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Applied ecology and management of a European barbel *Barbus barbus* population of a lowland river

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Abstract

Freshwaters represent some of the most degraded ecosystems in the world, with approximately 56% of the European rivers being sufficiently altered by human activities to modify the composition of their biological communities. River fish communities are often used to indicate this altered status due to their ecological, recreational and economic value. In lowland rivers, habitat alterations include impoundments and activities such as channel straightening, impacting aspects of fish behaviour and lifecycle completion. Species such as European barbel *B. barbus* are particularly affected due to their propensity for long-distance migrations and requirements of high quality gravels for spawning. Consequently, *B. barbus* populations throughout Europe are increasingly threatened.

Barbus barbus is indigenous to eastern flowing rivers in England, including the River Great Ouse that has been historically subjected to multiple alterations in channel morphology for flood defence and impoundments for land drainage. The river's *B. barbus* population is now restricted to the upper reaches where they represent a key resource for angling, yet temporal and spatial data on their populations suggest relatively low abundances in recent years. Over the last 30 years, the regulatory authority responsible for their management (Environment Agency) have managed the population through a combination of enhancement stocking using hatchery-reared fish and habitat improvement schemes, especially gravel jetting of spawning substrates. There is, however, little knowledge on the effectiveness of these. Consequently, this research investigates *B. barbus* in rivers in England generally and the Great Ouse specifically by assessing the efficacy of stocking and habitat works to enhance populations.

The initial research has focused on using scales from historical surveys on the fish communities of three rivers (including the Great Ouse) to determine the trophic relationships of the fishes using stable isotope analysis. Outputs indicated that scales can be used for this analysis and revealed that rather than sharing food resources with functionally similar species such as chub *Squalius cephalus*, *B. barbus* occupied distinct isotopic (trophic) space. Their diet was then assessed using stable isotope analysis on *B. barbus* scales from four English rivers to determine their major food resources. Results indicated that angling heavily modified *B. barbus* diet, with introduced bait (as pelletized fishmeal) being the most important dietary component. The next phase of the research built of these outputs of both these studies and assessed the impact and efficacy of enhancement stocking of hatchery-reared *B. barbus*. In both semi-controlled and wild conditions, analyses suggested that enhancement stocking with *B. barbus* has minimal detrimental consequences for other fishes such as *S. cephalus*, with strong patterns of trophic niche partitioning. Nevertheless, the efficacy of enhancement stocking might be limited, with low numbers of recaptured stocked *B. barbus* recorded in the study, with a concomitant genetic study revealing negligible introgression of stocked *B. barbus* genes into the population, despite the stocking activities.

Given that enhancement stocking has been of limited success to improve *B. barbus* population abundance in the Great Ouse catchment, their spawning habitats were assessed in the river, including whether the physicochemical properties of the sediments and hyporhic water were limiting. Whilst results indicated good quality of hyporehic water, the subsurface sediment was high in fine content, particularly sand. Gravel jetting, a method to clear spawning gravels of fine content, was shown to only provide short term benefits (e.g. 3 months) in reducing this content of fines, with this benefit only apparent in surface sediments and not in the subsurface. An *ex-situ* experiment to

assess the tolerance of *B. barbus* eggs and larvae to sand content in spawning substrata indicated no effect of high sand content on egg to emergence survival rates, but it did significantly decrease the timing of larval emergence from gravels. This early emergence of *B. barbus* larvae from substrates with high sand content could potentially impact their subsequent survival in the wild.

Thus, the current management strategies employed in the River Great Ouse to enhance the *B. barbus* populations appear to have limited success, largely failing to meet their objectives. Thus, more holistic management approaches are outlined and suggested for implementation.

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Author's declaration

I confirm that this thesis is all my own work, with the following exceptions:

