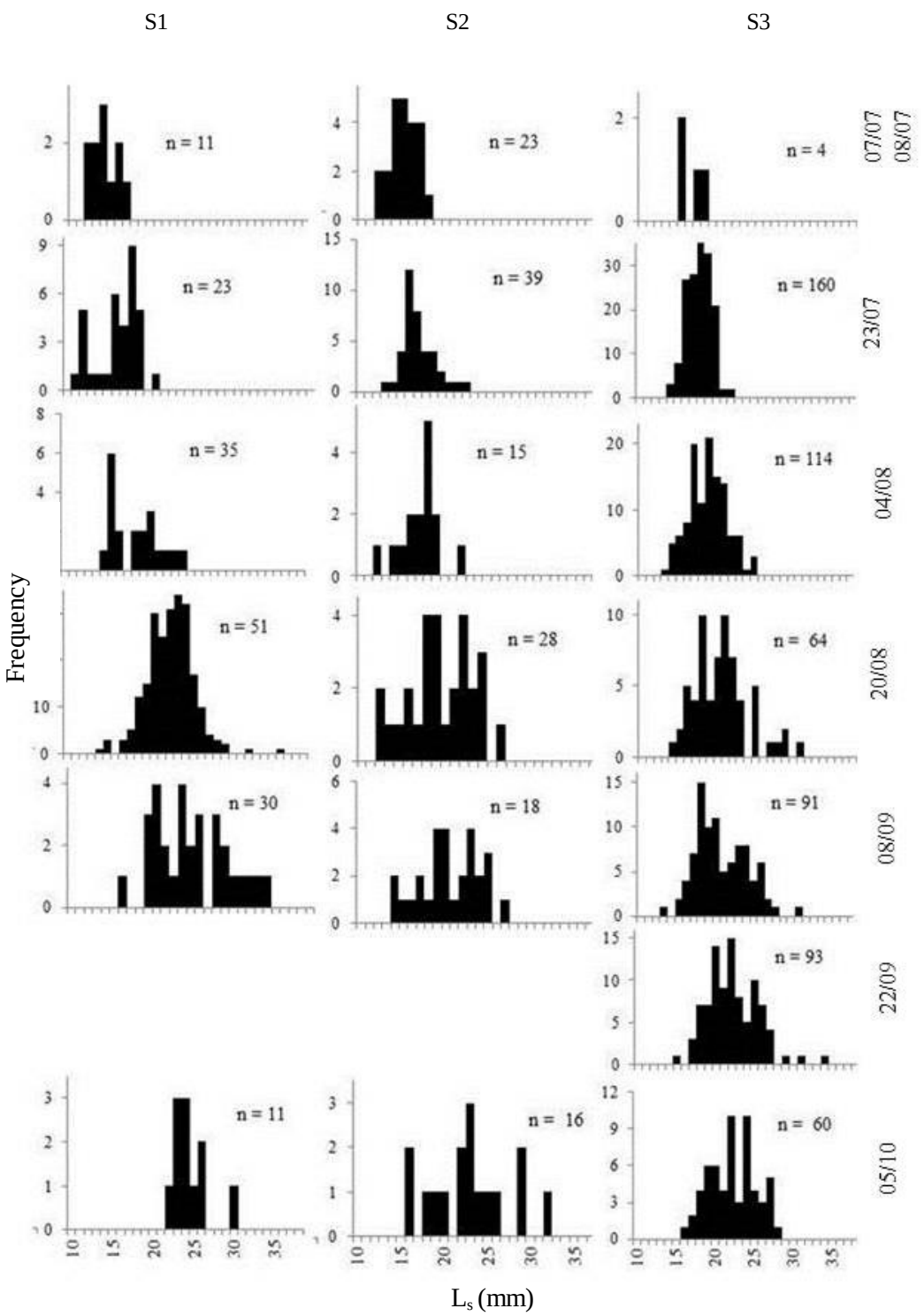

Figure 18. Standard length (L_S) distributions of *Squalius cephalus* at S1, S2 and S3, River Teme from July to October 2015. Note differences in values on the Y axis for comparative purposes.

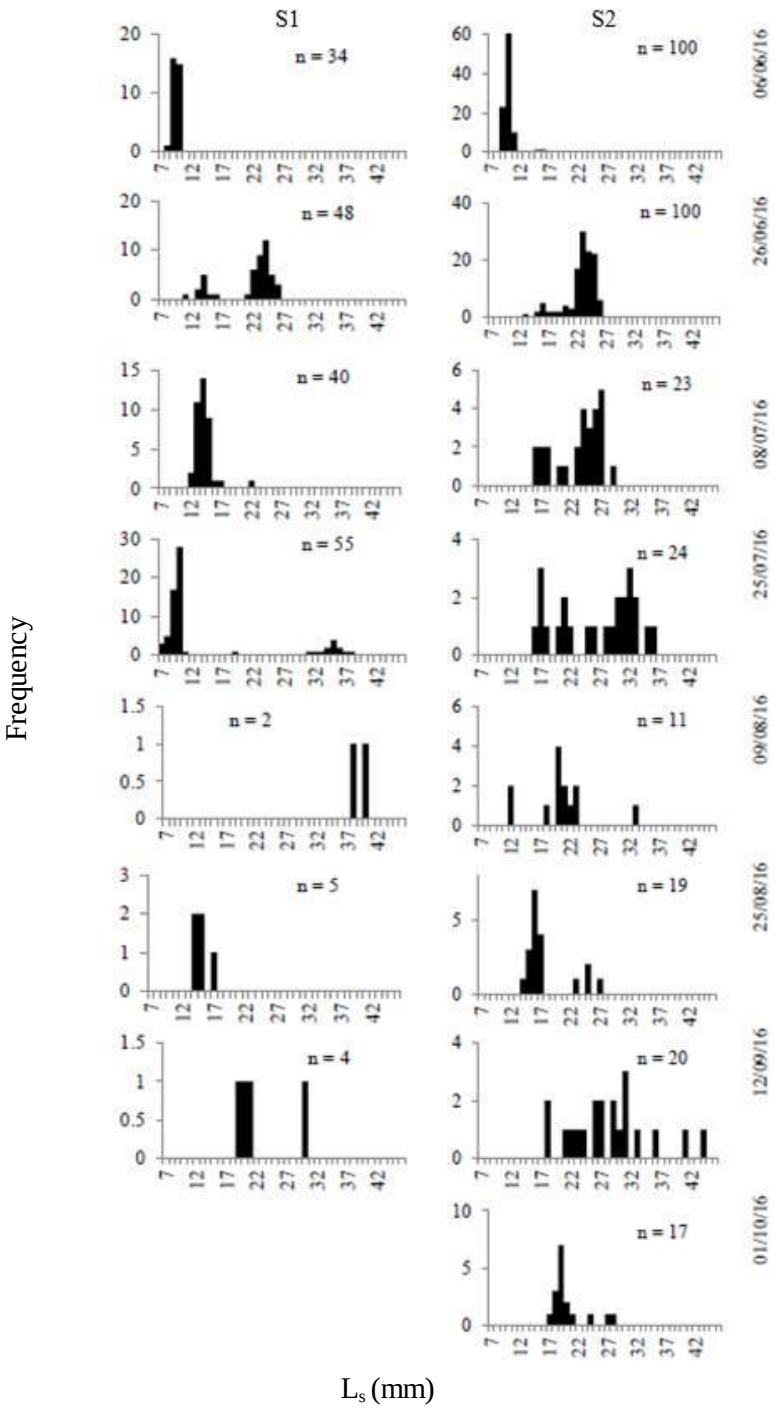

Figure 19. Standard length ($L_{S\ mm}$) distributions of 0+ *Squalius cephalus* at S1 and S2, River Teme 2016. Note differences in values on the Y axis for comparative purposes.

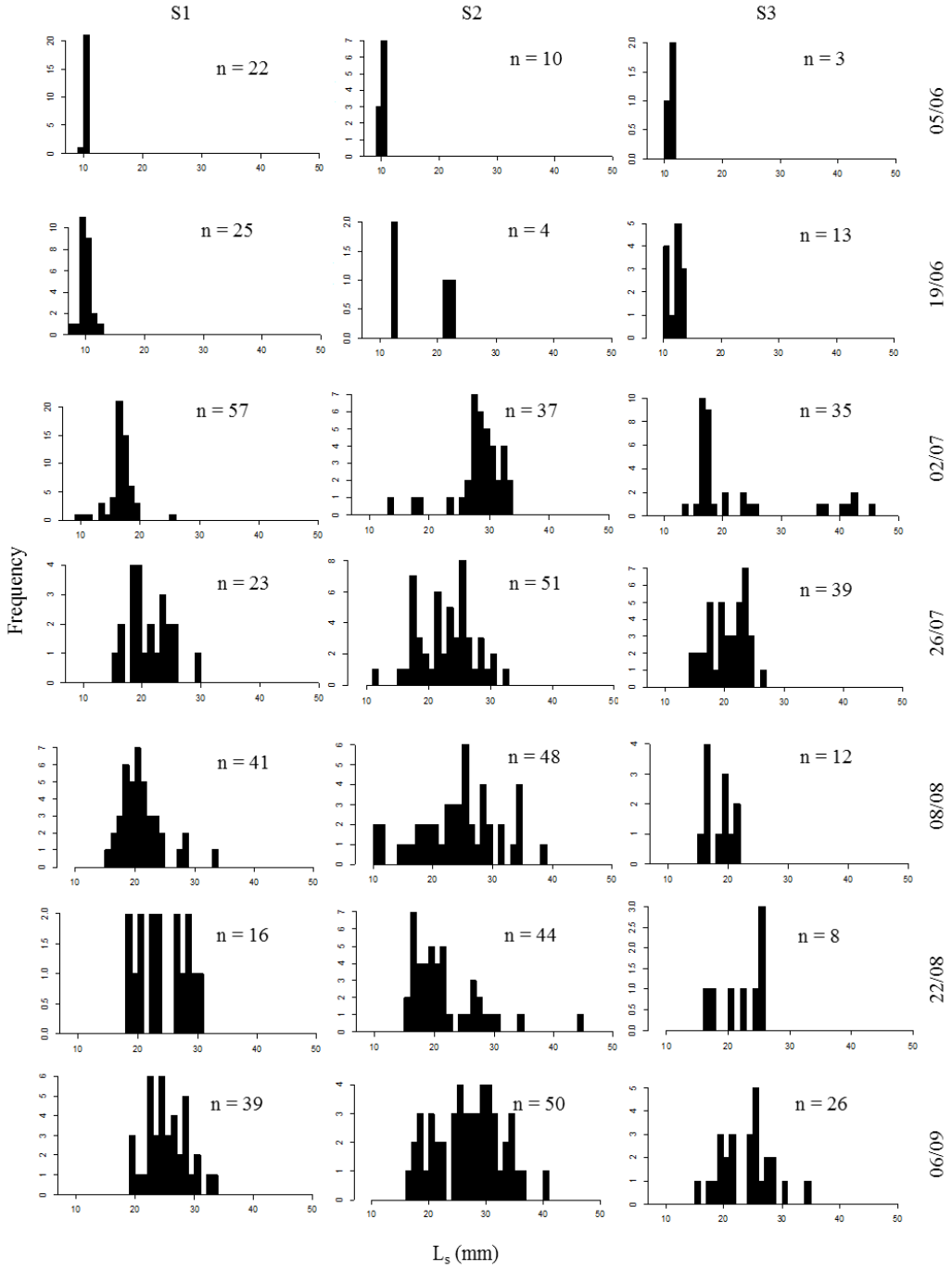

Figure 20. Standard length (L_S mm) distributions of 0+ *Squalius cephalus* at Site 1,2 and 3, River Teme 2017. Note differences in values on the Y axis for comparative purposes.

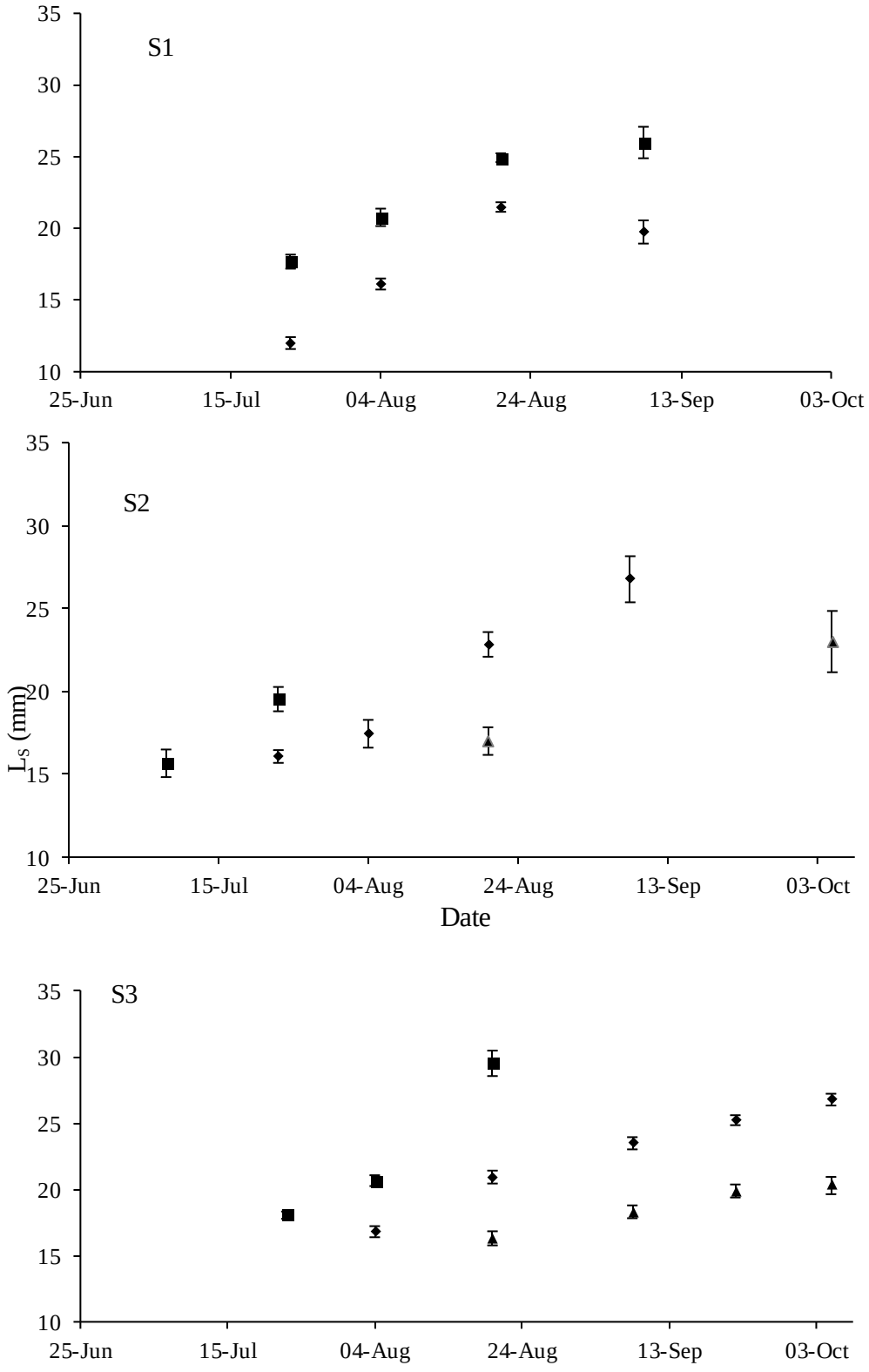

Figure 21. Mean length (mm, ± 95% CI) per mode of *Squalius cephalus* from Site 1, 2 and 3, River Teme from 2015, as identified by Modal Progression Analysis. The symbols represent each mode.

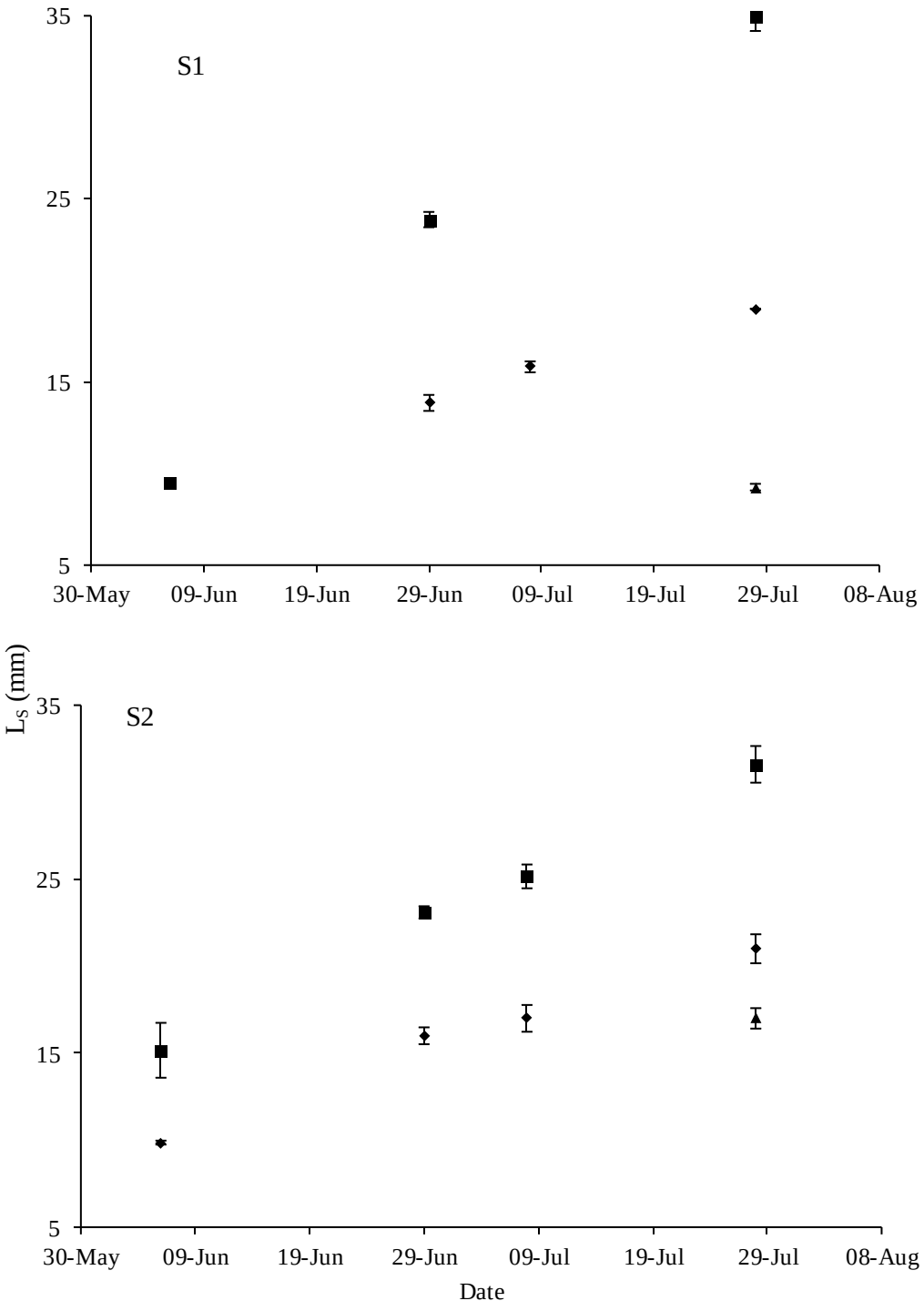

Figure 22. Mean length (mm, ± 95% CI) per mode of *Squalius cephalus* from Site 1 and 2, River Teme from 2016, as identified by Modal Progression Analysis. The symbols represent each mode.

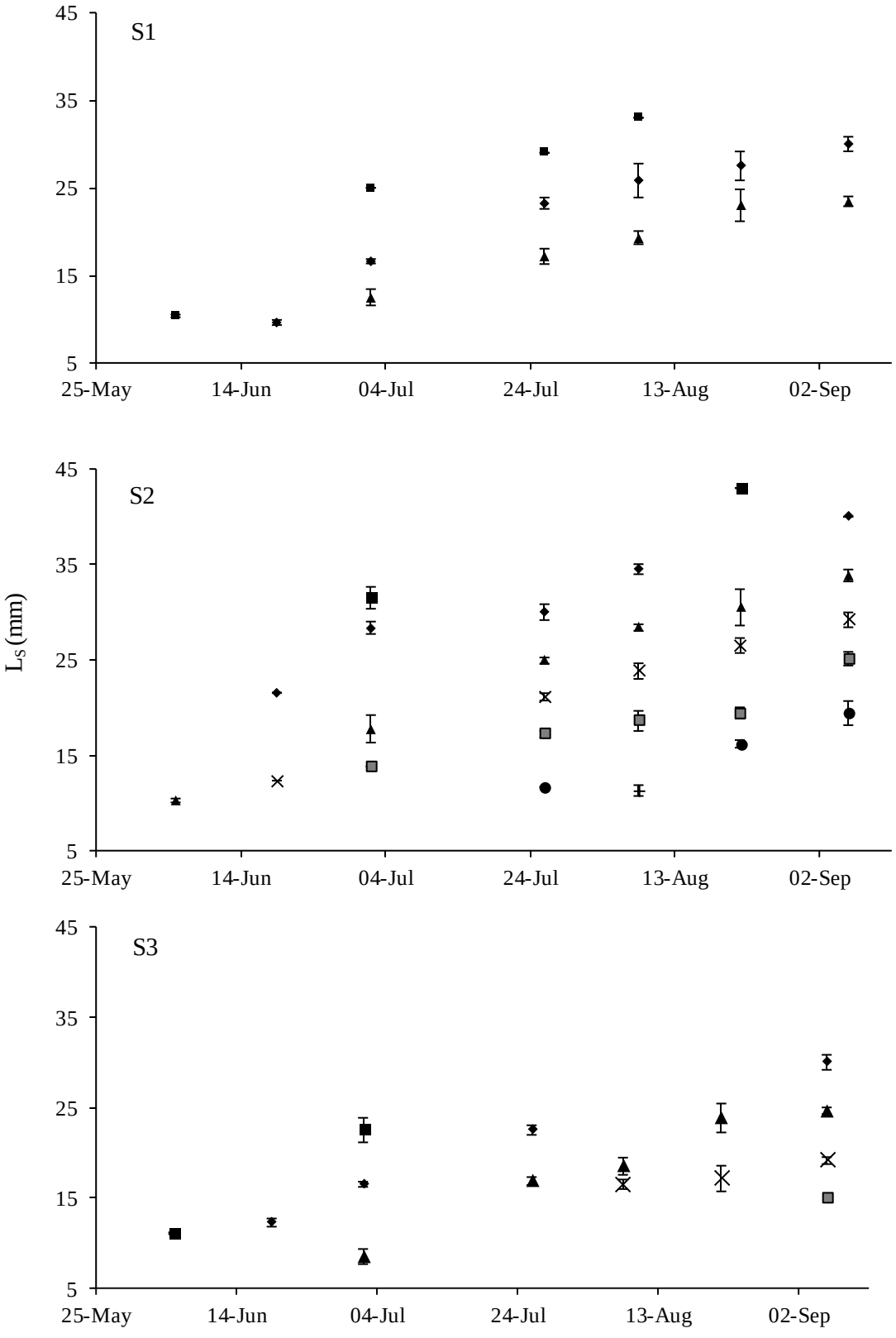

Figure 23. Mean length (± 95% CI) per mode of *Squalius cephalus* from Site 1, Site 2 and Site 3, River Teme from 2017, as identified by Modal Progression Analysis. The symbols represent each mode.

3.5.4 Phoxinus phoxinus

The length range of 0+ *P. phoxinus* varied from 7 to 50 mm across the samples (Fig. 24 to 26). Those that were larger than 27mm in May were expected to be 1+ fish, as this is the size they become juveniles (Simonović *et al*. 1999) and thus they were therefore excluded from subsequent analyses. During all three years of sampling, smaller fish (< 20 mm) appeared in samples collected throughout the summer and even in October. Fish < 10 mm were generally only captured from May to early August (Fig. 24 to 26). Four to six cohorts could be tracked over the sampling period, which suggests protracted spawning events occurred at all sites and in all years (Fig. 27 to 29).

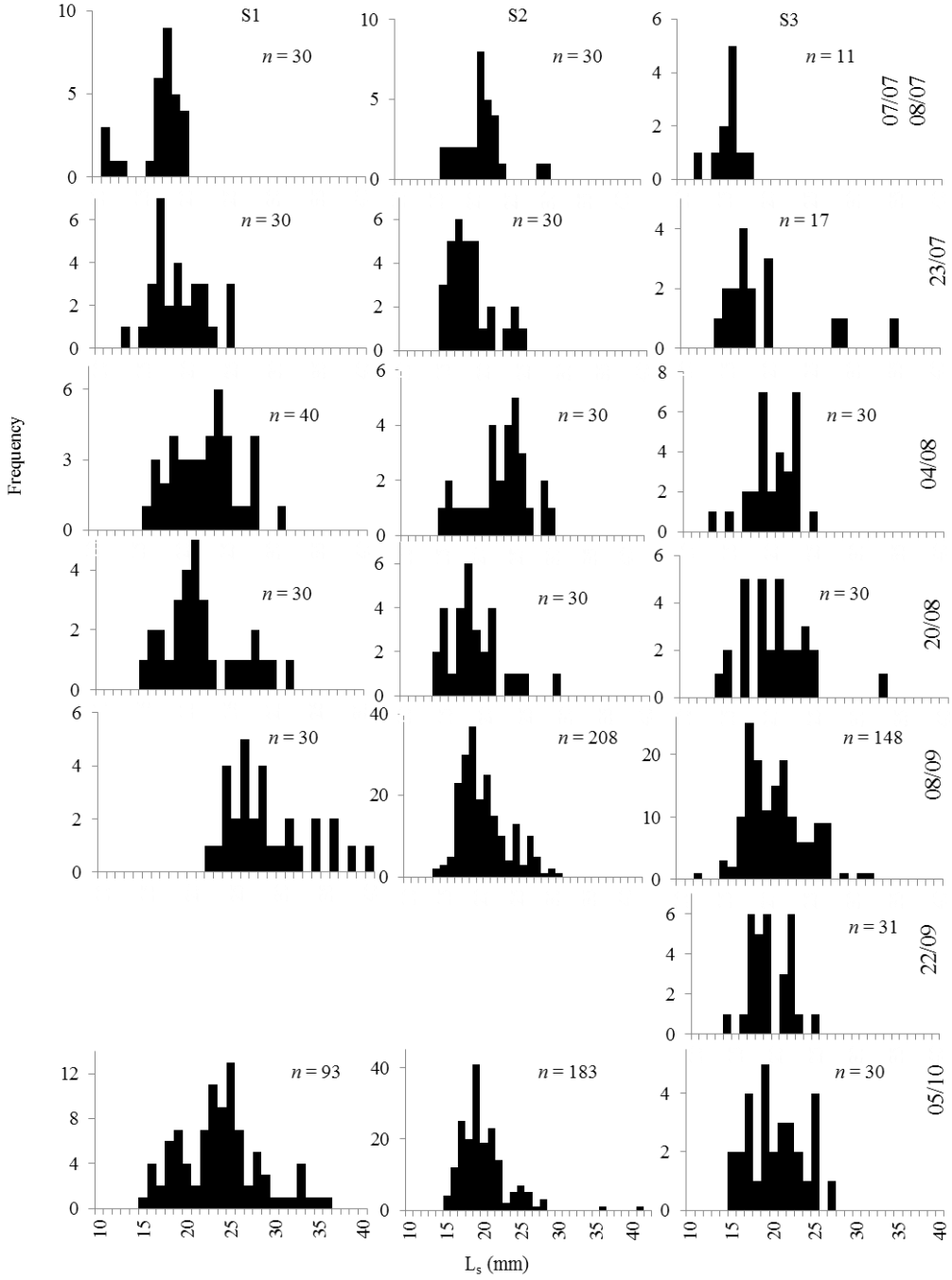

Figure 24. Standard length (L_S) distributions of *Phoxinus phoxinus* at S1, S2 and S3, River Teme from July to October 2015. Note differences in values on the Y axis for comparative purposes.

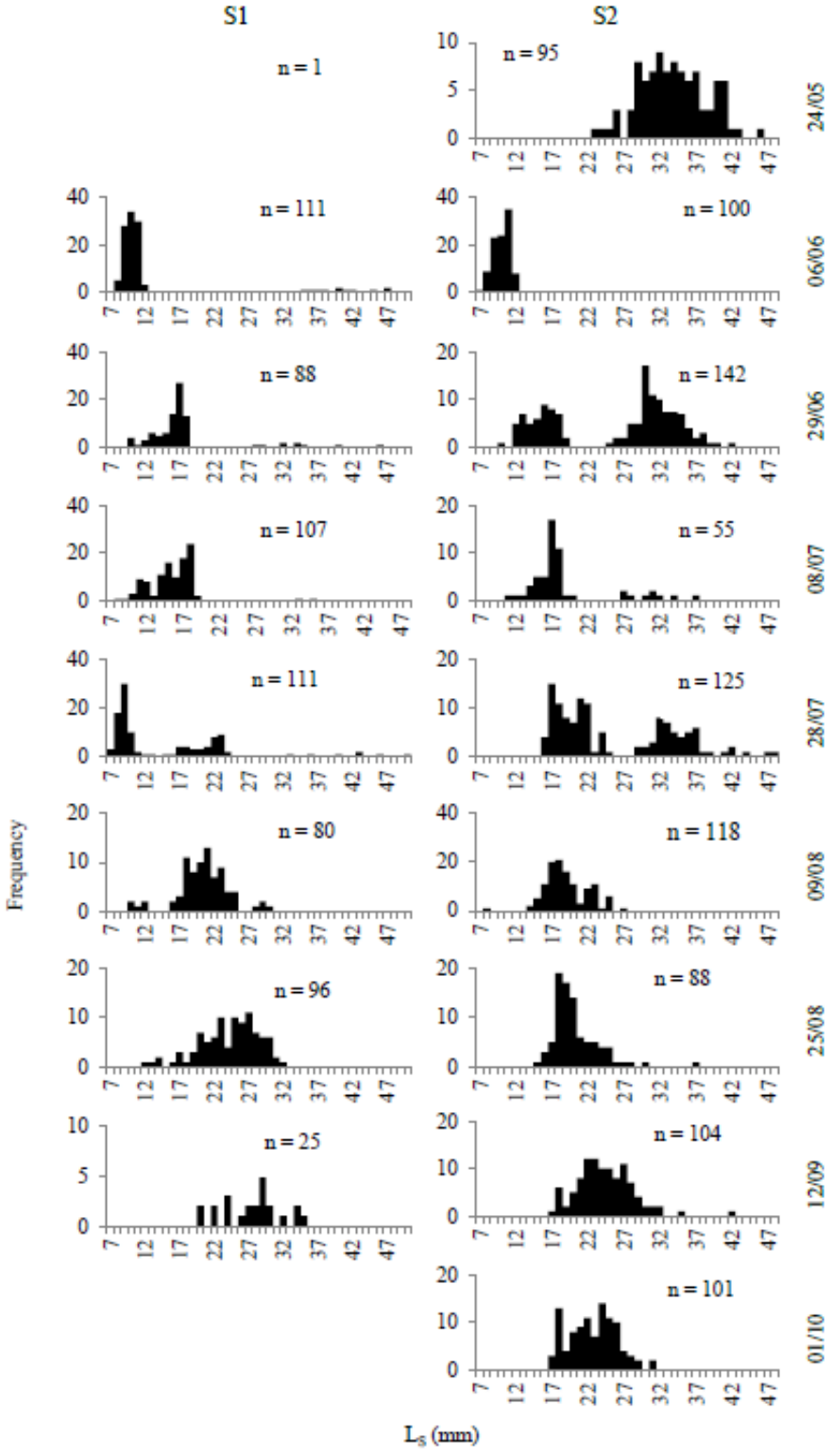

Figure 25. Standard length (L_S) distributions of *Phoxinus phoxinus* at S1 and S2, River Teme 2016. Note differences in values on the Y axis for comparative purposes.

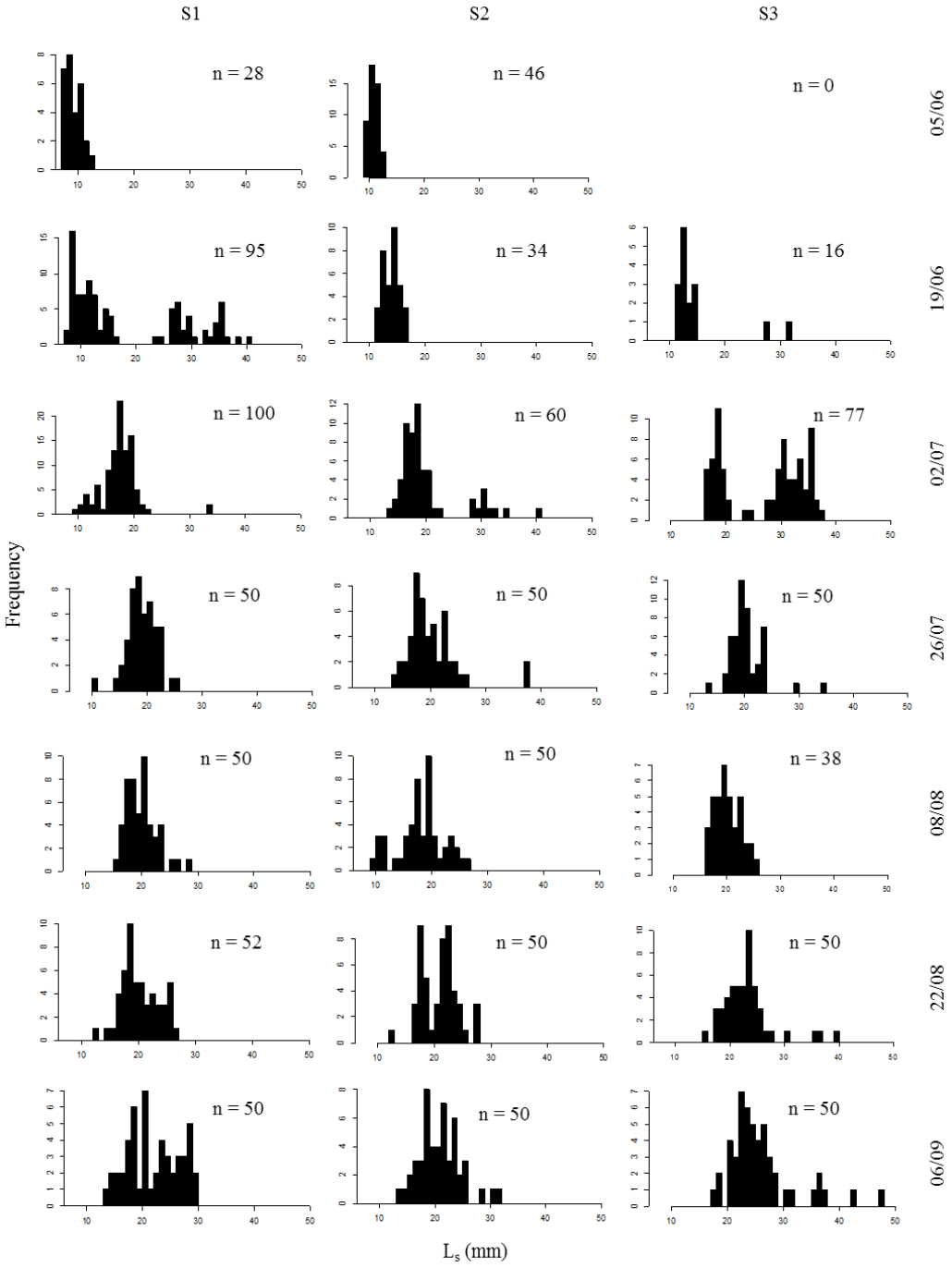

Figure 26. Standard length ($L_{S\ mm}$) distributions of *Phoxinus phoxinus* at Site 1, 2 and 3, River Teme 2017. Note differences in values on the Y axis for comparative purposes.

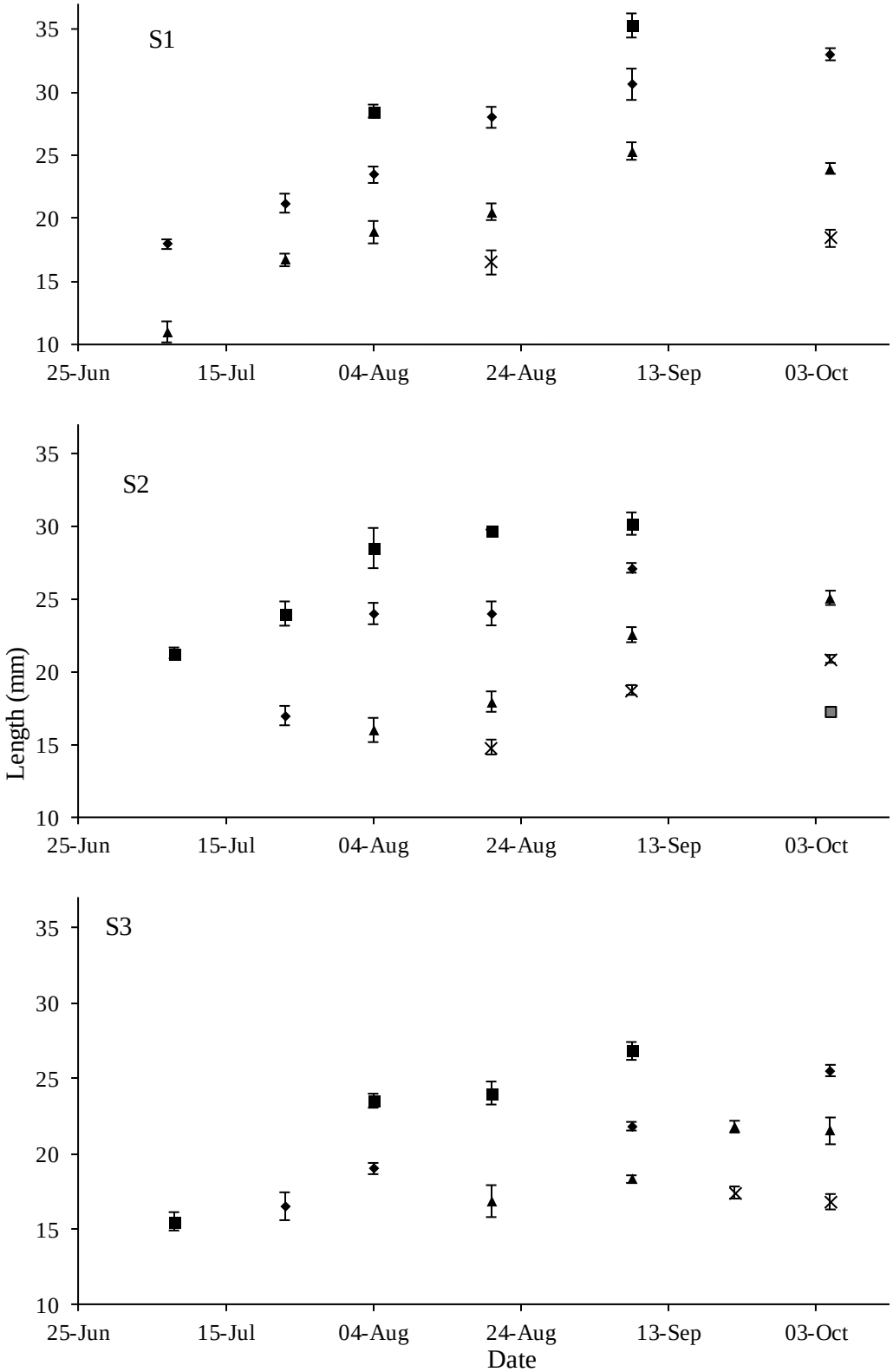

Figure 27. Mean length (± 95% CI) per mode of *Phoxinus phoxinus* from Site 1, 2 and 3, River Teme from 2015, as identified by Modal Progression Analysis. The symbols represent each mode.

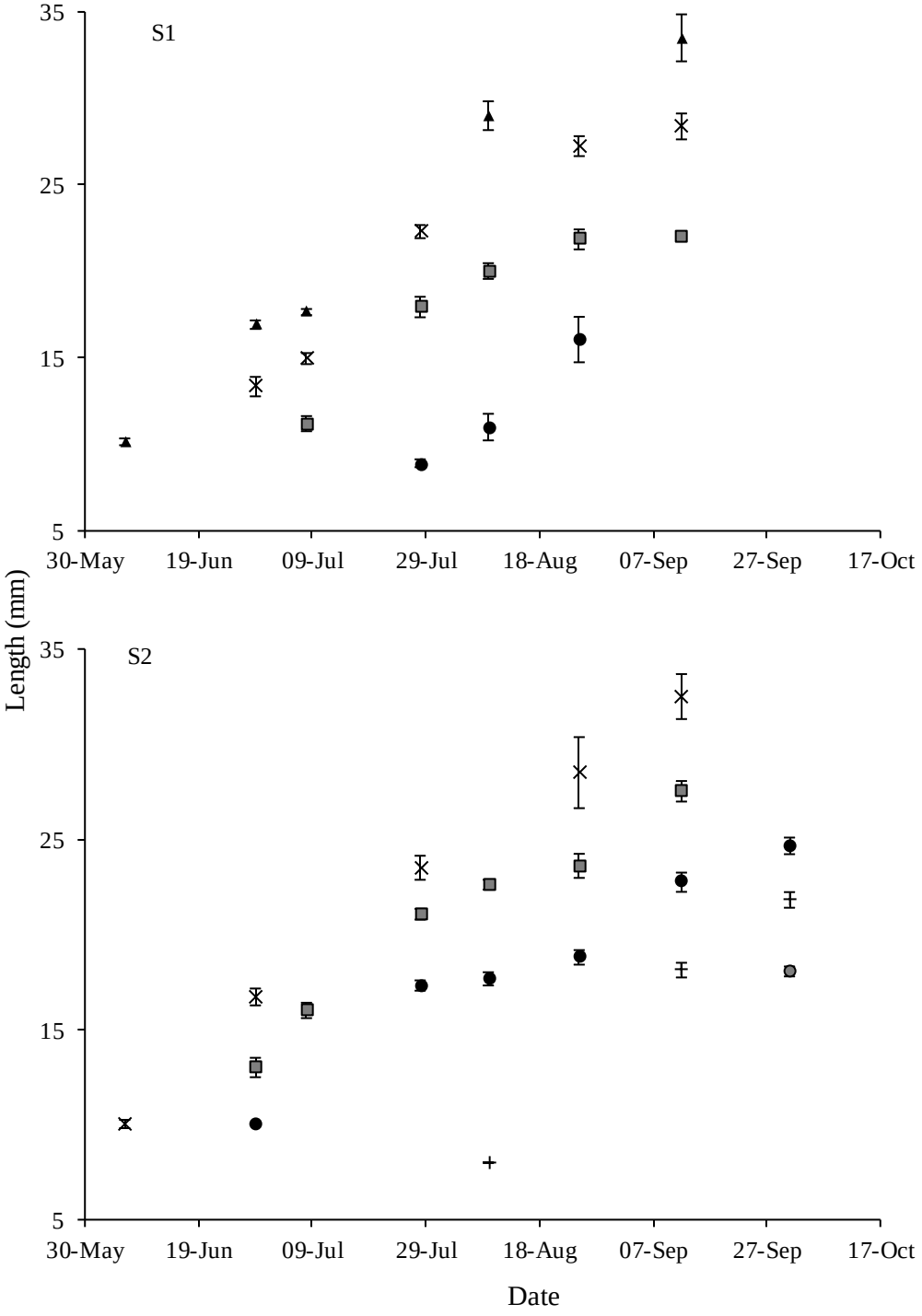

Figure 28. Mean length (± 95% CI) per mode of *Phoxinus phoxinus* from Site 1, and 2, River Teme from 2016, as identified by Modal Progression Analysis. The symbols represent each mode.

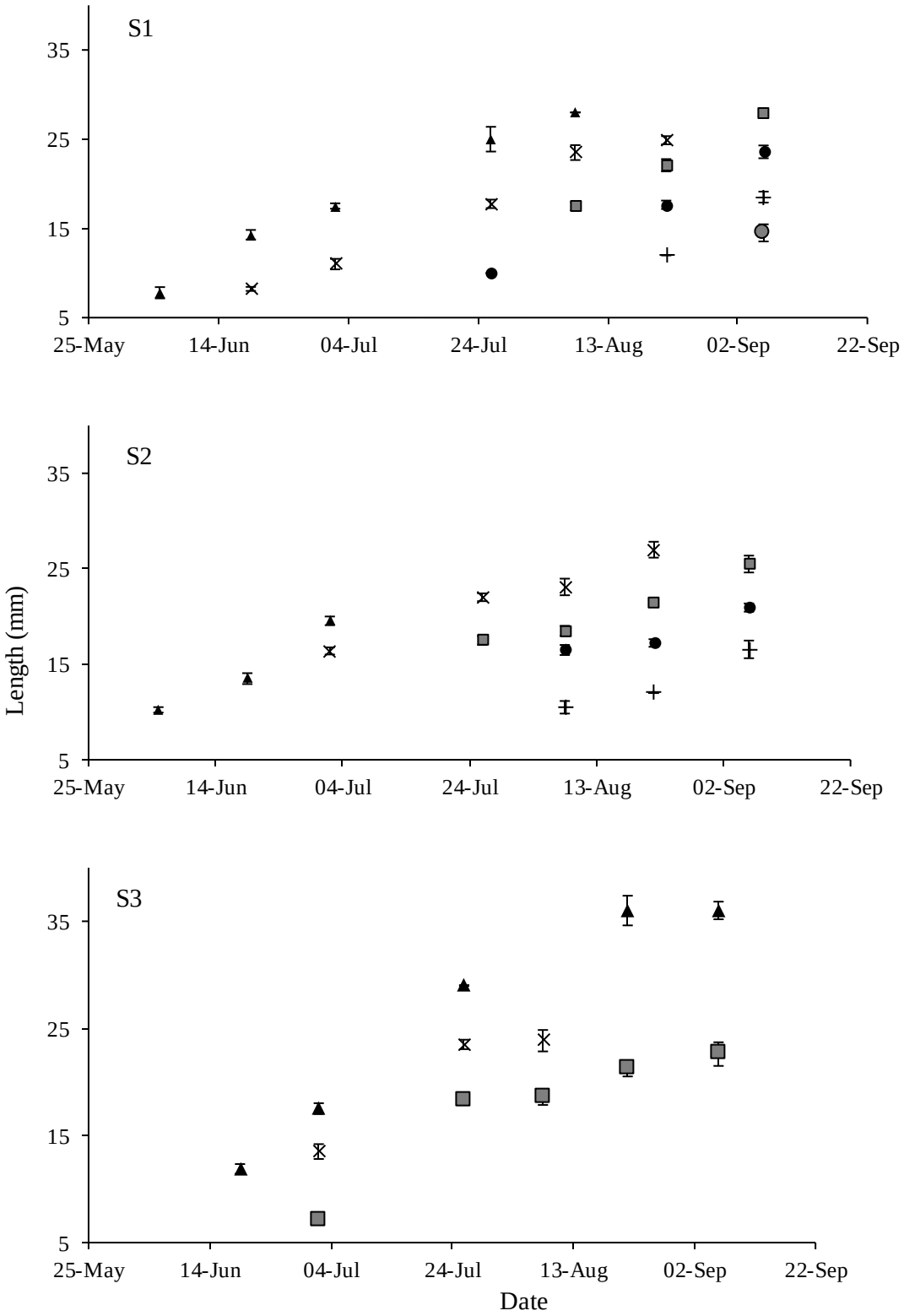

Figure 29. Mean length (± 95% CI) per mode of *Phoxinus phoxinus* from Site 1, 2 and 3, River Teme from 2017, as identified by Modal Progression Analysis. The symbols represent each mode.

3.5.5 Leuciscus leuciscus

Site 3 was the only site where *L. leuciscus* were sampled consistently. In samples collected after 1st July in all years, there was never the appearance of fish of < 20 mm as had been apparent for *B. barbus*, *S. cephalus* and *P. phoxinus*. This suggests that there had been a discrete spawning period rather than an extended spawning period, with no larvae or small juveniles appearing in the August or September samples (Fig. 30 to 32). Nevertheless, the length range of the cohort was relatively large by October 2015 (32 to 50 mm). In 2016, 0+ *L. leuciscus* ranged from 18 to 43 mm, with 0+ being caught as early as 26/06/16 (Fig. 31). In 2017, sample sizes were smaller than previous years but with a similar pattern of a single spawning event (Fig. 32 and 33).

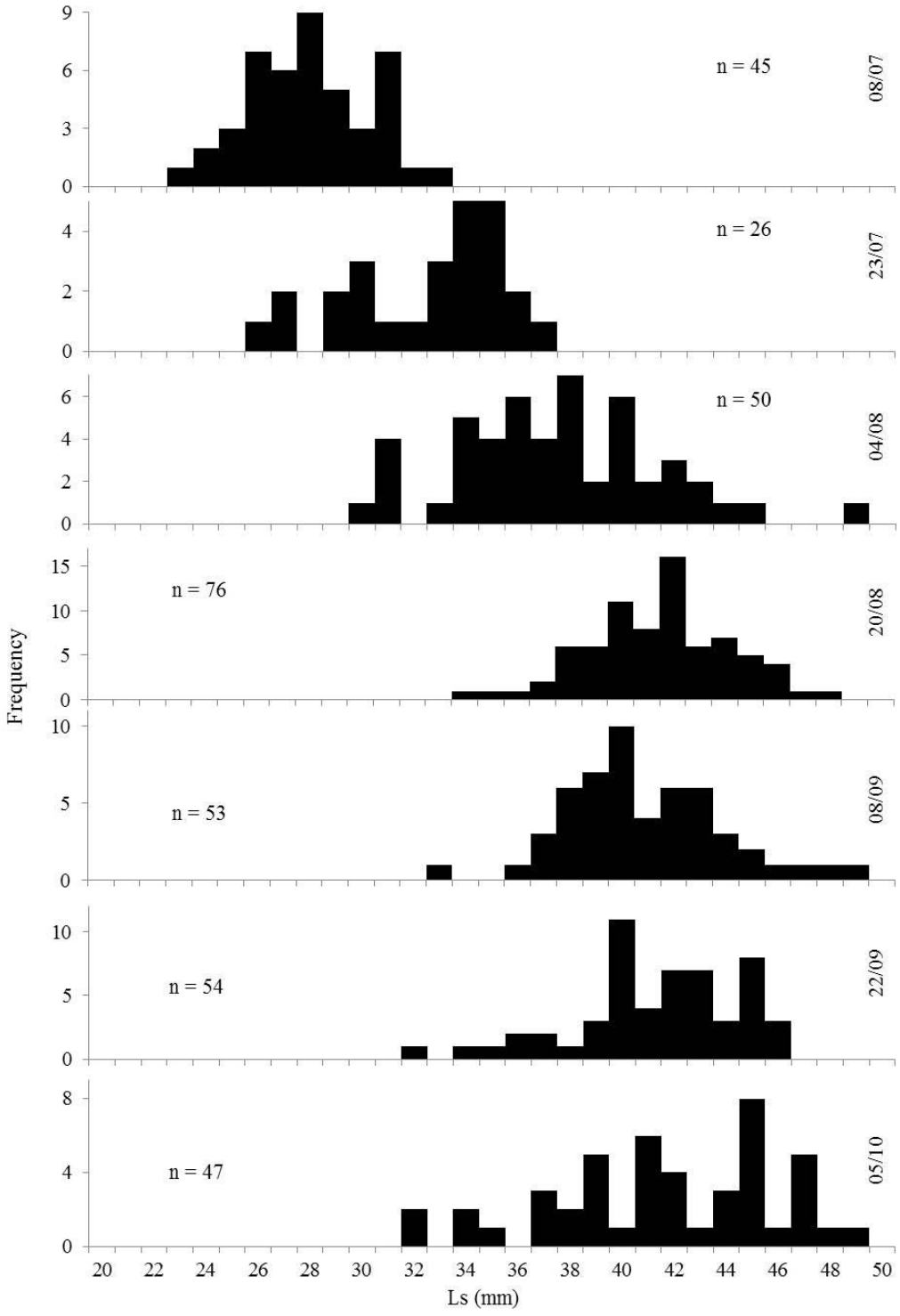

Figure 30. Standard length (L_S) distributions of *Leuciscus leuciscus* at S3, River Teme from July to October 2015. Note differences in values on the Y axis for comparative purposes.

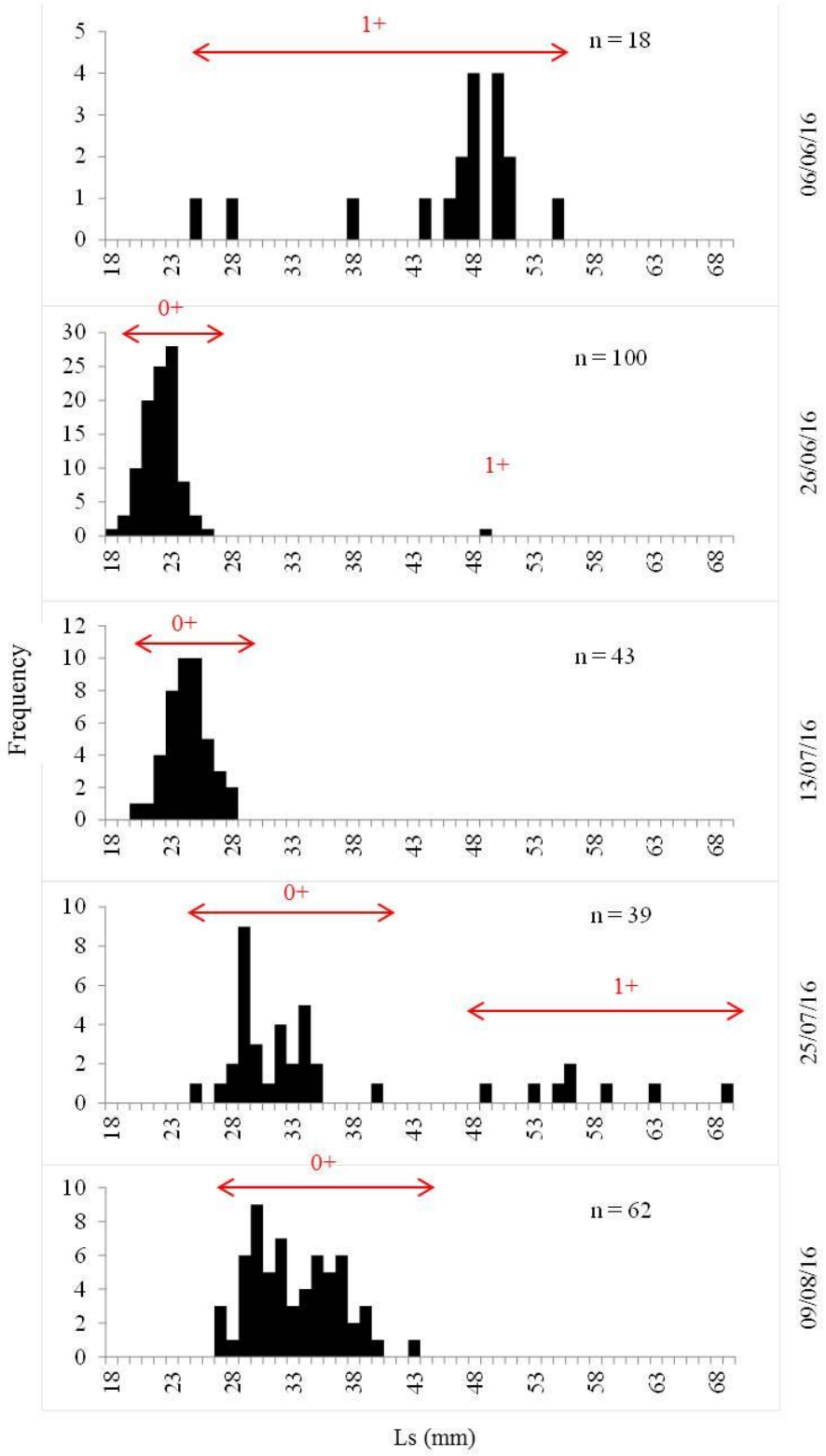

Figure 31. Standard length (L_S) distributions of *Leuciscus leuciscus* at Site 3, River Teme 2016. Arrows depicting 0+ or 1+ age groups. Note variable y-axis.

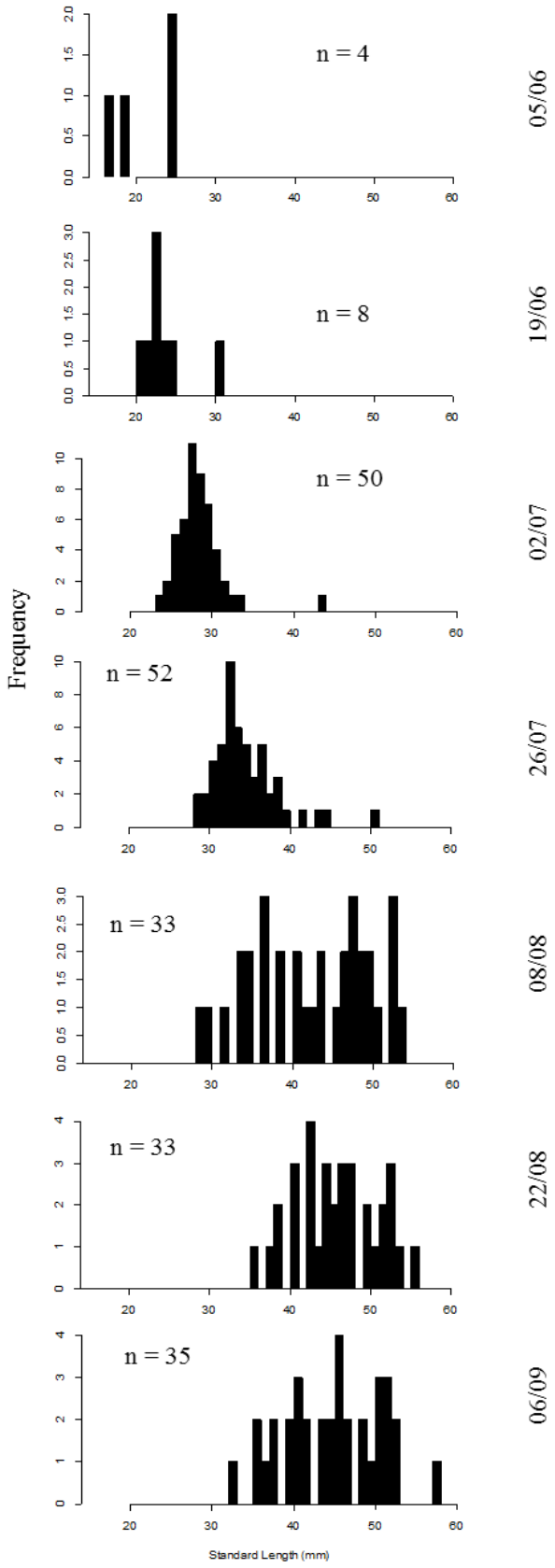

Figure 32. Standard length (L_S) distributions of *Leuciscus leuciscus* at Site 3, River Teme 2017. Note variable y-axis.

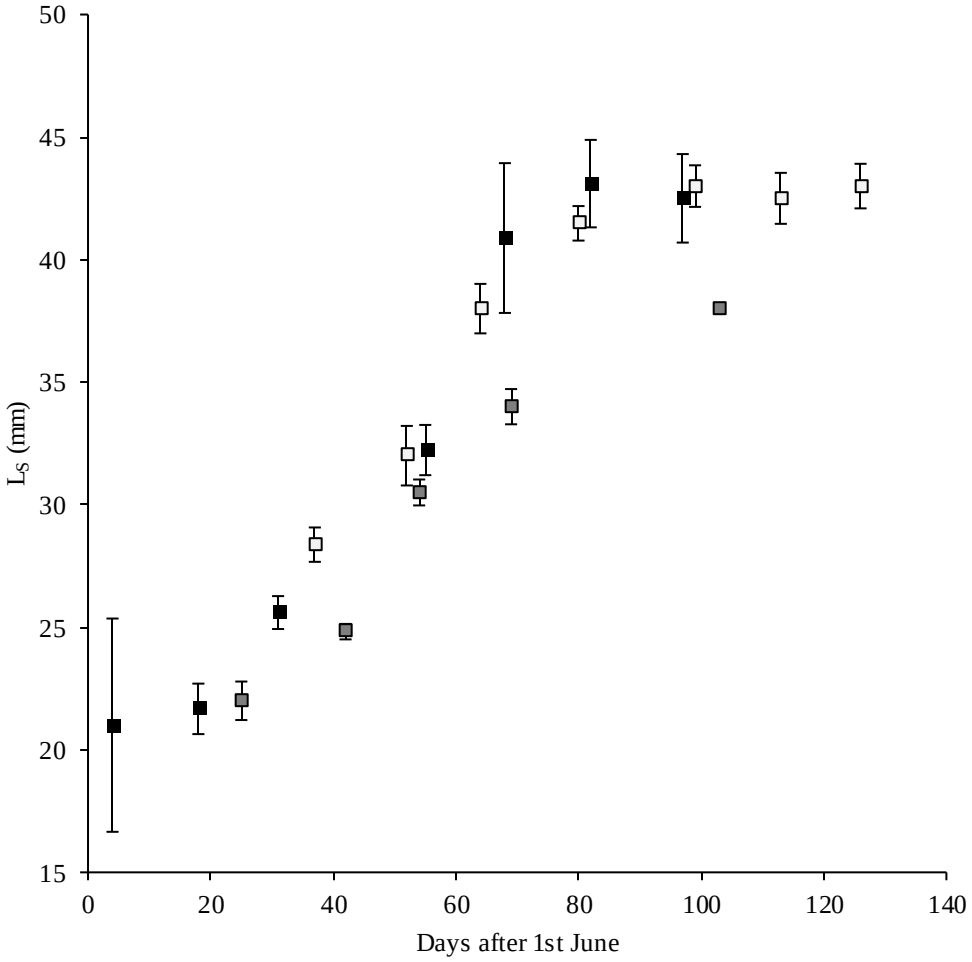

Figure 33. Mean length (± 95% CI) per mode of 0+ *Leuciscus leuciscus* from Site 3, River Teme from 2015 (white), 2016 (grey) and 2017 (black) with 0+ as squares, as identified by Modal Progression Analysis.

3.6 Discussion

The introduction of non-indigenous *B. barbus* into the River Severn and their subsequent dispersal into the River Teme has provided the opportunity, via their spawning strategies, to test the pre-adaptation hypothesis of invasion biology (Ricciardi & Mottiar 2006; Schlaepfer et al. 2010; van Kleunen et al. 2011). It was apparent from across the three sampling years, there was a consistent pattern of *B. barbus* having 0+ fish of < 20 mm regularly appearing in samples collected between June and late August, suggesting an adult spawning period that extended over several weeks (e.g. between May and July). This protracted spawning was also detected in the indigenous populations of *S. cephalus* and *P. phoxinus*, suggesting the non-indigenous *B. barbus* were utilising the same spawning strategy as these two fishes. Whilst this protracted strategy could have been mediated in *B. barbus* by plasticity in their reproductive traits, they utilise similar protracted spawning strategies in their native range. For example, individual adult *B. barbus* in the River Ourthe, Belgium, were detected as spawning as least twice per year (Baras 1995) and in captivity, *B. barbus* can spawn up to 15 times in one year under constant photoperiod and high thermal regimes (Poncin 1992). Moreover, this apparent pre-adaption is not just limited to their spawning strategies, but also includes their somatic growth rates, as there were no significant differences in adult *B. barbus* growth rates between populations in their British indigenous and non-indigenous ranges (Britton et al. 2013). In entirety, these results suggest that the invasion success of *B. barbus* in the study river, and potentially elsewhere in their non-indigenous range, is at

least partially related to their ability to express their life history traits and behaviours in a very similar manner to their indigenous range.

Testing of the pre-adaptation hypothesis has tended to focus on non-indigenous plants, where evidence suggests that where introduced species have traits that lead to a high performance in the native range, these are strong predictors of pre-adaptation as they enable these species to be invasive in the new range (Schlaepfer et al. 2010; van Kleunen et al. 2011). In introduced salmonid fishes, Buoro et al. (2016) suggested that native invaders, such those that result from stockings of hatchery-reared fishes, can result in greater ecological impacts than non-native invaders, a result of the native invaders having traits pre-adapted to their new environments. This enables these fishes to access to resources as per their native conspecifics, resulting in elevated intra-specific competition. Whilst pre-adaption is thus potentially important for some introduced species to be invasive, trait plasticity is also an important adaptive response of introduced fishes to new environments (Gozlan et al. 2010). For example, elevated growth rates and early maturity in individuals within establishing populations are often important in overcoming demographic bottlenecks that relate to low numbers of founders (Britton & Gozlan 2013). Given the non-indigenous *B. barbus* population under study here has been present in the River Teme since at least the 1970s, and were introduced into the River Severn basin in 1956 (Wheeler & Jordan 1990; Antognazza et al. 2016), then plasticity in growth and maturity could also have played an important role in their establishment process (Amat Trigo et al. 2017).

The differences in the spawning strategies between *L. leuciscus* (single spawning, spring) and the other fishes (protracted spawning, early to mid-summer) at least partially relate to differences in the water temperatures and photo-period required for initiating their spawning. For example, spawning in *L. leuciscus* usually commences when water temperatures exceed 10 °C (Kennedy 1969), with this generally occurring between March and April in British rivers (Britton 2007). A gravel spawning species, there are usually no other cyprinid fishes spawning at these times, thus they have minimal competition for spawning substrates and nursery habitats. Thus, a single spawning event could produce sufficient numbers of progeny to ensure some will survive any subsequent deleterious event, especially in conjunction with being able to achieve relatively large body sizes. In contrast, spawning of *B. barbus*, *S. cephalus* and *P. phoxinus* tends to occur at higher water temperatures, when temperatures are at least 11 to 12 °C (Varley 1967, Koç et al 2007, Mills 1987). With these cyprinid species all spawning at similar times (i.e. May to July), and with migratory fishes such as shad *Alosa* spp. and sea lamprey *Petromyzon marinus* also spawning in the River Teme at the same time and locations (Pinder et al. 2016a), then there is, potentially, relatively high competition for spawning substrates and nursery habitats. Thus, protracted spawning events not only increase resilience against deleterious stochastic events from impacting recruitment success, but also potentially reduce intra- and inter-specific competition for spawning and nursery habitats.

Although adult spawning behaviour was not assessed directly here, the length distributions of the 0+ *B. barbus* were generally consistent with the production

of progeny over prolonged periods (Nunn et al., 2002, 2007a,b). Multiple spawning events were also suggested in the data from the indigenous *S. cephalus*. This reproductive behaviour has also been detected in other native *S. cephalus* populations, such as in the River Spree, Germany, where individual adults were observed spawning twice in one spawning season (Fredrich et al., 2003). In the River Trent, England, protracted spawning in both *S. cephalus* and *P. phoxinus* has also suggested from 0+ fish length data (Nunn et al. 2002). Whilst *B. barbus* of above 40 mm were not present from samples collected from the River Teme, it was assumed that this was due to these individuals having attained body sizes that enabled them to utilise mid-channel habitats with stronger flows, as in their native range only when individuals attain body lengths above 50 mm can they withstand water velocities in excess of 10 cm s^{-1} (Bischoff and Freyhof 1999).

An issue with the protracted spawning events of *B. barbus* and *S. cephalus* in the River Teme was that in samples collected in September and October, i.e. at the end of their growth year, individuals were still present in samples at very small body sizes, such as below 20 mm. Thus, 0+ individuals produced late in the protracted spawning event were unable to compensate for their reduced length of the growth season by elevating their growth rates. In *S. cephalus* in other British rivers, this effect has been detected as having a life-long effect in the growth of individuals, with smaller fish at age 1 remaining relatively small for their age throughout life, although they tend to live longer than larger, faster growing individuals (Bolland et al. 2007). These small sizes of 0+ fish at the end of the growth season are also potentially important for recruitment, as length at

the end of the first growth season has been a successful correlate of recruitment strength in some riverine cyprinids, including *S. cephalus* (Nunn et al. 2007a,b). In the River Teme, winter spates can result in river levels increasing by over 4 m in several hours, with concomitant increases in flows (e.g. to over 60 m^3 s^{-1}). In conjunction with negligible off-channel refugia and the ability of fish to hold station in flows being a positive function of their body size (Müller et al., 1996), this suggests that over-winter survival rates of 0+ *B. barbus* at lengths below 20mm might be limited. This, however, remains speculative in the absence of knowledge of how *B. barbus* protracted spawning events translates into lifetime consequences for individuals and cohorts. Notwithstanding, Nunn et al. (2010) demonstrated that in the River Trent, 0+ *S. cephalus* as small as 13 mm can occasionally overwinter successfully (Nunn et al. 2010).

In summary, the protracted spawning events detected in this non-indigenous *B. barbus* population was a strategy also utilised by two native fishes and by *B. barbus* in their native range. Consequently, it was argued that this aspect of the population's invasive behaviour was consistent with the pre-adaption hypothesis of invasion biology. The potential implications of these spawning behaviours were increased resilience of 0+ cohorts from deleterious and stochastic events, and, potentially, reduced inter-specific competition for spawning and nursery habitats, but with individuals at the end of their first growth year often having relatively small body sizes.

4. Diet composition, feeding strategies and trophic niches of 0+ invasive *Barbus barbus* versus native cyprinid fishes

This chapter has been accepted as a manuscript in reduced form as:

Gutmann Roberts C. & Britton JR. Quantifying trophic interactions and niche sizes of juvenile fishes in an invaded cyprinid fish community. *Ecology of Freshwater Fish* (Accepted 2018)

4.1 Abstract

Quantifying the feeding ecology and trophic dynamics of fishes is a fundamental requirement of understanding their ecological interactions. For 0+ fishes, it also assists understanding of their functional ecology in respect of ontogeny. Here, the diet composition and feeding strategy of non-indigenous 0+ *B. barbus* was investigated at three sites along the River Teme, Worcestershire, and in relation to three native 0+ cyprinid fishes: *S. cephalus*, *P. phoxinus* and *L. leuciscus*. Analysis of stomach contents from samples collected between June and September of 2015 and 2016 revealed that the fishes were all generalist in their selectivity of prey items, with most prey having low frequency of selection. For some prey, there were strong relationships with fish length, indicating the importance of ontogenetic development as a key driver of their diet composition. Relationships of diet composition versus body length and gape height were species-specific, suggesting that feeding specialisms increased with ontogeny across the 0+ fish community. Analysis of dietary similarities revealed *B. barbus* diet was significantly dissimilar to *S. cephalus* and *L. leuciscus* and quantification of trophic niche sizes revealed that *B. barbus* also had a smaller niche than the other fishes at two sites, with only *P. phoxinus* having a smaller niche at the other site. These dietary analyses thus revealed that, in general, the diet of non-indigenous 0+ *B. barbus* differed significantly from two recreationally important confamilial fishes, with this potentially facilitating their integration into the 0+ fish assemblage.

4.2 Introduction

Quantifying the feeding ecology and trophic dynamics of fishes is a fundamental requirement of understanding their ecological and functional processes at individual to community levels (Nunn et al. 2007a,b, 2012). The ability to acquire and assimilate prey has potentially substantial impacts on fish growth, survival and recruitment rates, especially early in life when they are highly vulnerable to predation, competition, and environmental perturbations (Mills & Mann, 1985; Houde, 1997; Nunn et al. 2003, 2007a, 2010a). A range of factors regulates the growth and survival of fish in their first year of life (hereafter referred to as 0+ fishes), including their ability to capture and ingest the prey items and sizes available (Nunn et al. 2012). Should there be a lack of available prey then reduced growth rates and/ or starvation can occur, with potentially deleterious consequences for that 0+ cohort (Dickmann et al. 2007; Burrow et al. 2011). Where the 0+ fish community has been invaded by a non-indigenous species then there is also potential for the invader to share resources with native fishes, resulting in reduced access to prey, and subsequent impacts on food acquisition and assimilation, and growth and survival rates (Gozlan et al. 2010; Dick et al. 2014, 2017).

The feeding ecology of mature fishes is relatively well understood, including for temperate riverine cyprinid fishes (e.g. Mann, 1974; Nunn et al. 2012). Extant knowledge includes how plasticity in diet composition can assist the establishment of populations of introduced fishes (Basic et al. 2013; Tran et al. 2015). In contrast, the feeding ecology of fishes early in life, particularly larvae

and juveniles in their first summer of life, is often poorly understood (Nunn et al. 2012), especially within invaded communities (Britton et al. 2009). This is despite ontogenetic shifts in diet often being important for 0+ fish survival (DeVries et al. 1998). In general, most freshwater fishes are planktivorous at the onset of exogenous feeding, with zooplankton being an important larval prey resource (Nunn et al. 2007b, 2010). Thereafter, diets of juvenile riverine cyprinids in temperate regions tend to consist of a mix of cladocerans, copepods and insect larvae, with some species also exploiting adult dipterans and Aufwuchs (the periphyton and associated microfauna that grow on underwater surfaces) (Nunn et al. 2012). However, with the attainment of larger body and gape sizes, there tends to be a shift towards species developing specific dietary traits, often resulting in considerable differences in their diet composition and niche sizes (Nunn et al. 2007b, 2012).

Given the potential importance of attaining relatively larger body lengths for the over-winter survival and subsequent recruitment of 0+ fish in lowland rivers (Mills & Mann, 1985; Nunn et al. 2007a), understanding the feeding ecology of their cohorts in relation to length and ontogenetic stage is also ecologically significant. Whilst there is some knowledge of the feeding ecology of some 0+ riverine cyprinids in temperate lowland rivers, such as the River Trent in Eastern England, these fish communities tend to be dominated by roach *Rutilus rutilus*, with common bream *Abramis brama* and *S. cephalus* also prominent, but with indigenous *B. barbus* (L.) being relatively rare (Nunn et al. 2007b). Moreover, these studies have generally focused on sampling nursery areas in off-channel refuges and backwaters, such as boat marinas and connected side-channels

(Nunn et al. 2007b, 2010). The negligible flow of these habitats is advantageous as it prevents downstream displacement of the 0+ fishes, as there is a positive relationship of swimming ability with ontogenetic development (e.g. fin development) and body size (Keckeis et al. 2001). In contrast, where rivers have negligible off-channel habitats for 0+ fish then their nursery areas are limited to in-channel areas of relatively low flows, restricting the areas of suitable nursery habitat available and, consequently, potentially resulting in elevated competitive interactions within the 0+ fish community, especially if that community has been invaded by non-indigenous fishes.

Consequently, the aim of this Chapter was to quantify the diet composition of a community of 0+ cyprinid fishes invaded by *B. barbus* and where nursery habitats were restricted to in-channel habitats, using the River Teme as the study river. Applying stomach contents analyses (SCA) (Hyslop 1980) on samples collected in 2015 and 2016, the objectives were to: (1) quantify diet composition across the community of 0+ fishes, with assessment of inter-specific similarity and spatio-temporal patterns; (2) identify dietary shifts within each species and test these in relation to body length, gape size and ontogenetic development; and (3) quantify trophic niche sizes per species and according to ontogeny, with assessment of the extent of inter-specific niche overlap. It was predicted that the limited 0+ fish habitat and consequent limited opportunity for trophic niche partitioning would result in high dietary overlap between the species and result in high levels inter-specific competitive interactions. In the study, 'trophic niche' describes the diversity of diet composition of the group of fish being analysed; it can also be considered as the binomic axis of the ecological niche (Hutchinson 1978).

4.3 Materials and Methods

4.3.1 Sampling sites and methodology

The three sampling sites used for the study were Sites 1 to 3, as described in Section 3.3.1 and Figure 9. The sampling methodology and frequency was as described in Section 3.3.2. To provide inter-annual comparisons in diet, the sampling periods were July to September 2015 and June to September 2016 (Section 3.3.2; Table 10, 11). The diet of 0+ fishes was not assessed in winter due to elevated river levels that prevented safe access to sampling sites and thus no samples could be collected. The samples collated in 2015 were the primary data source for the study. The reduced number of fish analysed from samples collected in 2016 were used primarily as supplementary samples that enabled inter-annual comparisons of diet to be made across the fishes. Therefore, unless stated otherwise, dietary analyses were completed only on 2015 data.

Following each sampling occasion, the sampled 0+ fish were euthanised (MS222) before preservation in 70 % IMS. They were held at 5 ^{o}C prior to their processing in the laboratory.

4.3.2 Sample processing and data collection

In the laboratory, each fish was identified to species (Pinder 2001) and measured using digital callipers (standard length, L_S, to 0.01 mm). The ontogenetic stage was assessed under a dissecting microscope (x5 to x10 magnification) and was classified as larval stages 1 to 5 or juvenile stage 6 to 9, as per Pinder (2001), Simonović (1999) and Krupka (1988) (Appendix 1; Table A1). Gape height was

then measured as the height of the mouth when open at its widest angle (Lukoschek and McCormick 2001, Nunn et al. 2007), with this recorded using a stage micro-meter in combination with a pair of watchmaker's forceps and a hypodermic needle.

A maximum of 20 fish per site per sample date were dissected. A maximum of 30 were dissected per site per sample for *B. barbus*, as they were spread across more larval stages than the other fishes and so ensured greater balance in sample sizes across the juvenile stages. The initial step to remove the intestine (hereafter referred to as the 'gut'), with gut fullness (%) then estimated before the total gut contents were extracted, mounted on a glass slide and fixed using polyvinyl alcohol-lactic acid-glycerol (PVLG), with this mixed evenly using a hypodermic needle. The prey items were then identified to the lowest practicable taxonomic level using microscopy (to x100 magnification). Diatoms and similar material that was too small to identify were classed as 'Aufwuchs'. Some 0+ fish dietary studies have categorised the amount of Aufwuchs in juvenile fish guts on either a 0 (none) to 3 or 4 (full of Aufwuchs) scale (e.g. Garner 1996, Mann et al. 1997). Conversely, Nunn et al. (2007) estimated a numerical value based on the volume of Aufwuch versus non-Aufwuch prey items. Here, Aufwuchs were recorded in samples collected in 2015 as the estimated percentage cover of the cover slide area and then converted to numbers on a 0 to 5 scale using an exponential categories 0 (0 to 1 %), 1 (2 to 3 %), 2 (4 to 7 %), 3 (8 to 20 %), 4 (21 to 55 %) and 5 (56 to 100. A large number of prey items were encountered in the intestines.

For analytical purposes in prey frequencies in diet and feeding strategies, these were categorised into the following 16 groups according to their taxonomy and functional ecology: Chironomid larvae, Aufwuchs, amphipods, winged insects, chalcid wasp, copepods, Cladocera, nymphs (stonefly and mayfly), Arachindae, Hemipteroids, saucer bugs, caddis larvae, beetles, beetle larvae, springtail (hexapods), seed/ spore/ plant material, and fish. Maximum prey size was measured using an eyepiece graticule; for Chironomid larvae this always consisted on measuring the width of the head. During dissections, infections of the intestinal parasite *Pomphorhynchus* spp. was detected in some fishes, with the amphipods of the *Gammarus* genus (*Gammarus* spp. hereafter) being their intermediate host.

4.3.3 Data analysis

Overview

One-way ANOVA with a Tukey post-hoc test was used to initially test for differences in fish standard length between the sites. A range of methods (described below) were then used to analyse the dietary data, usually for each species site and sampling date. The vacuity index (%I_v) (i.e. the proportion of fish with empty guts) was calculated from: $\%I_v = S_0 S_1^{-1}$, where S_0 is the number of fish with empty guts and S_1 is the total number of larval and juvenile fish stomachs examined (Hyslop 1980).

Prey frequencies in diet

Frequency of occurrence for prey categories (F_i) represents the proportion of all guts that contain that prey category and was determined from: $F_i = N_i N^{-1}$, where

N_i is the number of guts in which that prey item i occurred and N is the total number of guts with prey present (Caillet 1977). Relative abundance of a given prey category ($\%A_i$) represents the proportion of total gut contents from all fish that comprised that prey category and was calculated from: $\%A_i = 100(\Sigma S_i S_t^{-1})$, where S_i is the number of prey items comprising prey i and S_t is the total number of prey in all guts regardless of whether they contained prey item i (Macdonald & Green 1983). Prey-specific abundance (P_i) represents the proportion of all prey that was comprised of a specific prey category and was calculated from data collated from only the guts in which prey items in that category were encountered. It was calculated from: $P_i = 100\Sigma S_i \Sigma S_{ti}^{-1}$ where P is the number of prey items comprising prey i and S_{ti} is the total number of prey items in guts that contained prey item i (Amundsen *et al.* 1996).

Feeding strategies

The calculation of frequency of occurrence and prey-specific abundance enabled feeding strategy plots to be produced (Costello 1990). These plots provided information about prey importance and feeding strategies of each species via examination of the distribution of points along the diagonals and the axes of the plot according to: prey importance (represented in the diagonal from the lower left (rare prey) to upper right (dominant prey), feeding strategy (represented in the vertical axis from the bottom (generalization) to top (specialization), and the relationship between feeding strategy and the between or within-phenotype contributions to the niche width (represented in the diagonal from the lower right (high within-phenotype component, WPC) to upper left (high between-phenotype component, BPC) (Amundsen et al. 1996; Leunda et al. 2008). To

test the assumption that fish with larger body sizes would consume different prey items to smaller conspecifics, and that smaller prey items may be selected against, linear regression was used with standard length as the independent factor and the percentage of specific prey items as the dependant factor. Where assumptions for the test were not met, the percentage of prey was transformed (natural logarithms).

Fish length versus gape height relationships and maximum prey size

Testing for spatial and temporal differences in gape height (µm) and standard length (mm) of the fishes then used generalised linear models with Gaussian error structure, where gape height or standard length was the dependent variable and the independent variables were year, site and species. To identify how ontogenetic stage, body length and gape height influenced the maximum prey size of each species, stepwise multiple regression was used to determine which of these variables explained most of the variability in the data. Differences in the maximum prey size per species were also tested using a generalised linear model but with a Gamma log link function. Maximum prey size was the dependent variable, species was the independent variable and standard length was the covariate. This model structure was also used to test differences in maximum prey sizes according to sampling year and site. The results of each generalised linear model were the significance of the independent variable and covariate on the dependent variable, the estimated marginal means (i.e. mean values per group, adjusted for effect of covariate), and linearly independent pairwise comparisons with Bonferroni adjustment for multiple comparisons.

Gape height versus trophic niche

The original scale of the stage micro-meter used to measure gape height was converted to µm using Equation 19. Therefore, these data were categorical rather than continuous data and so for subsequent ordination analyses, gape height was classified into five groups (Table 12). The gape range of *B. barbus* was 0.8 to 3.1 mm and as the relatively large gape of *L. leuciscus* had few fish overlapping with this range then two additional size classes were included above 3.1 mm. However, gape sizes above 4.8 mm were excluded from analyses as these were considered as not being ecologically relevant in comparisons with *B. barbus*.

$$y = 280x + 280 \qquad \text{(Equation 19)}$$

Table 12. Original stage micrometer units, their conversions into mm and their categorisation into five gape height size ranges, and the 0+ fishes in those gape height ranges that were used in subsequent ordination analyses.

Stage micro-meter units	2 to 4	5 to 7	8 to 10	11 to 13	14 to 16
Gape height (mm)	0.8 to 1.4	1.6 to 2.2	2.5 to 3.1	3.3 to 3.9	4.2 to 4.8
Species within gape height range	*B. barbus* *S. cephalus*	*B. barbus* *S. cephalus* *P. phoxinus*	*B. barbus* *S. cephalus* *L. leuciscus* *P. phoxinus*	*S. cephalus* *L. leuciscus* *P. phoxinus*	*S. cephalus* *L. leuciscus* *P. phoxinus*

To identify how the size of the trophic niche varied by gape height per species, dietary breadth was expressed as standard deviation ellipses (40%) that were calculated using de-trended correspondence analysis with basic reciprocal averaging, with this completed using the 'decorana' function in 'vegan' package

v2.4 in R (R Core Team 2016). This was completed within a Bray-Curtis similarity matrix where all data were square root transformed for normality (as they were percentages). Ellipse areas then compared the gape height classes of each species to test whether the size of the trophic niche increased as gape height increased.

Inter-annual, inter-specific and spatial similarities in diet composition

To determine the similarity of diets between sampling years, species, ontogenetic stages, sites and date of capture, a range of analyses were completed in PRIMER 7. All vacuous guts, and guts containing only diatoms were removed from the dataset prior to these analyses, plus three dietary items that only occurred once (fish, gastropod and worm). All prey items were included in their original form and not grouped as categories, to enable assessment of whether fish specialised on certain items within groups or if certain items were more abundant in particular sites or between years. As dietary composition data were expressed as percentages, they were square root transformed, followed by construction of a resemblance matrix with Bray-Curtis similarity that enabled analysis of similarities (ANOSIM) to be calculated between years, species, ontogenetic stages and sites. Where the results indicated there were significant dissimilarities between the independent variables then Similarity Percentages analysis (SIMPER) was used to determine the discriminatory dietary items.

To identify how the size of the trophic niche varied by species at each site, dietary breadth was expressed as standard deviation ellipses (40%) using the same methodology as outlined above in the 'decorana' function in 'vegan'

package v2.4 in R (R Core Team 2016), only 2015 data were used for this analysis to eliminate any inter-annual variations. For species comparison between sites only fish in the size range of *B. barbus* were chosen (12.3 – 37.6 mm), to account for the site variations in size and to ensure fish of similar sizes were being compared.

4.4 Results

4.4.1 Overview

Across the four 0+ species, stomach contents analysis was completed on 878 fish in 2015, with this reduced to 206 in 2016, given the focus was primarily on analysis of 2015 samples (2015, 2016 sample numbers: *B. barbus*: n = 431, 93; *S. cephalus*: n = 174, 40; *L. leuciscus*: n = 81, 30; *P. phoxinus*: n = 192, 43). Across all the samples, there were no fish that were identified at larval stage L1 and, as there was only one fish at larval stage 2, this individual was removed from subsequent analyses (Table 13, 14). As there were low numbers of fish sampled at larval stages 3 to 5, these fish were then grouped as 'larvae'. There were relatively high numbers of fishes in juvenile stages 6 to 9 across the samples and these were all analysed together as 'juveniles' (Table 13, 14).

Across the dataset, the standard length of *B. barbus* was significantly different between sites in 2015 (ANOVA; $F_{2,428} = 3.97$, $P = 0.02$, Table 15), with fish at Site 1 being significantly larger than those at Site 2. Similarly, *S. cephalus* at Site 2 in 2015 were significantly smaller than the other sites (ANOVA; $F_{2, 156} = 8.87$, $P < 0.001$, Table 15). *Phoxinus phoxinus* were significantly smaller at Site

3 than the other sites (ANOVA; $F_{2, 174} = 17.9$, $P < 0.001$, Table 15). As *L. leuciscus* was only sampled at Site 3 then no spatial comparisons were possible. In 2015, vacuity indices were low, with the highest values in *S. cephalus* (4 to 6 %) and lowest in *B. barbus* (0 to 0.6 %, Table 16). The vacuity index in 2016 was higher for *P. phoxinus* with 26 % but still low for *S. cephalus* (3 %), *B. barbus* (1 %) and *L. leuciscus* (0 %).

Table 13. Number (n) of larval / juvenile fish utilised for dietary analysis for 0+ fish in 2015 (*Barbus barbus, Squalius cephalus, Phoxinus phoxinus* and *Leuciscus leuciscus*) at Sites 1, 2 and 3. Fish were classed as larval stages L3, L4, L5 or juvenile (J).

	Site	Survey date	n	L3	L4	L5	J
B. barbus		07/07	19	4	4	10	1
		23/07	30		8	4	18
	1	04/08	30			2	28
		20/08	30				30
		08/09	30				30
		TOTAL	**139**	**4**	**12**	**16**	**107**
		08/07	30		1	29	
		23/07	30				30
	2	04/08	30				30
		20/08	30				30
		08/09	30				30
		TOTAL	**150**		**1**	**29**	**120**
		08/07	30		2	18	10
		23/07	30			2	28
	3	04/08	30		1	1	28
		20/08	30				30
		08/09	14				14
		TOTAL	**134**		**3**	**21**	**110**
S. cephalus		07/07	11		5	6	
	1	04/08	20			4	16
		08/09	20				20
		TOTAL	**51**		**5**	**10**	**36**
		08/07	20		4	16	
	2	04/08	15		1		14
		08/09	18				18
		TOTAL	**53**		**5**	**16**	**32**
		08/07	4			4	
		04/08	20				20
	3	08/09	20			1	19
		05/10	20				20
		TOTAL	**64**			**5**	**59**
P. phoxinus		07/07	20				20
	1	04/08	20				20
		08/09	20				20
		TOTAL	**60**				**60**
		08/07	20				20
	2	04/08	20				20
		08/09	20				20
		TOTAL	**60**				**60**
		08/07	11				11
		04/08	20				20
	3	08/09	20				20
		05/10	20				20
		TOTAL	**71**				**71**
L. leuciscus		08/07	20				20
		04/08	20				20
	3	08/09	20				20
		05/10	20				20
		TOTAL	**80**				**80**

Table 14. Number (n) of larval / juvenile fish utilised for dietary analysis for 0+ fish in 2016 (*Barbus barbus, Squalius cephalus, Phoxinus phoxinus* and *Leuciscus leuciscus*) at Sites 1, 2 and 3. Fish were classed as larval stages L2, L3, L4, L5 or juvenile (J).

	Site	Survey date	n	L2	L3	L4	L5	J
B. barbus	1	28/07	17	1	5	4	1	6
		25/08	10				3	7
		TOTAL	**27**					
	2	26/06	10			10		
		08/07	5				5	
		28/07	10					10
		25/08	11				1	10
		01/10	10					10
		TOTAL	**46**					
	3	25/08	10					10
		12/09	10					10
		TOTAL	**20**					
S. cephalus	2	26/06	10					10
		28/07	10					10
		25/08	10			1		9
		01/10	10					10
		TOTAL	**40**					
P. phoxinus	3	29/06	10					10
		25/07	11					10
		25/08	12					10
		12/09	10					10
		TOTAL	**43**					
L. leuciscus	3	29/06	10					10
		25/07	10					10
		12/09	10					10
		TOTAL	**30**					

Table. 15. Sample size (n), standard length range (Min L_S/ Max L_S) and mean standard length (mm) (± 95% confidence intervals) for *Barbus barbus*, *Squalius cephalus*, *Leuciscus leuciscus* and *Phoxinus phoxinus* between years.

	n	Min L_S (mm)	Max L_S (mm)	Mean L_S (mm)
B. barbus	517	12.3	37.6	21.5 ± 0.45
2015	427	12.3	36.8	21.7 ±0.49
2016	93	12.3	37.6	20.5 ±1.09
S. cephalus	183	11.2	35.7	19.9 ± 0.72
2015	147	11.2	33.9	19.7 ± 0.75
2016	36	12.7	35.7	21.0 ± 1.94
L. leuciscus	107	20.1	48.9	35.3 ± 1.42
2015	77	23.7	48.9	37.2 ± 1.46
2016	30	20.1	47.2	30.6 ± 2.77
P. phoxinus	168	12.7	40.4	22.4 ± 0.79
2015	142	12.7	33.8	21.4 ± 0.71
2016	26	13.3	40.4	28.4 ± 2.32

4.4.2 Relative frequency of prey

The following prey items were found across the four fish species stomachs; Chironomid larvae and adults (Chironomidae), fly larvae (Diptera), amphipods (*Gammarus spp.*), beetle larvae and adults (Coleoptera), mayfly nymphs and adults (Ephemeroptera), stonefly nymphs and adults (Plecoptera), dragonfly nymphs (Odonata), caddisfly nymphs (Tricoptera), snail adults and larvae (Gastropoda), unidentified fliers, chalcid wasps (Chalcidoidea), water treaders (Mesoveliidae), other water bugs (Hemiptera), water striders (Gerridae), saucer bug (Naucoridae), water mite (Hydrachnidia), water spider (Arachnid), booklice (Psocoptera), thrip (Thysanoptera), springtail (Collembola), water fleas (Cladocera), seed shrimp (Ostracoda), free-living copepods (Copepoda), rotifer (Rotifera), worms (Annelida) plant material, moss, seed/ spores, invertebrate eggs, fish and unidentified non-fliers.

According to relative frequency, Chironomid larvae were the most important prey item across the species, with values ranging between 44 % (*S. cephalus*) and 83 % (*B. barbus*) (Table 16). Aufwuchs were also a generally important item, being the second most abundant prey item for *P. phoxinus* (34 %) and the third most abundant prey item for *B. barbus* (6 %), *L. leuciscus* (10 %) and *S. cephalus* (15 %) (Table 16). There was some variability between species with, for example, Hemipteroids comprising of 7 % and 24 % of the diet of *S. cephalus* and *L. leuciscus* respectively, but less than 1 % for both *B. barbus* and *P. phoxinus* (Table 16). *Gammarus* spp. were found in the guts of all species but only in 12 individual fishes (1 % of the total sample), yet 13 % of all analysed fish (covering all species) had infections of *Pomphorhynchus* spp., suggesting

that *Gammarus* spp. were under-represented in the dietary analyses. The minimum gape height of *Pomphorhynchus* infected fish was 1.7 mm.

Spatially, there was low variability in the relative frequencies of prey items in *B. barbus* diet, with Chironomid larvae being the dominant item at all sites (S1: 90 %, S2: 76 % , S3: 80 %; Table 16). For Aufwuchs, values ranged between 4 and 14 %, and for *Cladocera* spp., between 2 and 11 % (Table 15). In contrast, prey items in *S. cephalus* had greater spatial variability, including in the proportion of hemipteroids (1 % at Site 3, > 10 % at other sites) and saucer bugs (0 % at Site 1 to 12 % at Site 2) (Table 16). For *P. phoxinus*, the major spatial differences were in the proportions of Chironomid larvae and Aufwuchs, although in the combination they comprised between 85 and 94 % of diet (Table 16). Chironomid larvae were more prominent at both Site 3 (65 %) and Site 1 (64 %), but Aufwuchs were more prominent at Site 2 (54 %) (Table 16).

4.4.3 Feeding strategies

Feeding strategy plots at the species level revealed all the fishes were relatively general in their diets, with the majority of prey items having prey specific abundances of < 50 % and relatively low frequency of occurrences (Fig. 34). The dominance of Chironomid larvae across the diet of each species was strongly reflected in the feeding strategy plots, where their prey specific abundances ranged between 52 and 83 % (Fig. 34). The most varied diet was in *L. leuciscus*, which comprised of 17 identified items, with the majority of these having low frequency of occurrence and low prey specific abundance (Fig. 34). Spatially, there was little variability in the feeding strategy plots for *B. barbus*

(Fig. 35), but with greater variability apparent for *P. phoxinus* and *S. cephalus* (Fig. 36, 37). At Site 2, *S. cephalus* appeared to have a more specialist diet than at the other sites, with this largely due to the high prey specific abundance of copepod, chalcid wasp and saucer bugs (Fig. 36). At Site 1, *P. phoxinus* only utilised four prey categories compared to nine at the two other sites (Fig. 37).

4.4.4 Size selection of specific prey items

There were some significant relationships between fish standard length and the proportion of specific prey items in diet, although these varied with fish species (Fig. 38 to 42). For example, the proportion of Aufwuchs decreased significantly with fish length for *B. barbus* (R^2 = 0.38, $F_{1,363}$ = 218.48; P < 0.001, Appendix 1; Fig. A1), increased significantly with fish length for *S. cephalus* and *L. leuciscus* (R^2 = 0.26, $F_{1,69}$ = 24.77, P < 0.001, Appendix 1; Fig. A2; R^2 = 0.16, $F_{1,78}$ = 14.43; P < 0.001, Appendix 1; Fig. A4 and Table 45 respectively), but showed little change over the lengths of *P. phoxinus* (R^2 = 0.00, $F_{1,173}$ = 0.06; P = 0.81, Appendix 1; Fig. A3). There was a significant relationship between the proportion of Hemiptera and increased *L. leuciscus* standard length (R^2 = 0.55, $F_{1,25}$ = 29.26; P < 0.001; Appendix 1; Fig. A4 and Table A2), and with significant decreases in chironomid larvae as length increased in *S. cephalus* (R^2 = 0.05, $F_{1,100}$ = 4.62; P = 0.03 Appendix 1; Fig A2).

Table 16. Relative frequency (%) of prey items, vacuity index (%I_v) and mean standard length (mm; ± 95% CI) for 0+ fishes in 2015 at Sites 1, 2 and 3.