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1. Introduction

1.1 Lowland rivers and their anthropogenic modification

Rivers are important components of the physical landscape and have been exploited by man for centuries as a means of transportation, recreation and water provision. However, their over-exploitation is common, leading to degradation and loss of habitat and biota (Dudgeon et al. 2006; Strayer and Dudgeon 2010). Environmental disturbances can have profound influences on freshwater systems, ranging from the alteration of ecosystem functioning through to biodiversity loss (Chapin et al. 2000). One of the most serious anthropogenic modifications to freshwater systems is the numerous interruptions of river connectivity that arise through the construction of, for example, hydropower plants, dams, weirs and culverts, which lead to major hydrological changes and the loss of lateral and longitudinal river connectivity (Yamamoto et al. 2004; McClure et al. 2008; Sabater and Tockner 2010). Human-mediated introductions of alien species and climate change represent further serious threats to biodiversity and ecosystem functioning (Cambray 2003; Leprieur et al. 2008; Rahel and Olden 2008; Jeppesen et al. 2010; Angienda et al. 2011). Inputs of allochthonous material can enhance in situ productivity of freshwater systems, as well as the stability of food webs when received in small to moderate amounts, especially in systems of low productivity (Jones et al. 1998; Jefferies 2000). However, increasing the amount of allochthonous nutrients in freshwater systems can lead to instability of the entire system, through changes in species abundance and food web dynamics (Jefferies 2000).

1.2 Measuring the impact of anthropogenic alterations on lowland rivers

In Europe, of 546 native fish species, 17% are in decline whilst only 1 and 6% of species are increasing in number and considered stable (Freyhof and Brook 2011).

Potential causes are numerous and, most likely multifactorial, but habitat degradation via pollutants (domestic, industrial or agricultural), habitat loss through water extraction, excessive competition or predation from invasive species, overfishing, and habitat fragmentation as a function of impoundments are all known to have detrimental impacts upon fish populations (Freyhof and Brook 2011). Moreover, approximately 56 % of classified river bodies in Europe have been reported below good ecological status due to some form of anthropogenic alteration (EEA 2012) that has impacted aspects of their biota (Dudgeon et al. 2006). Fish are generally strong indicators of ecosystem health and integrity, as reflected by their importance as a key ecological metric within the EU Water Framework Directive (Directive 2000/60/EC) (Pont et al. 2006). For example, in freshwater communities, fish provide strong indicators of the effects of environmental changes through showing responses through, for example, shifts in their community structure, life history traits and trophic ecology (Karr 1981; Rahel and Olden 2008). This is due to their high temporal and spatial variability, and strong linkages to structural parameters, as well as their high trophic positions in freshwater food webs (Karr 1981; Whitfield and Elliott 2002; EFI+ CONSORTIUM 2009). As one of the longest living organisms of the aquatic ecosystems, fish are generally exposed to individual and cumulative stressors much longer than other taxa, while their mobility provides a good indication of the river continuum (Karr 1981; Whitfield and Elliott 2002; EFI+ CONSORTIUM 2009). Allied to their ability to show responses to environmental change is their high socio-economic value. In the UK alone, freshwater angling has been assessed as contributing £3 billion to the economy (Environment Agency 2004), with anglers tending to prefer particular target species for exploitation, such as common carp *Cyprinus carpio* and European barbel *B. barbus* (Britton and Pegg 2011).

In temperate freshwater systems, population dynamics of fish communities are highly dependent on abiotic factors (Prokeš et al. 2006), especially those relating to climate (Nunn et al. 2007) and water quality (Amisah and Cowx 2000). However, biotic factors, especially competitive interactions and predation pressure, can also play a major role in affecting fish population viability and community structure (Pegg and Britton 2011; Britton et al. 2013). Thus, assessing long-term changes to fish community structure requires the use of ecological tools capable of quantifying the response of fish to long-term biotic and abiotic factors, including altered environmental conditions. For example, fish scales (the material from which fish age and growth rate data are derived) can provide a strong temporal record of fish growth that can indicate the response of fish populations and communities to environmental change (Britton 2007; Britton et al. 2013), such as improved water quality (Beardsley and Britton 2012). In addition, these scales can be used to assess the long-term changes in the ecology of the fish, including their trophic ecology, such as through the identification of how competitive relationships and trophic interactions between populations in a community have changed temporally (Wainright et al. 1993; Grey et al. 2009; Roussel et al. 2014). This is because they can be analysed for their stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ that provide information on their long-term (e.g. 6 month) assimilated diet (Jackson et al. 2012).