	S. cephalus				*B. barbus*				*P. phoxinus*				*L. leuciscus*
Prey items	1	2	3	Total	1	2	3	Total	1	2	3	Total	3
Chironomid larvae	32.3	20.6	59.5	43.5	80.4	75.7	90.1	83.3	64.0	31.0	65.4	57.7	51.8
Aufwuchs	15.6	3.9	19.3	15.4	3.8	13.7	4.6	5.9	29.5	54.4	27.2	33.7	10.3
Amphipods	0	0	0	0	0	0	0	0	0	0	0.2	0.1	0
Winged insects	22.8	40.2	7.4	18.2	1.5	1.0	0.9	1.1	4.2	10.2	1.8	4.4	6.8
Chalcid wasp	0	0.5	0.3	0.2	0	0	0	0	0	0	0	0	1.6
Copepod	2.3	2.0	5.2	3.7	2.3	2.0	1.8	2.0	0	0.4	0.4	0.2	0
Cladocera	5.3	0.5	3.4	3.6	11.1	6.0	1.8	6.5	0	0.4	0.4	0.3	0.8
Nymph	0.2	2.0	0	0.4	0.2	0.2	0.2	0.2	0	0.4	0.2	0.2	0.4
Water arachnids	7.9	2.5	0.3	3.3	0.2	0.4	0.3	0.3	2.2	0.9	0	1.0	0.3
Hemipteroid assemblage	10.9	13.7	1.2	6.7	0	0	0	0	0	0	0.2	0.1	24.3
Saucer bug	0	12.3	2.8	3.4	0	0	0	0	0	0	0	0	1.9
Caddisfly larva	2.3	2.0	0.3	1.3	0.5	0.8	0.3	0.4	0	1.8	0.2	0.5	0.4
Beetle	0	0	0	0	0	0	0	0	0	0.4	0	0.1	0
Beetle larvae	0.2	0	0	0.1	0.1	0.3	0.1	0.1	0	0	0	0	0.1
Springtail	0	0	0.3	0.2	0	0	0	0	0	0	0	0	0.5
Seed/spore/plant	0	0	0	0	0	0	0	0	0	0	4.2	1.8	0.3
Fish	0	0	0	0	0	0	0	0	0	0	0	0	0.1
%I_v	6.0	5.6	4.3	5.2	0	0	0.6	0.2	0	2.0	2.8	1.6	1.2
Mean L_S (mm)	20.5 ±1.5	17.6 ±1.2	21.0 ±0.8	19.8 ±0.7	22.6 ±0.9	20.9 ±0.7	21.3 ±0.9	21.6 ±0.5	22.5 ±1.2	23.1 ±0.8	19.1 ±0.9	21.5 ±0.6	27.4 ±1.4

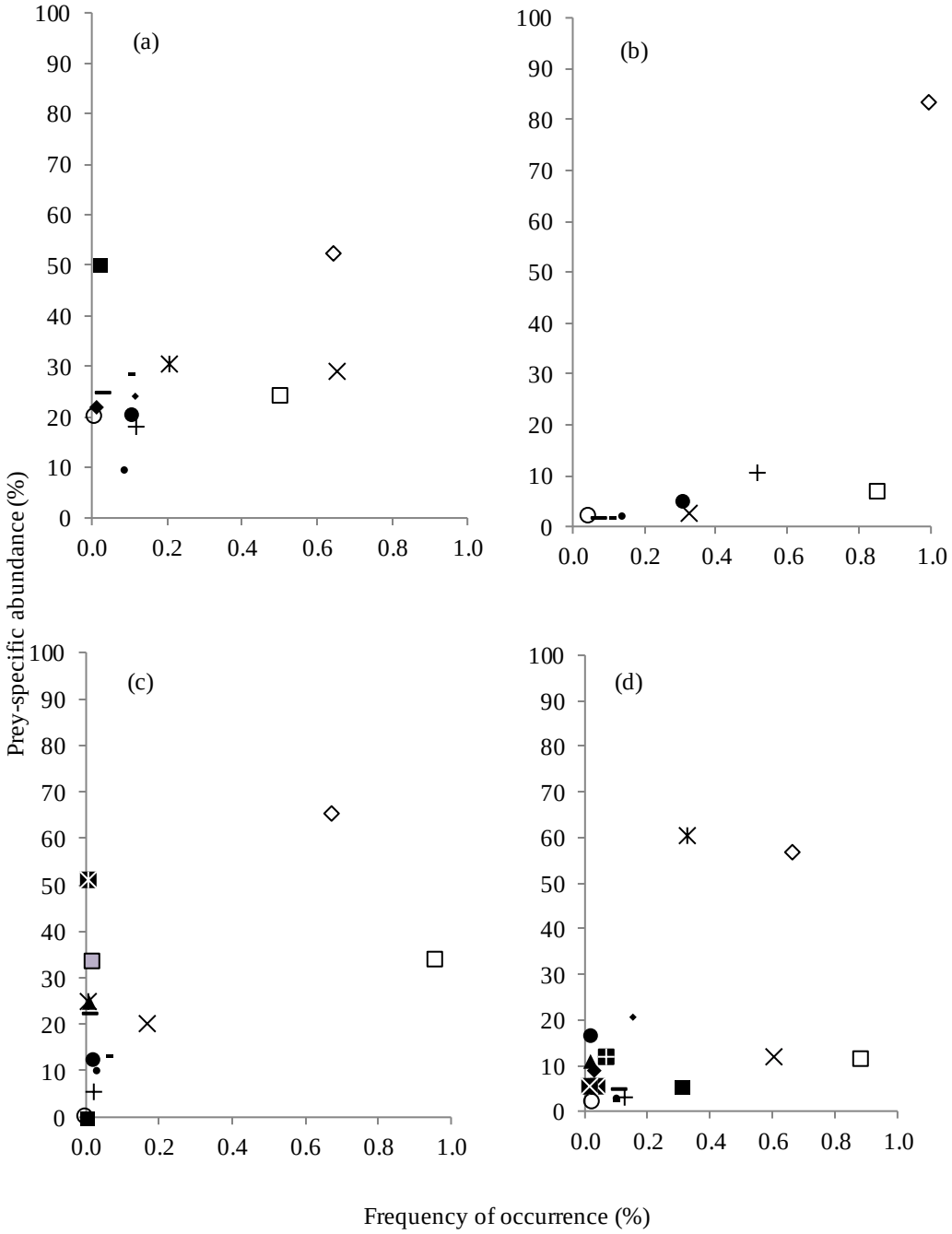

Figure 34. Feeding strategy plots for four 0+ fishes from the River Teme, where (a) *Squalius cephalus*, (b) *Barbus barbus*, (c) *Phoxinus phoxinus* and (d) *Leuciscus leuciscus*. Data combined across sites. Points represent prey categories: Aufwuchs (□); chironomid larvae (◊); amphipod (▣); winged insects (×); chalcid wasp (■); copepod (●); Cladocera (+); nymphs (▬); water arachnids (-); hemipteroid assemblage (✳); saucer bug (♦); caddisfly larvae (●); beetle (▲); beetle larvae (○); springtail (♦); seed/ spore (▩); fish (⊠) and UnID (✚)

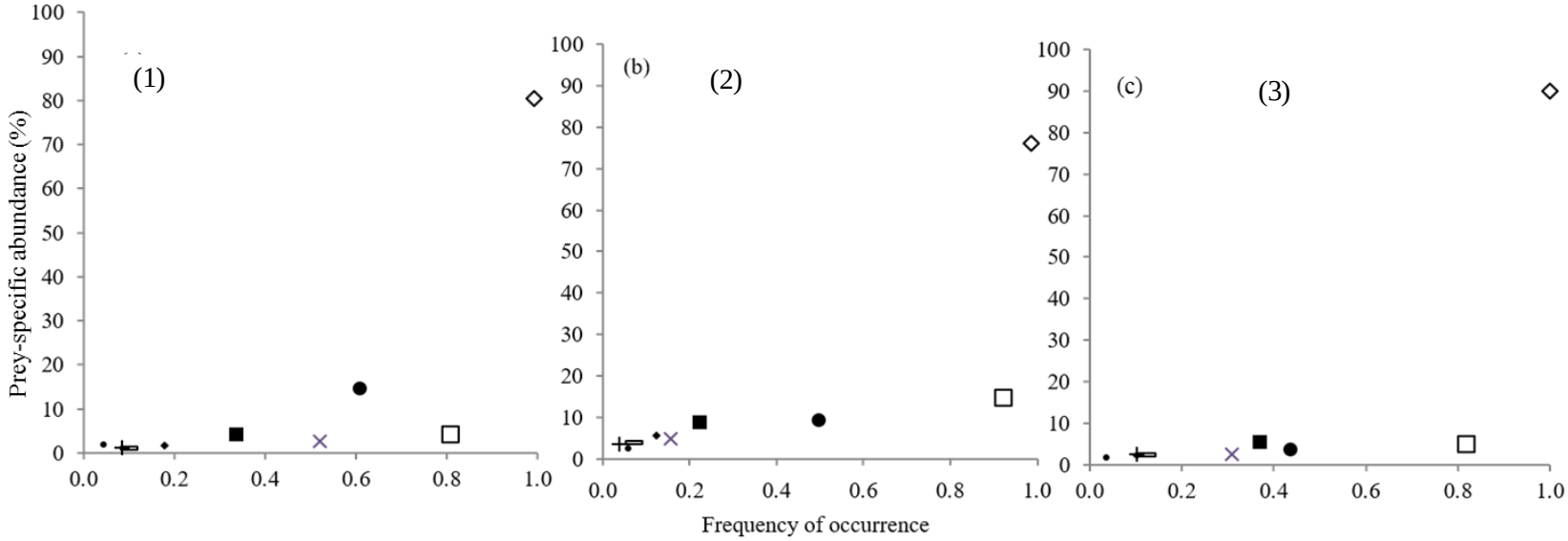

Figure 35. Feeding strategy plots for 0+ *Barbus barbus* at Sites 1 to 3. Points represent prey categories: Aufwuchs (□); chironomid larvae (◊); winged insects (×); copepod (■); Cladocera (●); nymphs (+); water arachnids (▬); caddisfly larvae (♦) and beetle larvae (●)

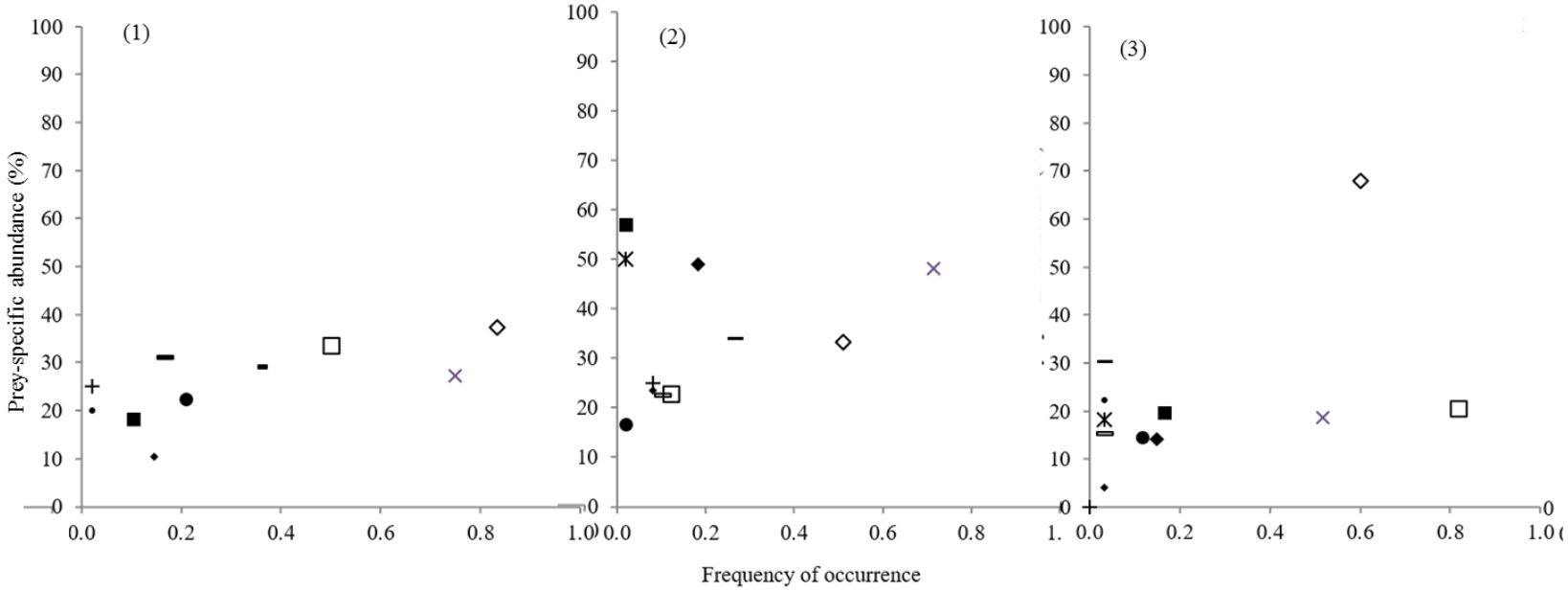

Figure 36. Feeding strategy plots for 0+ *Squalius cephalus* at Sites 1 to 3. Points represent prey categories: Aufwuchs (□); chironomid larvae (◊); winged insects (×); copepod (■); Cladocera (●); nymphs (+); water arachnids (▬); caddisfly larvae (♦); beetle larvae (●); hemipteroid assemblage (-); chalcid wasp (✳) and saucer bug (♦)

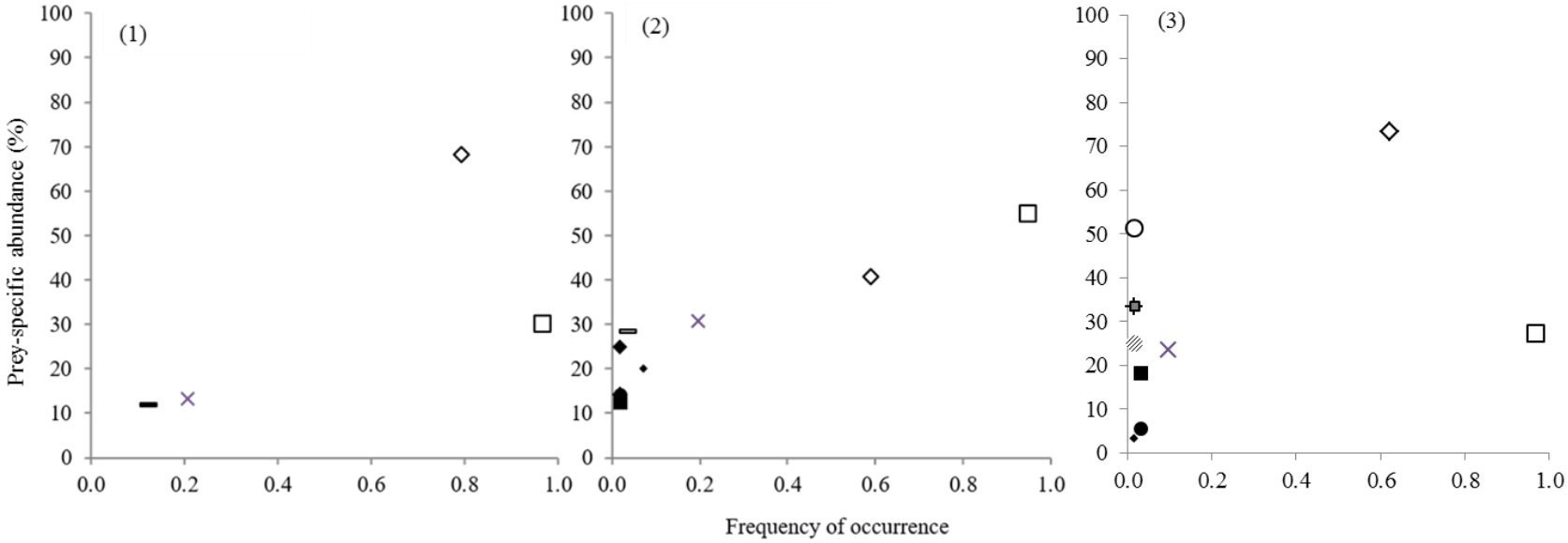

Figure 37. Feeding strategy plots for 0+ *Phoxinus phoxinus* at Sites 1 to 3. Points represent prey categories: Aufwuchs (□); chironomid larvae (◊); amphipod (▩); winged insects (×); copepod (■); Cladocera (●); nymphs (+); water arachnids (▬); caddisfly larvae (⬩); beetle (♦); hemipteroid assemblage (▨); seed/spore (○)

4.4.5 Fish length versus gape height relationships

The relationship of gape height versus fish length was significant for each species ($P < 0.001$; Table 17; Fig. 38). Between the species, there were significant differences in gape height (µm) (GLM: Wald $\chi^2 = 1080.84$, df = 3, $P < 0.01$; Table 18), where the effect of fish length as a covariate was significant ($P < 0.01$).

Table 17. Linear relationship between gape height (µm) and standard length (mm) for *Barbus barbus, Squalius cephalus, Leuciscus leuciscus* and *Phoxinus phoxinus*

	R^2	ANOVA
B. barbus	0.81	$F_{1,515} = 2247.0$, $P < 0.001$
S. cephalus	0.86	$F_{1,185} = 1095.0$, $P < 0.001$
L. leuciscus	0.89	$F_{1,106} = 738.4$, $P < 0.001$
P. phoxinus	0.73	$F_{1,158} = 435.4$, $P < 0.001$

Table 18. Mean gape height (GH) per species, adjusted for the significant effect of standard length as a covariate (fixed at 22.9 mm), for *Barbus barbus, Squalius cephalus, Leuciscus leuciscus* and *Phoxinus phoxinus*. *P* values from pairwise comparisons with *B. barbus*.

	n	Mean adjusted GH (µm) ± 95%CL	Significance of difference in GH with *B. barbus* (*P*)
B. barbus	517	2023	-
S. cephalus	187	2806	< 0.001
L. leuciscus	108	2379	< 0.001
P. phoxinus	157	2820	< 0.001

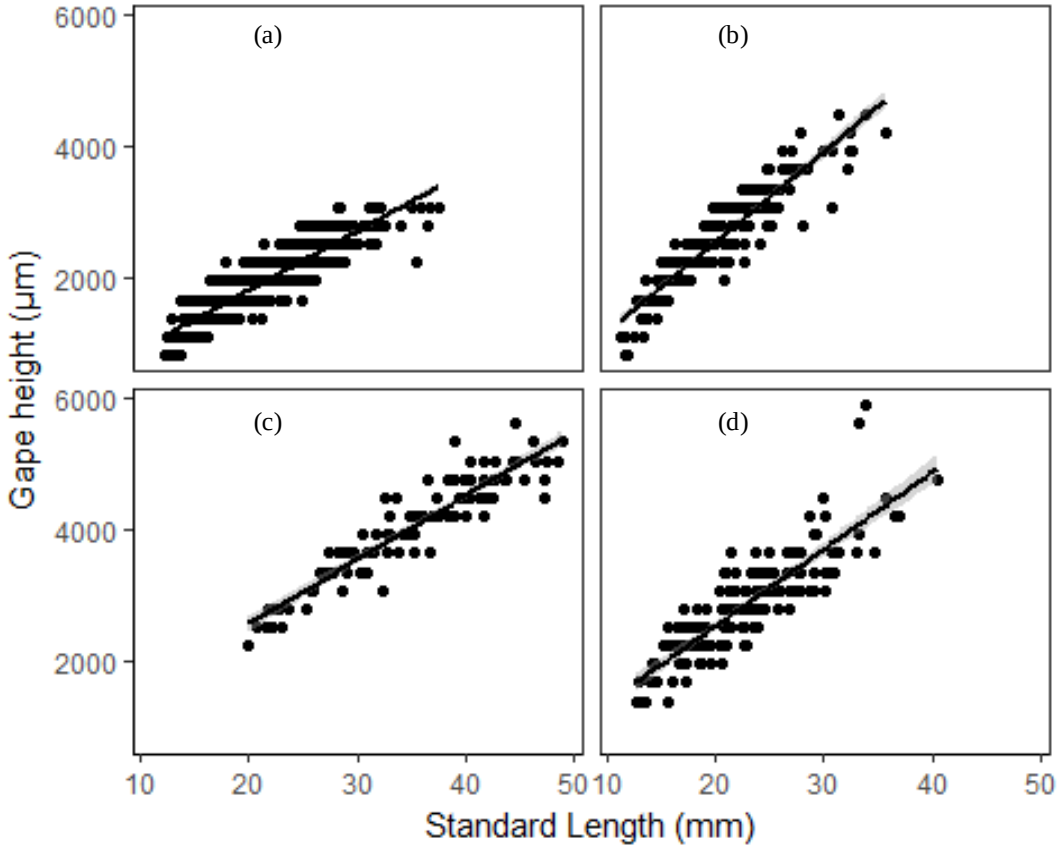

Figure 38. Relationships between standard length (mm) and gape height (µm) for (a) *Barbus barbus*, (b) *Squalius cephalus*, (c) *Leuciscus leuciscus*, (d) *Phoxinus phoxinus*; solid black lines represent the significant relationship according to linear regression between the variables ($P < 0.001$ in all cases; Table 17). Shaded grey area: 95% CI.

4.4.6 Maximum prey size

Maximum prey sizes differed significantly between the 0+ fishes (GLM: Wald $\chi^2 = 197.12$, df = 3, $P < 0.001$), where there was a significant effect of standard length as the covariate in the GLM ($P < 0.01$) (Table 19). The relationships between fish length, ontogenetic stage and gape size versus maximum prey size also differed between species, but the effect of ontogenetic stage was not significant for any species. In *B. barbus*, body length was the only significant variable explaining the variation in maximum prey size ($R^2 = 0.09$, $F_{1,515} = 53.23$, $P < 0.001$, Table 20, Fig. 40a). In *S. cephalus*, maximum prey size was only significantly related to gape height (Table 20, Fig. 39b). Both gape height and standard length significantly affected maximum prey size for *L. leuciscus* (Table 20, Fig. 39c, 40c). In *P. phoxinus*, however, fish length and gape height were not significantly related to maximum prey size taken (Table 20, Fig. 39d, 40d), with juvenile fish consuming much smaller prey than was feasible for the size of their gape (Fig. 39d).

Table 19. Mean maximum prey size (µm), adjusted for standard length (mm ± 95 confidence limits; L_S fixed at 22.9 mm) for *Barbus barbus*, *Squalius cephalus*, *Leuciscus leuciscus* and *Phoxinus phoxinus*. *P* values from pairwise comparisons with *B. barbus*.

Species	Mean adjusted maximum prey size (µm) ± CL	*P*
B. barbus	512.5 ± 20.4	-
S. cephalus	668.3 ± 45.8	<0.01
L. leuciscus	535.1 ± 59.6	0.47
P. phoxinus	350.6 ± 25.4	<0.01

Table 20. Output from stepwise multiple regression model choice to determine significant explanatory variables of maximum prey size for each study species (GH = gape height; OS = ontogenetic stage; L_S = standard length; none = no explanatory variable

Model step	Explanatory variables	AIC	F-value	*P*
Barbus barbus				
1	GH	5597	0.01	0.94
	OS	5597	0.07	0.79
	None	5599		
	L_S	5605	8.48	0.004
2	OS	5595	0.07	0.79
	None	5597		
	L_S	5614	18.86	<0.001
3	None	5595		
	L_S	5644	53.25	<0.001
Squalius cephalus				
1	OS	2142	0.05	0.82
	None	2144		
	L_S	2145	2.44	0.12
	GH	2146	3.21	0.07
2	None	2142		
	L_S	2143	2.53	0.11
	GH	2144	3.99	0.05
Leuciscus leuciscus				
1	OS	1187	0.26	0.61
	None	1189		
	GH	1190	3.67	0.06
	L_S	1195	7.58	0.01
2	None	1187		
	GH	1190	4.85	0.03
	L_S	1193	7.40	0.01
Phoxinus phoxinus				
1	OS	1680	0.01	0.92
	GH	1681	0.15	0.70
	L_S	1681	0.15	0.70
	None	1683		
2	L_S	1679	0.15	0.70
	GH	1679	0.16	0.69
	None	1681		
3	None	1677		
	GH	1679	0.02	0.89

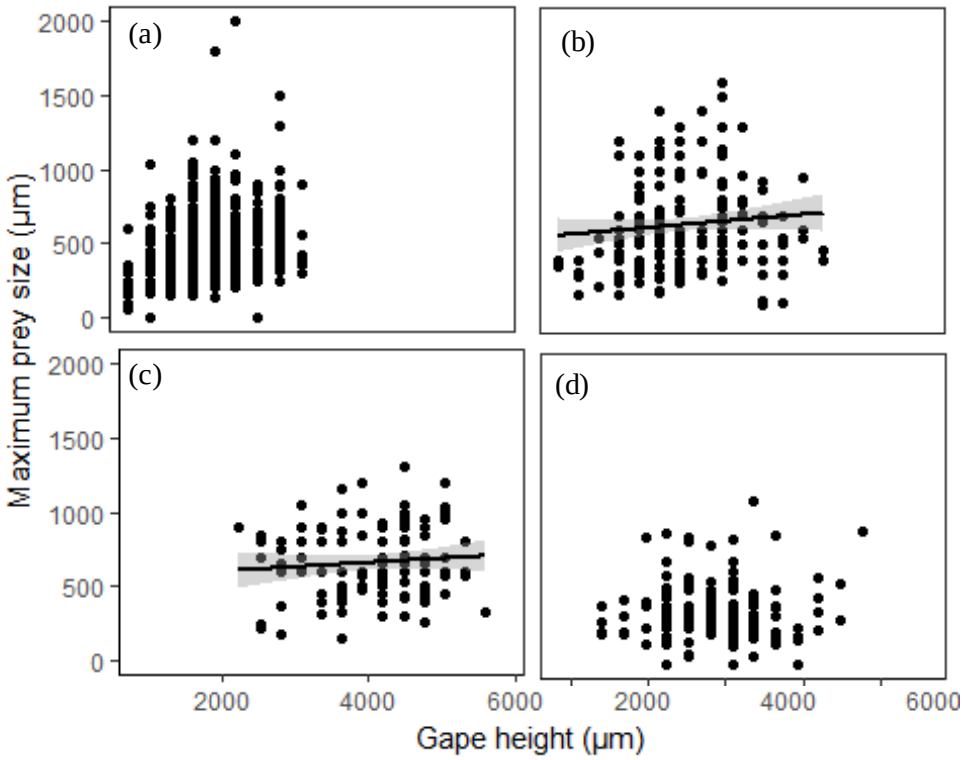

Figure 39. Linear relationship between maximum prey size (µm) with gape height (µm for (a) *Barbus barbus*, (b) *Squalius cephalus*, (c) *Leuciscus leuciscus*, (d) *Phoxinus phoxinus;* solid lines represent the significant relationship between the variables according to linear regression ($P < 0.05$; Table 20); shaded areas are the 95% CI.

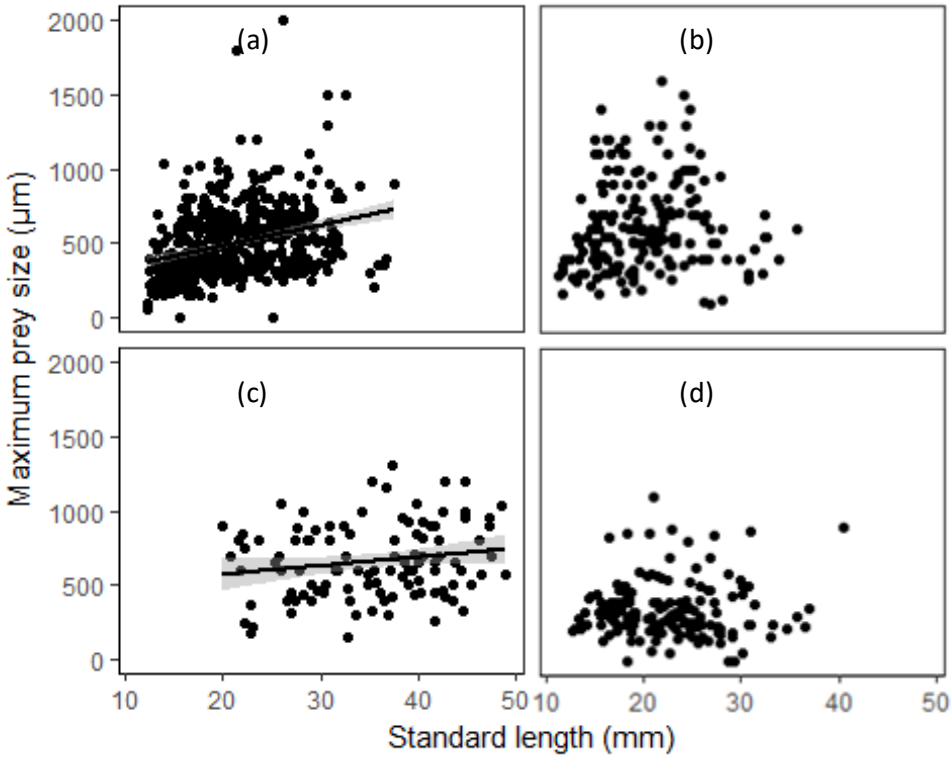

Figure 40. Relationships between maximum prey size with standard length for (a) *Barbus barbus,* (b) *Squalius cephalus,* (c) *Leuciscus leuciscus,* (d) *Phoxinus phoxinus;* solid lines represent the significant relationship between the variables according to linear regression ($P < 0.05$; Table 20); shaded areas are the 95% CI.

4.4.7 Gape height versus trophic niche

Across the data, it was apparent that when fish had larger gape heights, this did not necessarily result in a larger trophic niche, other than for *L. leuciscus* (Table 21, Fig. 41c). Whilst the trophic niche of *B. barbus* and *S. cephalus* niche did shift as gape height changed (Table 21, Fig. 41a and b), the largest niche size for *S. cephalus* was at gape height 2.5 to 3.1 mm (Table 21, Fig. 41b). The largest niche sizes in *P. phoxinus* occurred in the two smallest gape height classes, suggesting their diet became more specialised as gape height increased (Table 21, Fig. 41d).

Table 21. Ordination (nMDS) areas for *Barbus barbus, Squalius cephalus, Leuciscus leuciscus* and *Phoxinus phoxinus* between different gape height (mm) classes

	Gape height (mm)				
Species	0.8 – 1.4	1.6 – 2.2	2.5 – 3.1	3.3 – 3.9	4.2 – 4.8
B. barbus	0.33	0.39	0.37	-	-
S. cephalus	1.18	1.98	3.34	1.27	1.23
L. leuciscus	-	-	0.28	0.87	12.44
P. phoxinus	-	3.65	2.65	1.45	1.85

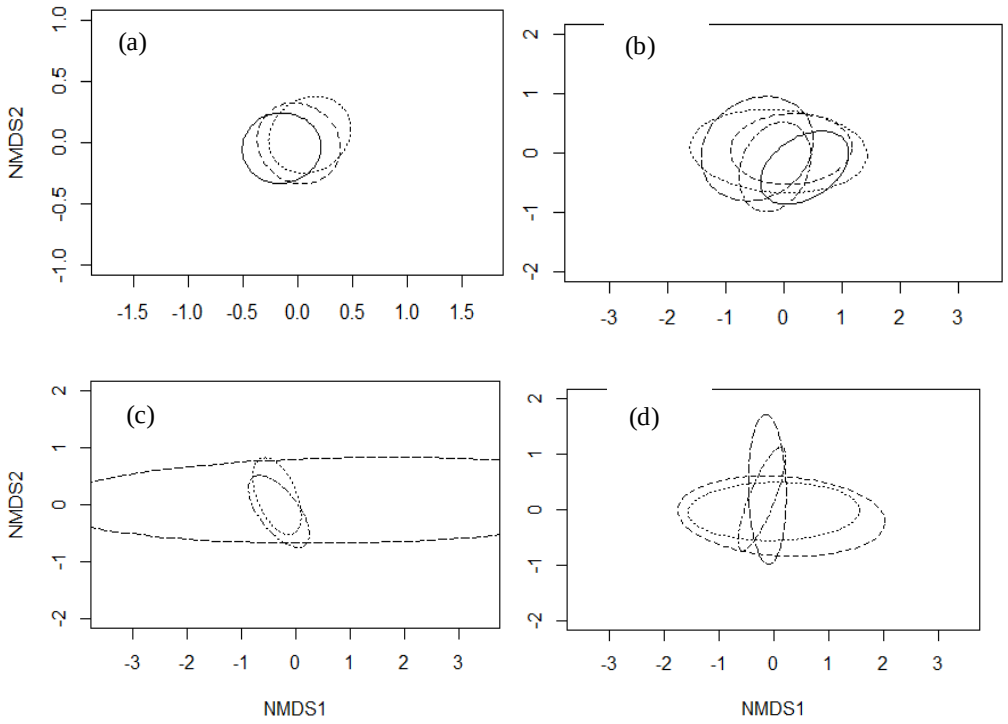

Figure 41. Trophic niche nMDS plots with 40% confidence interval ellipses for (a) *Barbus barbus*, (b) *Squalius cephalus*, (c) *Leuciscus leuciscus*, (d) *Phoxinus phoxinus* grouped by gape sizes. The lines surrounding the ellipses are: 0.8 – 1.4 mm (solid line), 1.7 – 2.2 mm (short dashes), 2.5 – 3.1 mm (dotted line), 3.4 – 3.9 mm (dash dot line) and 4.2 – 4.8 (long dashed lines).

4.4.8 Inter-annual, inter-specific, ontogenetic and spatial similarities in diet composition

There was significant inter-annual variation across the diet of all species when site-specific comparisons were accounted for (ANOSIM; *B. barbus* R = 0.18, *S. cephalus* R = 0.15, *L. leuciscus* R = 0.12, *P. phoxinus* R = 0.35, all $P < 0.05$). When data were pooled between years, then there were significant differences in the diet between each species (ANOSIM; R = 0.43, $P < 0.001$). Across the four species, the variation in diet between years differed according to SIMPER, with *B. barbus* having the highest similarity (74 % similarity), followed by *P. phoxinus* (57%), *S. cephalus* (33%) and then *L. leuciscus* (28%). The following analysis thus only included data from 2015 in order not to confound the results via including inter-annual variation. This revealed there was no significant difference in the diet between larval and juvenile stages in *B. barbus* (ANOSIM, R = 0.031, $P = 0.16$) or *S. cephalus* (ANOSIM, R = -0.056, $P = 0.89$). No larval stages were present in the dataset for *P. phoxinus* and *L. leuciscus* to enable comparisons.

Spatially, there was a significant difference in *B. barbus* diets across the three sites in 2015 (ANOSIM, R = 0.041, $P < 0.001$), with this also the case for *S. cephalus* (ANOSIM, R = 0.07, $p < 0.001$). Diet was significantly different for *P. phoxinus* between Site 1 and Site 2 (ANOSIM pairwise, R = 0.04, p = 0.02), but Site 3 was not significantly different (ANOSIM pairwise; (2 vs. 3) R = -0.004, p = 0.50; (1 vs. 3) R = 0.01, p = 0.07). The site-specific nature of aspects of the diet of the 0+ fishes meant that testing was completed at a site level and used only data collated in 2015. Diet composition of *B. barbus* differed significantly

to *S. cephalus* at all sites (Table 22), with some niche overlap at Sites 1 and 3 (Fig. 42, 43). The diet of *L. leuciscus* was also significantly different to *B. barbus* (Table 22), with low niche overlap (Fig. 42, 43). The diet of *P. phoxinus* was not significantly different to *B. barbus* at two sites; it was only significantly different at Site 2 (Table 22), where the *B. barbus* niche sat within the *P. phoxinus* niche (Fig. 42, 43). The trophic niche size of non-indigenous *B. barbus* was smaller than all of the native cyprinids at each site, apart from Site 1 where *P. phoxinus* had a smaller niche (Table 22). The largest niche was occupied by *S. cephalus* at all sites (Table 22).

The main drivers of dietary difference at Site 1 at 2 between *B. barbus* and *S. cephalus* were higher percentages of chironomid larvae, cladocera and copepods in *B. barbus* and higher amounts of chironomid adults and *Mesovellidae* in *S. cephalus*. The main drivers of dietary difference between *B. barbus* and *P. phoxinus* at Site 2 were higher percentages of chironomid larvae and cladocera in *B. barbus* diets and higher amounts of chironomid adults and copepods in *P. phoxinus* diet (Table 23). Dietary difference between *B. barbus* and *L. leuciscus* was driven by *L. leuciscus* preying on more *Mesovellidae* and chironomid adults, whilst *B. barbus* ate more chironomid larvae, cladocera and copepods.

Table 22. Sample sizes (n), mean standard length, calculated 40% standard error ellipse area calculated in vegan and the R statistic from ANOSIM comparison with *Barbus barbus* diet and its significance for each fish species (*Squalius cephalus, Phoxinus phoxinus and Leuciscus leuciscus*) and site.

Site (S)/ species	n	Average LS (mm) ± 95% CL	Within group similarity	40% Ellipse area	R statistic	*P*
S1						
B. barbus	140	22.6 ± 0.9	75%	0.28	NA	NA
S. cephalus	43	20.7 ± 1.6	47%	1.29	0.61	0.001
P. phoxinus	47	22.6 ± 1.3	83%	0.18	0.06	0.085
S2						
B. barbus	151	21.0 ± 0.7	76%	0.13	NA	NA
S. cephalus	44	17.9 ± 1.2	34%	8.72	0.83	0.001
P. phoxinus	51	22.9 ± 0.6	51%	1.29	0.28	0.001
S3						
B. barbus	136	21.5 ± 0.9	79%	0.18	NA	NA
S. cephalus	54	21.0 ± 0.9	40%	1.76	0.49	0.001
P. phoxinus	42	22.0 ± 0.7	74%	0.41	-0.01	0.50
L. leuciscus	33	30.9 ± 1.4	48%	1.35	0.56	0.001

Table 23. Prey species and their contribution to dissimilarity for *Barbus barbus* (n = 976) and *Phoxinus phoxinus* (n = 457). Total dissimilarity =37.5%.

	B. barbus average abundance	*P. phoxinus* average abundance	Average dissimilarity ± 95% CI	Contribution %	Cumulative %
Chironomid larvae	9.23	7.84	9.36 ± 0.04	25.21	25.21
Chironomid adult	0.64	1.19	6.39 ± 0.03	17.19	42.4
Cladocera	1.47	0.10	5.75 ± 0.04	15.47	57.87
Copepod	0.92	0.38	4.48 ± 0.03	12.05	69.92
Plant material	0.00	0.66	2.65 ± 0.02	7.14	77.06

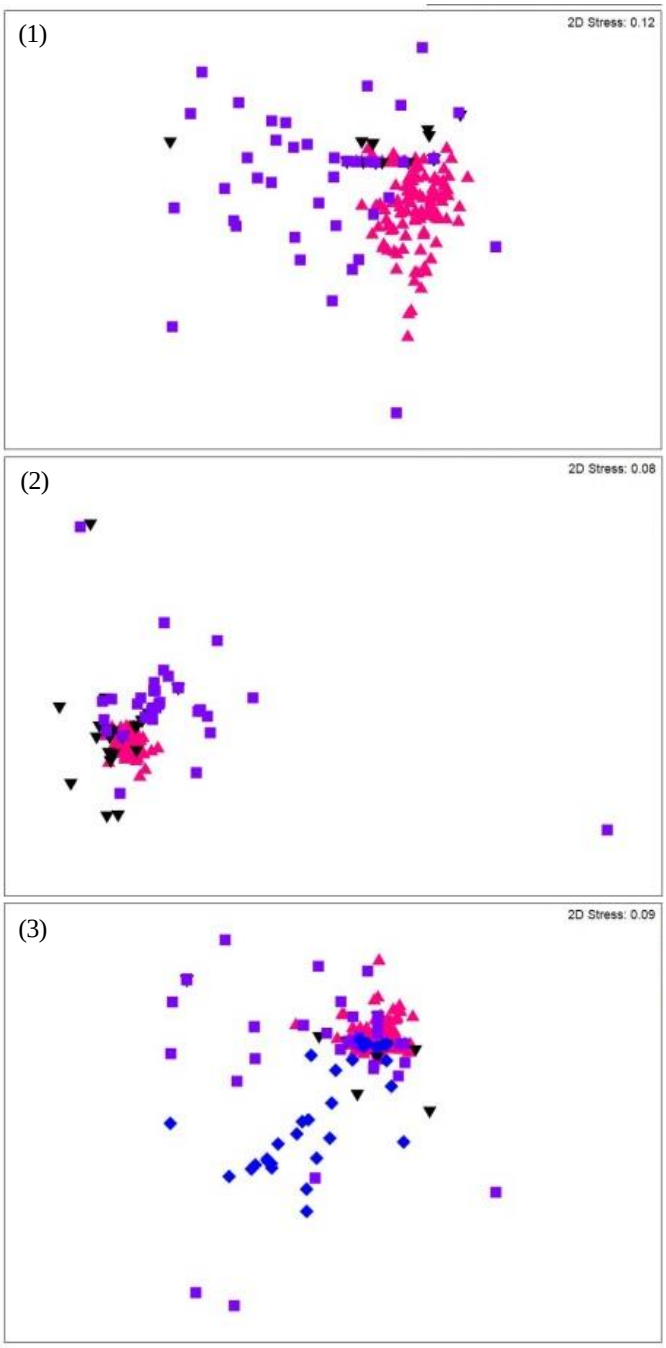

Figure 42. Non-metric MDS point plots (Square root transformation, Bray Curtis similarity) for *Barbus barbus* (▲), *Squalius cephalus* (■), *Phoxinus phoxinus* (▼) and *Leuciscus leuciscus* (♦) for 2015 from Site 1, 2 and 3.

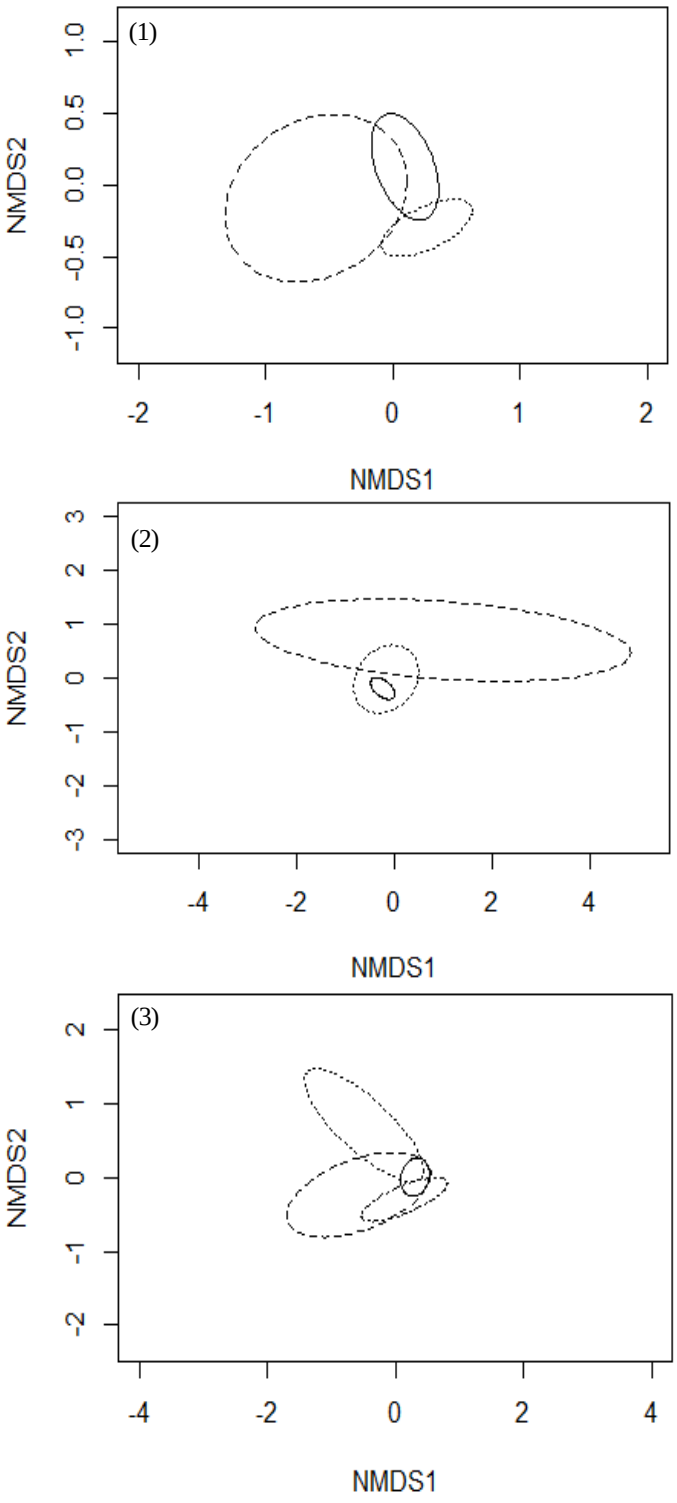

Figure 43. Non-metric MDS plots (Square root transformation, Bray Curtis similarity) showing the 40% ellipses for 2015 from Site 1, 2 and 3, where *Barbus barbus* (solid line), *Squalius cephalus* (long dashed line), *Phoxinus phoxinus* (dotted line) and *Leuciscus leuciscus* (short dashed lines), all of lengths between 12.3 and 37.6 mm

4.5 Discussion

This study successfully described the diet composition of 0+ fishes in this cyprinid fish community that was invaded by non-indigenous *B. barbus*. The results revealed the 0+ fishes were all primarily generalist in their diet, with most (but not all) prey categories having low selectivity, as defined by Costello (1990). For some prey in the diet, there were strong relationships with fish length, indicating the importance of increasing body size as a driver of diet changes. Whilst ontogenetic stage, length and gape height co-correlate and increase temporally, there were differences in how their effects manifested on diet composition, with shifts in diet influenced more by increases in fish length and gape height than ontogenetic stage.

Ontogeny in *B. barbus* did not result in significant dietary composition changes between larval stages 3 and juveniles, with this building on Nunn et al. (2007b) who also detected no significant differences in their diet between indigenous larval stages 5 and 6 in the River Trent, England. There were also no significant ontogenetic shifts in *S. cephalus* diet, a finding contrary to Nunn et al. (2007b). Whilst there was some overlap in the trophic niche of *B. barbus* and the other 0+ fishes, there were also some significant differences in their diet composition with *S. cephalus* and *L. leuciscus*, the other two fishes in the sample that have importance for recreational angling, with this contrary to the prediction. The trophic niche size and composition was most similar between *B. barbus* and *P. phoxinus*, with this more consistent with the prediction, although this pattern varied spatially. The main driver of trophic similarity between *B. barbus* and *P.*

phoxinus was their high dietary proportions of Chironomid larvae. Given that *P. phoxinus* were the most abundant 0+ fish at each site then there was thus potential for high inter-specific competition for resources with invasive *B. barbus* (Chase et al., 2016). However, both fishes had other items in their diet, suggesting that should high inter-specific competitive interactions have resulted in reduced food intake rates, they would have could have switched to alternative prey (Dill, 1983). Moreover, with *P. phoxinus* the most abundant 0+ fish at all sites and sampling occasions, there was no evidence to suggest their high dietary similarity with invasive 0+ *B. barbus* was having negative consequences at the population level.

The diet composition of the invasive 0+ *B. barbus* was relatively similar to their diets in rivers in their indigenous range. For example, in the River Seig, Germany, larvae of Chironomids, caddisfly and mayfly were also all present in 0+ *B. barbus* diet (Bischoff & Freyhof, 1998). Similarly, in the River Trent, Eastern England, the diet of *B. barbus* in their late larval stages was also strongly dependent on Chironomid larvae (Nunn et al., 2007b). In the River Lee, England, Copp et al. (2005) also reported 0+ *B. barbus* predating upon similar items, including larvae of caddis fly and Chironomid larvae. Thus, there appears to be high similarity in *B. barbus* diet between their native and invasive ranges. When coupled with their diet similarities to the native and abundant *P. phoxinus*, these results suggest some consistency with the pre-adaptation hypothesis of invasion biology. As outlined in Chapter 3, this hypothesis suggests that the probability of invasion by an introduced species is elevated when they share similar ecological traits and behaviours with native species due to, for example, a similar

ability to acquire resources, combined with expressing their traits and behaviours in a similar manner to their natural range (e.g. Duncan & Williams, 2002; Ricciardi & Mottiar, 2006; Buoro et al., 2016). These results here suggested that 0+ *B. barbus* had required minimal shifts in their foraging behaviours to adapt to the food resources available in the River Teme, given their diet similarities to both their natural range and the native species in the new range, with these likely to have assisted their establishment and invasion.

The results of the trophic niche analyses indicated that 0+ *B. barbus* had a niche with minimal overlap with *L. leuciscus*, with this at least partially explained by their contrasting spawning strategies, whereby *L. leuciscus* spawned only once each year in spring versus protracted spawning in *B. barbus* that commenced later in the year (Chapter 3). This resulted in minimal overlap in their 0+ size ranges, gape heights and, correspondingly, their diet composition and trophic niche. In addition, the higher specialisation of the feeding strategy of *B. barbus* versus *L. leuciscus* might be due to differences in their functional morphology (De Silva et al., 1979). For example, *B. barbus* has an inferior mouth suited for feeding on the benthos, whilst *L. leuciscus* has terminal mouth with a larger gape height that enables greater diversity in prey including, for example, drifting aerial insects. Whilst *S. cephalus* had a similar spawning strategy to *B. barbus*, with appearance of larvae and juveniles at similar times in samples (Chapter 3), there were some important differences in their diets, with *S. cephalus* being more generalist with a larger trophic niche. This suggested that the functional morphology of *B. barbus* was more limiting than *S. cephalus*, resulting in their narrower diet, and suggested the presence of invasive 0+ *B. barbus* was not having a detrimental impact on the trophic ecology of 0+ *S. cephalus*.

Of the native 0+ fishes studied, the larval and juvenile *P. phoxinus* were consuming similar prey items to their populations in other British rivers (Nunn et al., 2012). Of note was the consistency in the proportions of the prey items consumed by *P. phoxinus* over their length range, as this was contrary to what might have been predicted. This is because the species undergoes a rapid shift in the length of the intestine at approximately 27 mm body length and this can either intensify feeding or result in a shift to different prey items (Simonovic et al., 1999). Whilst only 10 % of *P. phoxinus* analysed here were larger than 27mm, no such dietary shift was detected. For *S. cephalus*, the decrease in the proportions of Chironomid larvae consumed with increasing fish length was likely to be relate to their ontogenetic development that enabled their predation on a wider range of prey items as they attained larger body lengths (Nunn et al., 2007b). This was also reflected in *L. leuciscus*, where proportions of aerial insects in their diet increased with fish length. This was, however, contrary to Weatherley (1987) who found that the percentage of aerial insects decreased within the similar length range (20 to 50 mm), suggesting some context dependencies in 0+ fish diet.

Improvements to this study would have been included the collection of samples sizes across a wider range of ontogenetic stages, especially in early larval stages, as these would have increased the ability to detect ontogenetic dietary changes, including on sizes of maximum prey and trophic niches. The lack of early larval stages in samples was likely to relate to sampling bias resulting from the micromesh seine net being inefficient at capturing fish of below 15 mm standard length (Cowx et al., 2001), with point abundance sampling using electric fishing

a potentially alternative technique to sample fish of above 5 mm length (Copp, 2010). Notwithstanding, at the free embryo stage and when they emerge from within spawning gravels, *B. barbus* can be between 8 and 13 mm (Vilizzi & Copp, 2013). Thus, for early larval stages to be captured might have required sampling methods capable of catching fish within the spawning gravels. An additional issue in the study was the potential for shrinkage of body lengths of the 0+ fish through their preservation (Fox, 1996). However, Leslie & Moore (2001) suggested shrinkage effects are relatively low when using similar preservation methods, providing samples are processed within a year of collection, as was completed here.

Selectivity measures could have been further quantified by comparing the prey items available in the environment compared with the prey items found in the fish's stomachs. This would require a variety of sampling techniques to sample invertebrates and other prey from the water surface, the water column and the benthos. This would allow for a 'log of the odds' calculation as used in Schabetsberger et al. (2003) which can determine if food items have positive or negative selection compared to their availability (Gabriel 1978).

In summary, these results indicated how invasive 0+ *B. barbus* were integrating into a 0+ cyprinid fish community via their diet and feeding ecology. The results highlighted that the 0+ *B. barbus* were consuming similar items to conspecifics in their native range and some confamilial fishes in the River Teme, suggesting some consistency with the pre-adaptation hypothesis of invasion biology, with this then consistent with the findings for their spawning strategies in Chapter 3.

However, the increasing species-specificity in 0+ fish diet as length and gape height increased meant their diets became increasingly dissimilar, especially in *S. cepahlus* and *L. leuciscus*. This was likely to assist the integration of the invasive *B. barbus* to the community and minimise their detrimental ecological impacts on native fishes. This apparent increasingly dissimilarity in fish diet with life-stage of the fishes is thus explored further in Chapter 5.

Chapter 5: Inter- and intra-specific patterns of isotopic niche partitioning in an invaded community of lowland river fishes

This chapter has been accepted as a manuscript as:

Gutmann Roberts C. and Britton JR. Trophic interactions in a lowland river fish community invaded by European barbel *Barbus barbus*. *Hydrobiologia* (Hydrobiologia 2018)

5.1 Abstract

Determinants of invasion success include how the introduced fishes interact trophically with native fishes, including whether they compete for food resources, and converge or partition in resource use. Here, invasive European barbel *Barbus barbus* was used as the model species across three life stages (0+, juveniles and adults) to test the null hypothesis that their trophic niches (as core isotopic niches) would be similar to native fishes at each life-stage, indicating dietary convergence and high potential for competitive interactions. Stable isotope metrics revealed that at each life stage, there was a general pattern of inter-specific partitioning in the core isotopic niche of invasive *B. barbus* and the native fishes, contrary to the null hypothesis, with this inter-specific partitioning being strongest between the fishes as 0+ and as juveniles. Within *B. barbus*, there was also complete partitioning in their isotopic niches between each life stage. Thus, rather than acting as a strong competitor, these results suggest that invasive *B. barbus* integrate into native food webs via exploiting different food resources to native fishes, facilitating their coexistence. These results contribute to the increasing evidence that suggest that rather than competing for food resources, invasive fishes more frequently partition their resource use with native fishes.

5.2 Introduction

Introductions of non-indigenous fishes can result in adverse impacts in the native fish community, including via increased inter-specific competition (Gozlan et al. 2010). Determinants of invasion success include how the introduced species interacts trophically with species in the native fish community, for example, whether they converge or partition in their exploitation of food resources (Tran et al. 2015). The extent and intensity of the trophic interactions are then important for determining the strength and symmetry of their competition (Cucherousset et al. 2012; Jackson et al. 2012; Copp et al. 2017). Quantifying the feeding relationships of invasive and native fishes is thus important for assisting understanding of the ecological risks the invader poses to the native communities (Cucherousset and Olden 2011), and facilitates assessment of the ecological impacts that might develop (Gozlan et al. 2010; Tran et al. 2015; Copp et al. 2017).

Ecological theory suggests that following an invasion by a non-native species, their trophic consequences for the recipient food web vary according to the ecological opportunities available that determine the extent of their feeding interactions with native fishes (Tran et al. 2015; Copp et al. 2017). Where food resources are not fully exploited in the receiving environment, these can be exploited by the invader, potentially facilitating their integration into the food web via trophic partitioning with native species that result in few inter-specific interactions (Shea and Chesson 2002; Tran et al. 2015). Where the invader must integrate into a community where the food resources are more limiting, the niche

variation hypothesis suggests that increased inter-specific competition will result in the trophic niche sizes of the competing species decreasing, potentially resulting in patterns of niche partitioning (Van Valen 1965; Olsson et al. 2009; Tran et al. 2015). Alternatively, this scenario can result in populations utilizing a great range of prey to maintain their energy requirements, increasing the size of their trophic niches and potentially resulting in greater resource sharing with other species (Svanbäck and Bolnick 2007). The trophic consequences of an invasion for native fishes can thus vary, with potential for patterns of trophic niche constriction and partitioning, as detected from invasions by some small bodied fishes, such as topmouth gudgeon *Pseudorasbora parva* (Jackson and Britton 2014; Tran et al. 2015), or trophic niche expansion and overlap, as detected from invasions of some salmonid fishes (e.g. Cucherousset et al. 2007). Where the invader attains relatively large body sizes then it is also important to understand how their trophic relationships with native species changes with ontogeny, given that increased body and gape sizes usually result in shifts in diet composition within species (DeVries et al. 1998; Bašić and Britton 2016).

European barbel *B. barbus* is now invasive in many European rivers outside of their native range (Britton and Pegg 2011; Section 1.5). Attaining lengths to approximately 800 mm and weights in excess of 8 kg (Amat Trigo et al. 2017), they are generally valued for sport angling, with this the primary driver for introductions (Britton and Pegg 2011). In Britain, they are indigenous to eastern flowing rivers in England due to previous connections with mainland Europe at the end of the last glacial period (Wheeler and Jordan 1990; Section 1.5). Many of these indigenous populations are, however, increasingly imperilled due to

habitat and connectivity loss (Bašić et al. 2017). These populations are now often supported by stocking with hatchery-reared individuals that are released at lengths between 120 and 250 mm and age 1+ and 2+ years (Britton et al. 2004; Antognazza et al. 2016). Studies on the trophic interactions of these stocked fish suggest substantial partitioning in their trophic niches with the trophically analogous *S. cephalus*, with this partitioning also apparent between adults of these species, and with other species such as *L. leuciscus* (Bašić and Britton 2016).

Knowledge on the trophic interactions of invasive *B. barbus* with native fishes is, however, more limited. Bašić et al. (2015) revealed that in rivers in both the invasive and native range of *B. barbus*, and where they were exploited by catch-and-release anglers, baits based on marine derived nutrients provided a strong trophic subsidy, with some individual *B. barbus* and native *S. cephalus* (generally > 400 mm) specialising on this allochthonous resource. This is also explored in Chapter 6. In areas of rivers where angling pressure is lower, however, there remains a distinct knowledge gap on the trophic ecology of invasive *B. barbus*, especially in relation to how their diet and trophic niche sizes might alter with changes in body size and in relation to native fishes. Previous research have shown that *B. barbus* adults may eat small fishes such as *P. phoxinus* and *C. gobio* (Basic et al. 2015) and therefore might pose a predation threat on larval and juvenile cyprinids such as *S. cephalus* and *L. leuciscus*, and there are also anecdotal reports of *B. barbus* eating eggs of other fish (The Herald 2003). The aim of this research was to quantify the trophic interactions of invasive *B. barbus* with native fishes, with focus on determining the extent of their niche sharing and how this alters across the extent of their body sizes. Using

the River Teme, western England, as the study river, where non-indigenous *B. barbus* have been present since the 1970s (Antognazza et al. 2016; Section 1.8, the objective was to determine the trophic niche sizes and overlaps between invasive *B. barbus* and native fishes at three different life-stages: young -of-the-year ('0+ fish'; 18 to 38 mm), juveniles (150 to 250 mm) and adults (> 380 mm). The null hypothesis was the trophic niches of invasive *B. barbus* and native fishes would be similar at each life-stage, with high niche convergence that indicated feeding on similar food resources. As the *B. barbus* population of the River Teme is considered an important angling resource (Amat Trigo et al. 2017; Section 1.8) then the use of stomach contents analysis via destructive sampling of the juvenile and adult fish was not feasible in the study and so it was based on stable isotope analysis (SIA). Ratios of heavy to light stable isotopes of Nitrogen indicate the trophic position of the items in the diet (Fry 1988) and the Carbon indicates the primary carbon sources indicating if they are marine, freshwater or terrestrial in origin (Chisholm et al. 1982). By using two separate isotopes it enable us to create bi-plots to determine interactions between species (Newsome et al. 2007; Jackson et al. 2011).

5.3 Methods

5.3.1 Sampling details and stable isotope analysis

Samples for the study were collected from the middle reaches of the river. The sampled area was between Tenbury Wells (52°19'N, -2°24'W) and Bransford (52°10'N, -2°16'W) (Fig. 3). Across these sampling areas, the cyprinid fish community was relatively limited in diversity, with only invasive *B. barbus*, and

S. cephalus, *L. leuciscus* and *P. phoxinus* present. The salmonid fish *T. thymallus* was also present at the upper end of the sampling area and so was also included in some analyses. Compared with the area of river located below Powick Weir and utilised on Chapter 6, angling pressure was relatively light in these areas of the river and thus inputs of pelletized fishmeal were considered as being comparatively low.

The 0+ fish utilised for stable isotope analysis were sampled from a single area of nursery habitat located at Knightwick using a micromesh seine net on 12th September 2016 as described in Section 3.2. The fish were euthanised via anaesthetic overdose (MS-222) and transported back to the laboratory on ice. In the laboratory, within 24 hours, they were identified to species, measured (standard length, nearest mm) and a sample of dorsal muscle tissue removed and dried to constant weight at 50 ^{o}C.

'Juvenile' and adult fish were sampled by angling and electric fishing during summer periods in 2015 and 2016. Electric fishing was completed in September 2016 in conjunction with the Environment Agency and focussed on using hand-held equipment powered by a generator, with the habitats sampled primarily being areas of riffle that enabled focus on collecting samples from fish in the 'juvenile' size range (150 to 250 mm length). Due to the issues of the sampling areas being used for catch-and-release angling, destructive sampling to collect dorsal muscle samples was not possible and so the stable isotope analysis of these fish was based on scales (Busst and Britton 2016, 2017). Correspondingly, for each captured fish, identification was to species level, followed by measuring

(fork length, nearest mm) and the collection of between three and five scales from the area between the base of the dorsal fin and above the lateral line. As scales grow in proportion to fish length, with the outer portion of scales reflecting their most recent growth (Hutchinson and Trueman 2006; Bašić et al. 2015), then only the outer portion of the sampled scales was used in subsequent stable isotope analyses. The scales were prepared in the laboratory, with preparation involving thorough washing of scales in distilled water followed by removal of the outer edge of the scale using dissection scissors. The scale material was then dried to constant weight as per the 0+ fish samples. In all cases, one scale was used per individual fish for the stable isotope analysis. Scale decalcification was not performed prior to isotopic analysis, since the removal of inorganic carbonates has no significant effect on scale $\delta^{13}C$ and $\delta^{15}N$ values (Sinnatamby et al. 2007; Ventura and Jeppesen 2010; Woodcock and Walther 2014). Concomitantly, samples of the putative food resource *Gammarus pulex* were collected from a number of areas within the sites in both sampling years (Table 24); this species was the most abundant macro-invertebrate species in samples and were assumed to be an important and consistent prey resource for the fishes. In the laboratory, the *G. pulex* samples were washed in distilled water and dried to constant weight as per the fish samples; note one sample comprised of between three and six individuals, with at least three replicate samples being analysed for each year (Table 24).

The dried muscle, scale and invertebrate samples were then submitted to the Cornell Isotope Laboratory in New York, USA, for stable-isotope analysis. This involved the samples being ground to powder, weighed in tin capsules (nearest

1,000 µg) and analysed on a Thermo Delta V isotope ratio mass spectrometer (Thermo Scientific, USA) interfaced to a NC2500 elemental analyser (CE Elantach Inc. USA). Standards were verified against international reference materials and calibrated against the primary reference scales for $\delta^{13}C$ and $\delta^{15}N$. The accuracy and precision were checked every 10 samples using a standard animal sample (mink). The outputs were values of $\delta^{13}C$ and $\delta^{15}N$ (‰) for each sample. As C:N ratios were below 3.5, indicating low lipid content, there was no need for $\delta^{13}C$ to be lipid corrected (Skinner et al. 2016, Post et al. 2007).

5.3.2 Data analysis

The 0+ fish utilised in the analysis were all between 17 and 38 mm, the juvenile fish were between 86 and 231 mm (note that in this length range, some *L. leuciscus* would have been sexually mature, but the *B. barbus* and *S. cephalus* would not be) and the adult fish were all above 386 mm (Table 25). By only completing inter-specific analyses within these length ranges, the comparative data were from fishes of relatively similar body sizes, with this more biologically relevant than comparing data between species of very different length ranges (Basic and Britton 2015). Testing for differences between lengths of species within the 0+, juvenile and adult fish groups used ANOVA, with Tukey post hoc tests used to determine the significance of any length differences.

As the stable isotope data of the *G. pulex* samples indicated some inter-annual and inter-area differences (as comparisons of 95% confidence intervals around the mean; Table 24), then the juvenile and adult fish stable isotope data required correction to enable their data to be combined across years and sampling areas.

For use in subsequent analyses, the $\delta^{15}N$ data were converted to trophic position (TP; Equation 20) and the $\delta^{13}C$ data were corrected to C_{Corr} (Equation 21) (Olsson et al. 2009; Jackson and Britton 2014).

$$TPi = \left[\frac{\delta^{15}N_i - \delta^{15}N_{base}}{3.4}\right] + 2 \quad \text{(Equation 20)}$$

$$\delta^{13}Ccorr = (\delta^{13}C_i - \delta^{13}C_{meaninv})/CRinv \quad \text{(Equation 21)}$$

Table 24. Mean (± 95% CL), minimum (Min), maximum (Max) and range of $\delta^{13}C$ and $\delta^{15}N$ values (‰) of *Gammarus pulex* from samples collected from within each sampling area and year.

			$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$		
Area	Year	n	Mean		Min	Max	Range
1	2015	6	-30.68 ± 0.89	10.78 ± 0.95	-32.29	-29.27	3.02
1	2015	3	-31.52 ± 0.95	10.95 ± 1.70	-32.29	-30.62	1.67
1	2015	3	-29.84 ± 0.56	10.61 ± 0.62	-30.21	-29.27	0.93
1	2016	4	-29.44 ± 0.25	8.73 ± 0.23	-29.76	-29.19	0.57
2	2015	3	-29.10 ± 0.50	10.22 ± 0.38	-29.41	-28.60	0.81
2	2016	6	-29.91 ± 0.53	9.13 ± 0.53	-30.90	-29.17	1.73

Following the conversion of the stable isotope data of the juvenile and adult fishes to TP and C_{Corr}, they were able to be combined across the years and sampling areas. Initial analyses then tested the relationships for each species and length group of body length versus TP and C_{Corr} using linear regression. Differences in TP and C_{Corr} for each species and length group were then tested using either ANOVA or Welch's test, with the latter used where the data were normally distributed but violated the assumption of homogeneity of variance.

For each group of fishes, their corrected SI data were used to calculate their isotopic niche size per species. The isotopic niche was used here as an approximation of the trophic niche, with it acknowledged that the isotopic niche varies slightly from the trophic niche due to it being influenced by factors other than diet (Jackson et al. 2011), such as growth and metabolic rate of individuals (Busst and Britton 2017). The isotopic niche was represented by the metric 'standard ellipse area' (SEA), a bivariate measure of the distribution of individuals in trophic space (Jackson et al. 2012). Each plotted ellipse enclosed 40% of the SI data and thus represented the 'core' niche, i.e. the typical resource use of the species. The core niche was used, as the fishes were sampled across a considerable spatial area of river, and thus were potentially relatively variable in their resource use, and so determination of their core niche provided more robust inter-specific comparisons of typical resource use and niche size (Jackson et al. 2011; Jackson et al. 2012). The ellipses were calculated within the R package SIBER v2.1.3 (Jackson et al. 2011; Jackson et al. 2012) and, due to some relatively small sample sizes, a corrected Bayesian estimate of Standard Ellipse Area (SEA_c) was calculated. This was followed by a calculation utilising a Markov chain Monte Carlo simulation with 10^4 iterations for each analysed group that provided 95% confidence limits (SEA_b) of the isotopic niche size (Jackson et al. 2011; R Core Team 2017). Using SEA_c, the extent of niche overlap (%) between species and life stages was then estimated; using the maximum likelihood fitted standard ellipses, the extent of the overlap between two groups was represented by the overlap of their core niches. The extent of the overlap was calculated using Bayesian modelling in the SIBER package, with

the denominator being the sum of non-overlapping area of the two ellipses (Jackson et al. 2011). Significant niche overlap was suggested when the extent of overlap was more than 60 % (Schoener 1968; Matley et al. 2017).

5.4 Results

5.4.1 Fish length and stable isotope relationships

For the 0+ fishes, there were no significant differences between the standard lengths of the species (ANOVA: $F_{3,60}$= 1.90, p = 0.14, Table 25). For the juvenile fishes, despite their relatively similar length ranges (Table 25), there were some significant length differences between the species (ANOVA: $F_{3,73}$ = 7.48, P < 0.01), where the differences were from *T. thymallus* being significantly smaller than *B. barbus*, *S. cephalus* and *L. leuciscus* (P < 0.01, P = 0.03, P < 0.01 respectively; Table 25). For the adult fishes, *B. barbus* were significantly larger than adult *S. cephalus* (ANOVA: $F_{1,40}$ = 91.08, P < 0.01, Table 25), with these fishes being the only fishes present in samples at lengths above 380 mm (Table 25). The length of fishes between life stages were significantly different for both *B. barbus* ($F_{2,64}$ = 2948, P < 0.01) and *S. cephalus* ($F_{2,49}$ = 576.2, P < 0.01).

For the 0+ fishes, the relationships of standard length versus $\delta^{13}C$ and $\delta^{15}N$ were not significant for *B. barbus* and *S. cephalus*, but were for *P. phoxinus* (Table 26*)*. For the juvenile fishes, *B. barbus* lengths were not significantly related to Ccorr, but there was a significant positive relationship with TP (Table 26). Length of *S. cephalus* and *T. thymallus* were not significantly related to Ccorr or TP (Table 26). The relationship of *L. leuciscus* length with Ccorr was also significant (Table 26). For the adult fishes, the relationsjips between *B. barbus* and *S. cephalus* lengths with Ccorr and TP were not significant (Table 26).

Table 25. The number (n), fish length ranges and mean lengths (95% CI) of each life stage of fish analysed for their stable isotopes across the two sampling areas.

Species	n	Length range (mm)	Mean length (mm) (± 95% CI)
0+ *B. barbus*	30	18 – 34	25.2 ± 1.8
0+ *S. cephalus*	15	17 – 36	27.3 ± 2.4
0+ *P. phoxinus*	16	17 – 38	27.3 ± 2.8
Juvenile *B. barbus*	16	105 – 231	158 ± 15
Juvenile *S. cephalus*	16	112 – 207	153 ± 11
Juvenile *L. leuciscus*	30	102 – 214	167 ± 11
Juvenile *T. thymallus*	15	86 - 205	122 ± 16
Adult *B. barbus*	23	540 – 690	584 ± 17
Adult *S. cephalus*	21	386 – 570	466 ± 22

Table 26. Outputs of linear regression of fish length versus corrected carbon (C_{corr}) and trophic position (TP) for each length group and fish species.

C_{corr}

Species	Group	R^2	df	F	*P*
Barbus barbus	0+	0.01	28	0.03	0.95
	Juvenile	0.09	14	1.41	0.26
	Adult	0.02	19	0.44	0.52
Squalius cephalus	0+	0.01	13	0.04	0.85
	Juvenile	0.13	14	2.12	0.17
	Adult	0.06	19	1.24	0.28
Phoxinus phoxinus	0+	0.36	14	7.95	0.01
Leuciscus leuciscus	Juvenile	0.15	28	4.87	0.04
Thymallus thymallus	Juvenile	0.14	13	2.10	0.17

TP

Species	Group	R^2	df	F	*P*
Barbus barbus	0+	0.08	28	2.57	0.12
	Juvenile	0.38	14	8.44	0.01
	Adult	0.04	19	0.78	0.39
Squalius cephalus	0+	0.14	13	2.19	0.16
	Juvenile	0.15	14	2.41	0.14
	Adult	0.05	19	0.91	0.35
Phoxinus phoxinus	0+	0.29	14	5.72	0.03
Leuciscus leuciscus	Juvenile	0.12	28	3.33	0.08
Thymallus thymallus	Juvenile	0.02	13	0.23	0.64

5.4.2 Stable isotope data within and between species

For *B. barbus* and *S. cephalus*, the only species present in all groupings, there was a pattern of enriched C_{corr} and decreasing TP as the body sizes of the fish increased (Table 27, 28). In *B. barbus*, C_{corr} was significantly higher in adults than the 0+ fish and juveniles ($P < 0.01$), whilst TP was significantly lower for adults versus the 0+ fish ($P < 0.01$, Table 27, 28). For *S. cephalus*, the 0+ fish had significantly lower C_{corr} than juveniles and adults ($P < 0.01$) and significantly higher TP ($P < 0.01$, Table 27, 28).

When compared between the species, the difference in C_{corr} between 0+ *B. barbus* and 0+ *S. cephalus* was not significant, but it was between both these 0+ fishes and 0+ *P. phoxinus*. The TP of 0+ *B. barbus* was significantly higher than both *S. cephalus* and *P. phoxinus*, whilst *S. cephalus* and *P. phoxinus* were not significantly different (Table 28). For the juvenile fishes, C_{corr} of *B. barbus* was significantly lower than all other fishes (Table 28). The TP of *B. barbus* was significantly higher than *T. thymallus*, significantly lower than *L.* leuciscus, but not significantly different to *S. cephalus* (Table 28). There were no significant differences in C_{corr} and TP between adult *B. barbus* and *S. cephalus* (ANOVA: Ccorr; $F_{1,40} = 2.09$, $P = 0.16$, TP; $F_{1,40} = 0.02$, $P = 0.90$).

Table 27. Mean stable isotope data (± 95% CI) per fish species and group and where C_{corr} = corrected carbon and TP = trophic position. Samples sizes as per Table 25.

Group/ species	C_{corr}	TP
0+ *B. barbus*	0.06 ± 0.38	3.30 ± 0.08
0+ *S. cephalus*	0.49 ± 0.65	2.92 ± 0.13
0+ *P. phoxinus*	-1.49 ± 0.49	3.02 ± 0.11
Juvenile *B. barbus*	0.39 ± 0.58	2.70 ± 0.13
Juvenile *S. cephalus*	2.57 ± 0.75	2.63 ± 0.14
Juvenile *L. leuciscus*	1.42 ± 0.39	3.03 ± 0.05
Juvenile *T. thymallus*	1.57 ± 0.20	2.13 ± 0.14
Large *B. barbus*	2.52 ± 0.40	2.62 ± 0.15
Large *S. cephalus*	3.22 ± 0.45	2.61 ± 0.15

Table 28. Outputs of ANOVA/ Welch's test of corrected carbon (C_{corr}) and trophic position (TP) for comparisons within length groups and between species and between length groups for *Barbus barbus* and S*qualius cephalus*. Note data for all sites and years are combined.

C_{corr}

Species	Test	Length group	df	F	*P*
Barbus barbus	ANOVA	Length	2,64	32.76	<0.01
Squalius cephalus	Welch's	Length	2,31	17.66	<0.01
0+	ANOVA	Species	3,60	10.01	<0.01
Juvenile	Welch's	Species	3,36	16.61	<0.01
Adult	ANOVA	Species	1,40	2.09	0.16

TP

Species	Test	Length group	df	F	*P*
Barbus barbus	ANOVA	Length	2,64	47.17	<0.01
Squalius cephalus	ANOVA	Length	2,49	6.52	<0.01
0+	ANOVA	Species	3,60	12.36	<0.01
Juvenile	Welch's	Species	3,30	60.53	<0.01
Adult	ANOVA	Species	1,40	0.02	0.90

5.4.3 Inter- and intra-specific differences in the isotopic niche

The 95% confidence intervals of the isotopic niches (as standard ellipse areas) of the 0+ fishes suggested that there were no significant differences in niche sizes between the species (Table 29). In general, the core isotopic niches of the 0+ fishes had low overlap, being 7 % between *B. barbus* and *S. cephalus*, 4 % between *S. cephalus* and *P. phoxinus,* and 0.2 % between *B. barbus* and *P. phoxinus* (Fig. 44). For the juvenile fishes, the 95 % confidence intervals of their isotopic niches also suggested they did not differ significantly in size between the fishes (Table 29) and there were no overlaps in their core isotopic niches (Fig. 45). For the adult fishes, there was also no significant difference in their niche sizes (Table 29), but their core niches did overlap by 55 % (Fig. 46).

Regarding intra-specific comparisons across the length groups, for *B. barbus*, there was no overlap in their core niches between the 0+, juvenile and adult fish (Fig. 47a), whereas for *S. cephalus*, there was a greater extent of niche overlap between the length groups, with no overlap between 0+ fish and juveniles, 2% between 0+ fish and adults and 31 % between juveniles and adults (Fig. 47b). Adult *S. cephalus* had a significantly larger isotopic niche than juvenile *S. cephalus* (Table 29, Fig. 47b), and if the juveniles had been the denominator for core niche overlap then it would be 100 % overlap.

Table 29. Standard ellipse areas (SEA_c) (± 95% CI SEA_b) for five fish species from the River Teme across three life stages; 0+, juvenile and adult. Samples sizes were as per Table 25.

Species	SEA_c (± 95% CL)
0+ *B. barbus*	0.77 ± 0.28
0+ *S. cephalus*	0.96 ± 0.51
0+ *P. phoxinus*	0.73 ± 0.36
Juvenile *B. barbus*	0.54 ± 0.28
Juvenile *S. cephalus*	0.59 ± 0.30
Juvenile *L. leuciscus*	0.44 ± 0.16
Juvenile *T. thymallus*	0.28 ± 0.14
Adult *B. barbus*	1.34 ± 0.58
Adult *S. cephalus*	1.89 ± 0.83

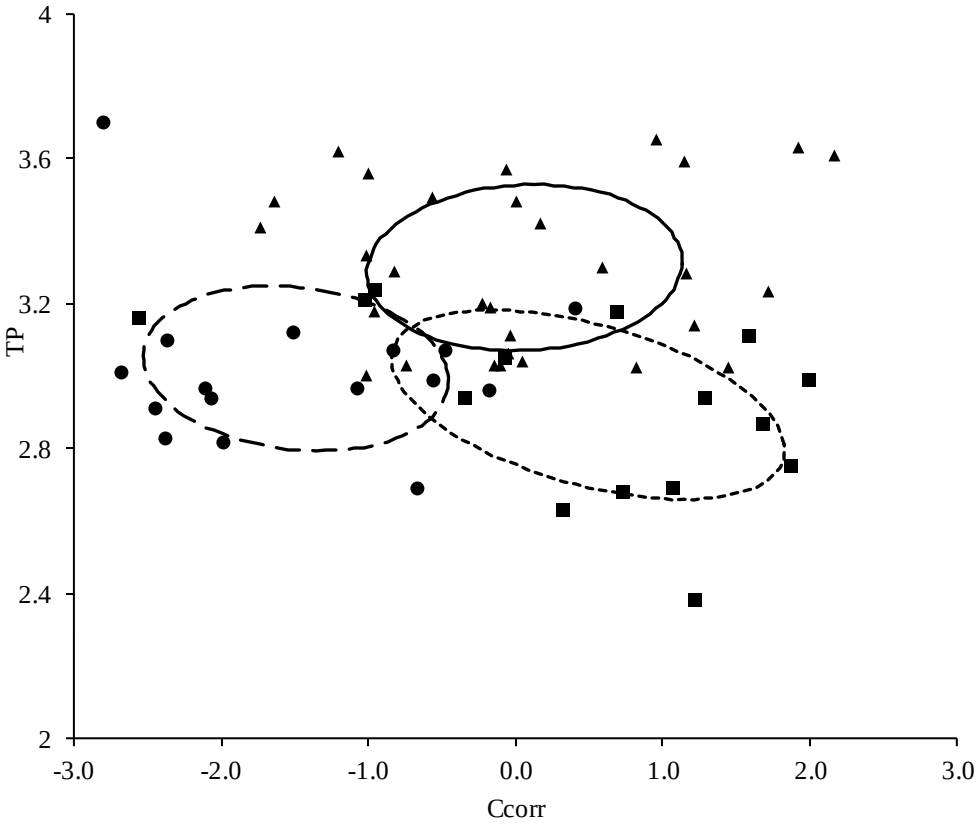

Figure 44. Corrected Carbon (C_{corr}) versus trophic position (TP) for 0+ *Barbus barbus* (▲), *Squalius cephalus* (■) and *Phoxinus phoxinus* (●) and the positions of their core isotopic niches (as SEA_c), where solid line: *B. barbus*, small dashed line: *S. cephalus*, and long dashed line: *P. phoxinus*.

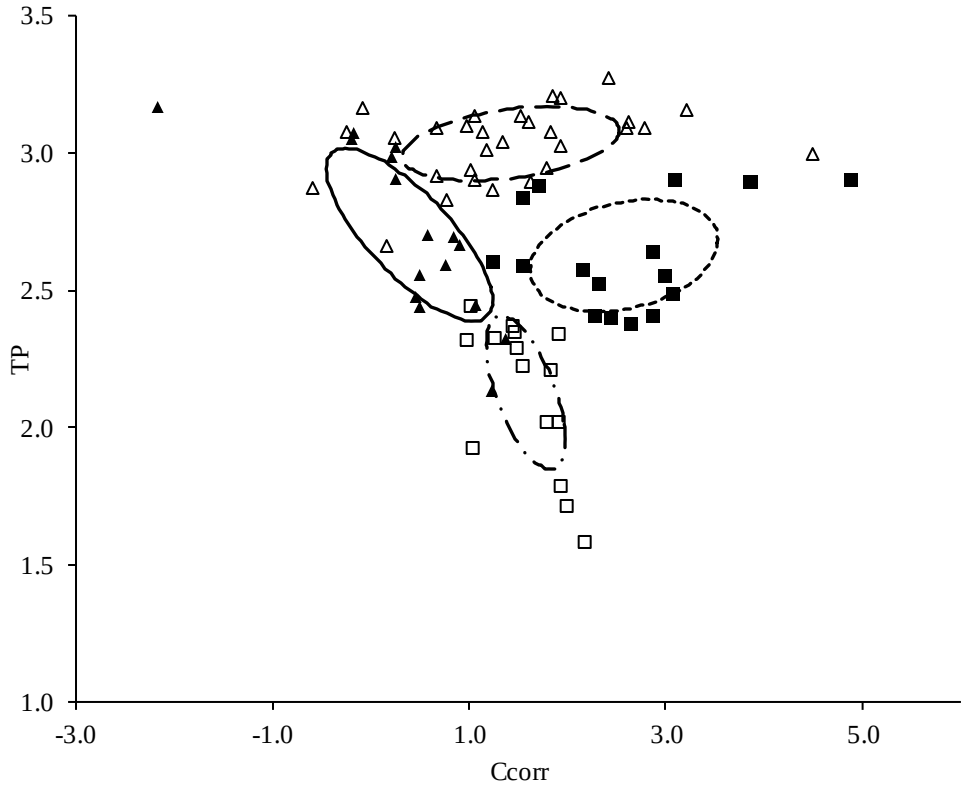

Figure 45. Corrected Carbon (C_{corr}) versus trophic position (TP) for juvenile *Barbus barbus* (▲), juvenile *Squalius cephalus* (■), *Leuciscus leuciscus* (Δ) and *Thymallus thymallus* (□), and the positions of their core isotopic niches (as SEA_c), where solid line: *B. barbus*, small dashed line line: *S. cephalus*, long dashed line: *L. leuciscus*, and dash/ dot line: *T. thymallus*.

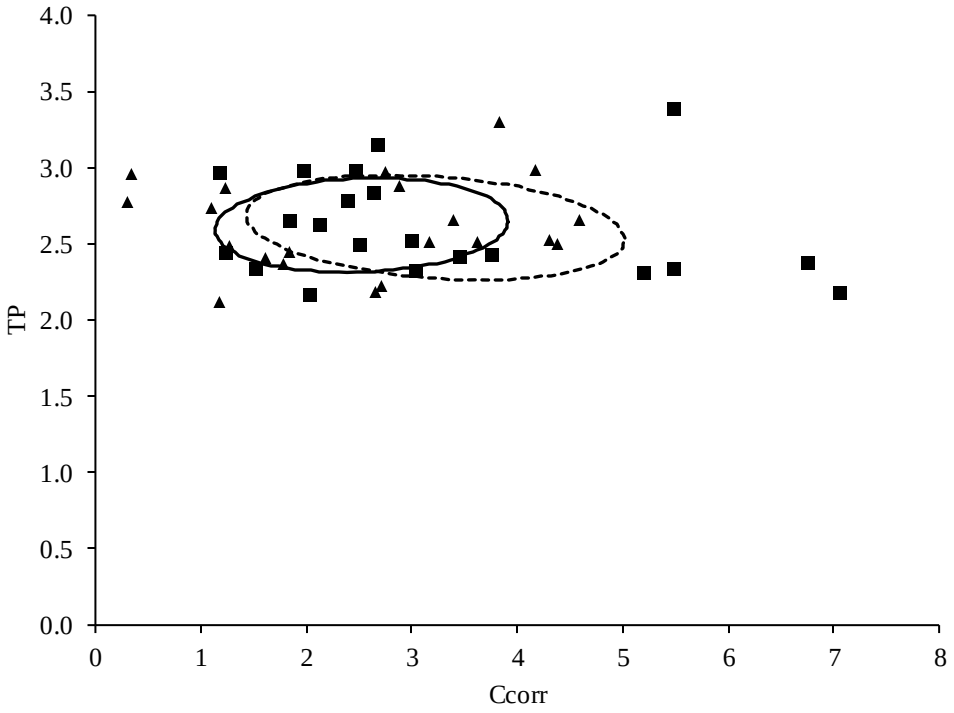

Figure 46. Corrected Carbon (C_{corr}) versus trophic position (TP) of adult *Barbus barbus* (▲) and adult *Squalius cephalus* (■), the positions of their core isotopic niches (as SEA_c), where solid line: *B. barbus*, short dashed line: *S. cephalus.*

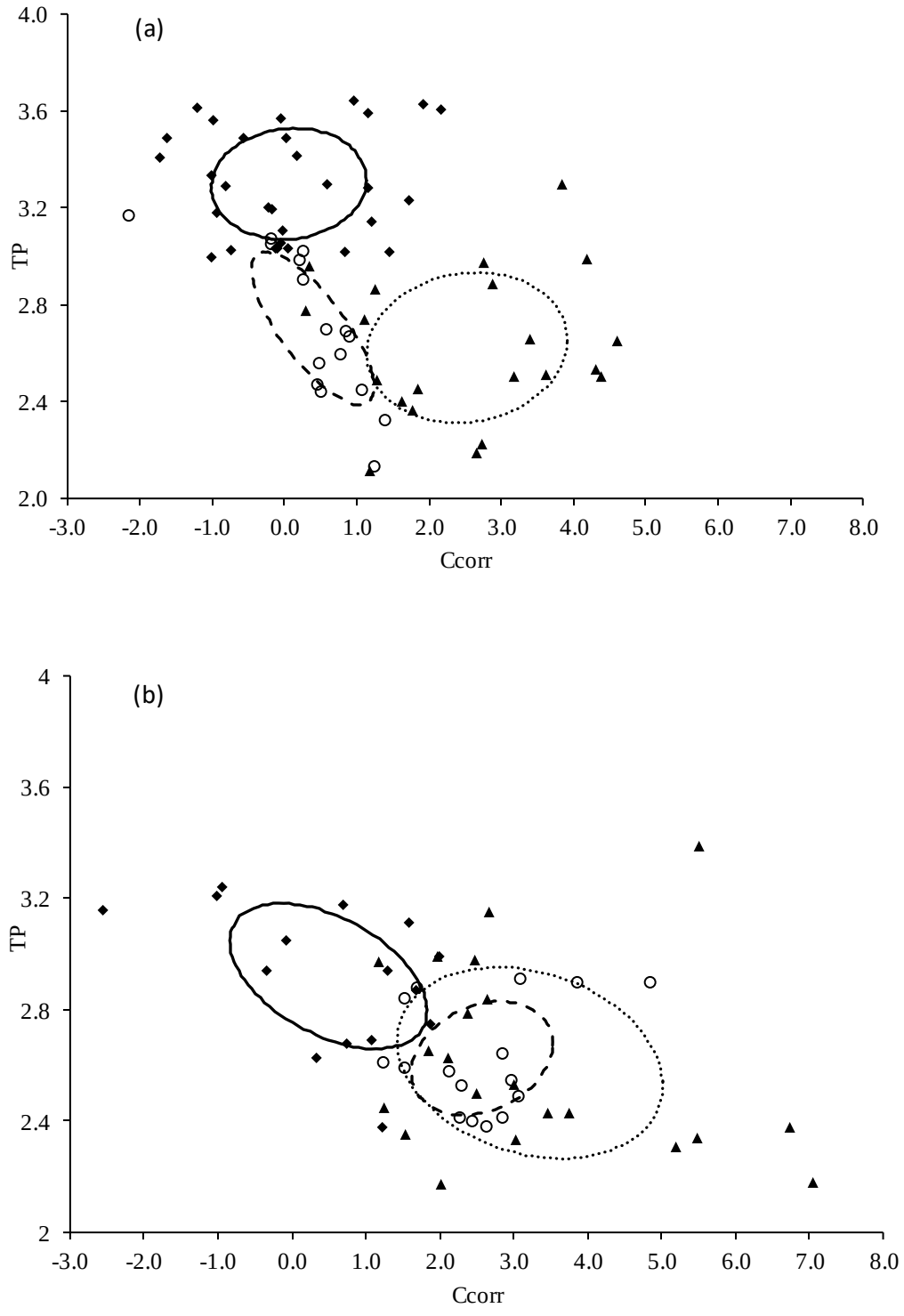

Figure 47. Intra-specific comparisons of Corrected Carbon (C_{corr}) versus trophic position (TP) and positions of core isotopic niches (as SEA_c) for (a) *Barbus barbus* and (b) *Squalius cephalus*, and where ♦, solid line: 0+ fish; ○, dashed line: juvenile fish; ▲, dotted line: adult fish.

5.5 Discussion

Across the three life stages, there was a general pattern of inter-specific partitioning in the core isotopic niche of invasive *B. barbus* and the other fishes, with this contrary to the null hypothesis. This partitioning was particularly strong between the juvenile fishes, where their core niches did not overlap, and was also evident in the 0+ fishes, where overlaps in the core niche were always less than 7 %. In contrast, there was some convergence in the core isotopic niches of the adult *B. barbus* and *S. cephalus*, but the 55 % overlap was less than the 60 % overlap that was suggested as being required to represent significant niche overlap (Schoener 1968; Matley et al. 2017).

This pattern of isotopic niche partitioning between *B. barbus* and other fishes was consistent with a number of isotopic studies completed on populations in their indigenous range (Bašić et al. 2015; Bašić and Britton 2014, 2016). These studies all suggested that *B. barbus* and *S. cephalus* have distinct core isotopic niches, with minimal inter-specific sharing of dietary resources. This pattern was evident in rivers that had been stocked with hatchery reared *B. barbus* at sizes below 250 mm and remained evident in adult fishes (Bašić and Britton 2016). Whilst in Chapter 6, there is suggestion of high overlap in the core isotopic niche of these adult fishes, this was primarily the result of individual fish specialising in the consumption of pelletized marine fishmeal utilised by anglers. Here, in stretches of the study river with less angling pressure, it was demonstrated this niche overlap was less evident, with the isotopic niche partitioning between invasive *B. barbus* and the other fishes becoming established very early in life

(age 0+), with this also supported by 0+ fish stomach contents data from Chapter 4. Moreover, in *B. barbus*, there was also strong core niche partitioning between their different life-stages, suggesting considerable ontogenetic shifts in their diet that resulted in their population having a relatively large core isotopic niche that was composed of at least three distinct sub-sets. In contrast, the isotopic niches of *S. cephalus* were more similar over their three studied life stages, with only the niche of the 0+ fish being distinct from the other life stages, with the juvenile and adult niches overlapping completely.

Stable isotope data of 0+ fishes can be confounded by issues of their data still showing a strong parental signal. For example, in anadromous brown trout *Salmo trutta*, newly emerged fry retained a strong parental, marine-based isotopic signal that enabled their differentiation from fry produced from non-anadromous parents, but this difference was much reduced after four months of feeding in freshwater (Briers et al. 2013). In 0+ smallmouth bass *Micropterus dolomieu*, post-hatch embryos had elevated $\delta^{15}N$ values that were associated with their parental origin, but these values subsequently decreased rapidly due to their exogenous feeding during their metamorphosis from larvae into juveniles (Vander Zanden et al. 1998). Here, the 0+ fishes utilised were all of lengths above 17 mm, were all fully formed juveniles rather than larvae and were likely to be up to 10 weeks old (*cf*. Chapter 3). Their stable isotope data were also very distinct from those of the adult fishes; in terms of uncorrected data, the 0+ fishes were depleted in $\delta^{13}C$ by up to 8 ‰ compared to adult conspecifics. Consequently, the strong patterns of core isotopic niche partitioning detected in these 0+ fishes were interpreted as resulting from their dietary differences

formed by their exogenous feeding within the river, rather than being a legacy of their parental isotopes.

A paradigm in fish invasion ecology is that adverse ecological impacts often develop through increased inter-specific competition for food resources between invasive and sympatric native fishes (Gozlan et al. 2010; Cucherousset et al. 2012). Given the relatively similar size ranges of the invasive *B. barbus* with other cyprinid fishes at each studied life stage (albeit with some significant inter-specific length differences within life stages that were unable to be avoided through sampling issues), this suggests there was considerable potential for inter-specific competitive interactions, especially given the fishes were all from relatively similar functional guilds (Bašić and Britton 2016). Despite this invasion paradigm, there was limited evidence to suggest inter-specific competitive interactions were occurring within the analysed fishes, with only the adult fishes showing some resource sharing. Schulze et al. (2012) suggested that species within the same ecological guild can only coexist when they respond differently to resource availability with, for example, specialised species only persisting if their competitors are generalists. Evidence in literature supports this, with reduced trophic niche sizes in many co-existing fishes when compared to allopatry (Bolnick et al. 2010; Tran et al. 2015). In the River Teme, however, whilst the isotopic niches of the fishes were partitioned, their niches were also similarly sized. Although this suggests there had not been any niche constriction in the native fishes in *B. barbus* presence, it is acknowledged that this is speculative given that isotopic niche sizes of the native fishes were unable to be measured in *B. barbus* absence. Notwithstanding, the inter-specific niche

partitioning evident in the study suggests that despite their similar ecological guilds and sharing similar habitats (especially the 0+ fishes), there were sufficient differences between the fishes in their functional traits and/ or habitat utilisation to result in substantial differentiation in their resource use (Robinson et al. 1993; Borcherding et al. 2013; Negus and Hoffman 2013).

These results suggested that the ecological impacts of invasive *B. barbus* are relatively minor, with little evidence to suggest there was increased inter-specific competition in the fish community, with this supported by other recent studies on native *B. barbus* that have revealed strong patterns of inter-specific core isotopic niche partitioning (e.g. Bašić and Britton 2014, 2016). These studies were, however, all limited to assessing trophic interactions via stable isotope analysis, with the impacts of invasive fishes also potentially including other ecological concerns, such as habitat disturbances (Gozlan et al. 2010). This is important, as recent work has demonstrated that in their native range, *B. barbus* act as 'zoogeomorphic agents' in rivers, where their benthic foraging activities can reduce bed material stability, increase bedload transport, and impact micro-topographic roughness and sediment structure (Pledger et al. 2014, 2015) and redd building also moves large quantities of sediment (Chapter 2). This benthic foraging and redd construction could then also impact upon aspects of the macro-invertebrate communities, such as decreased abundance via predation or reduced species richness via disturbance. However, impacts of the invasive *B. barbus* on these aspects were unable to be tested in the study and thus must remain speculative.

In summary, across three life stages of invasive *B. barbus*, there were some strong patterns of isotopic niche partitioning with native fishes, with this partitioning initially evident during their first growth season, at lengths between 17 and 38 mm, that then persisted through much of their life. These results contribute to the increasing evidence that suggest that rather than competing for food resources, invasive fishes tend to partition in their resource use with native fishes (e.g. Tran et al. 2015; Copp et al. 2017; Britton et al. 2017). Thus, rather than acting as a strong competitor, invasive *B. barbus* appear to integrate into native food webs via exploiting different food resources to native species that then facilitates their coexistence. However, the use of fishmeal pellets by anglers in the river, that contain high levels of marine derived nutrients, can potentially alter the trophic relationships of the adult fishes via individual trophic specialisation and thus this is studied in the following chapter (Chapter 6).

Chapter 6: Trophic consequences for riverine cyprinid fishes of angler subsidies based on marine derived nutrients

This chapter has been published in full as:

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6.1 Abstract

The crossing of freshwater ecosystem boundaries by marine derived nutrients (MDN) is usually associated with migratory salmonid fishes returning to natal rivers. An alternative source of MDN in freshwaters is the widespread use of pelletized marine fishmeal ('pellets') by freshwater anglers as they target large bodied cyprinid fishes, such as *B. barbus*. Here, the trophic consequences of MDN from pellets for riverine cyprinid fishes were tested using stable isotope analyses in controlled and wild scenarios and using *B. barbus* and *S. cephalus* as model species. The isotopic niche, measured as standard ellipse area, assessed trophic niche size, and mixing models predicted the extent to which MDN contributed to fish diet. In experimental mesocosms, *B. barbus* fed low volumes of pellets (approximately 3 per fish) for 130 days had isotopic niches that were up to four times larger than for a control, 'medium' (6 per fish) and 'high' pellet (12 per fish) treatment. Somatic growth rates were significantly higher in the 'medium' and 'high' treatments. In pond enclosure experiments, when juvenile *B. barbus* and *S. cephalus* were fed pellets daily for 100 days, there was a substantial and significant shift in the position of their isotopic niche compared to controls with no pellets fed. However, for each species, there were no significant differences in their somatic growth rates in the presence/ absence of pellets. In the River Teme below Powick Weir, high proportions of MDN contributed to the diet of *B. barbus* and *S. cephalus* captured by angling, but with substantial individual variability in those captured by electric fishing. Across all *B. barbus* > 400 mm, MDN dietary contributions ranged between 9 and 71 %. This suggested some individual diet specialisations within their population that

was associated with feeding on this angler subsidy and that also resulted in a significant increase in the size of their population isotopic niche. The results also suggested that the extent to which individuals specialise in feeding on pellets potentially influences their vulnerability to capture by anglers.

6.2 Introduction

Trophic fluxes of energy and nutrient resources can be ecologically significant when they cross the boundaries of ecosystems that differ in their productivity (e.g. Polis & Hurd, 1995; Zhang *et al.*, 2003; Richardson *et al.*, 2016). These cross-system fluxes can maintain the productivity, diversity, and community structure of recipient ecosystems (Schindler *et al.*, 2005). Anadromous salmonid fishes are well recognised as playing integral roles in these processes, as they accumulate the majority of their biomass in the ocean and import these into freshwaters during spawning, thus releasing marine derived nutrients (MDN) into the relatively nutrient-poor freshwater systems (Schindler *et al.*, 2003). However, this delivery mechanism is not the only MDN source in freshwaters, as aquaculture and angling activities can also elevate the quantity of MDN to freshwater ecosystems via the release of energy rich foods based on pelletized fishmeal ('pellets') that is derived from marine fishes (Bašić *et al.*, 2015).

The use of marine derived fishmeal pellets in freshwater aquaculture is an integral part of the husbandry process (Naylor et al. 2000). In recreational angling, marine derived fishmeal pellets of up to 21 mm in diameter are used as both an attractant and hook-bait, and thus they can supplement fish diet (Grey et al. 2004; Jackson et al. 2013; Bašić et al. 2015). These inputs of pellets can increase the productivity of freshwater systems due to their nutrient and energy fluxes (Jones et al. 1998; Jefferies 2000), and thus they can act as a strong allochthonous trophic subsidy (Marcarelli et al. 2011; Sato and Watanabe 2014). In doing so, they potentially alter food web structure via changes in the trophic

interactions of consumers (Jefferies 2000; Marzcak et al. 2007), and potentially result in resource partitioning between populations (Bašić et al. 2015). The pellets utilised by anglers tend to have high protein levels from fishmeal (typically 40 to 50%) and lipid levels from fish oil (typically 20%) (Naylor et al. 2000; Bašić et al. 2015). These pellets have been used widely for at least 20 years by European freshwater anglers for exploiting the cyprinid fishes *C. carpio* and *B, barbus* (Jackson et al. 2013; Bašić et al. 2015). Substantial quantities can be used, with individual anglers often using in excess of 1 kg per day, with at least 10 anglers often being present daily on some small (< 1 km) stretches of English rivers in summer (Bašić et al. 2015). Arlinghaus and Niesar (2005) estimated that the amount of bait used annually per freshwater angler in Germany was 7.3 kg, indicating that considerable volumes of angler bait might be introduced into freshwaters on an annual basis.

The provision of novel feeding opportunities, such as the seasonal availability of terrestrial insects for stream fishes (Syrjanen et al. 2011), can result in individual trophic niche specialisation developing within populations (Britton and Andreou 2016). This is where the population trophic niche consists of sub-groups of trophically specialised individuals that, in entirety, comprise the population niche (Araújo, Bolnick and Layman 2011). The attractiveness of pelletized marine-derived fishmeal to many fishes is likely to relate to their provision of an energy rich resource that is relatively easy to assimilate and maximises growth rates (Naylor et al. 2000; Bašić et al. 2015). It was recently established that in four rivers in England, the diet of adult *B. barbus* comprised considerable proportions of pelletized fishmeal (up to 80 %; Bašić et al. 2015). However, this

study was all based on samples collected from uncontrolled field conditions, with no consideration of how it impacted the population trophic niche of the fish or their somatic growth rates. The aim of this study was thus to quantify how MDN in pelletized fishmeal from angling modifies the population trophic niches, influences individual dietary specialisation, and affects the growth rates of riverine fishes. Following Grey et al. (2004) and Bašić et al. (2015), who established that MDN from pellets results in fish isotopic data being distinct within freshwater food webs, objectives were to: (1) assess how MDN modifies the trophic niche size and somatic growth rates of allopatric and sympatric fishes in controlled conditions; and (2) quantify the contribution of MDN to the diet of wild fishes, and assess its role in driving individual trophic niche specialisation and modification of the population trophic niche. It was hypothesised that where available, MDN pellets contribute substantial proportions of the diet of river fishes, resulting in individuals specialising on this trophic subsidy and having faster somatic growth rates.

6.3. Materials and methods

6.3.1 Model species, experimental designs and field study

The model species were *B. barbus* and its cyprinid trophic analogue *S. cephalus*. These fishes are sympatric in many European rivers and achieve relatively similar body sizes (Bašić and Britton 2016). A mesocosm experiment tested how the variable availability of pellets affected the trophic niche size and somatic growth rates of allopatric *B. barbus*. A semi-controlled pond experiment then determined how pellet availability affected the trophic niche position and size,

and somatic growth rates, of *B. barbus* and *S. cephalus* in allopatry and sympatry. A field study then tested the influence of pellets on the trophic niche and diet composition of *B. barbus* and *S. cephalus* in the lower River Teme. These studies utilised stable isotope analysis (SIA) to assess trophic niche sizes (as isotopic niches) and the diet composition of the fishes.