1.3 Stable isotope analysis as a tool for measuring long-term change

Stable isotopes are naturally occurring elements that differ in their nuclear mass due to their differing number of neutrons. As the ratios of stable isotopes of, for example, carbon (^{13}C: ^{12}C) and nitrogen (^{15}N: ^{14}N) vary predictably in the environment as they are transferred up food chains and food webs (Figure 1), they can be utilized as an indicator of perturbations and alterations to food web structure in light of biotic and/ or abiotic alterations, such as introduced species (Vander Zanden et al. 1999; Fry 2002) and water

quality improvements (Grey et al. 2009). Their applications include the analysis of dietary shifts (Hesslein et al. 1991; Hobson et al 1994; Vander Zanden and Rasmussen 1999; Kelly et al. 2000; Post 2002), trophic interactions (Hesslein et al. 1991; Hobson et al. 1994; Vander Zanden and Rasmussen 1999; Kelly et al. 2000; Post 2002), monitoring nutrient loadings and hydrological flushing (Fry 2002), establishing migration patterns (Hesslein et al. 1991; Hansson et al. 1997; Hobson 1999; Clegg et al. 2003; Rubenstein and Hobson 2004) and determining fish geographic origins (Marra et al. 1998; Rubenstein and Hobson 2004; Adev et al. 2009; MacKenzie et al. 2011).

Measured using a mass spectrometer (Peterson and Fry 1987; Rubenstein and Hobson 2004) and expressed in δ values (defined as the part per thousand (‰) in relation to international standards; Peterson and Fry 1987), the ratio of ^{15}N: ^{14}N can be used to predict the trophic position of consumer species due to predictable enrichment of 3 to 4 ‰ relative to its prey tissue (DeNiro and Epstein 1981; Minagawa and Wada 1984; Vander Zanden and Rasmussen 2001; Post 2002; Figure 1). Conversely, the ratio ^{13}C: ^{12}C shows less alteration between trophic levels (< 1 ‰) and are instead used as indicators of energy sources (DeNiro and Epstein 1978; Fry and Sherr 1984; Vander Zanden and Rasmussen 2001; Post 2002; Figure 1). Despite there being some discrepancies in using the isotopic position and isotopic niche as a proxy for trophic position and trophic niche respectively, there is a high incentive in using stable isotopes as a powerful tool to investigate trophic ecology (Bearhop et al. 2004; Layman et al. 2007; Newsome et al. 2007). Thus, hereafter, isotopic niche and isotopic position will be used throughout to represent the trophic niche and trophic positon of species and will be referred to as such.

The most commonly used tissues for stable isotope analyses include metabolically active tissues, such as muscles (Rubenstein and Hobson 2004; Cucherousset et al. 2012). These tissues can provide better insight regarding spatial information, for example, for differentiating between resident and newly arriving species (Rubenstein and Hobson 2004). However, there is an incentive to use less destructive approaches, which could provide long-term integrated data, such as fin tissue, mucus and scales (Rubenstein and Hobson 2004; Cucherousset et al. 2012). With the benefits of using stable isotopes including non-lethal sampling, smaller sample sizes and long-term integrated data compared to the more traditionally used gut content analysis (GCA) (Rybczynski et al. 2008; Cucherousset et al. 2012), there are still some constraints in using this approach. These constraints include the difficulty of estimating trophic fractionation factors between different tissues, consumers and prey items, while considering intraspecific variation and the spatial and temporal scale of sampling (Rubenstein and Hobson 2004; Davis et al. 2012; Layman et al. 2012). Therefore, there remains emphasis on coupling stable isotopes analysis in field studies with *ex-situ* experiments to get a better understanding of the underlying ecological processes (Cucherousset et al. 2012; Layman et al. 2012; Busst et al. 2015; Busst and Britton 2016.).