The mesocosm experiment was completed in 12 artificial ponds of 250 L volume, using hatchery-reared juvenile *B. barbus* across four treatments: control (no supplementary feeding), low (supplementary feeding of approximately three pellets per day per fish), medium (6 pellets per day per fish) and high (12 pellets per day per fish). Each treatment was replicated three times, with five fish used per replicate. The pellets were 2 mm diameter and comprised of 45 % protein (from marine fishmeal) and 20 % fish oil (Dynamite Baits 2017). Each mesocosm pond was outside, mounted on a concrete base with no overhanging trees nearby, and had a gravel substrate (6 mm diameter), aeration and a filter to maintain water quality. Feeding rates were achieved via automated feeders releasing pellets once per day at 20:00, as *B. barbus* are crepuscular (Britton and Pegg 2011). The mesocosms were set up in April 2015 and were seeded with macroinvertebrates collected from a local stream (*Gammarus pulex*; 20 per mesocosm). Chironomid larvae naturally colonised all mesocosms.

The fish were measured (fork length, nearest mm) and weighed (to 0.1 g) before their introduction into the mesocosms in June 2015 (Table 30). They were removed in October 2015, thus were exposed to their new diets for 130 days. Temperature loggers (TinyTag TGP-4017) in eight mesocosms (2 per treatment)

recorded water temperatures twice per day (0.00 and 12.00) revealed a mean water temperature (± 95% confidence limits) of 19.4 ± 0.7 °C, with no significant differences between mesocosms (ANOVA: $F_{1,6} = 0.56$, $P = 0.48$). For a consumer species of starting weight 10 g, estimated half-life at 20 °C is 36 days for $\delta^{13}C$ and 38 days for $\delta^{15}N$ (Thomas and Crowther 2015). These values equate to 92% replacement of both isotopes in the fish after 130 days, with consumers generally considered to have fully equilibrated to their food resources at 94% isotopic replacement (Hobson and Clark 1992).

On day 130, the mesocosms were drained and the fish removed, euthanized (over-anaesthesia; MS-222), re-measured, re-weighed and a dorsal muscle sample taken for SIA (Busst et al. 2015). Samples of putative prey resources were also collected from each mesocosm (*G. pulex* and Chironomid larvae); where possible, these represented triplicate samples per mesocosm (1 sample = 5 individuals). All samples were then oven dried to constant weight at 60ºC as preparation for SIA.

The pond experiment used mesocosms where *B. barbus* and *S. cephalus* were used in allopatry and sympatry. Thus, three treatments were used in pellet presence and absence: both species in allopatry (n = 10), and a final treatment where they were present in sympatry (n = 5 + 5), with three replicates per treatment. All fish were juveniles (starting lengths 60 to 88 mm, starting weights < 10 g) and hatchery reared. Each mesocosm comprised of an independent enclosure situated within one of two larger semi-natural, ex-aquaculture ponds (pond size: 30 x 12 m; consistent 1 m depth). Each enclosure comprised of

aluminium frames of 1.66 m (length) x 1.05 m (width) x 1.2 m (height) within a net of 7 mm square mesh that prevented fish ingress/ egress but enabled transfer of water and invertebrates. The enclosures provided uniform habitats across the treatments and replicates in which the fish were exposed to the same prey communities. The enclosures in which pellets were fed were located in a separate pond to those with no pellets fed to avoid risk of cross-contamination between treatments. Within their larger ponds, the enclosures were located randomly, with least 0.5 m distance between them for independence. Water temperatures were measured hourly using a temperature logger (TinyTag TGP-4017) placed in the centre of each pond; mean temperature (± 95% confidence limits) was 18.2 ± 0.3 ^{o}C in the non-pellet pond and 18.4 ± 0.4 ^{o}C in the pellet pond. Anti-predator netting (15 mm mesh) was also placed over the top of all enclosures. The enclosures sat on the substrate and macrophytes grew through each of them (primarily *Elodea* spp.)

The enclosures were placed into the ponds seven days before the fish were introduced, with the experimental period commencing in May 2014 and lasting 100 days. The estimated isotopic turnover was approximately 90% (Thomas and Crowther 2015). Feeding of pellets used two methods. Firstly, 2 mm pellets were fed via automated feeders (30 per day). Secondly, 3 mm pellets were fed once per week by hand (approximately 60 pellets per replicate). Other than size, the pellets were identical to those used in the first mesocosm experiment, with the same ingredients and constituents (i.e. fishmeal-based, with the same protein and lipid levels; Dynamite Baits 2017). Following the removal of the enclosures on day 100, the fish were recovered, euthanized (anaesthetic overdose, MS-222)

and placed on ice, with samples of macroinvertebrates taken from each enclosure. In the laboratory, fish were re-measured and dorsal muscle samples taken. Macroinvertebrate samples were sorted to species, enabling three samples per species to be dried for SIA (Bašić and Britton 2016). A random selection of fish dorsal muscle samples (n = 15 to 18 per species and treatment; minimum number of samples per replicate = 5) was then also selected and dried for SIA.

The field study used the *B. barbus* and *S. cephalus* populations of the River Teme below Powick Weir (Fig. 3, 52°10'13" N; 2°14'31" W) to test the influence of MDN from pellets on the diet composition and trophic niche size of wild fishes. The study stretch receives considerable angling pressure for *B. barbus* from both banks throughout the year, but especially between June and October when anglers are present daily, with the majority utilising pellets based on fishmeal. A previous study also indicated *B. barbus* diet elsewhere on the river (approximately 10 km upstream, with separation by Powick Weir) consisted of high proportions of pelletized fishmeal (Bašić et al. 2015). Here, SIA of the fishes utilised scales, as only catch and release angling is practised for cyprinid fishes on the river and so the collection of SIA material had to be rapid and non-destructive, but also appropriate for analysis (Hutchinson and Trueman 2006; Busst and Britton 2016; Chapter 5).

Samples of *B. barbus* were captured using a combination of boat mounted electric fishing on the 22nd September 2015 and angling on the 22nd and 23rd September. Samples of *S. cephalus* were captured by angling between 22nd and 30th September 2015. Fish were tagged with passive integrated transponder tags

before their release, and some were also tagged with acoustic tags (*cf*. Chapter 7), with no tagged fish recaptured. Each captured fish was measured (fork length (L_f), nearest mm) and three to five scales removed and stored in paper envelopes. Concomitantly, samples of angler bait were taken for SIA, with samples taken from two types used in the river ('fish pellet 1'; 'fish pellet 2'). Samples of macroinvertebrates for SIA were collected by kick-sampling. This also provided samples of minnow *P. phoxinus*, bullhead *Cottus gobio* and stone loach *Barbatula barbatula* for SIA (hereafter referred to as 'small fishes'; all were <40 mm). Triplicate samples were taken of each species, with dorsal muscle samples taken from each 'small fish'. For SIA, the large body size (> 270 mm) of the sampled *B. barbus* and *S. cephalus* meant that only material from the very outer portions of scales were used in analyses, i.e. material produced from recent growth (Hutchinson and Trueman 2006; Bašić et al. 2015; Section 5.2).

6.3.2 Stable isotope analysis

The analysis of all samples for SI was as already described in Section 5.3.1. Thus, the SI data comprised of values of $\delta^{13}C$ and $\delta^{15}N$ (‰) for each sample of fish and putative food resource. Prior to the use of the pond experimental data in analyses, their macro-invertebrate data were checked to ascertain whether their data needed correction to enable their comparison between ponds (Section 5.3.1). This suggested that the 95% confidence limits of the mean SI data for the macroinvertebrates had some significant differences between the two larger ponds ('pellet pond': $\delta^{13}C$: -31.86 ± 1.06, $\delta^{15}N$: 5.9 ± 0.66‰; 'non-pellet pond': $\delta^{13}C$: -34.68 ± 1.14, $\delta^{15}N$: 8.49 ± 0.60‰). Therefore, to enable appropriate comparison of SI data between the pellet and no pellet treatments, the $\delta^{15}N$ data

were transformed to trophic position (TP) using the Equation 20 and the $\delta^{13}C$ data were converted to $\delta^{13}Ccorr$ using Equation 21 (Section 5.3.2). As stable isotope data from dorsal muscle more closely reflects diet (Grey *et al.*, 2009), then for the fish samples from the field study, their SI scale data were converted to dorsal muscle tissue values before further analysis using conversion values from Busst et al. (2015) that are specific to *B. barbus* ($\delta^{15}N + 0.21$, $\delta^{13}C - 2.17$) and *S. cephalus* ($\delta^{15}N + 0.39$, $\delta^{13}C - 2.91$).

6.3.3 Testing of stable isotope analysis data

In all cases, the SI data were used to calculate the trophic niche sizes of the fishes, using the core isotopic niche, along with the extent of its overlap between species. The method used for this was already described in Section 5.3.2. Bayesian mixing models then estimated the relative proportions of different food resources contributing to fish diet using the MixSIAR package in R (Parnell et al. 2010; R Core Team 2016; Stock and Semmens 2016). Correction for isotopic fractionation between resources and consumers used species-specific and tissue-specific fractionation factors between fish and prey ($\delta^{15}N$: 3.4 ± 0.98‰; $\delta^{13}C$: 0.39 ± 1.3‰) (Busst, Bašić and Britton 2015; Busst and Britton 2016). All models were run using normal run length (chain length: 100,000 iterations with burn-in of 50,000, with posterior thinning (thin: 50) and 3 chains). Model diagnostics were based on Gelman-Rubin and Geweke, with sufficient convergence to accept the results (Stock and Semmens 2016). In mesocosm experiments, models were run with the resources as 'pellets' and 'macroinvertebrates'. The latter was primarily Chironomid larvae, as this was the only putative food resource sampled from each individual mesocosm.

However, it also covered *G. pulex*, as some samples were collected from a small proportion of the mesocosms. Their SI data overlapped with Chironomids and so the model could not separate their dietary contributions (mean SI values ± 95% confidence limits (‰): Chironomid: n = 18; $\delta^{13}C$: -24.08 ± 0.36, $\delta^{15}N$: 7.83 ± 0.38; *G. pulex*: n = 6; $\delta^{13}C$: -23.78 ± 0.46, $\delta^{15}N$: 8.29 ± 0.24). In the pond experiments, four putative food resources were used: 2 mm pellet, 3 mm pellet and the macroinvertebrate groups Corixidae and Odonata. In the field study, the putative food resources in the model were pooled according to 'fish pellet 1', 'fish pellet 2', small fishes and Arthropoda. In addition to the Bayesian mixing models already outlined, these field study data were then also used to assess individual variability using SOLOSIAR ('siarsolomcmcv4') in the SIAR package in R (Parnell et al. 2010; R Core Team 2016). In this model, fractionation values were (mean ± SD): $\delta^{13}C$: 2.57 ± 0.06 for 'small fishes' and both pellets, and 0.80 ± 0.30 for Arthropoda; $\delta^{15}N$: 2.4 ± 0.07 for 'small fishes' and both pellets, and 3.0 ± 0.02 for Arthropoda (Busst et al. 2015; Busst and Britton 2016).

6.3.4. Other data analyses

In the mesocosm and pond experiments, SI data were also tested in linear mixed effect models (LMEM). In the mesocosm experiment, differences were tested in the isotopic data of *B. barbus* between the four treatments. The dependent variable was $\delta^{13}C$ or $\delta^{15}N$, and each model was fitted with mesocosm number as a random effect on the intercept to prevent inflation of the residual degrees of freedom (Tran et al. 2015). The significance of differences in SI data between treatments used estimated marginal means and linearly independent pairwise

comparisons with Bonferroni correction for multiple comparisons. In the pond experiment, differences were tested between the species, their allopatric and sympatric treatments, and between the pellet and no pellet treatments. Species were entered into models according to their treatments so, for example, *B. barbus* was present in models as (1) allopatric *B. barbus*, (2) in sympatry with *S. cephalus*, and (3) in the presence and absence of pellets. The dependent variable was Ccorr or TP, with each model also fitted with mesocosm number as a random effect. The significance of differences in Ccorr and TP were also determined from the model outputs using linearly independent pairwise comparisons.

Somatic growth rates were estimated in the mesocosm experiments using incremental length (IL) and specific growth rate (SGR); IL was determined per replicate for each treatment and was expressed as the mean daily growth increment per fish, calculated from Equation 22.

$$[((\text{total } L_{t+1}) - (\text{total } L_{t+1}))/4]/_t \qquad \text{(Equation 22)}$$

Where total L_t and L_{t+1} was the total starting and end lengths of the fish in each replicate, 4 represents the number of fish per replicate and $_t$ = number of days. Mean specific growth rates (SGR) were determined from Equation 23.

$$100[((\ln W_{t+1}) - (\ln W_t))/4]/_t \qquad \text{(Equation 23)}$$

where W_t = total starting weight and W_{t+1} = total end weight. In the pond experiments, only incremental length was tested. Using generalised linear models, differences were tested in the growth rate of each species according to

their context (allopatric or sympatric) and treatment (pellet or no pellet). In the field study, the scales of the fish were viewed on a projecting microscope (magnification x 10 to x48) and an age estimate derived from counting of annual growth checks (Amat Trigo et al. 2017). Scales measurements of total scale radius (SR) and distance to the penultimate and final annulus (PA and FA respectively) were then taken to enable the last annual length increment (L_{fa}) of the fish to be calculated from Equation 24.

$L_{fa} = ([FA-PA]/SR) \times L_f$. (Equation 24)

Throughout the results, where error is expressed around the mean, it represents 95% confidence limits unless stated otherwise.

6.4 Results

6.4.1 Mesocosm experiments

There were no significant differences in starting lengths and weights of the fish across the experimental treatments (generalized linear models: length: Wald $\chi^2 = 0.91$, $P = 0.47$; weight: Wald $\chi^2 = 0.79$, $P = 0.51$). At the conclusion of the experiment, all of the fish were recovered, and their mean length and weight had increased to 120.4 ± 4.1 mm and 18.3 ± 2.0 g, with significant differences in final lengths and weights across the treatments (generalized linear model: Wald $\chi^2 = 50.64$, $P < 0.01$). Fish had higher lengths and mass in the Low, Medium and High treatments compared with the Control ($P < 0.01$). The generalized linear model for both SGR and IL was significant (Wald $\chi^2 = 263.9$, $P < 0.01$ and Wald $\chi^2 = 2776.3$, $P < 0.01$ respectively), with growth rates being significantly faster

in all treatments compared with the Control ($P < 0.01$; Fig. 48). Both SGR and IL increased as the proportion of pellets fed daily increased (Fig. 48).

The LMEM revealed significant differences in $\delta^{13}C$ between *B. barbus* in the control (mean -21.4 ± 0.17‰) and the other treatments (Low: -21.7 ± 0.2‰; Medium: -22.1 ± 0.1‰; High: -22.1 ± 0.1‰) ($P < 0.01$; Fig. 49). For $\delta^{15}N$, the LMEM revealed significant differences between the Control and High treatment (12.4 ± 0.6 vs. 10.6 ± 1.0‰; $P < 0.01$), but not between the Control and the Low and Medium treatments (12.4 ± 0.6 vs. 12.0 ± 1.6 and 11.6 ± 1.6‰ respectively; $P = 1.0$ in all cases; Fig. 49). The 95% confidence limits of the estimates of isotopic niche size (SEA_b) indicated that the niche of the *B. barbus* in the low treatment was significantly larger than the Control, Medium and High treatments (Table 30; Fig. 49). The isotopic niche of the Control overlapped with that of the Low treatment by 76%, but did not overlap at all with the Medium and High treatments (Table 30; Fig. 49). In the Control, macroinvertebrates were the principal contributor to *B. barbus* diet, whereas in the Medium and High treatments, pellets contributed up to 48% of diet (Table 30). In the Low treatment, pellets only contributed 23% to estimated diet (Table 30).

6.4.2 Pond experiments

Across the treatments, the mean starting lengths of the *B. barbus* were 77.5 to 82.0 mm and *S. cephalus* 73.9 to 81.7 mm (Table 31). At the conclusion of the experiment, 97 % of the fish present at the start of the experiment were recovered at the end (174 from 180 fish), with no more than one fish per replicate missing. The length range of the fish had increased to 113.7 to 119.4 mm (*B. barbus*) and

124.6 to 131.1 mm (*S. cephalus*). The generalized linear model testing differences in IL across the species and treatments was significant (Wald χ^2 = 105.4, P = 0.02), with the effect of starting length being a significant covariate (P = 0.04). Pairwise comparisons revealed, however, that there were no significant differences in growth rates across the species and their treatments (P = 0.09 to 1.0; Fig. 50).

The LMEM revealed that the significant differences in the corrected $\delta^{13}C$ data (Ccorr) were primarily between the pellet and no pellet treatments, including between allopatric *B. barbus* (pellet: 1.92 ± 0.09; no pellet: 0.68 ± 0.09; P < 0.01) and allopatric *S. cephalus* (pellet: 1.84 ± 0.09; no pellet: 0.25 ± 0.09; P < 0.01) (Fig. 51). The same differences were also apparent for TP, but with additional differences between the two fishes in the presence and absence of pellets (P < 0.02 in all cases), where *B. barbus* were at a higher TP than *S. cephalus* (Fig. 51). Isotopic niche estimates revealed that there was no overlap in the niches of the two fishes in allopatry or sympatry, or in the presence and absence of pellets, but the availability of pellets caused a substantial shift in the position of the isotopic niche of both fishes in both allopatry and sympatry (Fig. 51). This shift was caused by the presence of the pellets in fish diet; where present, their contribution to fish diet was 43 and 58 % (Table 32). In terms of isotopic niche size, however, there was considerable overlap in the 95 % confidence limits of estimates of SEA_b for the species in the presence/ absence of pellets in their allopatric and sympatric contexts, thus the pellets did not affect isotopic niche size (Table 33).

6.4.3 Wild fishes

A total of 31 *B. barbus* were sampled from the River Teme in September 2015. Of these, 19 were captured by electric fishing (mean length 512.1 ± 63.8 mm) and 12 by angling (mean length 616.8 ± 72.7 mm), with the differences in their lengths being significant (ANOVA: $F_{1,29} = 5.56$, $P = 0.03$). Across this dataset, there was also a significant relationship between fish length and SI data ($\delta^{13}C$: $R^2 = 0.42$, $F_{1,29} = 20.61$, $P < 0.01$; $\delta^{15}N$: $R^2 = 0.32$, $F_{1,29} = 13.50$, $P < 0.01$). To remove this length influence on the SI data, the six fish captured by electric fishing of < 400 mm length were removed from the dataset, resulting in the relationships between fish length and SI data now being non-significant ($\delta^{13}C$: $R^2 = 0.10$, $F_{1,23} = 2.30$, $P = 0.13$; $\delta^{15}N$: $R^2 = 0.09$, $F_{1,23} = 2.18$, $P = 0.15$). This also increased the mean length of the electric fished *B. barbus* to 585.8 ± 55.9 mm (n = 13), with this not significantly different to the angler caught fish (ANOVA: $F_{1,23} = 0.96$, $P = 0.34$). In addition, 6 *S. cephalus* were sampled by angling (length range: 400 to 540 mm; mean length 456.7 ± 51.3 mm), with none sampled by electric fishing. Regarding the age of the *B. barbus* > 400 mm, there was only one individual age at 8+ years, with the reminder all between 11+ and 18+ years. At these ages, their annual length increments were relatively low (mean last annual length increment: 18.7 ± 4.1 mm), with the relationship between length increment and the SI data being non-significant ($\delta^{13}C$: $R^2 = 0.04$, $F_{1,23} = 0.67$, $P = 0.42$; $\delta^{15}N$: $R^2 = 0.08$, $F_{1,23} = 1.56$, $P = 0.23$.

For the *B. barbus* > 400 mm sampled by electric fishing, their isotopic niche was significantly larger than the angled fish (95 % CL SEA_b: 2.54 to 6.66 vs. 0.66 to 2.30‰; Fig. 52). The angled sub-set of *B. barbus* shared 83 % of their isotopic

space with those that were electric fished (Fig. 52). The angled *S. cephalus* had an isotopic niche in a similar position to the angled *B. barbus* and they also had a similar niche size (95% CL SEA_b: 0.63 to 4.28‰; Fig. 52). The estimated dietary contributions from the Bayesian mixing models suggested that the angled *B. barbus* and *S. cephalus* had total contributions of pellets of 59 and 44 % respectively, whereas this was reduced to 39 % for the electric fished *B. barbus* of > 400 mm (Table 34a). At the individual level, estimated dietary proportions varied by sampling method, but with generally lower proportions of pellets in the diet of electric fished *B. barbus* (range 9 to 62 %) than angled (range 40 to 71 %) (Table 34b). The coefficient of variation was also higher for all food items for electric fished *B. barbus*, but this was especially strong for pellets (electric fished: 0.45; angled: 0.17; Table 34b). The overall range of the contribution of pellets to *B. barbus* diet, irrespective of sampling method, was 9 to 71 % (Table 34b).

Table 30. Mean lengths and weights, isotopic niche size (as 95% CI of standard ellipse area, SEA_b) of *Barbus barbus* per treatment and the extent of their overlap between treatments, and the estimated contributions of putative foods to their diet (0 – 1 scale), as predicted in MixSIAR (±95% CI). Sample sizes were n = 15 per treatment.

							Estimated contribution to diet (%)	
Treatment	Mean length (mm)		Mean weight (g)		SEA_b (‰)	Overlap in isotopic niche with Control (%)	Macroinvertebrate	Pellet
	Start	End	Start	End				
Control	106.5 ± 8.5	108.2 ± 8.3	9.9 ± 1.8	11.2 ± 2.2	0.06 – 0.21	n /a	0.97 ± 0.02	0.03 ± 0.02
Low	103.8 ± 5.9	113.3 ± 6.6	10.2 ± 1.2	14.7 ± 2.5	0.39 – 1.31	76	0.77 ± 0.02	0.23 ± 0.02
Medium	105 ± 3.9	127.3 ± 3.9	12.3 ± 1.0	22.9 ± 2.5	0.10 – 0.33	0	0.52 ± 0.02	0.48 ± 0.02
High	106.6 ± 4.1	132.7 ± 6.6	11.6 ± 0.9	24.3 ± 3.4	0.08 – 0.28	0	0.54 ± 0.02	0.47 ± 0.02

Table 31. Number of fish per species and treatment analysed for stable isotope analysis from the pond enclosure experiment, their start and end mean lengths (± 95% CI), and mean stable isotope values (± 95% CI).

Treatment	Species	n	Mean starting length (mm)	Mean end length (mm)	Mean $\delta^{13}C$ (‰)	Mean $\delta^{15}N$ (‰)
Allopatry/pellets	*B. barbus*	18	80.1 ± 0.3	117.83 ± 1.99	-24.70 ±0.21	9.39 ± 0.10
Allopatry/pellets	*S. cephalus*	18	81.7 ± 0.4	131.06 ± 1.38	-25.10 ± 0.23	8.44 ± 0.04
Allopatry/no pellets	*B. barbus*	18	77.6 ± 0.2	113.67 ± 1.32	-28.20 ± 0.20	11.18 ± 0.05
Allopatry/no pellets	*S. cephalus*	17	73.9 ± 0.3	124.59 ± 1.69	-30.31 ± 0.19	10.72 ± 0.05
Sympatry/pellets	*B. barbus*	15	82.0 ± 0.4	119.4 ± 1.84	-25.45 ±0.18	9.25 ± 0.09
Sympatry/pellets	*S. cephalus*	15	76.3 ± 0.4	125.27 ± 1.69	-24.94 ± 0.20	8.34 ± 0.04
Sympatry/no pellets	*B. barbus*	15	77.5 ± 0.3	118.94 ± 1.91	-29.05 ± 0.11	10.79 ± 0.05
Sympatry/no pellets	*S. cephalus*	15	76.1 ± 0.4	126.73 ± 1.64	-30.67 ± 0.14	10.81 ± 0.03

Table 32. Estimated contributions (0 – 1) of each putative food item to fish diet in the 'pellet' treatments of the pond enclosure experiment. Values represent mean estimated dietary proportions (± 95% CI) from MixSIAR.

	Corixidae	Odonata	2mm pellet	3mm pellet	Total pellet*
Allopatric *B. barbus* (n=18)	0.34 ± 0.11	0.21 ± 0.13	0.27 ± 0.06	0.18 ± 0.06	0.45
Allopatric *S. cephalus* (n=15)	0.26 ± 0.04	0.16 ± 0.05	0.33 ± 0.04	0.25 ± 0.04	0.58
Sympatric *B. barbus* (n=18)	0.32 ± 0.11	0.22 ± 0.12	0.25 ± 0.06	0.22 ± 0.07	0.47
Sympatric *S. cephalus* (n=15)	0.25 ± 0.09	0.15 ± 0.10	0.33 ± 0.09	0.27 ± 0.11	0.60

* derived from summing the modal estimations of the 2 mm and 3 mm pellet and so no estimate of error around the values can be provided.

Table 33. Isotopic niche size, as 95% CI of SEA_b (‰) for *Barbus barbus* and *Squalius cephalus* in the different treatments of the pond enclosure experiment, and as calculated from corrected stable isotope data. Sample sizes were as per Table 32.

	n	No fishmeal pellet	Fishmeal pellet
Allopatric *B. barbus*	18	0.02 – 0.05	0.03 – 0.09
Sympatric *B. barbus*	18	0.01 – 0.03	0.02 – 0.04
Allopatric *S. cephalus*	15	0.02 – 0.05	0.02 – 0.05
Sympatric *S. cephalus*	15	0.01 – 0.02	0.01 – 0.04

Table 34. (a) Mean contributions to fish diet of putative food resources (0 – 1 scale; ± 95% CL) of *Barbus barbus* and *Squalius cephalus* in the River Teme by sampling method, estimated by MixSIAR; (b) minimum, maximum, mean (± 95% CI) and coefficient of variation (CV) of estimates of contributions to individual *B. barbus* diet (0 – 1) of the putative foods per sampling method (EF: electric fishing; A: angling), estimated by SOLOSIAR, where mean pellet data represents the sum of mean Pellet 1 and mean Pellet 2 per individual fish. Only *B. barbus* of > 400 mm length were used in analyses.

(a) Species	n	Arthropoda	'Small fishes'	Pellet 1	Pellet 2	Total pellet*
Electric fished *B. barbus*	13	0.39 ± 0.10	0.26 ± 0.09	0.10 ± 0.04	0.26 ± 0.04	0.36
Angled *B. barbus*	12	0.22 ± 0.07	0.20 ± 0.06	0.11 ± 0.03	0.48 ± 0.04	0.59
Angled *S. cephalus*	6	0.23 ± 0.11	0.24 ± 0.10	0.15 ± 0.06	0.39 ± 0.08	0.54

* derived from additional of the modal estimations of the 2mm and 3mm pellet and so no estimate of error around the values are provided.

(b)	Minimum		Maximum		Mean		CV	
Dietary item	EF	A	EF	A	EF	A	EF	A
Arthropod	0.07	0.13	0.45	0.30	0.19 ± 0.09	0.18 ± 0.05	0.82	0.68
Small fish	0.18	0.16	0.50	0.43	0.23 ± 0.10	0.24 ± 0.05	0.81	0.69
Pellet	0.09	0.40	0.62	0.71	0.38 ± 0.09	0.59 ± 0.06	0.45	0.17

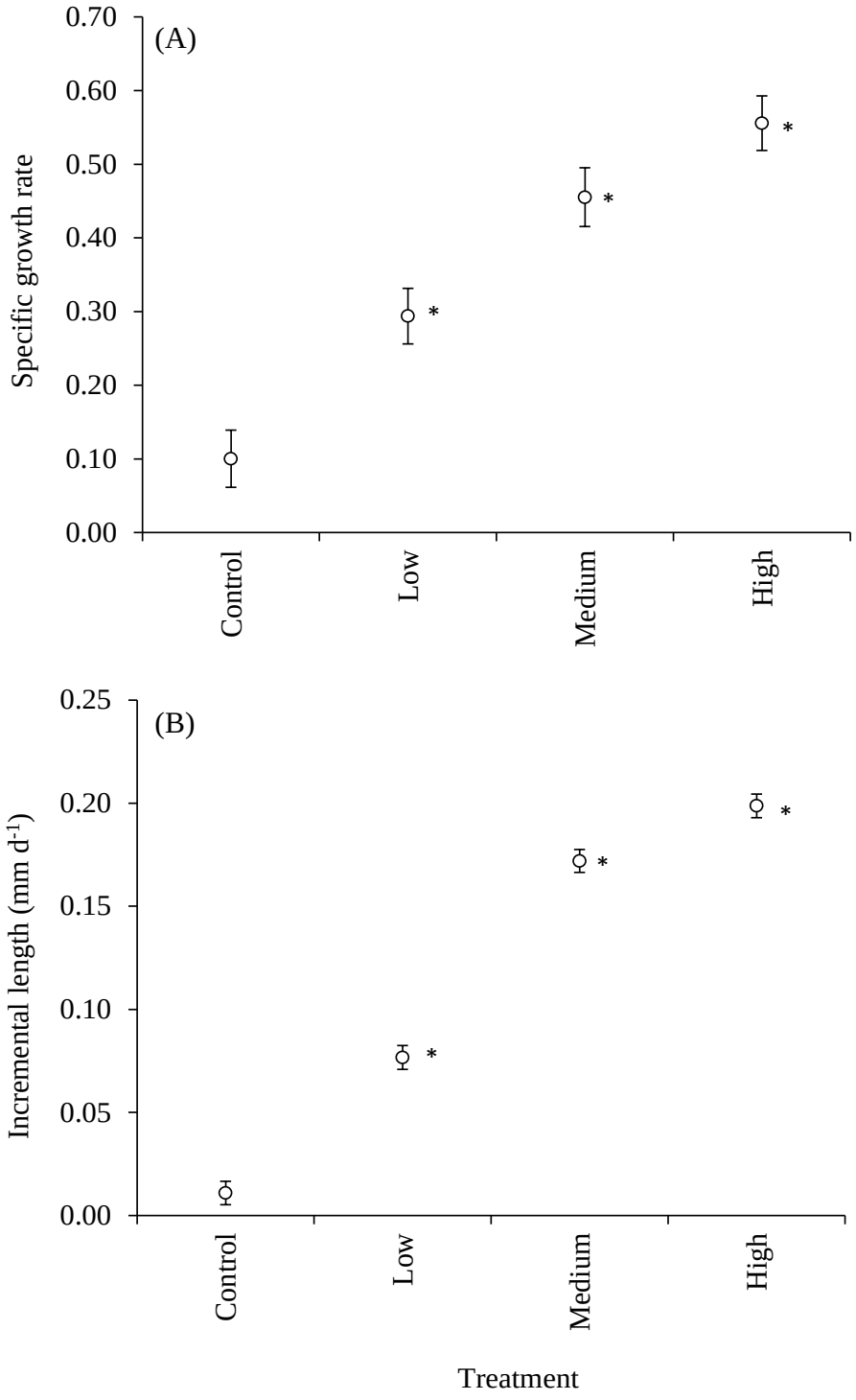

Figure 48. Somatic growth rates, as specific growth rate (A) and incremental length (B) per treatment for *Barbus barbus* in the mesocosm experiment. Values represent estimated marginal means from the generalized linear models and * indicates the difference in growth rate is significant at $P < 0.001$) between the treatment and the control according to linearly independent pairwise comparisons. Error bars represent 95% confidence intervals.

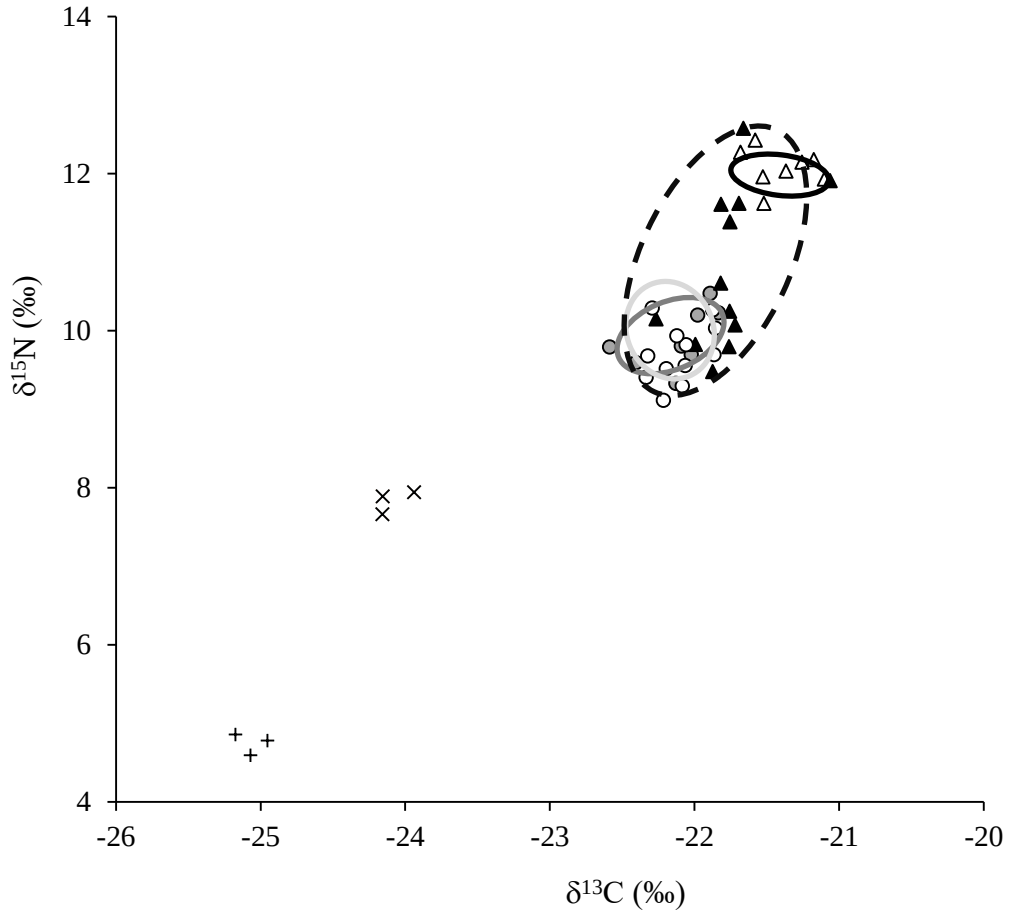

Figure 49. Stable isotope bi-plot of *Barbus barbus* in the 250 L mesocosms and their isotopic niche (as standard ellipse area, SEA_c), where clear triangles are the control fish and solid black line is their isotopic niche, filled triangles are the low treatment fish and the dashed black line is their isotopic niche, clear circles are the medium treatment fish and the solid light grey line is their isotopic niche, and grey circles are the high treatment fish and the dark grey line is their isotopic. × represents Chironomid larvae and + represent the fishmeal pellets fed daily.

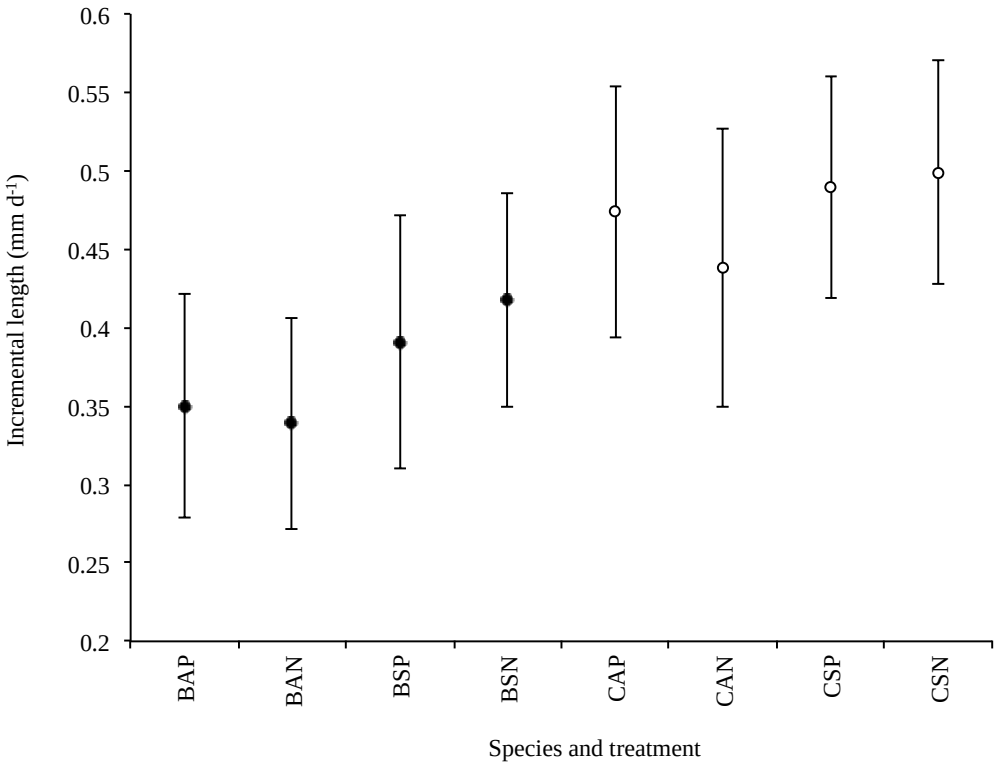

Figure 50. Somatic growth rates, as incremental length, of *Barbus barbus* (filled circles) and *Squalius cephalus* (clear circles) per treatment in the pond enclosure experiment. BAP: allopatric *B. barbus* with pellets; BAN: allopatric *B. barbus*, no pellets; BSP: sympatric *B. barbus* with pellets; BSN: sympatric *B. barbus*, no pellets; CAP: allopatric *S. cephalus* with pellets; CAN: allopatric *S. cephalus*, no pellets; CSP: sympatric *S. cephalus* with pellets; CSN: sympatric *S. cephalus*, no pellets. Error bars represent 95% confidence limits.

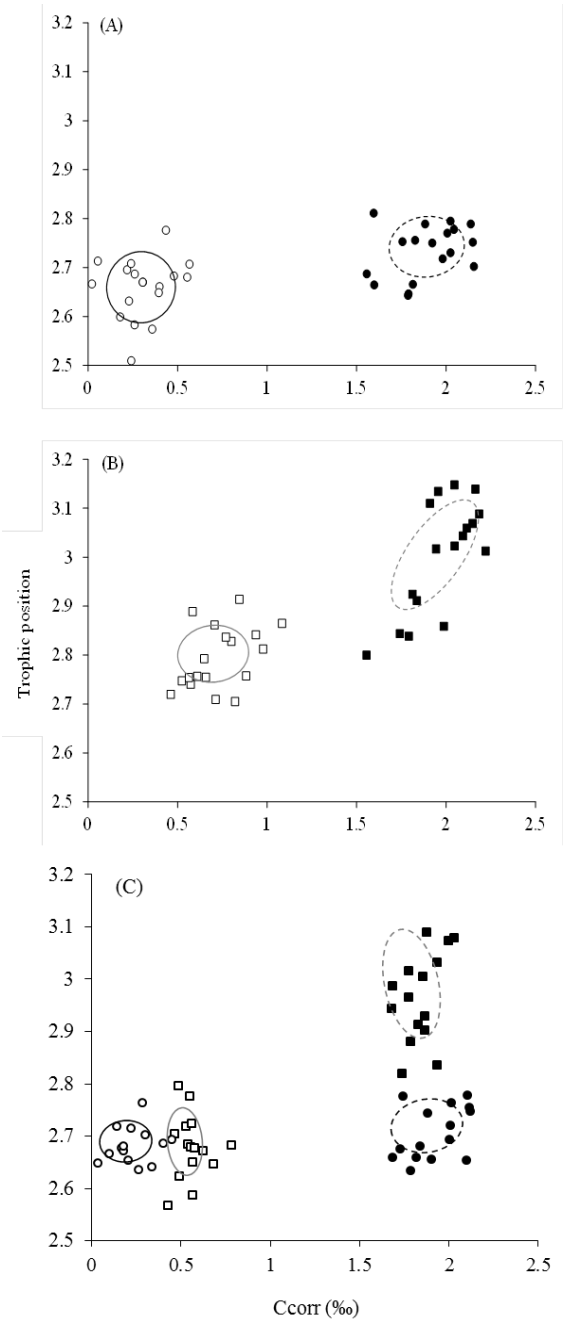

Figure 51. Stable isotope biplots (of corrected stable isotope data to trophic position and corrected carbon, Ccorr) showing individual data points (as symbols) and the isotopic niche (as standard ellipse area, SEA_c) for (A) allopatric *Squalius cephalus* in the no pellet (clear circle, solid black line) and pellet treatment (filled circle, dashed black line); (B) allopatric *Barbus barbus* in the no pellet (clear square, solid grey line) and pellet treatment (filled square, dashed grey line); and (C) sympatric *S. cephalus* in the no pellet (clear circle, solid black line) and pellet treatment (filled circle, dashed black line), and sympatric *B. barbus* in the no pellet (clear square, solid grey line) and pellet treatment (filled square, dashed grey line).

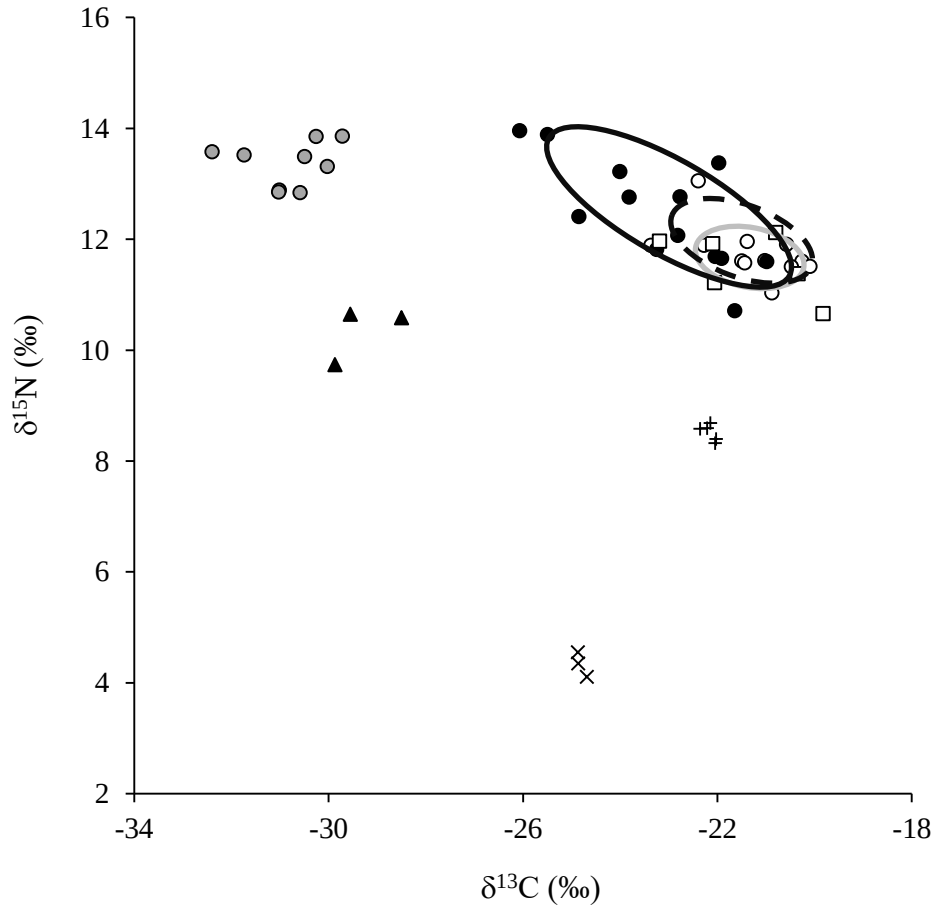

Figure 52. Stable isotope bi-plot of the lower River Teme, showing individual data points and isotopic niches (as standard ellipse areas). *Barbus barbus* (electric fishing; length range 401 to 770 mm; n = 13): data points: black circles, solid black line: isotopic niche; *Barbus barbus* (angling, length range 520 to 721 mm; n = 12): data points: clear circles, dashed black line: isotopic niche; *Squalius cephalus* (angling, length range 400 to 540 mm; n = 6): data points: clear squares, solid grey line: isotopic niche, Grey circles are combined data for 'small fishes' (*Cottus gobio, Barbatula barbatula, Phoxinus phoxinus*); + fishmeal pellet 1; × fishmeal pellet 2; black triangle: Arthropoda.

6.5 Discussion

The two experiments revealed that where fishmeal pellets were present as a food resource for *B. barbus* and *S. cephalus*, these were generally consumed in sufficient proportions to alter the SI signatures of their tissues, as per the hypothesis, and resulted in major shifts in the position of their population isotopic niche. In the lower River Teme, where *B. barbus* were sampled by both angling and electric fishing, there was considerable individual variability in the contribution of pellets to diet, ranging between 9 and 71 %; where only angled fish were considered then the range was 40 to 71 %. High estimates of contributions of pellets to *S. cephalus* diet were also apparent, with these all captured by angling. The largest isotopic niches were apparent in the 'Low' treatment of the mesocosm experiment and in the wild *B. barbus* captured by both angling and electric fishing. This was likely to be the result of the diets of the individual fish comprising of a greater variety of dietary items, in which MDN pellets were important items for only some individuals. Regarding somatic growth rates, whilst these were significantly higher in the 'medium' and 'high' treatments compared to the control and 'low' treatment in the mesocosm experiment, there were no significant differences in the growth rates of the fishes detected in the pond experiment, and there was no relationship between annual length increments and the SI data for the wild fishes. Thus, despite the pellets being consumed and assimilated into the fish tissues across the study approaches, it was only in very controlled conditions where feeding on pellets facilitated faster growth rates, and then only when they were available in relatively high quantities. This finding was generally contrary to the hypothesis.

Recent studies have suggested that where *B. barbus* populations are enhanced with hatchery reared individuals via stocking then there are strong patterns in isotopic niche partitioning between these fish and other wild fishes, including *S. cephalus* (Bašić and Britton 2016). This partitioning is also evident between larger individuals, suggesting functional differences between the species result in these trophic differences (Bašić and Britton 2015, 2016). The influence of a pellet based diet in the wild appears to have converged adult *B. barbus* and *S. cephalus* niches, for the angler caught fish, which previously showed much greater isotopic niche partitioning across all life stages (*cf*, Chapter 5) than detected here. Nevertheless, in the pond experiment, even where both fishes consumed high proportions of pellets, they still had some differences in the positions of their isotopic niches. Reasons for these inter-specific isotopic niches differences might relate to differences in the proportions of macroinvertebrates consumed between the species and differences in the stable isotope ecology between *B. barbus* and *S. cephalus*, for example through differences in their fractionation factors (Busst, Bašić and Britton 2015; Busst and Britton 2016). Irrespective, in this pond experiment, the growth rates and the isotopic niche sizes of both fishes were not significantly different between their allopatric and sympatric contexts in both pellet presence and absence, suggesting that the fishes were accessing sufficient food resources to maintain their growth rates without having to further alter their diet.

It was apparent that all of the fish sampled by angling in this section of the River Teme below Powick Weir had diets comprising relatively high proportions of

MDN, yet for *B. barbus* sampled by electric fishing, there was much greater variability in this MDN contribution, with this independent of body size. This suggests that despite the attractiveness of fishmeal pellets to *B. barbus* generally, resulting in some individuals developing trophic specialisations, other individuals primarily consumed other items, perhaps through avoiding consuming pellets due to previous angler capture experiences that lead to avoidance (Raat 1985; Askey et al. 2006). This also emphasises the potential bias that can result from samples collected by angling alone, as individual variability in the behaviour of individuals can affect capture susceptibility (Klefoth et al. 2013; Chapter 7).

It was apparent that the MDN from the pellets was being consumed directly by the fishes, with the stable isotope data of the macroinvertebrates and fish suggesting there was no indirect transfer via prey populations. This is in contrast to the transfer of MDN into freshwaters via anadromous salmonid fishes, where the nutrients are more freely available and facilitate the increased production of benthic algae and macroinvertebrates (Schindler et al. 2003). This then enhances the food resources available for the larvae and juveniles of the adult migrants, facilitating their feeding, growth and survival in the early life stages (Wipfli et al. 2003). The MDN from salmonids can thus be traced through freshwater food webs, enabling assessment of the links between the aquatic and terrestrial food webs. For example, Tonra et al. (2015) reported on the removal of Elwha River dam in the USA, which resulted in migratory salmonids returning to the river within 12 months. Following reproduction and death of these fishes, their MDN could be traced through the macroinvertebrate community and then into a bird

that preys upon these, the American dipper *Cinclus mexicanus*. Indeed, there are now numerous studies that have traced MDN into terrestrial food webs (e.g. McLoughlin et al. 2016; Richardson et al. 2016), with its influence even affecting the behaviour of terrestrial predator and scavenger species (Schindler et al. 2013).

In contrast, the apparent direct transfer of MDN from fishmeal pellet to *B. barbus* and *S. cephalus* in this study suggested that this nutrient subsidy might have only minor impacts on the non-fish communities. In the lower River Teme, the fish consuming these pellets were large-bodied and thus are only likely to be predated upon by large piscivores, including otter *Lutra lutra*, although otters tend to prefer to consume high abundances of smaller bodied fishes (Britton et al. 2006). Unlike salmonid fishes, *B. barbus* and *S. cephalus* are relatively long-lived generally (> 15 years; Britton 2007; Britton et al. 2013), with this also the case in the River Teme (Amat Trigo et al. 2017). They also reproduce annually (Britton and Pegg 2011), and thus there is no large post-spawning die-off. Consequently, they might be acting as MDN sinks, with low rates of nutrient transfer to higher trophic levels. However, determining the extent of MDN transfer to higher trophic levels requires further work.

These results add to an increasing literature base on the role of subsidies from fishery activities in the trophic ecology of freshwater communities. For example, Grey et al. (2004) demonstrated that approximately 65 % of *Daphnia* spp. and over 80 % of roach *Rutilus rutilus* body carbon was ultimately derived from pellet material originating from an *in situ* fish farm in Esthwaite Water, England. These

data suggest that the MDN were more freely available within the lake via the breakdown of the pellets, with a number of other studies also revealing their integration into the food web more generally (Fernandez-Jover et al. 2011a,b; Demétrio et al. 2012; Jackson et al. 2013). Thus, further work is suggested in riverine systems where fishmeal pellets are used by anglers to identify whether there is greater transfer of MDN in the food web than suggested here.

In summary, across three spatial scales of increasing complexity, it was apparent that the release of fishmeal pellets into freshwaters as an allochthonous trophic subsidy based on MDN had a substantial influence on the isotopic niche (as a proxy of the trophic niche) of riverine fishes. Results from *B. barbus* in the River Teme below Powick Weir, with some support from the experiments, indicated that individual isotopic niche specialisation resulting from this trophic subsidy was strongly apparent, with its development potentially associated with behavioural differences between individual fish that leads to variability in their avoidance/ consumption of pellets and thus their likelihood of angler capture. Comparison of these results for *B. barbus* isotopic niche with Chapter 5 also indicates a stronger role of fishmeal pellets in their diet below Powick Weir, with this likely to be associated with relatively high angling pressure below this weir; observations over three summers indicated much lower angling activity in stretches upstream of the weir due to the declines in *B. barbus* catches that has resulted in low angling pressure and thus reduced inputs of fishmeal pellets (Section 1.10). In addition, the differences in the proportion of fishmeal pellets to the diet of *B. barbus* might also relate to more general behavioural differences between individuals. Consequently, the following chapter investigates the

individual movements of the fishes tagged by acoustic tags in this study over a 12 month period (Chapter 7).

Chapter 7: Factors affecting individual movements of invasive European barbel *Barbus barbus* in an impounded river

7.1 Abstract

The impacts of anthropogenic activities on river ecosystems include those resulting from introductions of non-indigenous species and river engineering that reduces habitat diversity and river connectivity. Here, to understand the movements of a non-indigenous fish in a river reach impacted by impoundments and channelization, a tracking study based on acoustic telemetry was completed over a 12 month period on the invasive *B. barbus* of the lower River Teme and Severn (n = 18). The tagged fish generally spent more time in the Teme than the Severn, with weirs at the upstream end of both river reaches providing impediments to movement; only three fish traversed the weir on the Teme and none traversed on the Severn. Home ranges were highly variable between individuals, ranging between 670 to >12,000 m, although total movements were not significantly different between individuals. Net movements were mainly in an upstream direction in spring and in a downstream movement in autumn and winter. Relationships of daily movements were asynchronous between both individuals and time of day, with minimal evidence suggesting crepuscular activity. The 18 fish were captured by a combination of angling and electric fishing; those captured by angling (n = 8; mean 2,739 ± 1,229 m) had significantly smaller home ranges than those captured by electric fishing (n = 10; mean 6,112 ± 2,075 m). This might relate to fish with smaller home ranges being more vulnerable to angler capture due to higher spatial encounters. In summary, there was considerable individual variation in the movement

behaviour of the tagged fish, with some behavioural differences relating more strongly to the initial capture method rather than responses to changes in abiotic conditions.

7.2 Introduction

The impacts of anthropogenic activities on river ecosystems include those resulting from river engineering that reduce habitat diversity and river connectivity (Britton and Pegg 2011). The loss of habitat heterogeneity and longitudinal connectivity has considerable implications for fish communities, with the potential for loss of key habitats, including spawning gravels and off-channel nursery areas (Mouton et al. 2007; Ziv et al. 2012). These issues are frequently associated with anadromous salmonid fishes, with extensive research completed on the population impacts of river engineering (e.g. Beechie et al. 1994; Buddendorf et al. 2017). It is, however, becoming increasingly apparent that even relatively minor engineering schemes can have implications for the movements and behaviours of fishes more generally (Lucas and Frear 1997; Ovidio and Phillipart 2002; Birnie-Gauvin et al. 2017).

Other anthropogenic impacts on river ecosystems include the manipulation of the composition of the fish community, with fish frequently introduced and stocked for the enhancement of recreational angling (Cowx 1994; Britton et al. 2004; Basic and Britton 2016). In many European rivers, hatchery-reared *O. mykiss* originating from North America are frequently released in large numbers for angling (Britton and Gozlan 2013). They have, however, yet to establish invasive populations in many rivers, with most released fish captured soon after release and removed from the system, coupled with sterile fish increasingly being stocked (Fausch 2007). In contrast, the release of fishes of the Cyprinidae family into freshwater systems potentially have longer term ecological

consequences, especially as their life-spans often exceed 15 years and they are mainly exploited by catch-and-release angling (Bašić and Britton 2016). Following an introduction, these fishes can persist and, potentially, establish viable and invasive populations, even after a long lag period (Crooks et al. 1999; Crooks 2005). How introduced fish behave relative to their native range, and how they interact with native species, is then important in determining their ecological impacts (Gozlan et al. 2010). Therefore, understanding the long-term behaviours of these fishes is crucial for assisting understandings of their integration into the fish community, including their dispersal in relation to potential dispersal barriers, such as weirs.

Integral to understanding these long-term behaviours of invaders is also understanding their intra-specific behavioural variability, given that many taxa, including fishes, often show distinct personalities or behavioural syndromes within populations, especially the 'bold/ shy continuum' (Ward et al. 2004; Bergmüller and Taborsky 2010; Nyqvist et al. 2012). Boldness tends to be characterised by individuals taking greater risks to gain higher returns. For example, they may spend more time foraging and exploring open water than shy individuals, but in doing so have elevated probabilities of being predated (Ward et al. 2004). Studies have suggested individual differences in behaviours can be apparent between capture methods, with shy fish being captured more frequently by angling compared with seine netting (Wilson et al. 2011). Whilst other studies have suggested boldness can increase vulnerability to capture by angling, especially in hatchery reared fish (Harkonen 2014), this remains equivocal, with perch *Perca fluviatilis* capture rates being influenced more by body size than

boldness (e.g. Vainikka et al. 2016). If bold individuals are assumed to have larger home ranges than shy fish through increased exploratory behaviours, then this might also increase their vulnerability to capture by passive and active fishing gears (Biro and Post 2008; Alos et al. 2016).

In their indigenous range, telemetry studies have revealed that European barbel *Barbus barbus* populations mainly comprise of individuals that are relatively sedentary, characterised by relatively small home ranges (< 1 km) (Britton and Pegg 2011). A small proportion of individuals, generally around 10 % of the population, tend to be more mobile, with regular movements within a relatively large home range (e.g. > 10 km) (Britton and Pegg 2011). The reasons for this individual variability in movement remain unclear and have yet to be associated with behavioural syndromes (Britton and Pegg 2011). Irrespective, given that *B. barbus* inhabit the middle and lower reaches of European lowland rivers, then their individual movements can potentially be disrupted by engineered structures such as weirs (Baras et al. 1994; Lucas and Frear 1997; Bunt 2001; Freyhof and Brook 2011). Populations of non-indigenous *B. barbus* are also present in some European rivers, where fish were originally released for enhancing recreational angling (Wheeler and Jordan 1990; Antognazza et al. 2016). Thus, in these rivers, knowledge on their movements have high utility for understanding both their ability to by-pass river engineering structures that enable dispersal and invasion, and for comparing their behaviours between their indigenous/ non-indigenous ranges and, potentially, in relation to behavioural syndromes.

Consequently, the aim of this study was to quantify the individual variability in the long-term movement patterns of a non-indigenous and invasive *B. barbus* population in an engineered lowland river system. The study area was the lower River Teme and Severn that provided a continuous riverine habitat that was, potentially, delimited from areas further upstream in both rivers by two weirs, with extensive river channelization also apparent in the Severn (Fig. 54b). Using acoustic telemetry methods over a 12 month study period, the objectives were to: (1) assess the extent of individual fish residence in each river and the impact of the weirs on *B. barbus* upstream movements; (2) quantify the extent of individual differences in their home range size, total and net movements, and diel activity; and (3) test the hypothesis that fish captured by angling would demonstrate distinct behaviours from those captured by electric fishing, with differences potentially associated with the bold/ shy continuum of behavioural syndromes.

7.3 Materials and methods

7.3.1 Study area

The primary area of study was downstream of Powick Weir on the River Teme (52°10'N, -2°14'W) through to its confluence with the River Severn, and then in the River Severn between Diglis Weir (at the upstream end of the study section) and Severn Stoke (at the downstream end) (Fig. 53c). An array of 14 fixed acoustic receivers (VR2, Vemco Ltd, Halifax, Nova Scotia, Canada) were deployed in this area, including upstream of both Powick and Diglis Weirs to test whether these were passable to *B. barbus*. All receivers were in place for the

duration of the 12 months, although the receiver at 'Boro' was moved to Bransford on the 07/07/16 to record fish movements more effectively at the upstream end of the array (Table 35). The total river length within the array of acoustic receivers was 17 km, covering 6 km in the River Teme and 11 km in the River Severn (Fig. 53b). The acoustic receivers were mainly deployed in the River Teme to facilitate the collation of movement data at a relatively fine spatial scale (n = 8), with one receiver at the confluence of the two rivers and then the remainder in the River Severn to facilitate the collation of movement data at a wider spatial scale (n = 5) (Fig. 53c). In the study area, the River Teme primarily comprised of sequences of pools and riffles within a river channel of up to 18 m width and depths < 2 m, with overhanging trees (primarily *Salix* spp.) being abundant in the riparian zone. Instream macrophyte growth was minimal. In contrast, the River Severn downstream of Diglis Weir was highly impounded and navigable, with heavy boat traffic in summer. With the exception of the weir pool at Diglis, depths were consistently > 4 m, with widths generally > 35 m. The two rivers thus provided highly contrasting riverine habitats within the study area.

7.3.2 Fish sampling and tagging procedures

The 22 *B. barbus* tagged in the study were sampled by a combination of electric fishing (n = 12) and rod and line angling (n = 10), and all were captured within the River Teme (Table 38). Electric fishing was completed from a boat, with fish captured between the weir pool at Powick and then downstream for approximately 1 km (4 - 9; Fig. 53c). Captured fish were initially held in large water-filled containers before being transferred to aerated holding tanks prior to

tagging. The fish captured by angling were generally caught in the same area as the electric fishing; where fish were captured further downstream, they were always from areas in excess of 1 km from the River Severn confluence. These fish were initially held in fish keep sacks before also being transferred to aerated holding tanks prior to tagging. All fish were sampled and tagged on 22/09/15 and 23/09/15.

Each fish was tagged with a Vemco V9 acoustic transmitter (hereafter referred to as 'acoustic tags'), with each tag being 9 x 45 mm and approximate weight 3 g, and operated on 69 kHz (Vemco, 2017). A 21 mm passive integrated transponder tag was also inserted to enable individual identification in case a fish was recaptured in future. The acoustic tags were coded to allow individual fish identification and were set to pulse randomly once every 60 to 180 s, providing a battery life of each tag of up to 22 months. Random repeat pulse rates allowed multiple individual *B. barbus* to be monitored simultaneously within a given area and without continuous signal overlap. Upon reception of a signal from a V9 tag, the VR2 receivers identified the tag number by its unique coded transmission pattern and recorded its time of detection. Range testing revealed that detection distances for V9 transmitters were generally 70 m in the River Teme and 100 m in the River Severn; in subsequent analyses, a nominal detection distance of 100 m was thus utilised. V9 tag insertion was into the peritoneal cavity, with the incision then closed with a single stitch. Throughout this procedure, the fish were always under general anaesthesia (tricaine methanesulfonate; *MS-222*). They were then transferred to recovery tanks where they were held until their return to normal swimming behaviour. All fish were then returned to the river within

500 m of their capture site. Additional information recorded for each fish was their fork length (nearest mm) and method of capture (electric fishing/ angling). All surgical procedures were completed following ethical approval and UK Home Office project licence 70/8063, and were undertaken by a competent and experienced practitioner.

Following the return of the fish to the river, all VR2 receivers were initially downloaded for their data on 29/10/15 and 30/10/15, before then being re-deployed for the winter period when access to receivers was inhibited by high water levels. The next data download was 14/07/16 (the first time since the winter period when all receivers were accessible) and then again at the end of the study period (30/09/16). They were then redeployed in the same locations for the purposes of a different study and so some fish remained being detected for a further 18 months, although these data were not utilised here unless explicitly stated. All of the receivers remained operable in the study period and none were lost, thus they provided continuous data throughout this period. Tiny tag temperature loggers were also deployed in both rivers (one in the Teme at Temeside Cottage, one in the Severn at Diglis Lock; Table 35, Fig. 53c), with recording of temperature (to 0.1 ^{o}C) every three hours. Flow data (m^3 s^{-1}) were available for both rivers from the Environment Agency, with data for the Teme available from the Knightwick flow monitoring station, approximately 6 km upstream of Powick Weir (52°10'N, -2°14'W), and the River Severn from the Saxons Lode station (51°59'N, -2°10'W), located within the study area. Complementary to this was a river level gauging station located 2 km upstream from receiver 1 (52°10'N, -2°17'W; Fig, 53c).

7.3.3 Data and statistical analyses

Environmental data

The influence of environmental conditions on movement patterns in the tagged fish utilised water temperature and flow data. For flow, data recorded by the Environment Agency were utilised. For the Severn, the Saxons Lode discharge (m^3 s^{-1}) data were used directly (Table 36). No discharge data was available at the River Teme study site, so river level data from Bransford, which was the closest river level monitor to the River Teme study site, was used. For the River Teme data, the river level at Bransford were converted to discharge (m^3s^{-1}) values, as it is more ecologically relevant as a measure of river flow, for use in the study area. This was done via Equation 25 the linear regression equation from the significant relationship between discharge (m^3 s^{-1}) at Knightwick and river level at Bransford ($R^2 = 0.98$; $F_{1,271} = 9{,}063.8$, $P < 0.01$).

River flow (m^3s^{-1}) = (39.468*Level) – 9.6865 (Equation 25)

Throughout analyses, the flow data (m^3s^{-1}) were categorised into three groups: flows exceeding Q_{10}, flows between Q_{10} and Q_{50}, and flows less than Q_{50} (where Q_{10} = flows exceeded on 10 % of occasions and Q_{50} = flows exceeded on 50 % of occasions, Table 36). This was to quantify a threshold to determine if fish moved during high, medium or low flows, with data representing the long-term flow regime of the river to enable transferability of the relationship between fish movements and flow beyond the study period. However, these Q values were not significantly different between the study year and long-term data (t-test; Teme; $t_2 = -1.75$, $P = 0.22$ and Severn; $t_2 = -1.30$, $P = 0.32$, Table 36). For testing

of daily fish movements, flow was used as a continuous variable. In all cases, only flow data from the River Teme was used, although the River Teme and River Severn discharge was significantly and positively related ($R^2 = 0.89$, $F_{1,365} = 1487.3$; $P < 0.01$, Fig. 55).

For water temperature, the data collated from the data loggers were used to calculate daily means. Occasionally during summer low river levels, the water showed substantial diurnal fluctuations during periods of low flow, suggesting it was partially exposed to air. As the flow monitoring station at Knightwick also recorded water temperature then linear regression of the two datasets enabled these anomalous data to be removed; once completed, the regression relationship of these two temperature datasets were highly significant ($R^2 = 0.991$, $F_{1,357} = 37623.85$, $P < 0.01$). The water temperature data were used in two ways; in categorical temperature groups ≤ 10 °C, 10.1 to 15°C, and ≥ 15.1 °C and as a continuous variable (as daily means). These temperature groups were chosen in this manner as behaviour of *B. barbus* has previously been found to vary above and below a temperature threshold of 10 °C (Baras 1995a). There was only a single day during the study period below the reported thermal limit of *B. barbus* activity (4°C; Baras 1995b) and so a separate temperature class was not included for this. When grouped, the number of days in each temperature group was 161 (≤ 10 °C), 91 (10.1 to 15°C), and 114 (≥ 15.1 °C). Despite fish moving between two rivers, only water temperature from the River Teme were used, as the temperatures from the two river were significantly and positively related ($R^2 = 1.00$, $F_{1,365} = 4.06*e^{21}$; $P < 0.01$) (Fig. 56).

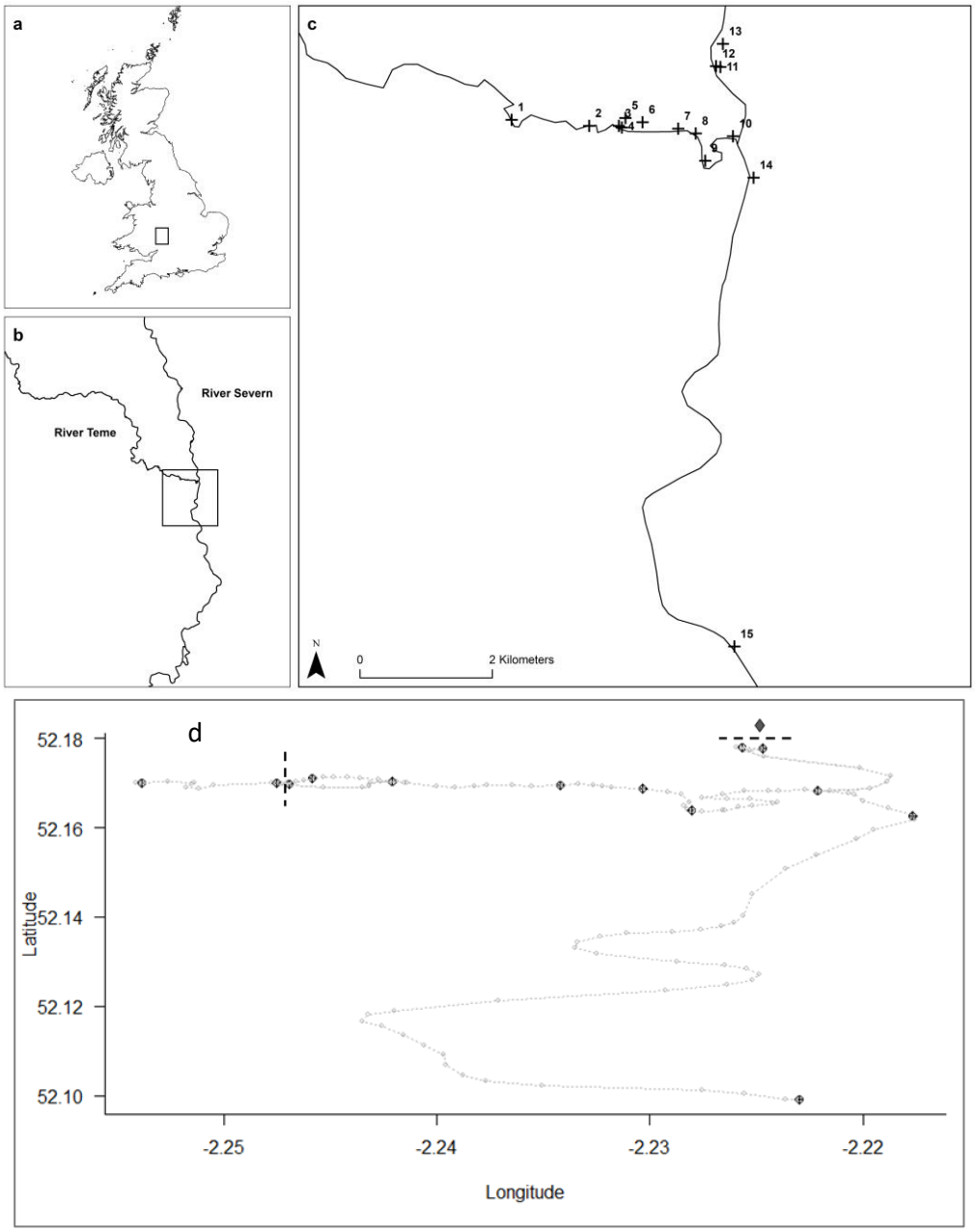

Figure 53. Maps showing: a) the position of the study area within the UK; b) the study area within the River Teme and Severn; c) the acoustic array, with the 15 receiver locations; d) the river as a dotted line and receivers as grey points, not including receiver 1, with weirs marked as dashed lines, highlighting the positions of four receivers in the array in and around Powick Weir.

Figure 54. Aerial images, taken from a drone, of (a) Powick weir, River Teme, and b) Diglis weir, River Severn, that in entirety present the two potential barriers to upstream movement in the study area.

Table 35. Receiver number (*cf*. Fig. 53), and location coordinates (Degrees, minutes, seconds), river and position (U/S: upstream of Powick or Diglis Weir; D/S: downstream of Weir), and location name, and the total number of detections ('Detections') from all fish in the 12 months of study. All receivers in place for 12 months of study except Boro and Bransford as the receiver at Boro was moved to the Bransford position on 07/07/16 (*).

Receiver	Location (Northings and Eastings)		River position	Location	Detections
1	52° 10' 15.18"	-2° 16' 15.51"	Teme, U/S	Bransford*	13
2	52° 10' 11.86"	-2° 15' 13.91"	Teme, U/S	Daweshill	75
3	52° 10' 11.71"	-2° 14' 50.29"	Teme, U/S	Upstream of Powick weir	10,559
4	52° 10' 10.85"	-2° 14' 47.94"	Teme, D/S	Powick weir	38,989
5	52° 10' 15.49"	-2° 14' 44.99"	Teme, D/S	Mill leat	15
6	52° 10' 13.37"	-2° 14' 31.38"	Teme, D/S	Old Bridge	140,337
7	52° 10' 10.02"	-2° 14' 3.12"	Teme, D/S	Manor Farm	25,945
8	52° 10' 7.56"	-2° 13' 49.17"	Teme, D/S	Boro*	31,892
9	52° 9' 54.22"	-2° 13' 41.88"	Teme, D/S	Temeside Cottage	470,761
10	52° 10' 6.06"	-2° 13' 19.63"	Confluence	Confluence	86,044
11	52° 10' 39.94"	-2° 13' 29.03"	Severn, D/S	Diglis Lock	1,489
12	52° 10' 40.33"	-2° 13' 32.48"	Severn, D/S	Diglis Weir	4,391
13	52° 10' 51.23"	-2° 13' 26.88"	Severn, U/S	Diglis	0
14	52° 9' 45.68"	-2° 13' 3.58"	Severn, D/S	Carrington Bridge	4,724
15	52° 5' 56.62"	-2° 13' 22.87"	Severn, D/S	Severn Stoke	62

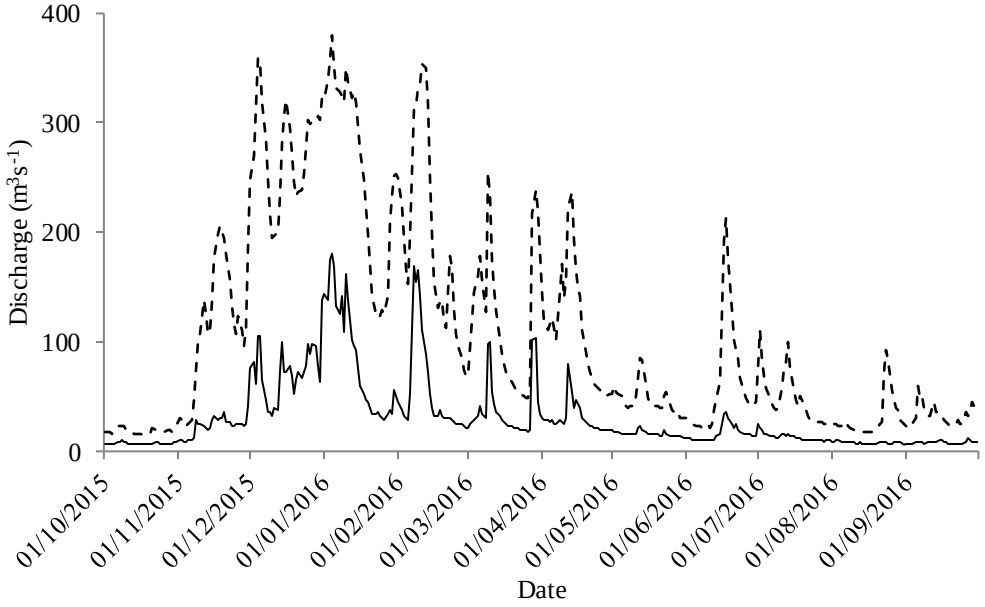

Figure 55. Water discharge (m^3s^{-1}) for the River Teme (solid line) and River Severn (dashed line) over the study period.

Table. 36. Gauged daily river flow (as Q values, m^3 s^{-1}) from the River Teme and Severn gauging stations, representing long-term flow regimes between 1970 – 2016 and the current flow regime during the study period.

Site	Timeframe	Q_{10}	Q_{50}	Q_{95}
Teme at Knightsford Bridge	1970 - 2016	42.4	10.2	2.0
Severn at Saxons Lode	1970 - 2016	222.0	53.7	15.4
Teme at Bransford	2015 - 2016	76.7	19.3	6.9
Severn at Saxons Lode	2015 - 2016	282.0	62.3	17.8

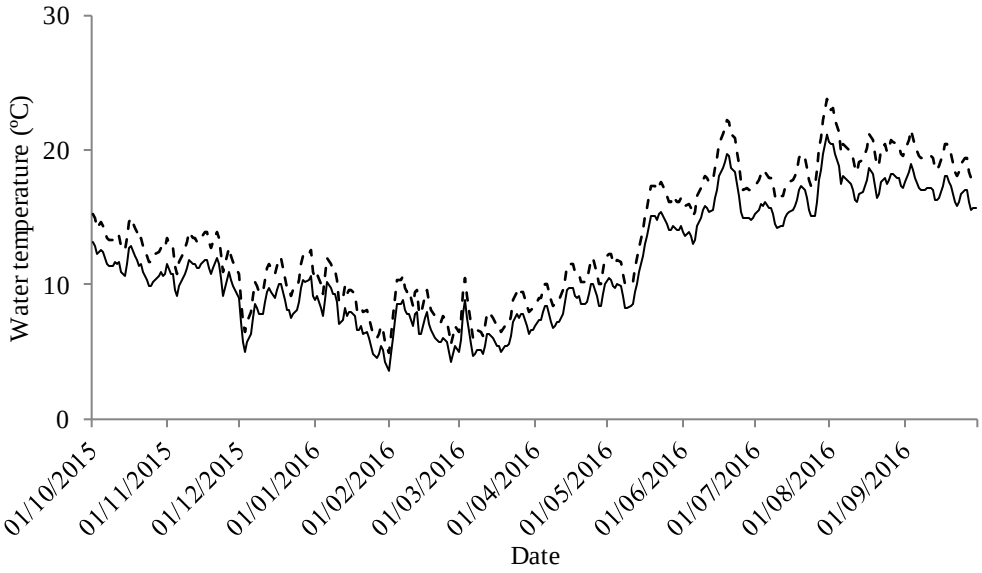

Figure 56. Water temperature (ºC) from the River Teme (solid line) and River Severn (dashed line) over the study period

Fish movement data from telemetry

Following the collation of over 12 months of continuous acoustic tag data collection, analyses on fish movements were completed for the period of 01/10/15 to 31/09/16 (n = 366 due to the leap year). The initial days of movement between tagging and 30/09/15 were not utilised to avoid analysis of initial post-tagging behaviours, when fish removed from a specific area were displaced by their return to the river in a slightly different area, thus they potentially undertook an enforced movement (rather than a natural movement). Utilising the fish movement data from all receivers (all expressed in terms of metres of river length moved), the data for each individual fish were initially manipulated within the software 'Vtrack' (Campbell et al. 2012), a package written within the R-programming language (R Core Team 2017), prior to being analysed for the following movement metrics:

Residence indices: The residency index (number of days detected by at least one receiver/ total number of days of study) and the linearity index (total range/ total movement) were initially calculated (Acolas et al. 2017). Total range was calculated as the length of river between the furthest upstream and furthest downstream detections, where this covered two rivers it was the sum of the distance between upstream and downstream in the Teme and the distance between upstream and downstream detections in the Severn. The total movement differs by including all the distances of overlapping movements and the multiple upstream and downstream movements within the range that occur. To overcome the issues that a tagged fish could be within the receiver array on a given day but were not necessarily detected on a VR2 receiver, then a further index was calculated, residency within the array, which was calculated from: [number of tracking days within the array (as determined by receiver detections at the upstream and downstream limits of the array)/ 366 (the total number of tracking days)].

River residence: The VR2 receivers located in both the Teme and the Severn meant that the duration of residence of the individual fish in each river could be determined, with this duration rounded to the nearest day. The 12 month period was then split into an 'autumn/ winter' period (01/10/15 to 29/02/16; 01/09/16 to 30/09/16) and 'spring/ summer' period (01/03/16 to 30/08/16) to determine broad temporal differences in residence by testing for differences between the two periods using paired samples t-tests. River residence was then tested according to the three water temperature data groupings, with the residence

tested between each temperature group using paired t-tests. In both cases, the test was to determine the significance of the difference in the proportional number of days spent in the Teme and Severn from 1:1.

Influence of weirs on movement: The upstream limits to *B. barbus* movement in both rivers was potentially the two weirs (Powick and Diglis; Fig. 54). Consequently, the movement data for each individual fish were analysed to determine the number of movements into each weir pool (where a VR2 receiver was deployed in each; Table 35) and whether this was then followed by successful weir ascent (as detected by the VR2 receivers upstream) (Fig. 53d). In the River Teme, a successful ascent of Powick Weir was defined as when the ascending fish was detected at both the initial upstream receiver and then next one upstream (680 m). This was due to some detections of the same fish on the upstream and downstream receivers occurring at extremely high-water levels, when the weir was flooded out and thus no longer prevented the upstream receiver from detecting fish downstream. The flow and temperature data (as continuous data rather than grouped) were used to test for differences between successful (detected in weir pool and then upstream soon after) and unsuccessful ascents (detected in weir pool only). Testing used binary logistic regression (no ascent: 0; ascent 1) to determine the significance of time of year (as date of detection), water temperature and flow as the predictors of successful ascent.

Home range: this was calculated for each individual fish as the distance between the most upstream to the most downstream site detection within the receiver array (Fig. 53). As the array covered two rivers, then for fish that were detected

in both rivers, their home range was determined as the sum of the distance between the upstream/ downstream site in the Teme and the upstream/ downstream site in the Severn (Fig. 53). The distance between the VR2 receivers were determined as river length (m) to the nearest 100 m (as further accuracy is limited by the tag range). Home range size was then tested against fish length (linear regression) and fish capture method (ANOVA). Home range was then split between the 'spawning' (01/03/16 to 30/08/16) and 'non-spawning season' (01/10/15 to 29/02/16 and 01/09/16 to 30/09/16), with linear regression used to determine if the relationship between these seasonal home ranges was significant.

Total movement: this referred to the total distance (m; nearest 100 m) moved by an individual *B. barbus* in the study period, irrespective of whether it was in an up- or downstream direction. Note total movement is a minimum estimate of actual total movement, as it cannot account for fine-scale movement in between receivers, when the fish is not close enough to be detected. Total movement was tested against fish length (linear regression) and fish capture method (ANOVA). Total movement was then split between the 'spawning' (01/03/16 to 30/08/16) and 'non-spawning season' (01/11/15 to 29/02/16), with linear regression used to determine if the relationship between the seasonal home ranges was significant. A Generalized Linear Mixed model was used to test the effects of flow and temperature on total movement by month, with individual fish used as a random factor using the package glmm (Knudson 2017) in R (R Core Team 2017). Only fish with a full 12 months of movement data were used, to reduce false zeros in the dataset (n = 13; Table 38). This model also accounted for the

interaction between flow and temperature, and a Poisson distribution was used, as this was the best fit of the distribution of the response data.

Daily movement: Only fish with a full 366 days of movement were included in this analysis (n = 13; Table 38). As each day of movement could not be treated as being independent to the movement on the following or previous day, then the movement (m) and environmental (temperature and flow) time-series data were tested for temporal autocorrelation using a Box-Pierce test, from package 'tseries' (Trapletti et al. 2017), where the test results are reported as χ^2 values with significance values (*P*). Correlation between individual daily movement time series and both environmental time-series were then tested for with cross-correlation function (ccf) estimation from the package 'tseries', which also accounts for the possibility of time-lagged effects (Trapletti et al. 2017).

Net movement: this refers to the specific distance (m; nearest 100 m) of the net difference between movement in an upstream and downstream direction. Thus, an individual that moves 200 m upstream and then 200m downstream has a total movement distance of 400 m but a net movement of 0 m. It therefore indicates, for any given period of time, whether the overall pattern of movement was in an up- or downstream direction. Net movement per month was tested for differences using an ANOVA and post- hoc Tukey test. Net movement was tested between flow and temperature categories using ANOVAs.

Diel activity patterns: for each individual fish, the number of movements commencing per hour, as determined from the first receiver detection, of at least

two receiver detections, and extracted using VTrack (Campbell et al. 2012) in R (R Core Team 2017), was assessed over the tracking period to determine diel activity patterns. The time of each movement recorded on the first receiver for the 13 fish with a full 366 days of movement data (n = 13; Table 38) was rounded up to the closest hour (00.00 to 23.00). Chi-square goodness of fit tests were then performed on the data for each individual to determine whether the observed proportion of movements per hour differed from an expected distribution of the number of movements per hour being equal. If a significant difference from the expected distribution was recorded, then that individual moved significantly more at one or more specific hours of the day.

There were four fish that were limited to a small home range within the River Teme only (detected consistently during their movements across five VR2 receivers (receivers 6 to 10; Table 35). Their data enabled their diel patterns of detections to be assessed overall, as per the 18 fish described above, and then by season. Seasons were defined by autumn (September to November), winter (December to February), spring (March to May) and summer (June to August). Studies suggest *B. barbus* only display crepuscular activity when temperatures are above 10 °C (Baras 1995a), so the summer and winter categories used previously were too broad. The revised seasons here had the summer period above 10 °C and the winter mostly below, with two intermediate seasons (Table 37). If there was a significant diurnal movement pattern in an individual fish, i.e. it significantly differed to an equal distribution of movement during the 24-hour period, it was then tested whether more movements occurred during daylight, twilight or night-time hours. This was completed by dividing the 24-hour period

into daylight, twilight and night on a seasonal basis, with the onset times of each of these taken from the median date of each season and rounded to the nearest hour (Table 37; UK Weather Cams 2017). Day, twilight and night movements were summed for each fish that showed significant diel trends and divided by the number of hours during that period. These data were then tested to determine if the frequency of movements were significantly different to equality in each period using Chi square goodness of fit.

Table 37. Four seasons used to discriminate between diurnal movement patterns, the median date used for twilight and daylight timings and the mean water temperature ± 95% CI (°C) and range in the river Teme during those periods.

Season	Median date	Dawn	Sunrise	Sunset	Dusk	Mean temp ± 95% CI (°C)	Temp range (°C)
Autumn	15th Oct	07:00	07:34	18:14	18:49	12.17 ± 0.34	5.00 – 18.07
Winter	15th Jan	07:31	08:10	16:26	17:05	7.35 ± 0.37	3.60 – 10.70
Spring	15th Apr	05:34	06:10	20:06	20:42	9.87 ± 0.65	5.00 – 15.80
Summer	15th Jul	04:20	05:06	21:23	22:09	17.06 ± 0.32	14.20 - 21.16

7.4. Results

7.4.1 Overview of tracking data and river residency

During the tracking period, the mean water temperature of the River Teme study reach was 11.6 ± 0.5 °C (range 3.6 to 21.2 °C) and Severn was 13.6 ± 0.5 °C (4.9 to 23.8 °C). Mean flow at Knightwick (Teme) was 31.14 ± 3.50 m^3s^{-1} (range

6.10 to 180.55 m^3s^{-1}) and at Saxons Lode (Severn) was 109.83 ± 10.18 $m^3 s^{-1}$ (range 15.20 to 21.16 m^3s^{-1}). The mean number of detections across all fish was 37,155 ± 22,483, and ranged between individuals from 2 to 202,856 (Table 38). The 22 tagged fish were detected for a total of 5956 days. There were 18 fish being detected regularly (n = 5838 days). The 18 fish had an 'array residency index' of 0.96, thus mostly remained within the receiver array during the study period (Table 38). Values of the 'residency index' were between 0.00 (0.12 for the 18 fish with an 'array residency index' of 0.96) and 0.98 (mean 0.31 ± 0.12); only one fish had a linearity index > 0.50 (Table 38). Given the short detection period of four fish (ID 7, 12, 18 and 73), their data were omitted from all subsequent analyses (Table 38). For analysis involving monthly or daily comparisons of movement, a further five fish that had less than 366 days of detection from the start of the study were also omitted (n = 13 for these analyses; Table 38).

Of the 5,838 days on which the 18 analysed fish were detected, they were detected for 4,490 days on the River Teme receivers and 1,348 days on those in the River Severn; there were four fish that were only ever detected in the Teme and one fish that was never detected within the Teme during the summer months (Fig. 55b). Only four fish were detected on more days in the Severn than the Teme (Table 38). There was no significant difference between the number of days that fish were detected in the Teme between summer and winter (t_{17}=-1.99, P = 0.06), or the amount of days detected in the Severn between summer and winter (t_{13}=-2.04, P = 0.06). When analysed within groupings of water temperature, the tagged fish were always detected on significantly more days in

the Teme than the Severn when temperatures were above 10.1 °C, but with no overall pattern in river residence below 10.1 °C (paired t-tests: ≤ 10°C: $t_{17} = 1.71$, $P = 0.11$; 10.1 – 15.0 °C: $t_{17} = 5.93$, $P < 0.01$; ≥15.1 °C: $t_{17} = 6.38$, $P < 0.01$) (Fig. 55c).

Table 38. Summary of data for each fish (ID) including fork length (mm), method of capture; electric fishing (EF) or angler caught (AC), the number of days from first to last detection, number of days detected by a receiver, number of detections, receiver residency index, array residency index, linearity index, annual home range (m), total annual distance moved (m), mean daily distance moved (m) and River preference (T = Teme, S = Severn and NS = no significant preference)

ID	Length (mm)	Capture method	Days from first to last detection	Days of detection	Number of detections	Receiver residency index	Array residency index	Linearity index	Home range (m)	Total distance moved (m)	Mean daily distance (m)	River preference
18	495	EF	0	0	10	0.00	1.00	-	-	-	-	-
73	721	AC	18	1	2	0.00	1.00	-	-	-	-	-
7	397	EF	41	1	46	0.00	1.00	-	-	-	-	-
12	552	EF	56	44	25,614	0.12	0.15	-	-	-	-	-
13	665	EF	191	76	27,068	0.20	1.00	0.54	12,210	22,470	-	T
17	545	EF	204	97	49,401	0.26	0.51	0.10	2,930	29,220	-	T
11	644	AC	222	108	34,250	0.29	1.00	0.22	5,040	22,950	-	T
9	677	AC	286	37	4,541	0.10	1.00	0.06	1,090	17,750	-	T
14	591	EF	298	191	27,068	0.51	1.00	0.15	5,950	40,040	-	T
21	557	AC	366	362	202,856	0.98	1.00	0.02	1,450	68,160	139	T
71	394	EF	366	67	10,207	0.18	1.00	0.24	4,100	17,040	46	NS
70	529	AC	366	45	2,607	0.12	1.00	0.25	5,010	19,860	50	T
68	480	EF	366	65	15,459	0.18	0.83	0.22	5,250	24,020	26	T
72	602	AC	366	108	24,169	0.29	1.00	0.05	1,870	38,220	105	NS

Table 38 (cont.).

ID	Length (mm)	Capture method	Days from first to last detection	Days of detection	Number of detections	Receiver residency index	Array residency index	Linearity index	Home range (m)	Total distance moved (m)	Mean daily distance (m)	River preference
10	698	AC	366	327	183,332	0.88	1.00	0.02	670	34,670	84	T
69	495	EF	366	116	12,803	0.31	1.00	0.11	3,340	30,110	82	NS
67	691	EF	366	97	31,918	0.26	1.00	0.11	4,580	41,790	114	T
19	582	AC	366	188	23,124	0.51	1.00	0.14	4,240	30,090	77	T
16	401	EF	366	34	10,934	0.09	1.00	0.49	12,210	24,840	68	S
20	371	EF	366	71	8,162	0.19	1.00	0.12	5,010	40,520	102	S
15	593	EF	366	269	70,399	0.73	1.00	0.11	5,550	48,470	133	T
8	565	AC	366	254	53,435	0.68	1.00	0.16	2,570	16,460	43	T

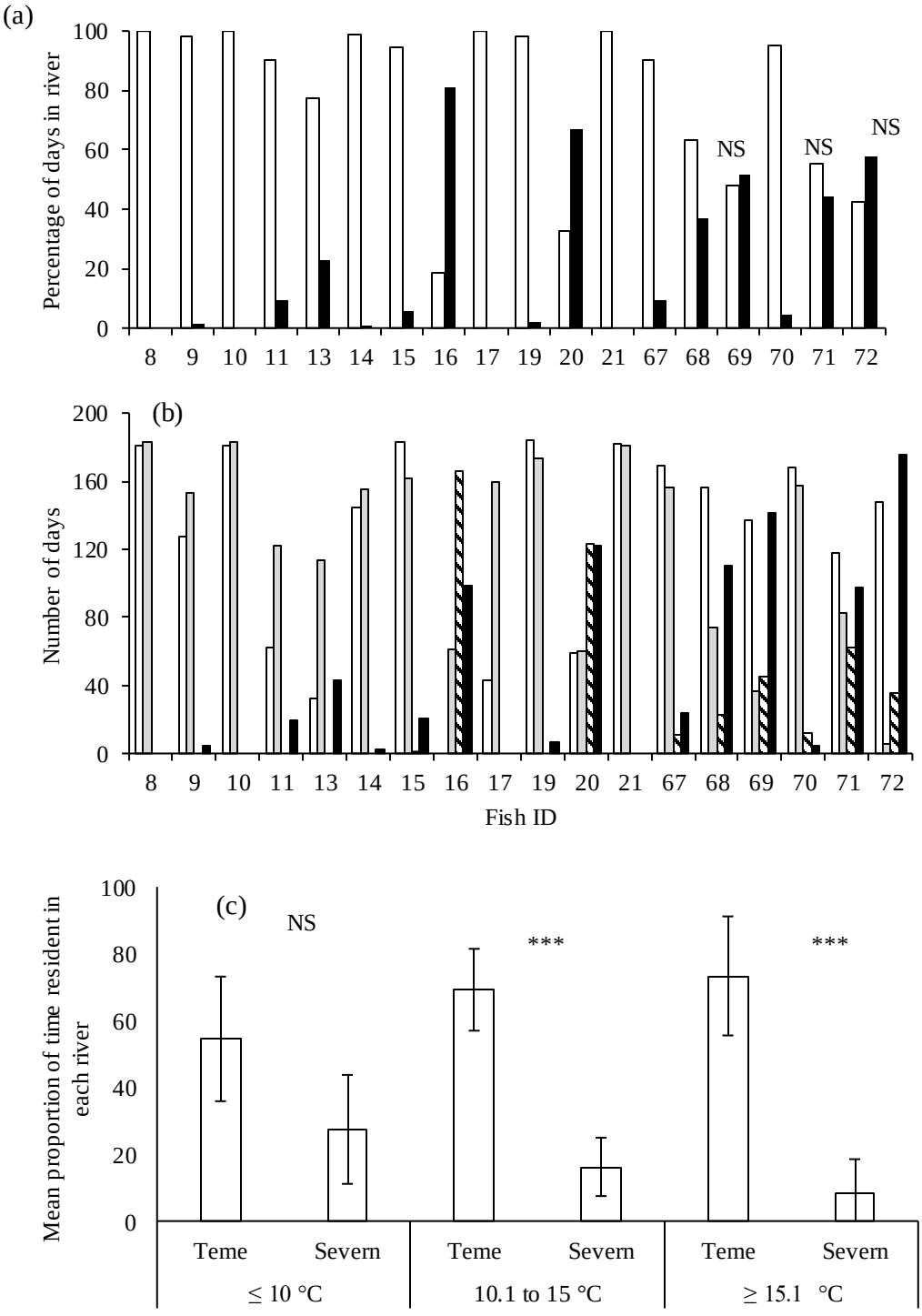

Figure 57. (a) Proportion (%) of tracking days of each tagged fish (n = 18) in the River Teme (clear) and River Severn (black), NS: non-significant differences between the rivers; all other fish, differences between the rivers at $P > 0.01$; (b) Seasonal residence by river, as number of days, where River Teme summer = clear, River Teme winter = light grey, River Severn summer = striped, and River Severn winter = black; (c) Mean proportion of time spent resident between River Teme and River Severn during three different temperature classes ≤10°C, 10.1 – 15°C and ≥ 15.1°C.

7.4.2 Barriers to movement

There were two potential barriers to fish movement within the receiver array, Powick Weir (Teme) and Diglis Weir (Severn) (Fig. 53, 54). During the study period, six fish approached Powick Weir and three ascended it, and five tagged fish approached Diglis Weir and none ascended it. Successful ascensions at Powick Weir only occurred during March and April 2016, and when flows exceeded Q_{50} (Table 36) and water temperatures ranged between 6.8 and 11.6 ^{o}C (Table 39). The times of day of when the fish ascended were 09:30, 15:25 and 00:03. Of the ascended fish, only one ascended the weir on its first approach, with the others approaching the weir on multiple occasions before ascending. Conditions for successful/ unsuccessful ascent of Powick weir were significantly affected by day length and water temperature but not by year, flow or individual fish (Table 40). Of the three ascended fish, only one fish returned back downstream of the weir in the period of the study, after 62 days (16th June 2016). One fish descended the following year, on 24th November 2016 (after spending 225 days upstream), whilst the final ascended fish remained upstream (441 days upstream by the time the tags were no longer active).

Table 39. Environmental conditions under which tagged fish were detected as being within the Powick weirpool (W) for more than one detection or on the date of ascending Powick weir (A), and then month during which the fish was present there, denoted by the first three letters and the number of days (D) spent in the weirpool. Only fish ID in bold ascended the weir.

	Water flow (m^3s^{-1})				Water temperature (°C)				D	Months	
		W		A		W		A		W	A
Fish ID	Min	Mean	Max		Min	Mean	Max				
71	6.5	15	36.5	-	10.7	17.4	21.2	-	25	Jun, Jul, Aug	-
15	46.8	120.3	175.4	-	7.4	8.6	10.1	-	3	Dec, Jan	-
14	138.7	153.9	169.1	-	7.4	7.7	8.0	-	2	Jan	-
68	46.4	46.4	46.4	46.4	10.1	10.1	10.1	10.1	1	Apr	Apr
67	51.5	109.7	165.9	54.3	4.6	5.9	7.4	9.3	13	Jan, Feb, Apr	Apr
17	33.3	95.4	180.5	102.8	4.6	7.0	10.2	6.8	43	Dec, Jan, Feb, Mar	Mar

Table 40. Results of binary logistic regression testing the significance of variables affecting the ability of tagged fish to traverse Powick Weir *variable had significant influence on the successful traverse of the weir ($P < 0.05$).

	B	S.E.	Wald	*P*
Fish ID	0.01	0.01	1.38	0.24
Flow	0.01	0.02	0.20	0.66
Temp	-0.96	0.47	4.22	0.04 *
Day length	1.34	0.60	4.91	0.03 *
Year	-2.11	1.76	1.44	0.23
Constant	-547.88	459.07	1.42	0.23

7.4.3 Home range

Mean home range size of the 18 fish was 4,600 ± 1,500 m, with a range of 700 to 12,200 m (Table 38). The relationship between fish length and home range size was not significant ($R^2 = 0.04$, $F_{1,21} = 0.90$, $P = 0.35$, Fig. 57a). There was, however, a significant difference between the size of the home range of the tagged *B. barbus* that were sampled by electric fishing (n = 10; mean 6,112 ± 2,075 m) and by angling (n = 8; mean 2,739 ± 1,229 m) (t test: t = -2.742; $P = 0.02$; Fig. 57b). There was also a significant and positive relationship between the home range size in the spawning season and non-spawning season ($R^2 = 0.21$, $F_{1, 18} = 4.40$, $P = 0.05$, Fig. 58 a,b).

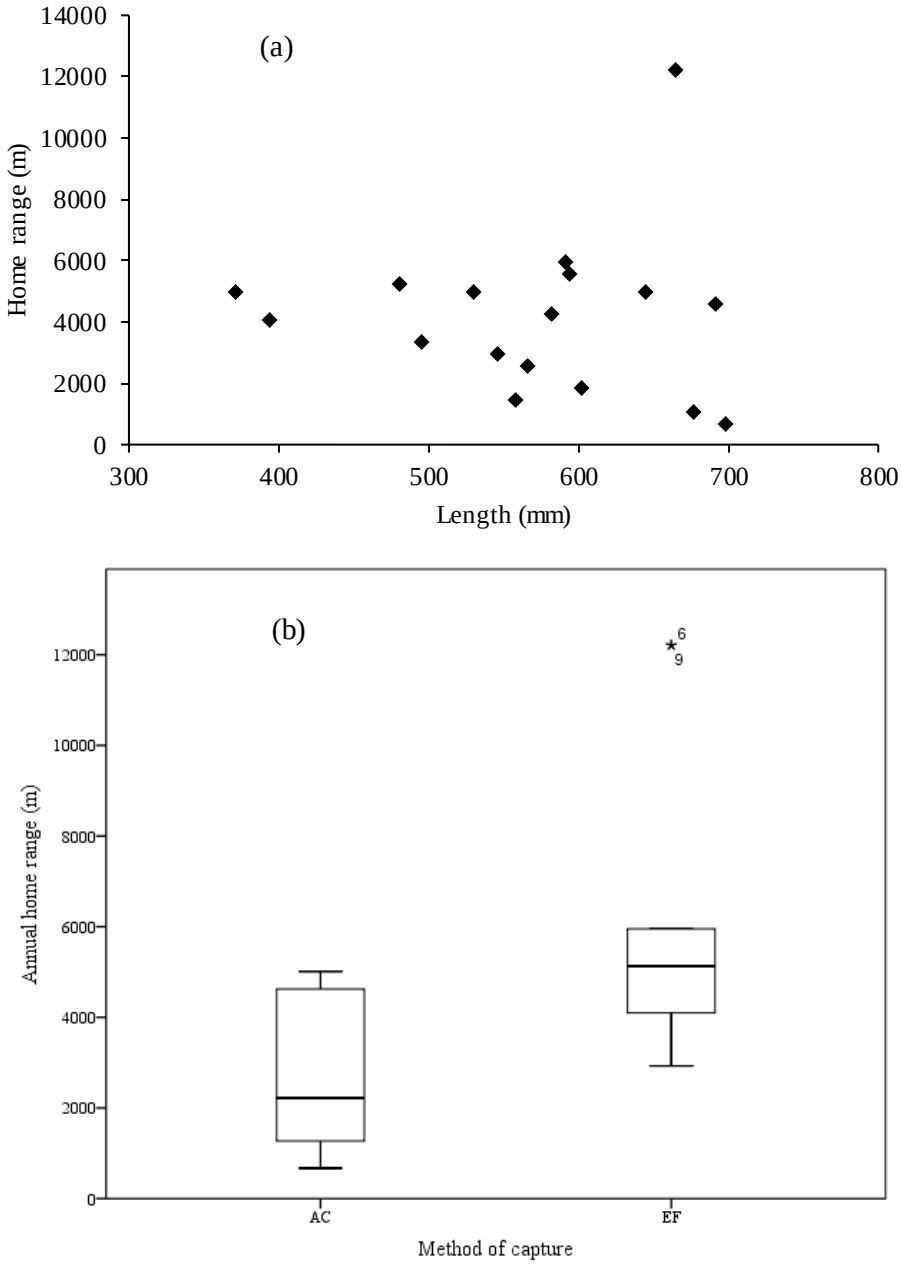

Figure 58. (a) Relationship of home range size and fish length. (b). Boxplots of home range size of tagged *B. barbus* sampled by angling (AC) and electric fishing (EF), where horizontal lines represent 10, 25, 50, 75 and 90th percentiles, with outliers as stars.