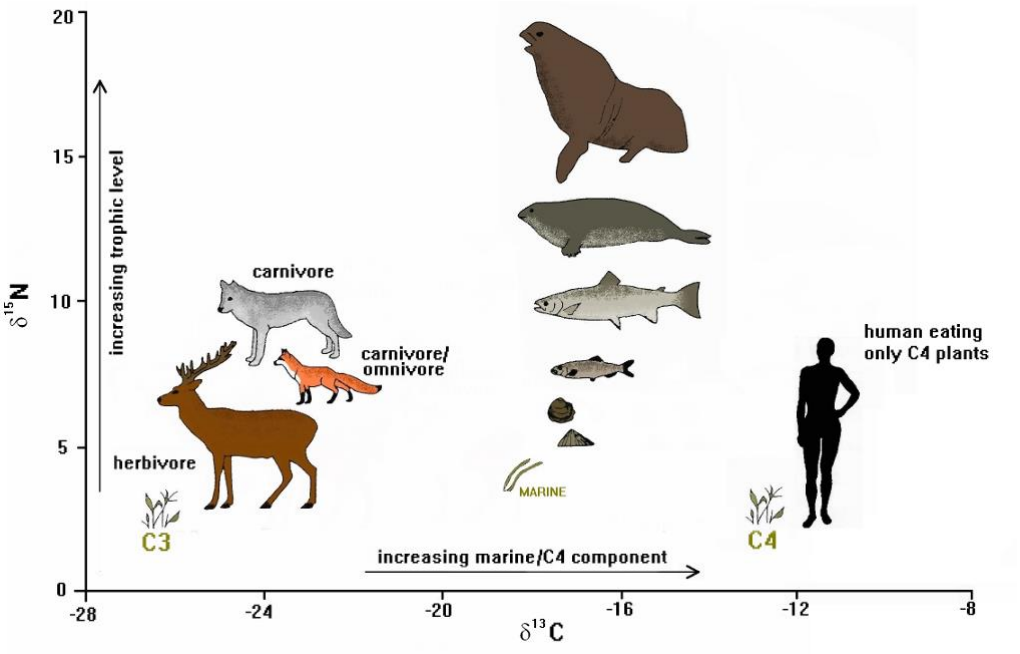

Figure 1 Simplified version of stable nitrogen and carbon values across different systems and organisms (Schulting 1998).

1.4 Focal fish species and river system

Barbus barbus is a lithophilous and rhephilous fish of the Cyprinidae family, usually inhabiting middle reaches of rivers, also known as the "barbel zone" (Huet 1949; Aarts and Nienhuis 2003; Britton and Pegg 2011; Figure 2). The species is considered of importance in Europe as it represents a strong socio-economic resource through its popularity amongst recreational anglers, particularly in England and Poland (Britton and Pegg 2011). It is also considered as a 'flag species' of high conservation importance due to its preference for fluvial habitats of relatively low anthropogenic disturbance (Britton and Pegg 2011; Britton et al. 2013). Therefore, it represents a strong focal species for assessing how alterations to the habitats of lowland rivers have impacted the fish community more generally.

Figure 2 Adult *Barbus barbus* (photo taken by Dr Andrew Pledger).

Barbus barbus is distributed all around Europe, from eastern England and France in the west to the Black Sea basin in the east. They have attributes of aggregative behaviour (Britton and Pegg 2011), as well as the migration activities during spawning which makes populations very sensitive to anthropogenic disturbances. They tend to spawn in late spring (e.g. May to June), with spawning migrations of distances to over 20 km, usually in an upstream direction (Britton and Pegg 2011). Spawning occurs on shallow riffles where depths are around 0.4 m and velocity between 0.3 and 0.5 m/s, over small pebbles and gravels (Wijmans 2007; Kemp et al. 2011). Light intensity and water temperature tend to synchronize *B. barbus* activity, while water temperature also effects their activity duration and it is the key parameter regulating *B. barbus* growth, reproduction and recruitment (Britton and Pegg 2011; Britton et al. 2013).

Barbus barbus is native to eight east flowing river catchments in England, between the Yorkshire Ouse and River Thames, and the species is also present in a number of other rivers due to introductions for angling into rivers in its non-indigenous range that has been completed by regulatory authorities (Britton et al. 2013; Antognazza et al. 2016). Even though their status across the entire range of distribution is not considered to be threatened, there have been various reports of numerous population declines in Europe,

classifying them threatened or endangered with extinctions in some rivers due to organic and chemical pollution, overfishing, and habitat fragmentation and loss (Vilizzi et al. 2006; Britton and Pegg 2011). Consequently, the combination of their importance to recreational angling, natural behaviours that include relatively large spawning migrations, spawning in gravels in late spring and their overall sensitivity to disturbances, makes them a strong focal species for research investigating the consequences of environmental changes in lowland rivers in England.

With *B. barbus* being the focal research species, the focal study river is the River Great Ouse in eastern England, as this river has historically been subjected to multiple alterations in channel morphology for flood defence purposes and impoundments in its lower reaches for land drainage. The river's indigenous *B. barbus* population is now restricted to the upper reaches of the river above Bedford where they represent a key resource for angling, yet temporal and spatial data on their populations suggest relatively low abundances in recent years coupled with a loss of spawning adults (Figure 3). Over the last 20 years, the regulatory authority responsible for their management, the Environment Agency, have managed the population through a combination of enhancement stocking using hatchery-reared fish and habitat improvement schemes. Throughout the research, data on populations of *B. barbus* from other rivers are, however, also utilised to provide greater replication and representation, and ensure outputs have more general application.