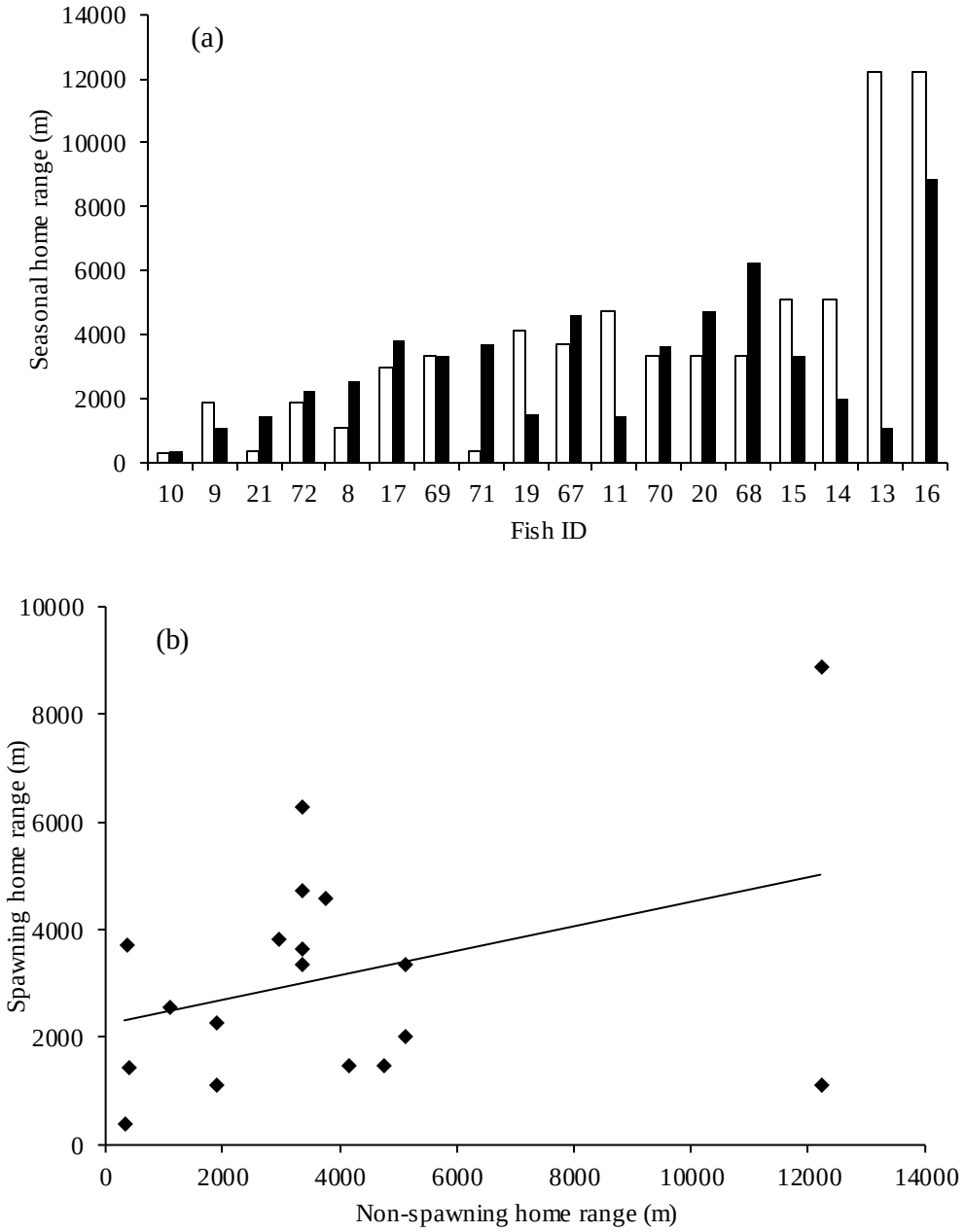

Figure 59. a) Home range in the 'spawning' (white bars) and 'non-spawning' (black bars) seasons in the individual fish (ID as per Table 38). b). Mean home range between "spawning" and "non-spawning" seasons across the 18 tagged fish, where the solid lines represents the significant relationship according to linear regression.

7.4.4 Total movements

Across the 18 fish, the mean total distance moved during the tracking period was 27,327 ± 4,919 m (range 9,582 to 48,470m) (Table 38). The relationship between body length and total distance moved was not significant ($R^2 = 0.01$, $F_{1,17} = 0.19$, $P = 0.67$, Fig. 60a). There was a significant negative relationship between total movement in the non-spawning versus spawning season (linear regression: $R^2 = 0.27$, $F_{1,17} = 5.98$, $P = 0.03$), with fish that moved less outside of the spawning season moving significantly more during it (Fig. 59a). There was no significant difference between the total movement of the tagged barbel that were sampled by electric fishing (n = 10; mean 31,850 ± 6,350 m) and by angling (n = 8; mean 31,020 ± 11,780 m) (t test: $t_{14.2} = 2.017$; $P = 0.06$; Fig. 60b).

Mean movements of fish per month differed significantly (Table 41), with peak movements in November, March, May and June (Fig. 61). Individual fish had significantly different total monthly movement patterns to each other, which are not explained by environmental variables (Table 41). Both flow and temperature had a significant negative effect on total movement (Table 41), with months of high flow (e.g. December to February) having relatively low fish movements, and months with high temperature (e.g. July and August) having relatively low movements (Fig. 62). There was also a significant interaction effect of flow and temperature (Table 41), hence total movement is high when flow is low and temperature is high (Fig. 61). When temperature and flow are plotted separately, the middle flow and temperature classes have the highest total movement (Fig. 63 a,b).

Table 41. Generalized linear mixed model results for monthly total movement, with individual fish as random effects

	Estimate	SE	z value	*P*
Intercept	10.82	0.03	432.20	< 0.01
Month	-0.09	0.00	-149.50	< 0.01
Flow	-0.09	0.00	-226.20	< 0.01
Temperature	-10.86	0.00	-201.60	< 0.01
Flow: Temperature	0.01	0.00	177.70	< 0.01
Fish ID (random)	0.20	0.08	2.60	< 0.01

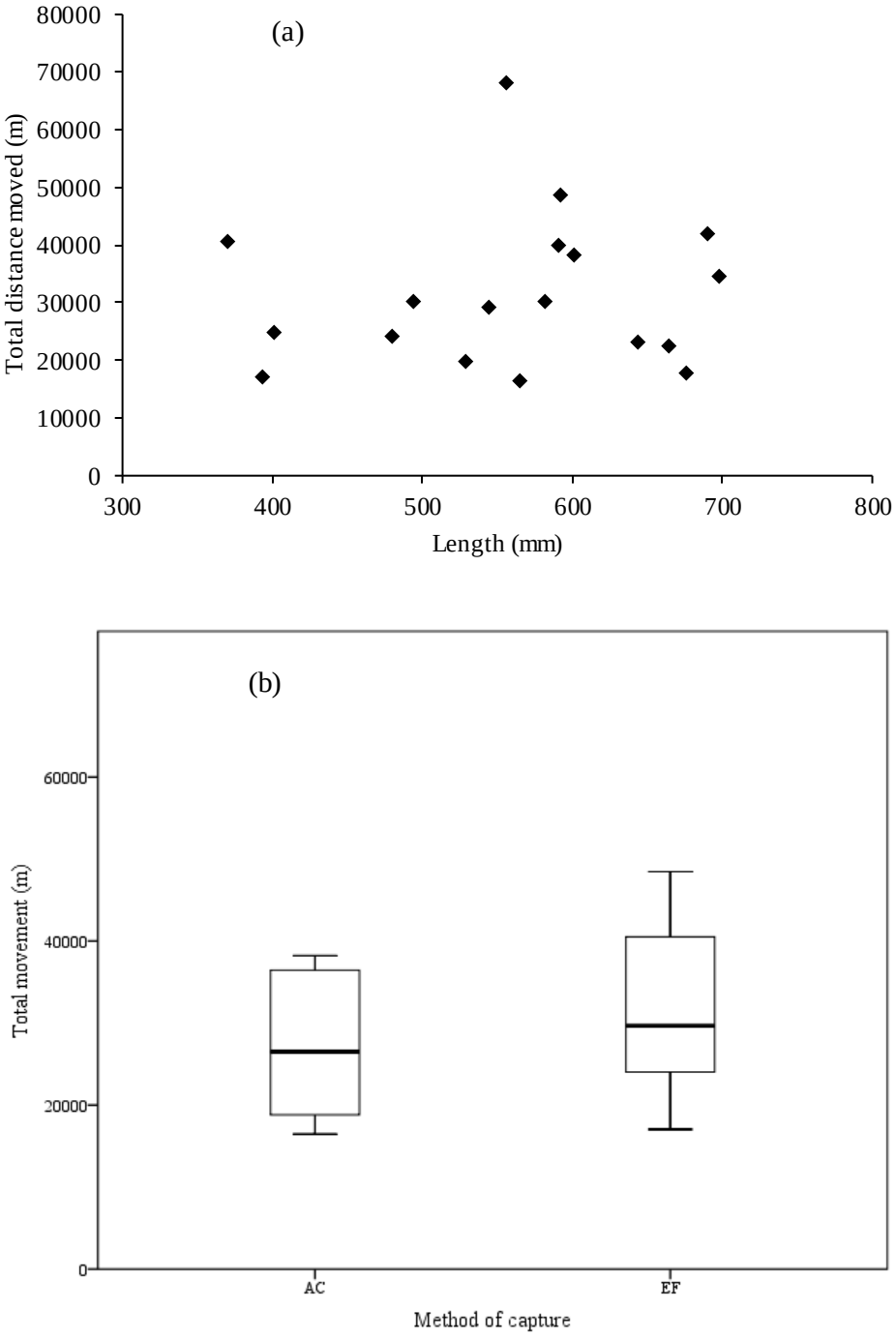

Figure 60. (a) relationship of total movement versus fish length (mm); and (b) boxplots of home range of tagged *Barbus barbus* (n = 18) sampled by angling (AC) and electric fishing (EF), where horizontal lines represent the 10, 25, 50, 75 and 90th percentiles, with outliers as circles.

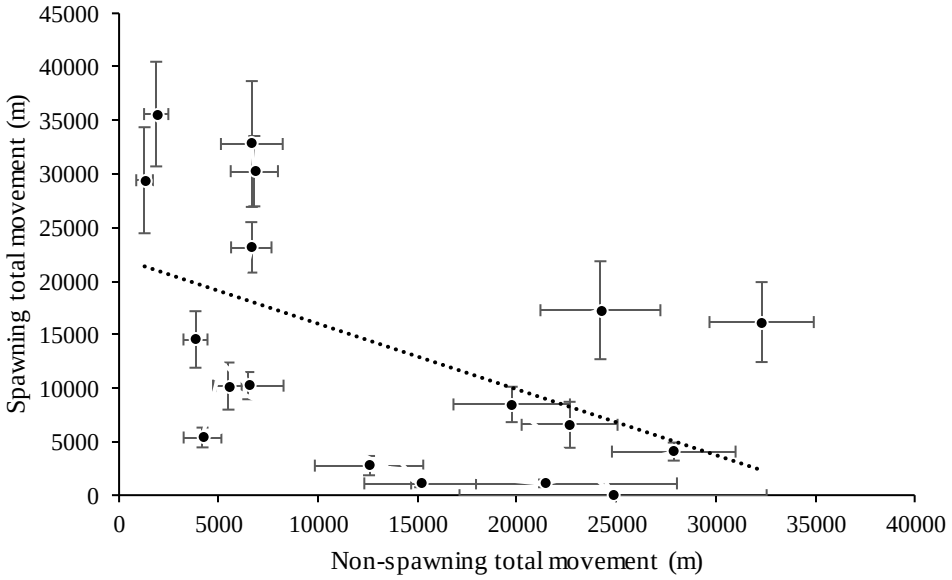

Figure. 61. Comparison of total movements of *Barbus barbus* (n = 18) between non-spawning and spawning seasons, with 95% confidence intervals. Dotted line represents their significant relationship according to linear regression (R^2=0.27, $F_{1,17}$ = 5.98, P = 0.03).

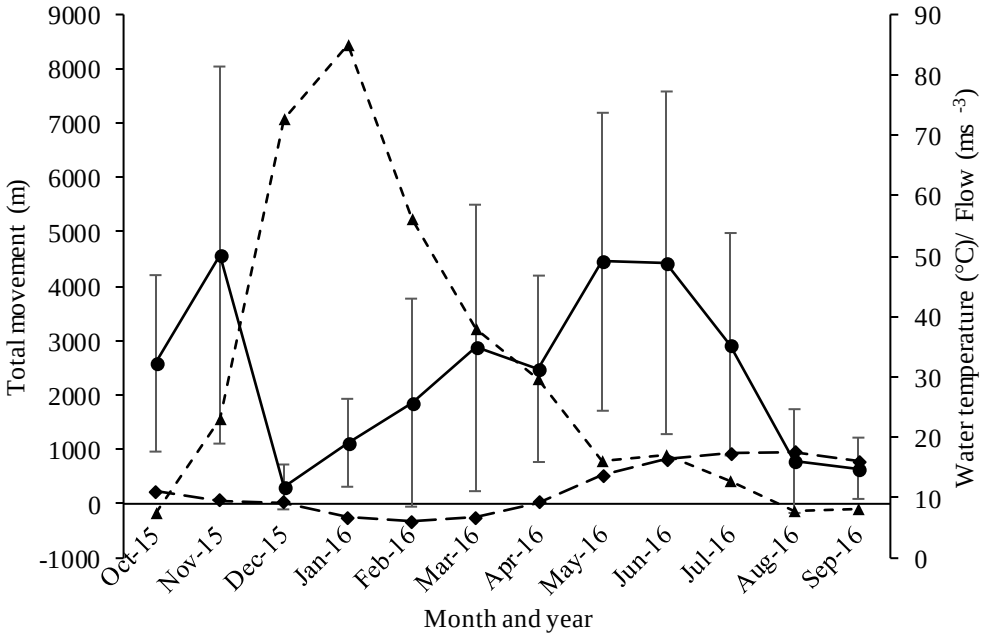

Figure 62. Monthly total movements (m) of *Barbus barbus* (n = 13, ●, solid line) with 95% confidence intervals on primary axis and mean water temperature (°C, ♦, long dashed line) and mean water flow (ms^{-3}, ▲, dashed line) on the secondary axis

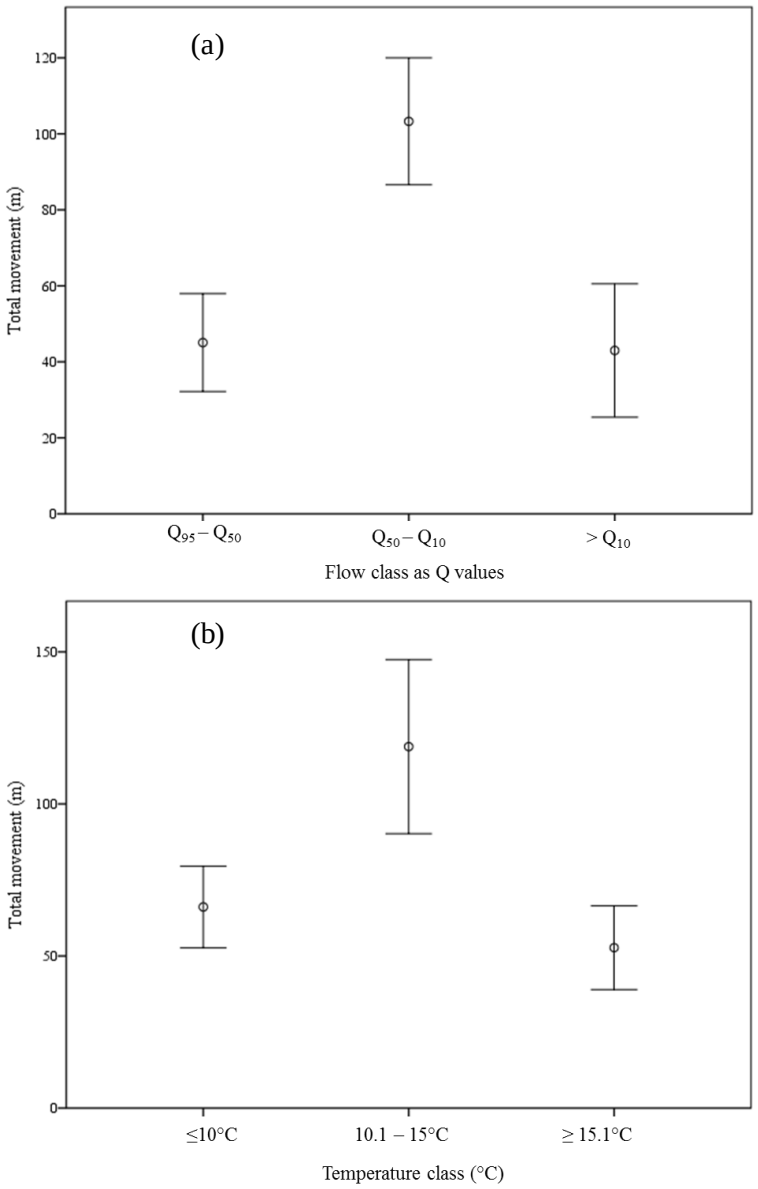

Figure 63. (a) Mean total movement of *Barbus barbus* (n = 18) at three different flows; Q10 and above, Q50 to Q10 and Q95 to Q50, no flows this year were below Q95. Error bars as 95% CI; and (b) mean total movement at three different temperature classes' ≤10 °C, 10.1 – 15 °C and ≥ 15.1°C. Error bars as 95% CI.

7.4.5 Daily movements

Across the 13 fish with 366 days of detection (Table 38), their total daily movements (hereafter, 'daily movements') ranged between 0 and 1311 m for fish 10 (minimum movement range) and 0 to 4256 m for fish 21 (maximum movement range); mean daily movement was 83 ± 10 m (Table 38). Time series analysis revealed that the daily movements of individual fish were not significantly autocorrelated, suggesting fish were moving independently from each other with no synchronicity (Appendix 2; Fig. A5), as supported by their low values of the linearity index (Table 38). Autocorrelation was then significant for both flow ($\chi^2 = 309.81$, df = 1, $P < 0.01$) and temperature ($\chi^2 = 356.80$, df = 1, $P < 0.01$), with these also significantly correlated with each other temporally (CCF > 0.10) and with a negative correlation of -0.52 at 0 time lag. Individual fish were tested separately for autocorrelation and 8 out of 13 fish showed significant temporal autocorrelation. Most fish (n = 10) had a significant correlation with flow and, for 9 of these, this was a negative relationship (Table 42). Most fish (n = 11) also showed a significant correlation of movement with temperature, but with 5 having a negative relationship and 6 being positive.

Table 42. Summary of autocorrelation tests between daily total movement time series for 13 *Barbus barbus* in the study period and whether those fish movements were cross-correlated with environmental factors; flow and temperature. NS = not significant, * = significant at $P < 0.05$, **$P < 0.01$; ***$P < 0.001$. Total number of fish with significant relationship for each variable summed in final row.

Fish ID	Temporally autocorrelated (df = 1)		Correlated with flow		Correlated with temperature	
08	$\chi^2 < 0.01$	NS	-0.04	NS	-0.05	NS
10	$\chi^2 = 104.09$	***	-0.16	*	0.32	*
15	$\chi^2 = 5.35$	*	-0.01	*	-0.25	*
16	$\chi^2 < 0.01$	NS	-0.02	*	-0.11	*
19	$\chi^2 = 1.48$	NS	-0.06	*	-0.15	*
20	$\chi^2 = 13.43$	**	-0.13	*	0.21	*
21	$\chi^2 = 127.42$	***	-0.15	*	0.22	*
67	$\chi^2 = 0.25$	NS	0.01	*	-0.24	*
68	$\chi^2 = 0.71$	NS	0.04	NS	-0.04	NS
69	$\chi^2 = 5.08$	*	-0.01	*	0.05	*
70	$\chi^2 = 22.98$	***	-0.02	NS	-0.09	*
71	$\chi^2 = 24.92$	***	-0.08	*	0.07	*
72	$\chi^2 = 90.04$	***	-0.07	*	0.17	*
Total		**8**		**10**		**11**

7.4.6 Net movement

Net movement was significantly different between months for the 18 fish (ANOVA: $F_{11, 215} = 5.47$, $P < 0.01$, Fig. 64a). Tukey post hoc analysis revealed the most upstream movements were in March and then May, with a mean upstream movement of 978 ± 497 m and 546 ± 453 m respectively. The greatest downstream movements were made during February and November (Fig. 64a), with mean downstream movement of 690 ± 464 m and 634 ± 780 m respectively. The months with the lowest net movement were January and August, with 17 ± 75 m downstream and 39 ± 115 m upstream respectively (Fig. 64a). When the data were split into spawning and non-spawning seasons, there was no significant difference (paired t-test: $t_{17} = -1.90$, $P = 0.08$, Fig. 64b), with mean upstream movement 145 ± 481m in the spawning season and mean downstream movement of -666 ± 569m in the non-spawning season. Comparison of net movements between flow categories revealed no significant differences between flows exceeding Q_{10}, between Q_{10} and Q_{50}, and between Q_{50} and Q_{95} (ANOVA: $F_{2, 365} = 2.207$, $P = 0.11$, Fig. 65a). There were also no significant differences in net movement between the three temperature classes (ANOVA: $F_{2,365} = 0.03$, $P = 0.97$, Fig. 65b).

7.4.7 Diurnal movement

When all seasons were grouped, only 3 out of the 13 tested fish showed a significant pattern in their diurnal movements (Fish 10, 67 and 72; Table 43a). These fish were between lengths of 602 to 698 mm, with low linearity indices (0.02 to 0.49) and receiver residency index values from 0.09 to 0.88 (Table 38). Fish 10 showed peaks of movement at 04.00 and 10.00, with no movement

during 22.00 and 23.00. Fish 67 started most movements at 19.00 and 23.00, with no movements started at 06.00, 07.00 and 12.00. Fish 72 started most movements at 18.00 and 22.00, with no movement at 12.00.

The four fish that had a small home range only within the River Teme had linearity indexes from 0.02 to 0.16 (Table 38). By season, significant diel patterns were only detected for one fish in autumn, one in spring and two in summer (Table 43b); further testing of their movement patterns by daytime, twilight and night showed no significant differences (Table 43c). Only one fish had some movements to test during winter, where there was no significant diel pattern apparent (Table 43b, Fig. 66a). Movements during spring occurred across the 24 h cycle, with three of the fish having peak movement in daylight, whilst in summer, most fish had peak movements at dusk and night (Fig. 66).

Table. 43. a) χ^2 analysis results of 13 individual *B. barbus* hourly movement, across a whole year, against the expectation that movement at each hour is equal (df = 23). b) χ^2 analysis results of individual *B. barbus* hourly movement, by season, against the expectation that movement at each hour is equal (df = 23). c) χ^2 analysis results of individual *B. barbus* proportion of movement per hour in daytime, twilight and night, for seasons that showed significant diurnal patterns, against the expectation that movement at each time of day is equal (df = 2). Significant results are highlighted in bold.

a)	Fish ID	χ^2	*p*
	08	18.33	0.74
	10	**53.57**	**< 0.01**
	15	23.99	0.40
	16	25.00	0.35
	19	26.86	0.26
	20	17.90	0.76
	21	18.39	0.74
	67	**76.27**	**< 0.01**
	68	23.20	0.45
	69	22.00	0.52
	70	30.00	0.15
	71	25.93	0.30
	72	**43.88**	**< 0.01**

b)		Aut		Win		Spr		Sum	
	Fish ID	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*
	08	32.00	0.10	21.00	0.58	26.67	0.37	22.00	0.52
	10	32.00	0.10			25.86	0.31	**40.57**	**0.01**
	21	**40.63**	**0.01**			29.89	0.15	20.73	0.60
	72	21.00	0.58			**36.77**	**0.03**	**48.32**	**< 0.01**

c)	Fish ID/ Season	χ^2	*p*
	21 Aut	1.51	0.47
	72 Spr	0.07	0.97
	72 Sum	1.46	0.48
	10 Sum	0.68	0.71

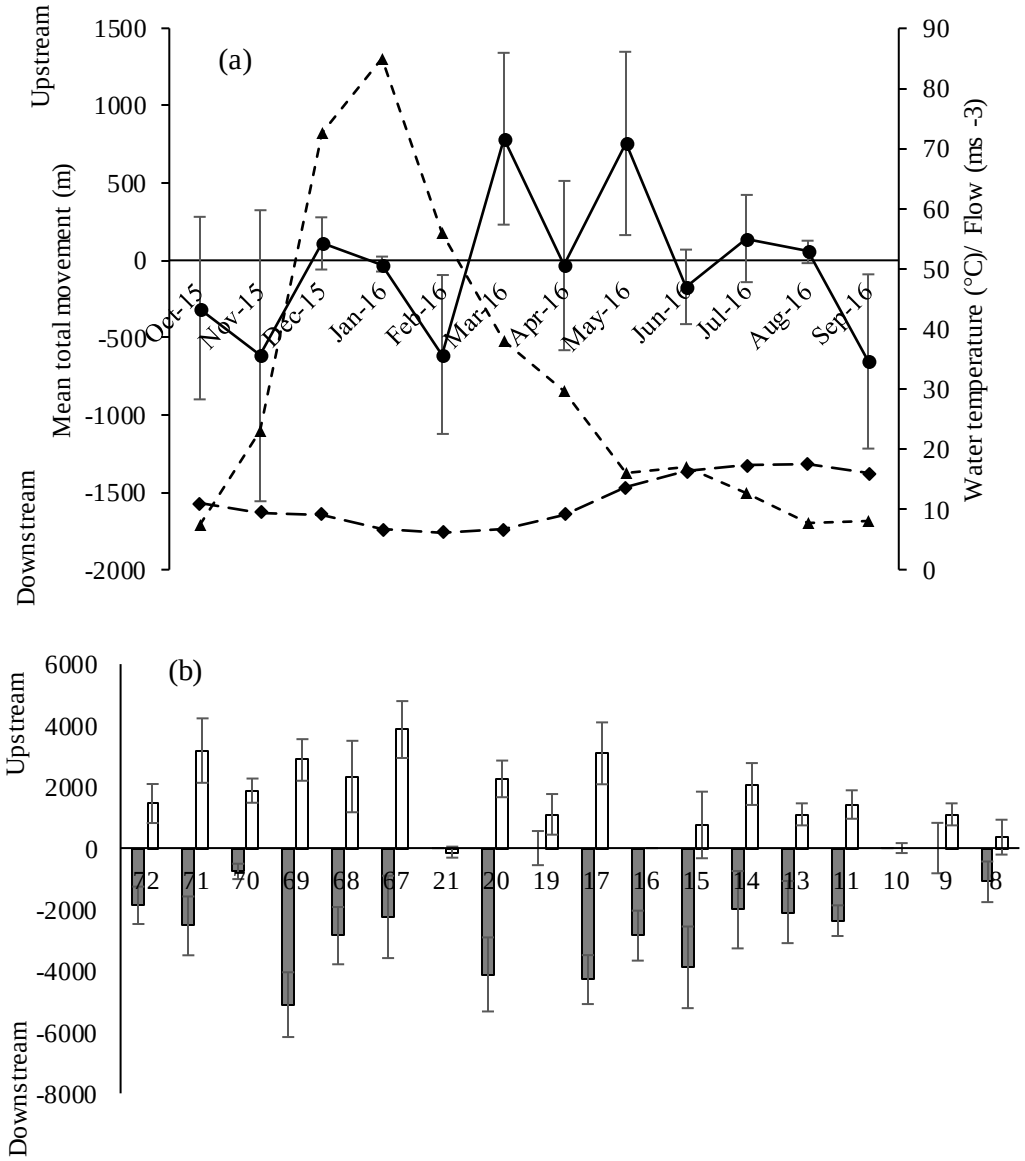

Figure 64. a) Monthly net movements (upstream/ downstream, m) of *Barbus barbus* (n = 13),●, solid line) over a year with 95% confidence intervals on primary axis and mean water temperature (°C, ♦, long dashed line) and mean water flow (ms^{-3}, ▲, dashed line) on the secondary axis b) Net movement of *B. barbus* (n = 18) by spawning (clear) and non-spawning (grey) season

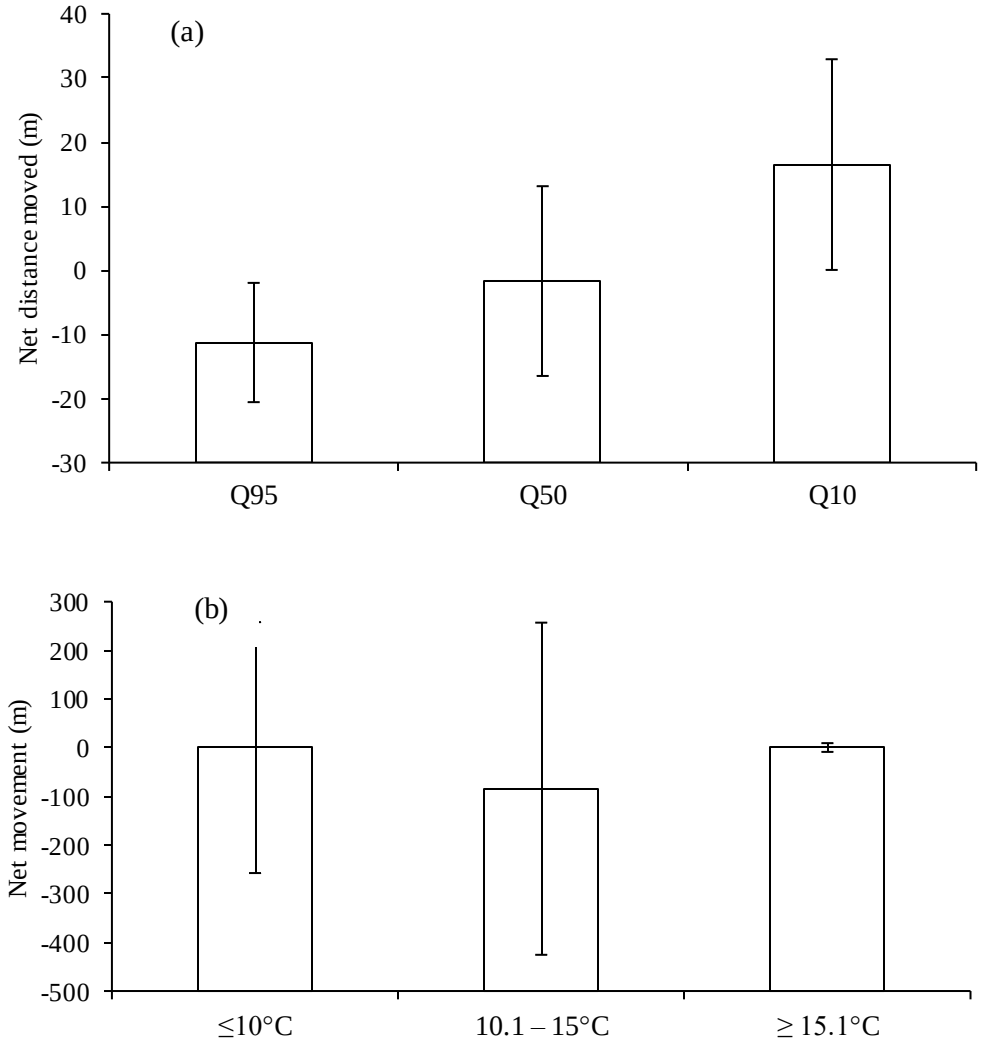

Figure 65 a) Net movement of *Barbus barbus* (n = 18) at three different flows from the River Teme; Q10 and above, Q50 to Q10 and Q95 to Q50, no flows this year were below Q95 and b) Net movement at three different temperature classes; ≤10°C, 10.1 – 15°C and ≥ 15.1°C. Error bars as 95% CI.

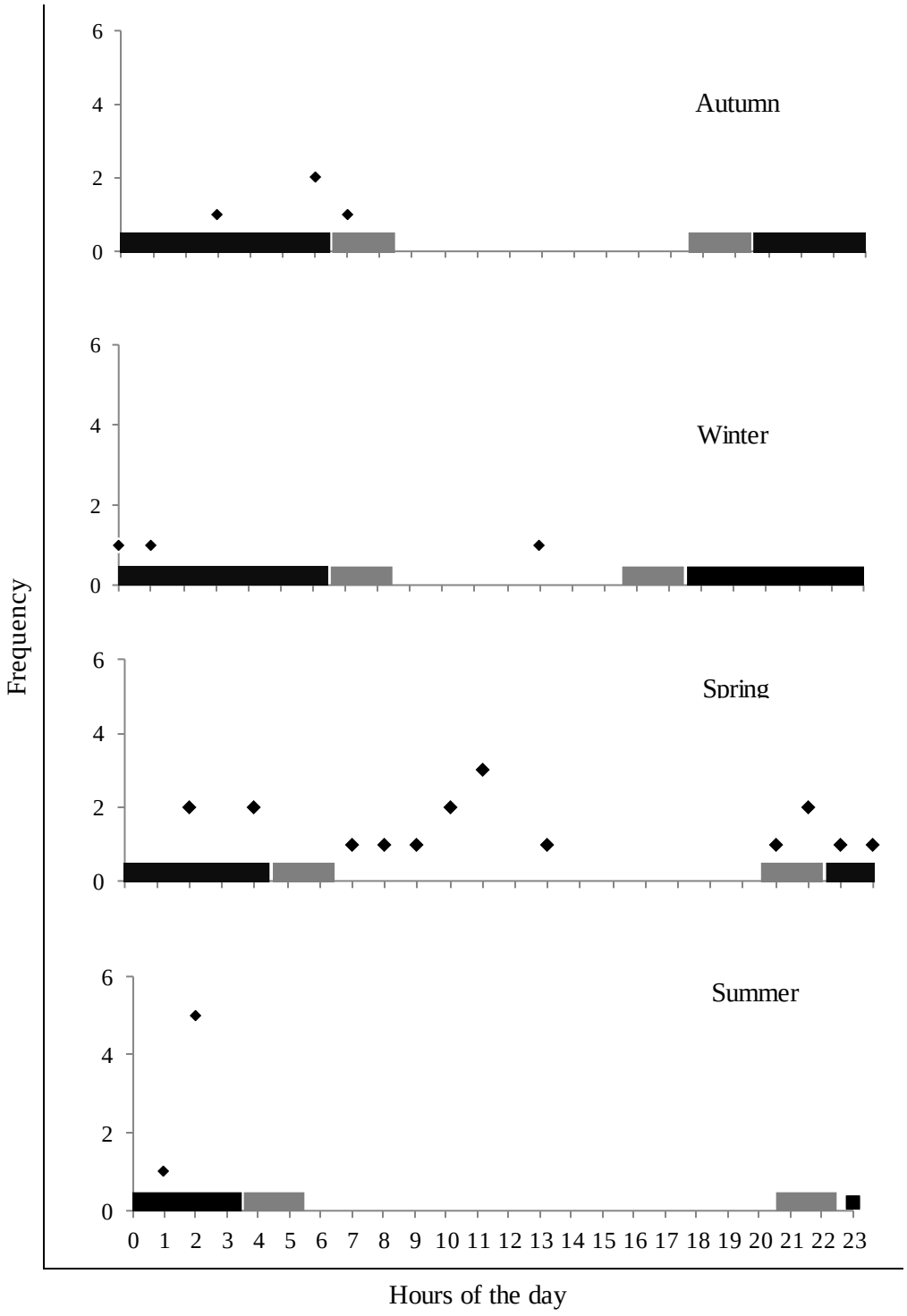

Figure 66a. Fish 08 Frequency of movement initiation over 24 hours between four seasons: Autumn, Winter, Spring and Summer

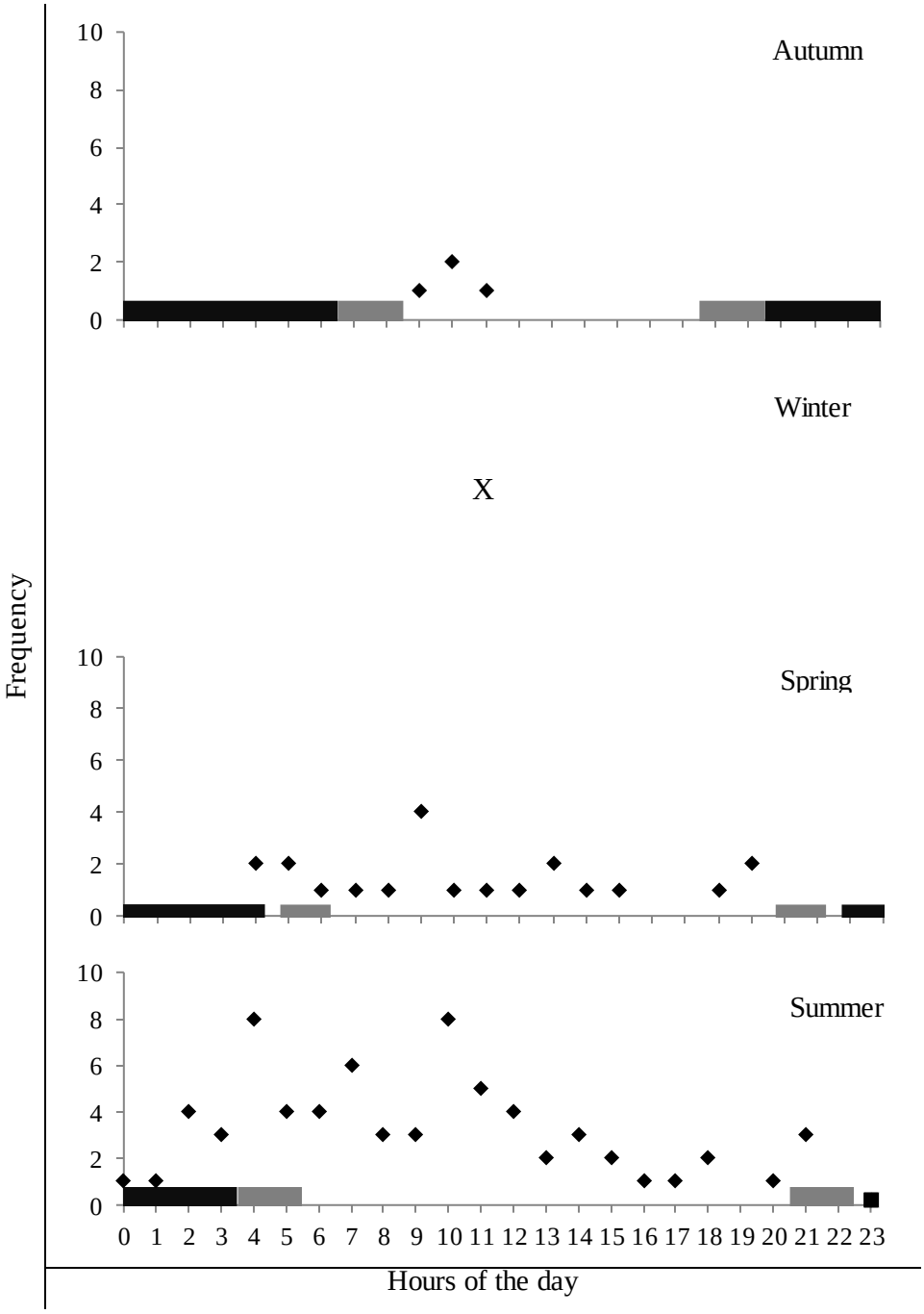

Figure 66b. Fish 10 Frequency of movement initiation over 24 hours between four seasons: Autumn, Winter, Spring and Summer. X represents no movement in that season.

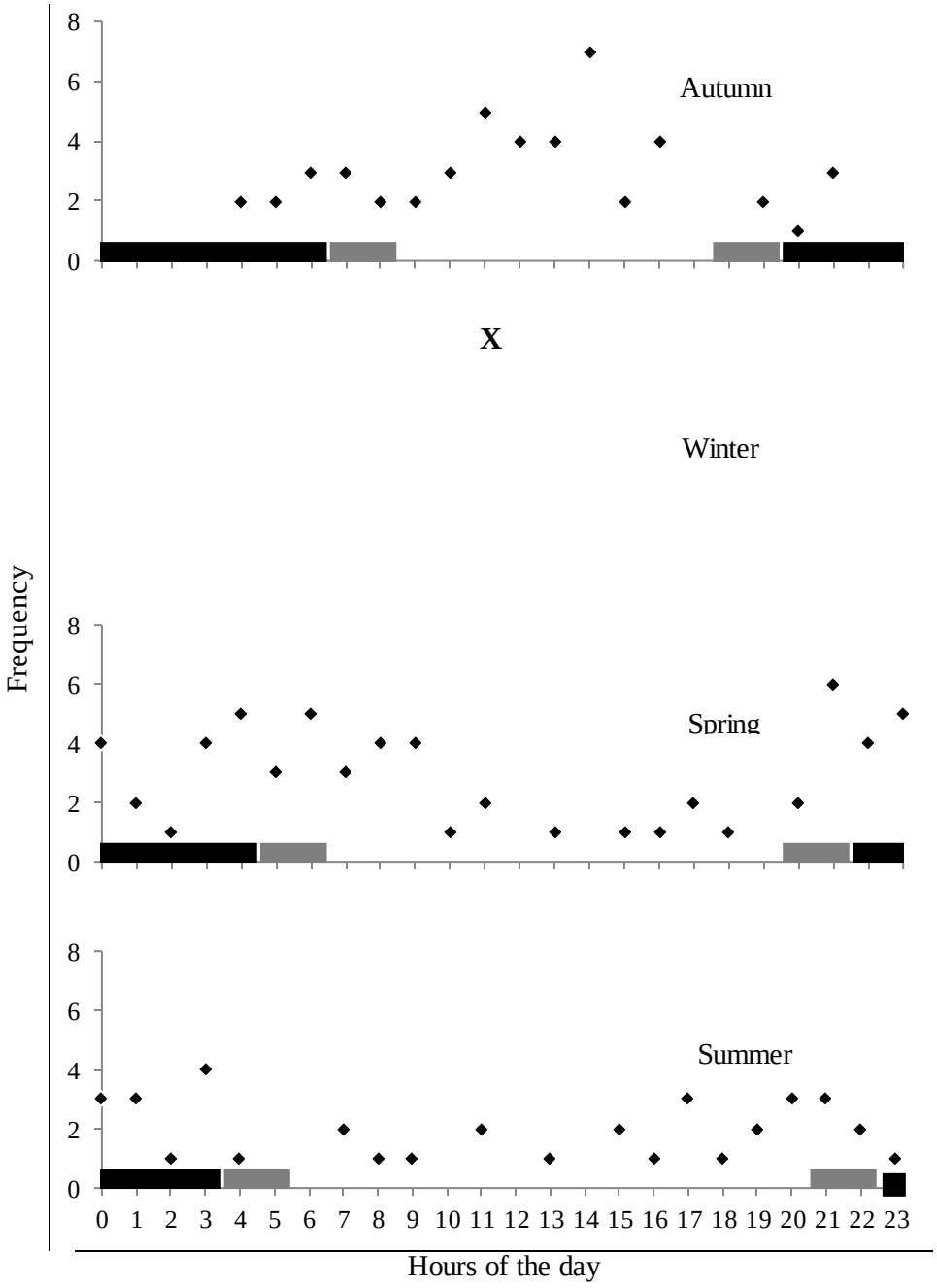

Figure 66c. Fish 21 Frequency of movement initiation over 24 hours between four seasons: Autumn, Winter, Spring and Summer. X represents no movement in that season.

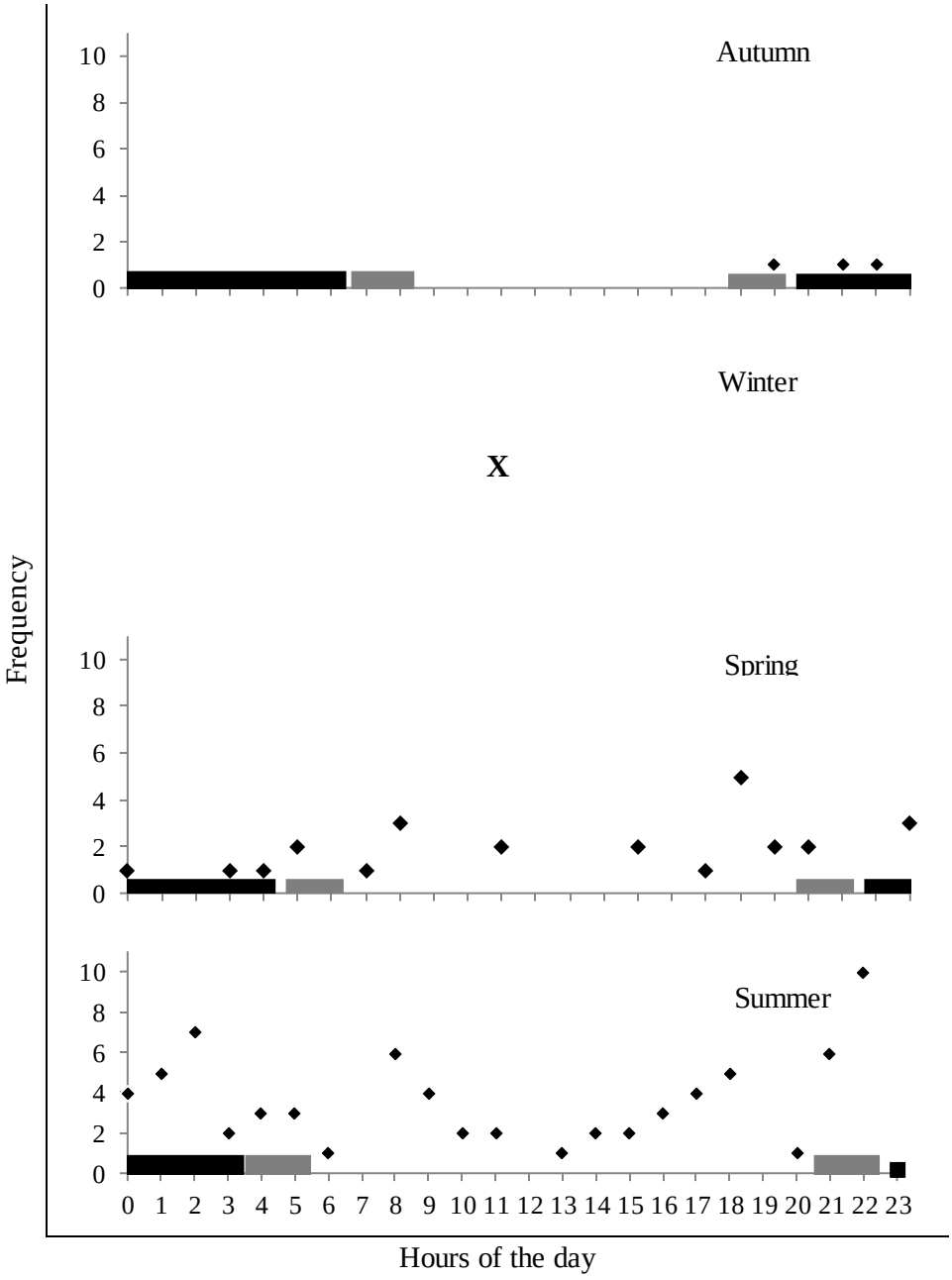

Figure 66d. Fish 72 Frequency of movement initiation over 24 hours between four seasons: Autumn, Winter, Spring and Summer. X represents no movement in that season.

7.5 Discussion

The tracking via acoustic telemetry of 18 *B. barbus* over a 12 month period in the study rivers revealed some distinct patterns in aspects of the data, but high individual variability in others. With the two rivers providing contrasting habitat typologies, then 13 of the tagged fish were primarily resident in the River Teme, being detected significantly more often there than in the River Severn. Whilst this might relate to their preference for the habitats provided by the Teme, such as the pool/ riffle sequences and substantial overhanging riparian vegetation, all the tagged fish were initially captured from the Teme. Thus, this preference might be a general reflection of their area of capture and might have differed had fish been also captured and tagged from within the Severn.