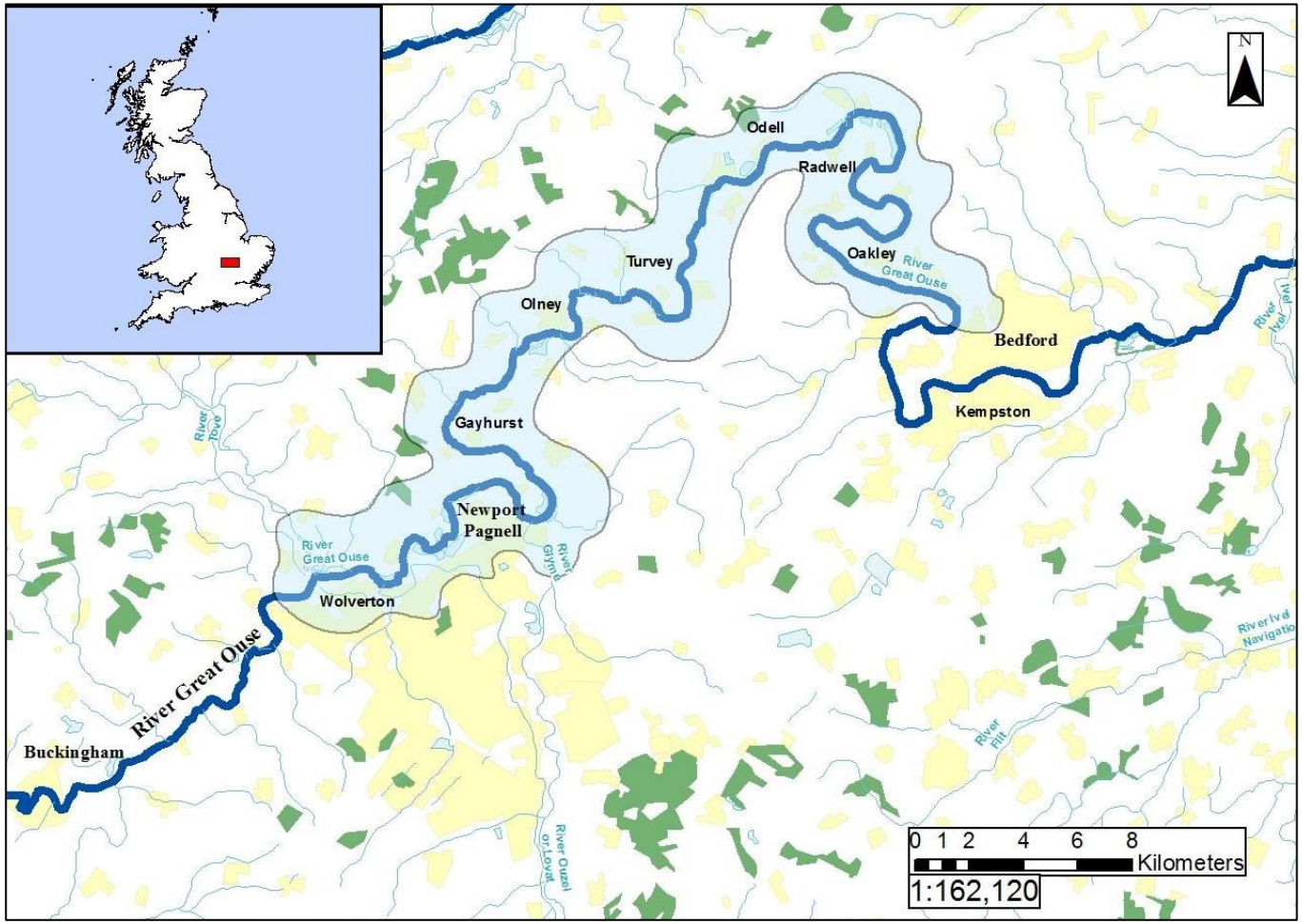

Figure 3 Approximate location of the study site in the UK, highlighting barbel zone (light blue polygon) in the River Great Ouse (Ordnance survey 2005; Ordnance survey 2015a).