The influence of the weirs at the upstream ends of the study area was marked for the movement of *B. barbus* in both rivers. Of six individuals approaching Powick Weir in the Teme, three successfully traversed but only during very high flow events; no fish managed to traverse Diglis Weir on the Severn, despite five individuals approaching it. Indeed, other studies have indicated that even relatively minor obstructions can inhibit the movement of *B. barbus* (Baras et al. 1994; Lucas and Batley 1996). For example, in the River Nidd, Northeast England, whilst spawning movements of up 20 km occurred, of 15 *B. barbus* (of 23 tagged) that approached a flow gauging weir, only six successfully traversed it, with these individuals then moving substantial distances upstream to spawn, while those that were unsuccessful moved back downstream (Lucas and Frear 1997). In the River Meuse, Belgium, individuals that attempted to migrate into

spawning tributaries were prevented from entering due to the presence of even relatively minor physical obstacles that inhibited their movement (Ovidio and Philippart 2002). A small proportion of individuals often do manage to successfully traverse these structures, with this often coincident with episodes of elevated discharge (Lucas 2000; Vilizzi et al. 2006). The engineered structures of Powick and Diglis Weirs in the study area were thus consistent with these studies, with the results showing they generally impeded the natural movements of *B. barbus*, especially in pre-spawning, early spring period as the fish naturally start to move upstream. These results then suggest that these weirs result in individuals being restricted in their spawning habitat choice, generally to areas within 1 km of Powick Weir (personal observations), as they cannot easily access gravels further upstream. Whilst the implications of this restricted spawning habitat were unable to be explored further here, it meant spawning individuals were frequently utilising the same spawning gravels as *S. cephalus*, *P. marinus* and *Alosa* spp. Thus, future management options for the rivers could include structures to improve the passage of cyprinid fishes (including *B. barbus*) above these weirs in order to facilitate their access to spawning gravels further upstream (*cf*. Chapter 8).

The general movement behaviour of *B. barbus* tends to involve cyclical migration patterns through the year, with movements downstream in autumn and upstream in spring and early summer (Lucas and Batley 1996; Lucas and Frear 1997). The downstream movements are often associated with flood events and upstream movement for spawning (Lucas 2000). Post-spawning, individuals often display strong homing behaviours (Baras 1996; Lucas and Batley 1996;

Ovidio et al. 2007). These patterns were generally evident with the tagged *B. barbus* of this study, with net movements being primarily upstream from March to May, minimal net movement in June to August, and downstream net movements during the winter months. Given that the availability of spawning gravels was limited in the study reach above the impoundment (Harrison et al. 2017), with the primary spawning areas observed to be within 1 km of Powick Weir, then movements upstream in the pre-spawning period were assumed to relate to their attempts to locate suitable spawning areas in these areas.

In summer, *B. barbus* tends to display daily peaks in activity associated with dusk and dawn when they move onto riffles for feeding (Baras and Cherry 1990); in autumn a trimodal pattern is more apparent with the additional emergence of a phase of diurnal movements. As temperatures decrease in winter then these activity peaks diminish, with fish entering a dormancy period in particularly cold temperatures (Baras 1995a). In contrast to the net movement data that showed consistency with other studies, these daily peaks in activity were less evident here, with a general pattern across the dataset of asynchronous diel movements. Whilst this might be an inherent feature of this invasive population, with a wide range of abiotic and biotic factors interacting to influence individual daily movements and so masking consistent diel patterns, it might instead be an artefact of the tracking methodology. Whilst mobile radio-tracking studies tend to enable the triangulation of positions of tagged individuals, enabling the relatively fine-scale movements of individuals to be recorded (e.g. White and Garrott 2000), acoustic telemetry can only detect movements according to issues such as range detection and receiver location. Given range detection in the study area here varied between the rivers and was given as a nominal 100 m, with

movement primarily detected on the fixed VR2 receivers due to the steep riparian zone inhibiting access for use of a mobile V100 acoustic receiver, then this method was likely to be less suited to detecting the more fine scale movements associated with crepuscular feeding movements from daytime refugia to riffles for feeding, especially in summer.

A common feature of *B. barbus* populations is considerable intra-population differences in movement distances and home ranges (Baras 1997). This has resulted in their populations being described as comprising of 'resident' and 'mobile' fish (Hunt and Jones 1974; Penaz et al. 2002; Britton and Pegg 2011). For example, in a study on the middle River Severn in the 1970s, 86 % of tagged fish were recaptured within 5 km of their point of release (Hunt and Jones 1974). However, the other fish moved more widely, with some recorded up to 34 km from the tagging area, with the total area covered by mobile fish being 54 km. In the River Jihlava, Czech Republic, resident fish had ranges of 250 to 780 m versus movements of mobile fish of up to 1,680 m downstream and 2,020 m upstream (Penaz et al. 2002). Elsewhere, home ranges of up to 2,200 m have been recorded (Baras and Philippart 1989; Pelz and Kastle 1989; Baras and Cherry 1990; Baras 1997). These studies thus indicate considerable differences in home range sizes between rivers and between individuals in the same river, with Lucas and Baras (2001) revealing a continuum of annual individual movements of < 1 to > 30 km. Consequently, the mean home range size of the 18 tracked fish of this study of 4,600 ± 1,500 m, with a range of 700 to 12,200 km, is relatively typical for the species. Moreover, the home ranges at the higher end of the range of values might have been larger but for the position of the VR2

fixed receivers at the extremes of the array (potentially limiting detection of the extremities of their home range). Plus, the presence of Powick and Diglis Weirs blocked at least some of the fish from moving further upstream and so further increasing their home range.

This consistency between rivers of *B. barbus* populations comprising of resident and mobile fish then raises the question as to why some individuals have very large home ranges compared to others, i.e. what are the fitness and selection advantages of this (Steingrímsson & Grant 2003), and what are the underlying differences between resident and mobile fish? Here, the differences were not related to body length and there was no evidence to suggest the fish with larger home ranges gained advantages in traits such as condition and growth (Amat Trigo et al. 2017). However, it was apparent that fish that had been captured by electric fishing had a significantly larger home range than those captured by angling (6,112 ± 2,075 m versus 2,739 ± 1,229 m), with this consistent with the hypothesis in Objective 3. Using the assumption that individuals with larger home ranges have higher exploratory behaviours than those with smaller home ranges, then it is tentatively suggested the exploratory, mobile individuals were more likely to have bold personality traits (Ward et al. 2004). These home range results by sampling method are then consistent with Wilson et al. (2011), who revealed individual differences in bluegill *Lepomis macrochirus* behaviours resulted in shy fish being captured more frequently by angling compared with seine netting. Empirical studies that have related the catchability of fish by passive fishing techniques, including angling, suggest that fish in highly exploited situations are generally characterised by low swimming activity (Alos

et al. 2012). Thus, in entirety, these studies arguably suggest the individual differences in home range sizes in the *B. barbus* of this study resulted from differences in their behavioural syndromes on the shy-bold continuum (Nyqvist et al. 2012). The individuals with small home ranges were likely to be towards the shy end of this continuum and were correspondingly more vulnerable to angling capture (Wilson et al. 2011). This vulnerability would have related to their likelihood of increased spatial encounters with anglers, elevating their probability of capture (and, likely, resulting in multiple captures) (Alos et al. 2012). Indeed, angler-captured fish have been hypothesised as having consistent selection towards low activity phenotypes that can have small home ranges (Alos et al. 2012). Moreover, anglers on the study section tend to fish from recognised areas which *B. barbus* inhabit during the daytime, with these fish likely to be those that generally have smaller home ranges, thus reinforcing this apparent relationship between home range and vulnerability to angler capture.

In summary, the tracking of these 18 *B. barbus* over a 12 month period revealed strong patterns in river residence, consistent patterns in net movements that related to the pre-spawning movements and the negative consequences of river impoundment for their movement generally. In contrast to other studies, there were no consistent patterns in their daily activities. However, as with other tracking studies on *B. barbus*, there was high variability in their home range sizes, suggesting a continuum of individual movements that are argued as likely to result, at least partially, from differences in their behavioural syndromes (bold-shy continuum) that then influence their vulnerability to angler capture. Consequently, the sampling method for individuals being used in tracking

studies can have important and inherent influences on the tracking data, especially in a vagile species such as *B. barbus*.

Chapter 8: DISCUSSION

8.1 Overview of thesis

The intentional introduction of fish species into new environments for enhancing recreational angling remains a common management practice (Gozlan et al. 2010a,b). For introduced species such as *O. mykiss*, their residence in fisheries can be brief, as the majority of released fish are captured by angling and then removed within a short period of time (Miko et al. 1995). For some cyprinid fishes, however, their long-life span and the utilisation of catch and release angling means that following their introduction, their presence is likely to be prolonged (> 10 years; Bašić and Britton 2016), increasing the probability of their establishment of a self-sustaining population and subsequent invasion due to their ability to withstand a long lag period (Azzurro et al. 2016).

The intentional translocation of 509 adult *B. barbus*, from the River Kennet (indigenous range; River Thames catchment) into the middle River Severn (non-indigenous range) in 1956 provides a strong example of where a translocation has resulted in an invasion (Antognazza et al. 2016). Following their release, these fish established a population that became dominant in angler catches in the middle reaches of the River Severn in the 1970s (Hunt and Jones 1974), with their subsequent dispersal throughout much of the Severn catchment, including the River Teme (Amat Trigo et al. 2017) and the Warwickshire Avon (Antoganzza et al. 2016). Whilst much of this dispersal was from the natural movement of fish, unregulated transfers of fish around the catchment (and into neighbouring catchments) by anglers were also likely to have occurred, but this remains unsubstantiated (Wheeler and Jordan 1990). This intentional

introduction into the River Severn for establishing a *B. barbus* population for the purposes of enhancing angling was thus highly successful in its primary objective of enhancing angling.

Following their subsequent colonisation of much of the River Teme, *B. barbus* supported high catch rates in fisheries along the middle and lower river in the period between the 1980s and mid-2000s, with catches in general comprising of relatively large numbers of fish (e.g. > 10 fish per angler day) in the size range of 450 to 650 mm, and 2 to 4 kg (unpublished data). Anecdotal evidence suggested, however, that there were large declines in catch rates from 2007. However, data to evidence this decline were lacking, allied with minimal ecological data available on their population. The aim of this research was thus to generate new knowledge on the ecology of this translocated and invasive fish in order to provide baseline information that could be used as a basis for more informed fishery and river management decision making.

The focus of the research was initially on understanding the reproduction of *B. barbus* in the river, with focus on the quality of the spawning substrate (Chapter 2) and the temporal and spatial production of 0+ fish in the river via their reproduction (Chapter 3). Then, understanding the inter-specific interactions of *B. barbus* in the river was completed through assessment of their diet and trophic relationships with other cyprinid species and in relation to angling bait (Chapters 4 to 6). In addition, the tracking, via acoustic telemetry, of *B. barbus* in the lower river provided insights into how individuals utilised the river in relation to its confluence with the River Severn and also in relation to river management

structures, specifically the weirs at Powick (Teme) and Diglis (Severn). Thus, the aim of this Discussion chapter is to synthesise the results of these chapters in order to draw more general conclusions on the current ecological status of the *B. barbus* population, make recommendations in relation to their management specifically and river management more generally, and to highlight the remaining knowledge gaps that could not be filled by this work.

8.2 Spawning substrate and the production of 0+ fish

In Chapter 2, the spawning habitat utilised by *B. barbus* in its non-indigenous range was characterised, complementing work completed by Bašić (2016) who characterised their spawning habitat in the River Great Ouse (indigenous range). The majority of the spawning gravels analysed in the River Teme had lower amounts of fines versus those sampled in the River Great Ouse (Bašić 2016). This is potentially important, as Bašić (2016) revealed through a controlled experiment that increased fine content in spawning gravels resulted in significant decreases in the emergence time of *B. barbus* larvae from gravels. Where fine content was above 20 %, it resulted in larvae prematurely being present in the water column and prior to commencing exogenous feeding. This finding was then related to the Great Ouse catchment, where Twine (2013) revealed repeated *B. barbus* spawning failures in the river, whereby no 0+ *B. barbus* were recorded in the majority of juvenile fish samples collected in the river in the mid-2000s. Moreover, genetic analyses of adult *B. barbus* in the Great Ouse by Antognazza et al. (2016) revealed that adult barbel were primarily the result of stocking of adults from the River Kennet in the 1970s, with minimal evidence of any

catchment-specific lineage. Consequently, the indigenous *B. barbus* population of the Great Ouse catchment appears imperilled due to repeated spawning failures that relate, at least in part (given observations of spawning adults), to high fine content in spawning gravels that result in premature larval emergence and thus low survival rates (Bašić 2016).

In contrast, in the larval and juvenile fish surveys completed in the River Teme between 2015 and 2017, as reported in Chapter 3, there were virtually always 0+ *B. barbus* recorded, with in excess of 150 individuals captured in some seine net hauls from some sites. Between July and September, 0+ *B. barbus* were generally present in all sampled areas, despite the sampled nursery areas being of very limited size. This was not only a contrast to the situation in the River Great Ouse (Bašić 2016), but also to the indigenous population of the River Trent, where juvenile fish surveys completed throughout the 2000s by Nunn et al. (2002, 2007a, 2010) revealed, in general, very low contributions of 0+ *B. barbus* to samples. In the River Teme, *B. barbus* were, after *P. phoxinus*, the most numerous 0+ fish captured at all sites (Chapter 3). Therefore, the relatively low fine content of spawning gravels in the River Teme might facilitate high *B. barbus* spawning success and the production of large numbers of 0+ fish. Thus, it is concluded that reproductive failure via highly degraded spawning gravels are not causal factors in any population decline that might have occurred in the River Teme *B. barbus* population during last decade.

In analysing the spawning substrate characteristics of *B. barbus* in the River Teme, the zoo-geomorphic impact of their spawning was also assessed. Across

six spawning redds, *B. barbus* were revealed to have moved 74,754 cm^3 of sediment, despite there being no significant effects on sediment characteristics, and revealed an apparently high potential reproductive capacity for *B. barbus*. This research adds to a growing body of research on the zoogeomorphic effects of spawning fishes, which currently have focused primarily on salmonid fishes (Gottesfel et al. 2004; Hassan et al. 2015; Riebe et al. 2014; Chapter 2).

The spawning strategy of this non-indigenous *B. barbus* population was also able to be assessed through the temporal collection of samples over three reproductive periods. This revealed that, as per sustainable populations in their native range, they generally utilised a protracted spawning strategy. Rather than the production of 0+ fishes in a single, large reproductive peak, it was more apparent that larval fishes were appearing in samples collected over a number of weeks and, generally, between late June and early August. The data suggested that *B. barbus* spawning occurred throughout June and July, with no obvious peak in effort. It could not, however, be determined whether this protected spawning involved individual fish spawning on one occasion only but with high variability in their spawning times, or whether it involved individuals reproducing on several occasions (i.e. fractional spawning). Moreover, this protracted spawning was also evident in native *S. cephalus* and *P. phoxinus*, and has also been reported in native *B. barbus* populations (Nunn et al. 2007a). In combination, this suggests some consistency with the pre-adaptation hypothesis, whereby the non-indigenous *B. barbus* utilised traits in the new range that it utilises in their indigenous range and thus there was no requirement for adaptive responses during their establishment (Buoro et al. 2016). This protracted spawning

behaviour is theorised as providing some bet-hedging in the reproduction and recruitment processes, with a trade-off between the risk of early summer flood events incurring high mortality rates in cohorts versus the risk that 0+ fish produced later in the summer achieving only small body lengths prior to the winter period (Nunn et al. 2002). The consequence of this for *B. barbus* recruitment in the River Teme was, however, unable to be assessed in this research and remains an outstanding requirement.

8.3 Trophic ecology of non-indigenous *B. barbus*

There were a number of aspects of the trophic ecology of this non-indigenous *B. barbus* population that were investigated in conjunction with the native cyprinid fishes: diet composition and trophic niche size of 0+ fish via stomach contents analyses, isotopic niche size and overlap of 0+, juvenile and adult fishes via stable isotope analysis, and the influence of marine derived nutrients via fishmeal pellets on *B. barbus* diet, as also indicated by stable isotope analysis. In general, the results consistently revealed that there was some partitioning in the trophic/ isotopic niche of *B. barbus* with other fishes, suggesting there is little evidence to suggest competitive interactions with native fishes. Although not directly assessed here due to an absence of historical data, there was no evidence to suggest that there has been any shifts in the trophic interactions between the fishes that could have been causal factors in factors in any *B. barbus* population decline that might have occurred in the last decade.

Stomach contents analyses revealed that whilst the 0+ fishes were all primarily generalist in their diet, *B. barbus* was the most specialist out of the four analysed fishes. The results revealed that the relationship between gape height and diet was species-specific and that inter-specific trophic overlap altered with ontogeny. Overall, the trophic niche of invasive *B. barbus* was dissimilar to the other two recreationally important cyprinids, *S. cephalus* and *L. leuciscus*, but it did overlap with the highly abundant *P. phoxinus*. Unlike the study by Nunn et al. (2007b), the effect of ontogeny in this study was not a significant driver of diet composition, although this might have been an artefact of the low sample size in larval stages. Notwithstanding, length was a significant indicator of the prey taken by *B. barbus*, with gape height also a significant predictor of diet composition for the other three species. Increasing length and ontogenetic development that occur during the early life-stages of cyprinid fishes are associated with development of their swimming abilities (e.g. fin development) and muscle development (Pinder 2001), and thus should facilitate the capture of more mobile and larger prey items (Nunn et al. 2007b). The prey items taken by *B. barbus* and the native cyprinids were largely similar to those found in other studies (Bischoff and Freyhof 1999; Nunn et al. 2012), apart from a lack of rotifers in the diet of the fish in the Teme. This suggests, similar to the previous chapter, that *B. barbus* were largely pre-adapted to utilise the prey at their non-indigenous range, and that no adaptive responses in foraging behaviours or diet composition was required during their colonisation and establishment.

The overlap in trophic niche between the 0+ *B. barbus* and the native 0+ cyprinids revealed that there was no significant resource sharing between the

recreationally important *S. cephalus* and *L. leuciscus*, but that there was significant resource sharing with the abundant *P. phoxinus* at two of the three sites. Despite this resource overlap, intra-specific competition would only be an issue if the prey items were limited in availability (Chase et al. 2016). The initial feeding periods of 0+ fishes can be important for promoting over-winter survival, such as through ensuring there are sufficient lipid reserves (Mills and Mann 1985; Nunn et al. 2007b). Correspondingly, these dietary analyses, including low vacuity index values, suggest a lack of suitable food resources for 0+ fish was not a causal factor in any decline in the *B. barbus* population with, for example, it not being a factor in any recruitment failures.

The application of stable isotope analysis to samples collected from a number of different life-stages revealed that whilst the extent of niche sharing varied between life stages, there was no significant niche overlap between the fishes at any stage. The niche overlap between juvenile invasive *B. barbus* and *S. cephalus*, *L. leuciscus* and *T. thymallus* were similar in this study to a study in the *B. barbus* native range (Bašić 2016). This pattern of resource partitioning between *B. barbus* and the native conspecifics thus avoids competitive interactions and so might have been a factor in facilitating the establishment and invasion of *B. barbus* (Tran et al. 2015). Similar to their spawning patterns and 0+ fish diet, the similarity between their trophic niche partitioning with other cyprinid fishes in their indigenous and non-indigenous ranges suggests consistency with the pre-adaptation hypothesis, with no adaptive response requited to the new conditions of the River Teme in order to maintain their natural foraging behaviour.

Angler baits containing high levels of marine derived nutrients (from fishmeal (up to 45%) and oil (up to 20%) are used in high quantities in the lower Teme and Severn. The stable isotope analysis in Chapter 6 revealed that some individual *B. barbus* and *S. cephalus* had diets that strongly relied on these baits, although this varied between individuals, indicating trophic specialisations and individual variability in exploiting novel food resources (Basic et al. 2015; Scharnweber et al. 2016). The result was some isotopic niche convergence in these fishes when they consumed high proportions of pellets in their diets. The issue of marine derived nutrients acting as a subsidy to freshwater production generally involves the movement of adult anadromous fishes into rivers for spawning, such as in *S. salar* and *P. marinus* that usually die post-spawning thus releasing the marine nutrients (Childress et al. 2014; Samways 2017). Numerous studies have shown the beneficial impact of marine derived nutrients being cycled from salmonid fishes into freshwater biota, including macroinvertebrates and 0+ salmonids, with considerable increases in productivity occurring (e.g. Naiman et al. 2002; Nislow et al. 2004; Williams et al. 2009; Moore et al. 2011; Guyette et al. 2013; Childress et al. 2014; Samways et al. 2017). The released nutrients can also facilitate selection pressure on offspring, with rivers lacking MDN nutrients from *S. salar* parent carcasses having a stronger selection on their egg size and juvenile metabolic rates (Auer et al. 2017).

This eco-evolutionary feedback of marine derived nutrients from anadromous *S. salar* reveals its fundamental importance to population processes, selection and, ultimately, fitness. In contrast, the marine derived nutrients from angling bait

here appear to be locked into the adult fishes that consume them, thus they appear to act as nutrient sinks, with little or low benefit to biota at other trophic levels. Consequently, unlike marine nutrients from alternative sources, it remains unclear at present whether any wider ecological benefits might accrue or whether the exploitation of these angler baits by some individual fish is merely an interesting artefact of individual trophic specialisation coupled with high angling exploitation on some reaches of the study river.

8.4 Fish movements

The vagility of *B. barbus* in the lower reaches of the study river and River Severn shows insights into their behaviour in relation to their ability to traverse weirs and the influence of environmental parameters. Whilst the ability of *B. barbus* to overcome barriers has been studied in its native range (e.g. Baras et al. 1994; Lucas and Frear 1997), there are no comparative studies available from their invasive range, despite these potential barriers to dispersal being important in their ability to colonise new reaches of river. No fish traversed Diglis Weir on the River Severn, suggesting it was completely impassable, with three fish traversing Powick Weir on the Teme. Environmental conditions affected the ability of fish to traverse this weir, with it only occurring in high water conditions in early spring, with this in agreement with studies of *B. barbus* in their native range (Lucas and Frear 1997). When these upstream movements are delayed, which are generally associated with accessing spawning grounds (Baras 1993; Ovidio et al. 2007), then there are potential implications for energy expenditure and thus subsequent reproductive effort might be reduced.

The tagged fish were captured by electric fishing and angling, with fish captured by angling having reduced home range sizes. This was tentatively related to the fish demonstrating different behaviours in the bold-shy continuum (Mittelbach et al. 2014). This is potentially important, given that studies generally indicate *B. barbus* populations comprise individuals that are 'movers' that travel large distances and 'stayers' which have a more sedentary life style (Twine 2013; Hobbs et al. 2017). However, few movement studies on fish have investigated the link between these intrinsic behaviours, individual movement patterns and their relationship with environmental conditions (Rasmussen and Belk 2017). Thus, this link between bold and shy in behavioural syndromes requires further work and research to determine how they map on to patterns of movements detected in the wild. It is, however, important from a *B. barbus* perspective, as many aspects of their ecology reported here, such as individual differences in diet composition based on angler baits, might also relate to these intrinsic behaviours. This also might affect their vulnerability to angler capture, with ('shy') fish of small home ranges likely to be more vulnerable to capture due to increased spatial encounters with anglers.

8.5 Management implications and recommendations

This thesis has begun to rectify the issue of a lack of data on non-salmonid fishes in the River Teme, and so should commence the process whereby the management of these cyprinid fishes is enhanced. Despite the anecdotal reports of lack of recruitment in the River Teme (Angling Trust 2013), the spawning substrates were in relatively good condition with generally low fine content, with

this coupled with the high production of 0+ fishes. Thus, reproductive success was not interpreted as a limiting factor for *B. barbus* specifically and cyprinid fish generally. In their initial summer of life, the growth and diet of these fishes was interpreted as being relatively normal and in line with populations elsewhere in their range and so, again, there were no concerns relating to the initial months of life of 0+ cohorts.

It was more apparent, however, that between age 0+ and the lengths at which *B. barbus* appear in angler catches (~ 400 mm; Amat Trigo et al. 2017), there are concerns of deleterious impacts relating to low numbers of surviving fish from the end of their first summer of life. Indeed, even when targeted electric fishing surveys were undertaken to target fish of between 100 (age 1+/ 2+ years) and 250 mm (age 3+/ 4+ years), these were generally unsuccessful, capturing only low numbers of fish (*cf*. Chapter 5). Of particular concern is the survival of 0+ fish during the winter, due to the propensity of the river to be subjected to large flood pulses in conjunction with negligible off-channel refuges for juvenile fishes (Fig. 67). It is thus hypothesised that this is a major limiting factor influencing the population abundance of *B. barbu*s in the River Teme and it is recommended that this should be a priority in future work, but requires the collection of long-term data-sets on both the fish and environmental parameters.

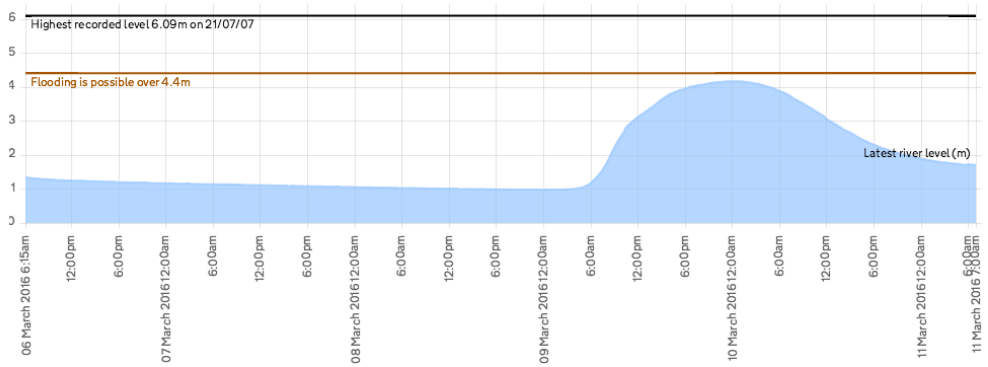

Figure 67. A river level recording taken at Knightwick on the River Teme on 11/03/2016 showing a flood pulse coming through the river from 06.00 on 09/03/16 at a river level of ~ 1m and peaking at 4.2 m at 00.00 on 10/03/2016 (Environment Agency 2016).

A further issue identified in the research was the inhibition of *B. barbus* movement by weirs. To facilitate *B. barbus* population restoration in the middle reaches of the River Teme, it is suggested that the ability of non-salmonids to traverse the weirs needs to be increased. It is thus arguably fortunate that this coincides with engineering efforts that seek to remove Powick Weir in late spring 2018 (Sharpe 2017) and insert a fish pass on Diglis Weir (The Guardian 2016). There is, however, opposition against the removal of Powick Weir from the angling community (Angling Trust 2016). However, the movement data suggest that the removal of this blockage will increase the home range size of some *B. barbus* in the lower river and enable their progression to spawning areas upstream. It could also impact the sediment composition upstream and downstream of the weir, with the highest fine contents of spawning riffles measured downstream of this weir. It is thus strongly recommended that changes in both the movement behaviour of the cyprinid fishes and in the fines content of spawning riffles are evaluated spatially and temporally over the next five years. It should be noted, however, that there is currently there negligible

spawning substrate directly upstream (2km) of Powick weir due to the impoundment, as it is too deep, with minimal gravel available (personal observation). Thus, removal of the weir could also create a series of new spawning areas for *B. barbus* in the river and this should also be evaluated.

This study has also revealed anglers, as citizen scientists, can help gather tissue samples to analyse trophic consequences of invasive fish and native fish. However, it has also shown that there can be both trophic (Chapter 6) and behavioural (Chapter 7) differences between fish caught by anglers and electric fishing. Whilst angling data and samples can add to the data collection for research and monitoring, these results suggest they should not be relied on as the sole method for collecting samples in species that potentially show considerable differences in individual behaviours. However, it also shows the potential to measure these differences and to map them to personality types on the basis of the sampling method used. Consequently, it is recommended that further research is completed on this to identify its transferability to other species and systems (Rasmussen and Belk 2017).

This has been the first study completed on the zoogeomorphic effect of spawning barbel, and revealed that individuals can move large quantities of sediment to create their redds, although this does not significantly alter the sediment characteristics. However, this study was limited to six redds and so it is recommended that future work involves higher sample sizes in the Teme and samples collected from other rivers to indicate more general patterns and the significance of any zoo-geomorphic alterations. Whilst this study represents an

initial attempt in characterising *B. barbus* redds, further study is also recommended to characterise the subsurface sediments in the redds to identify the conditions that surround the incubating eggs. This study has also shown large number of 0+ fish are produced during reproduction, so relating the reasons for this within redds versus rivers where there is reproductive failures (e.g. the Great Ouse; Twine 2013) would have high utility for *B. barbus* restoration in their indigenous range. As such, it is suggested that this is also a recommendation of future work that needs urgent attention, given the perilous state of some *B. barbus* populations in their indigenous range in Eastern England, including the River Great Ouse.

8.6 Conclusions

This once abundant and highly angler-exploited non-indigenous *B. barbus* population of the River Teme may have declined in population since the mid-2000s (Angling Trust 2013). However, the production of 0+ fish remains high (at least in 2015 to 2017), with spawning habitat quality being satisfactory and better than some rivers in their indigenous range in Eastern England. Adult fish appear to have access to suitable food resources and also behave as per fish in their indigenous range. If there is a management desire for this population to be restored to its former status, such as on the basis of its angling importance, it is thus recommended that future work on the river focuses on two principal areas. Firstly, some focus on recruitment and its association with high flow events in winter is required. Secondly, the impact of weir removal and fish passage construction on improving the access to upstream spawning habitats on both the Rivers Teme and Severn requires assessment for its benefits and negative

consequences. These schemes should also measure the impacts and responses of other fishes, including anadromous fishes, but also considering the native cyprinid fishes. This work would then be measuring the ecological integrity and status of the fish assemblage more generally. In doing so, the non-indigenous *B. barbus* can then act as a strong flag species as an indicator of the status of the assemblage, as it already does throughout its native range.

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Appendix 1

Testing of Equation 18 on B. barbus studies

Mean values from Policar (2011): L_T (mm) = 235, eggs/ female/ stripping = 1240

We used the conversion from Herrara et al. (1988) to convert the total length to fork length:

FL = (TL - 0.1)/1.1	calculated L_F (mm) = 213

The L_F above was then used in Equation 18 and estimated 8,398 eggs. This value is 6.8 times larger than the actual 1240 eggs per female per stripping. This study reported an average of 2.65 strippings, if these eggs were delivered over a natural recruitment cycle, such as two spawnings this would have been 1,643 eggs that would still leave the estimate from Herrara et al. (1988) overestimating by 5.1 times. However, Policar (2011) does note that the study shows lower reproductive capacity than other studies, and that could be due to the fish being anaesthetised.

Mean values from Poncin (1989): L_F = 333 mm, eggs/ female/ stripping = 8,000

The L_F above was then used in Equation 18 and estimated 32,918 eggs, which is 4 times larger than the actual number of eggs reported per female at 8,000 eggs. However, the fish in this study were stripped 10 times, giving a total of 80,000 eggs per female over the reproductive season, this maybe be higher that their natural activity which suggests two spawnings (Chapter 3). Therefore if the total number of eggs were delivered over two equal batches that would be 40,000, which is only an overestimation of 7,082 eggs.

Appendix 2

Table A1. Morphological features to determine ontogenic stages larval (1 – 5) and juvenile (6 – 9) stages of *Barbus barbus, Squalius cephalus, Phoxinus phoxinus* and *Leuciscus leuciscus. Barbus barbus* features as per Krupka (1988), Pinder (2001) for L1 – 5 general features and Simonović (1999) for general features 6 – 9.

Developmental stage	*Barbus barbus* Features	General features
Larval stage 1 (Referred to as stage 1, in Pinder 2001 as free embryo)	Yolk sac reduced but still visible. Gut is relatively straight and elongate, without loops. The embryonic fin-fold reaches all the way around to the back of the yolk sac.	Yolk sac present
Larval stage 2	Gas bladder develops. Yolk sac completely disappeared. Dorsal fin mostly separated from the fin-fold and reduced around the caudal fin. Mouth becomes inferior.	Dorsal fin rays not yet visible. Mouth terminal.
Larval stage 3	Dorsal fin completely separated from the fin-fold. Anal and ventral fins developing. Melanophores become more pronounced.	Dorsal fin developing but not yet detached posteriorly from the fin-fold.
Larval stage 4	Anal fin becomes separated from the fin fold and	Dorsal fin completely separate from fin-fold. Fin fold still surrounding pelvic fin.
Larval stage 5	Upper barbels are developing. Disappearance of embryonic fin fold. Fin apparatus completed including pelvic fin.	No fin fold remaining, all fins developed.

Juvenile stage 6	Development of a second pair of barbels, the nasal septum and forked rays in all the fins. Gut starts to form the first loop with a bend in the medial section.	The nasal septum develops. Onset of bifurcation of fin rays.
Juvenile stage 7	Scales begin to appear in the caudal area. Three loop formation in the gut.	Scales begin to appear in the caudal area.
Juvenile stage 8	Body fully pigmented and only the outline of the intestine visible from outside of the body. The gas bladder is covered in a fatty lining.	Scales on the lateral line.
Juvenile stage 9	Scales on the lateral line and dorsal area.	Complete scale cover including dorsal area.

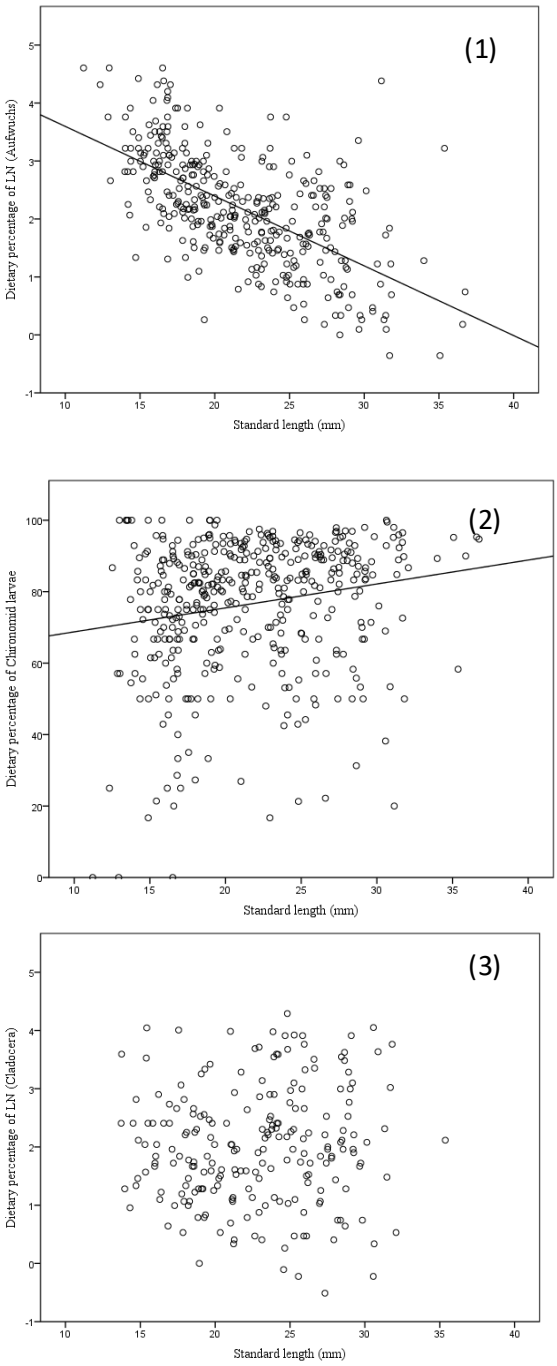

Figure A1. Standard length (mm) of *Barbus barbus* and the percentage of diet composed of prey items; Aufwuchs, chironomid larvae and Cladocera from 2015. In (1): LNAuf $R^2 = 0.38$, $F_{1, 363} = 218.48$, $P < 0.01$. (2) LN makes assumptions worse. $R^2 = 0.03$, $F_{1, 428} = 15.03$, $P < 0.01$ (3) LNCladocera $R^2 = 0.00$, $F_{1, 219} = 0.77$, $P = 0.38$

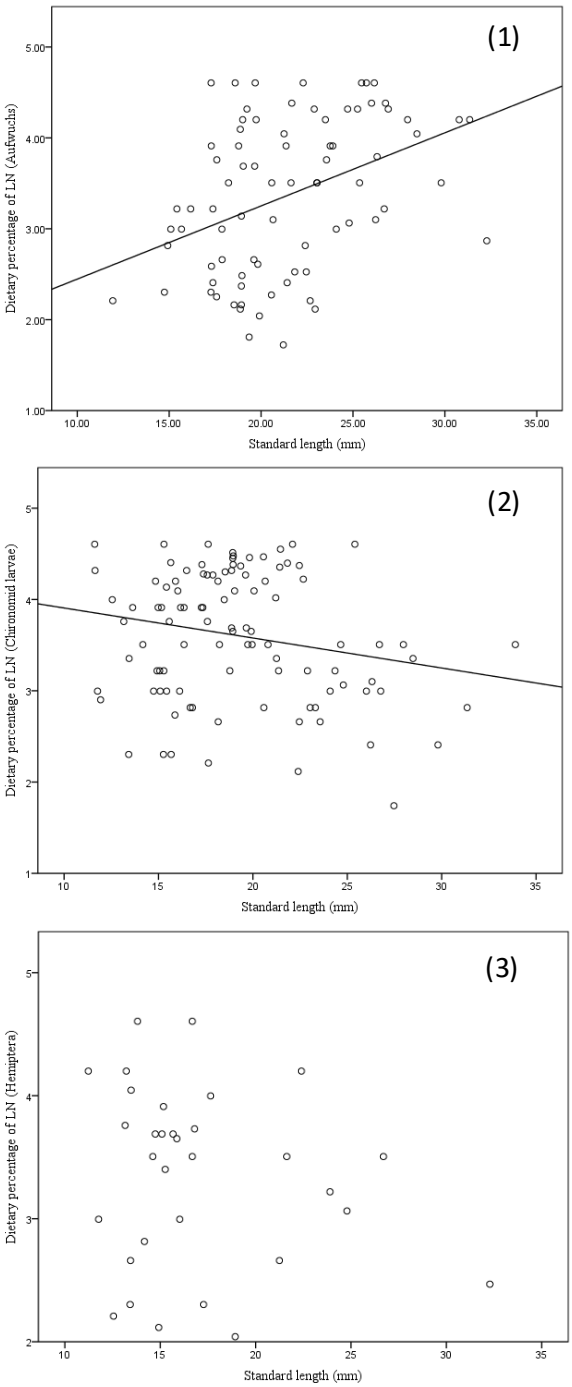

Figure A2. Standard length (mm) of *Squalius cephalus* and the percentage of diet with Natural Log transformation to conform to regression assumptions) composed of prey items; Aufwuchs, chironomid larvae and the hemipteroid assemblage from 2015. In (1) $R^2 = 0.16$, $F_{1, 78} = 14.43$, $P < 0.01$ (2) $R^2 = 0.05$, $F_{1, 100} = 4.62$, $P = 0.03$ (3) $R^2 = 0.09$, $F_{1, 31} = 0.85$, $P = 0.36$.

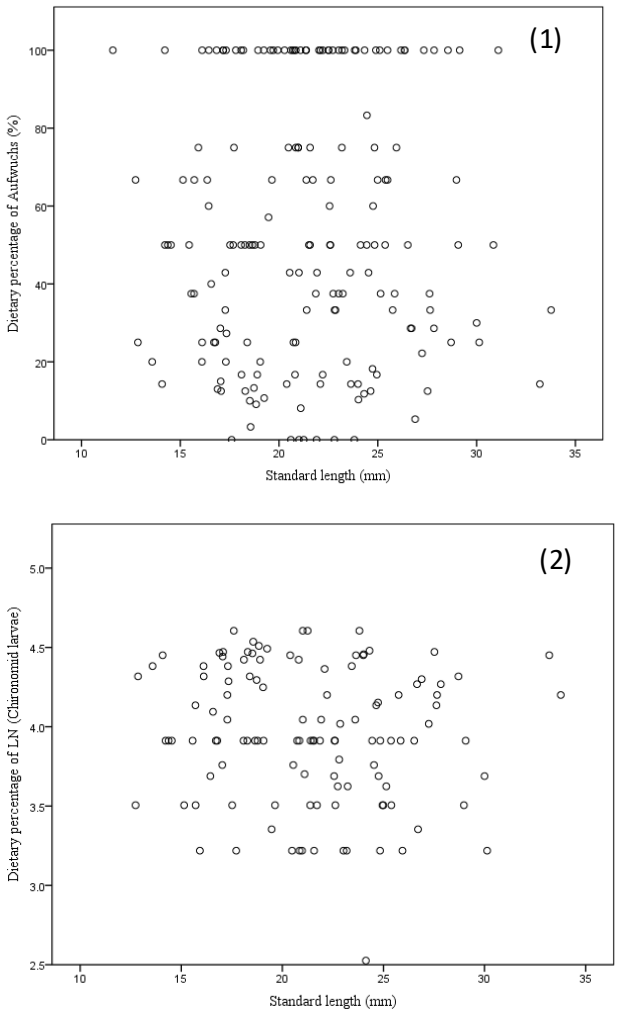

Figure A3. Standard length (mm) of 0-year *Phoxinus phoxinus* and the mean percentage of diet composed of prey items; Aufwuchs and chironomid larvae (Natural Log applied to conform to regression assumptions) from 2015. Both regressions are non-significant ($P > 0.05$). In (1), $R^2 = 0.00$, $F_{1, 173} = 0.06$, $P = 0.81$, (2) LNChiron $R^2 = 0.01$, $F_{1, 115} = 1.26$, $P = 0.26$.

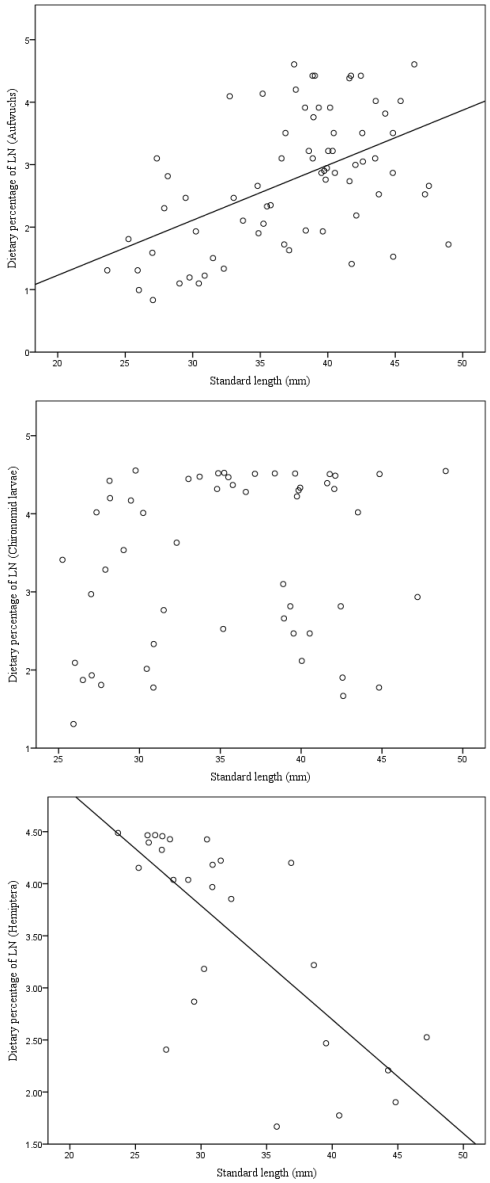

Figure A4. Standard lengths (mm) of 0-year *Leuciscus leuciscus* and the percentage of diet composed of prey items; Aufwuchs, chironomid larvae, hemiptera, winged insects and chalcid wasps from 2015. All Natural Log transformed to meet regression assumptions.

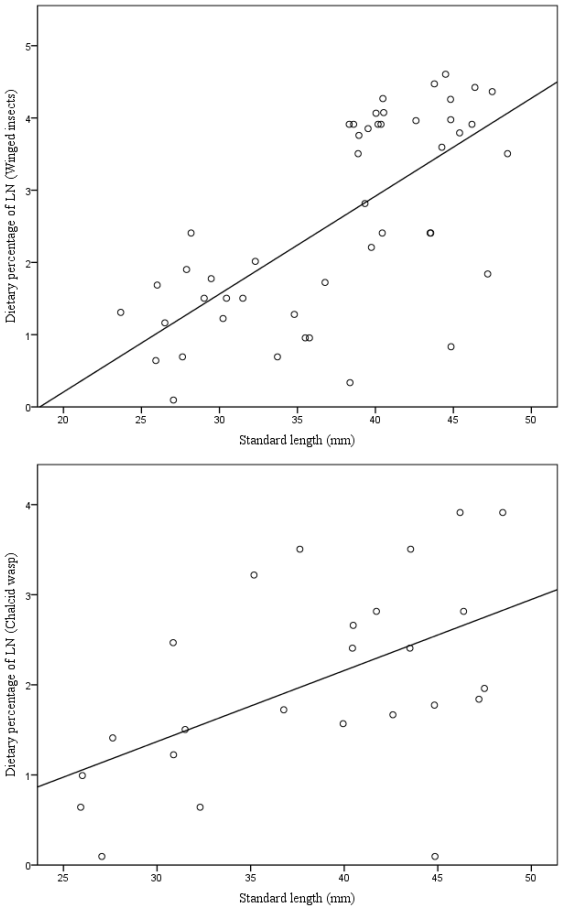

Figure A4 cont. Standard lengths (mm) of 0-year *Leuciscus leuciscus* and the percentage of diet composed of prey items; Aufwuchs, chironomid larvae, hemiptera, winged insects and chalcid wasps from 2015. All Natural Log transformed to meet regression assumptions.

Table A2. Regression outcomes for percentage of prey items in diet of 0+ *Leuciscus leuciscus* from Site 3, River Teme, 2015. All Natural log transformed to meet regression assumptions.

Prey item	R^2	df	F	*P*
Aufwuchs	0.264	69	24.77	< 0.001
Chironomid larvae	0.038	52	1.02	0.317
Hemiptera	0.549	25	29.26	< 0.001
Winged insects	0.472	47	41.04	< 0.001
Chalcid wasps	0.282	24	9.05	0.006

Appendix 3

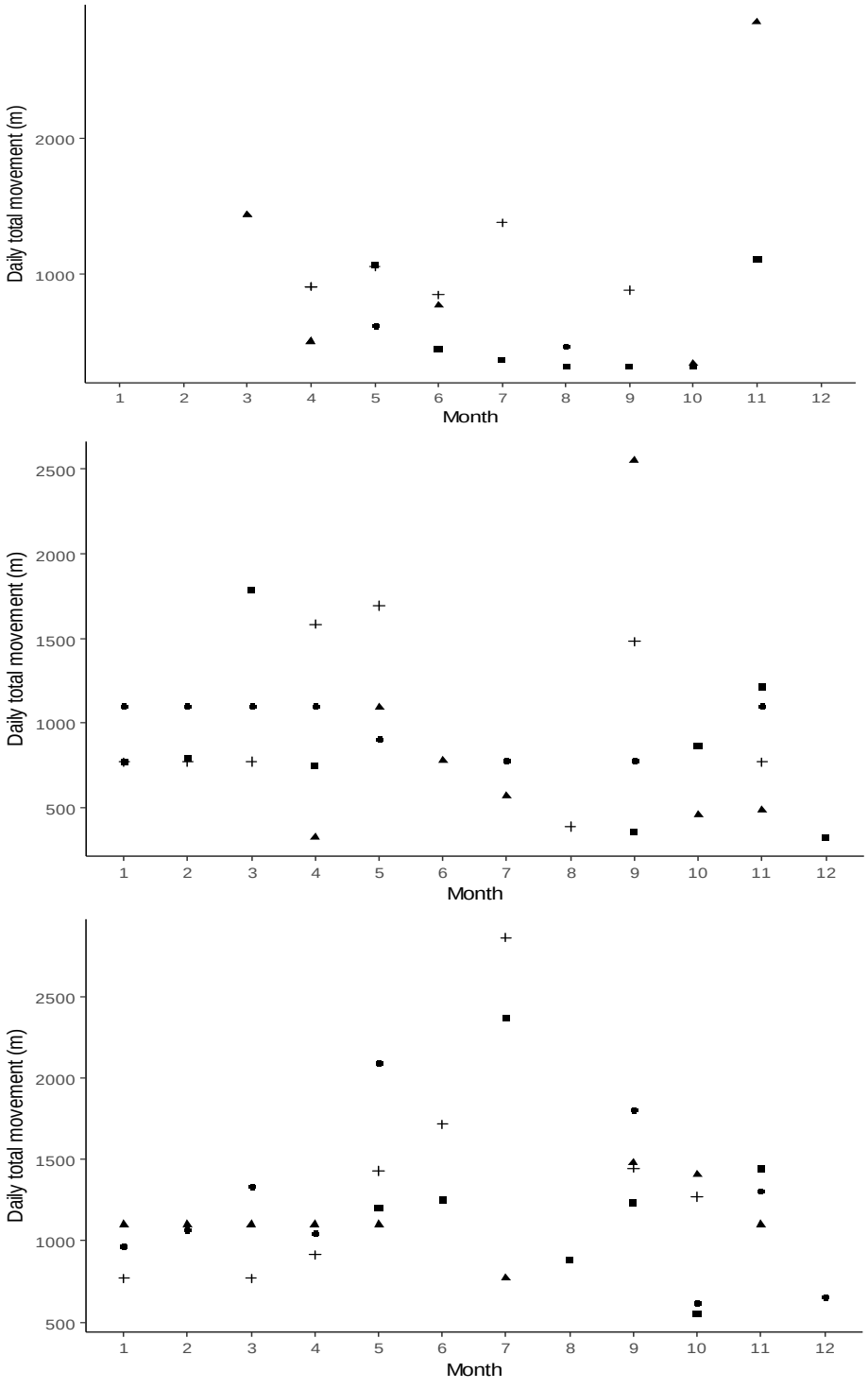

Figure A5. Point plot of mean daily total movement across months of the year (1 – January to 12 December) for 12 fish. Showing data from September 2015 – October 2016. (a) ●10, ▲68, ■71, +72 , (b) ●08, ▲21, ■67, +70 , (c) ●15, ▲19, ■20, +69