Although the assessment of different taxonomic groups during environmental assessments is preferable to provide more holistic approaches, this is often not feasible due to temporal and economic restrictions (Karr 1981). In these situation, the use of focal or target species is acceptable and thus why *B. barbus* are being used in this manner here. Nevertheless, there can be strong complementarity between use of single species and the ecosystem management approach, as species introductions and releases via stocking also carry implications at higher levels of biological organisation (Lindenmayer et al. 2007). Thus, combining approaches from levels of single species to communities to food webs can help in understanding the underlying issues arising from anthropogenic disturbances, providing key information for policy and managers that are also applicable to other species and systems (Lindenmayer et al. 2007).

In the ecological studies of freshwater fishes, traditional approaches have tended not to account for the holistic view of the system, as small-scale investigations with limited temporal aspects often do not scale-up adequately when applied to larger management and time scales (Fausch et al. 2002). Therefore, to better understand the major global stressors in freshwater systems, there is a need to increase the temporal and spatial scale of investigations whilst also integrating the key biotic and abiotic parameters (Fausch et al. 2002). Moreover, there are issues when studies are based only on field data, as these often lack the control and replication required to be able to quantify the factors that are influencing the ecology of the fish and thus studies that incorporate experiments completed in aquaria, mesocosms and/ or field conditions, as well as field studies more generally, can provide strongly complementary approaches (Korsu et al. 2009; Spivak et al. 2011; Tran et al. 2015).

1.5 Mitigation strategies to enhance river fish communities

Given that environmental disturbances, such as loss of longitudinal connectivity, can incur major changes in river biota and fish community structure, then fish population and fishery management schemes often have to work within the constraints of extant structures and engineering to enhance the fish communities (King et al. 2016). Such mitigation actions on lowland rivers tend to focus mainly on two possible strategies: restoration of habitats to alleviate some of the stressors and/ or mitigating the population consequences for fishes via artificial population enhancement through stocking programmes using hatchery reared fishes (Vilizzi et al. 2006; Britton and Pegg 2011; Arlinghaus et al. 2014; Tummers et al. 2016). Stocking fish into freshwater systems has become a frequently used management tool in enhancing recreational fisheries all over the globe (Molony et al. 2003), with activities including additional stocking of extant species as well as the introduction of 'sporting' non-native species that are usually preselected for their traits such as large body size or recreational value (Hickley and Chare 2004). However, suitable habitat conditions during different life stages of fish species represent prerequisites for viable populations (Kondolf 2000). Therefore, restoration measures have been emphasized as an important ecological tool for the improvement of the physical and hydraulic environment (Bond and Lake 2003a).

1.5.1 Fish stocking

Fisheries management programmes have historically tended to focus on fish stocking to enhance recreational fisheries (Cowx 1994, Aprahamian et al. 2004; Eby et al. 2006; Satake and Araki 2012; Thaulow et al. 2013; Von Lindern and Mosler 2014). These programmes utilise releases of both extant and non-indigenous species (Hickley and Chare 2004), with only few of these releases being for conservation purposes (Eby el al. 2006). The estimates of the number of fish stocked into European freshwaters reach

approximately 40 billion fish per year (Cooke and Cowx 2006), with species from Salmonide family representing the most valuable source in angling and aquaculture (Cowx 1994; Eby et al. 2006; Baer and Brinker 2010). As these fish are usually apex predators, they can subsequently influence the functioning of the receiving ecosystem (Radomski and Goeman, 1995; Eby et al. 2006; Cucherousset et al. 2012), through, for example, cascading effects arising from the increased species richness high in the food web that disrupts food-web linkages and overall complexity (Radomski and Goeman, 1995; Eby et al. 2006; Potthoff et al. 2008).

Some of the benefits of stocking include higher abundances of good quality fish caught during recreational angling which attracts more anglers and increases the capital value of fisheries (Aprahamian et al. 2004; Satake and Araki 2012). Regarding the impacts on native fish communities, some studies show non-detrimental effects of stocking on fish abundance, survival, recruitment, growth or genetic structure, possibly due to poor competitive abilities of sterilized triploid hatchery fish that tends to disperse further from the stocking location (Bohlin et al. 2002; Meyer et al. 2012; Weaver and Kwak 2013). However, this will also depend on stocking densities (Bohlin et al. 2002; Meyer et al. 2012) and the long-term history of stocking practises in that fishery (Meyer et al. 2012). Baer et al. (2007) reported a successful stocking programme where stocked adult brown trout dominated the catch with little effect on natural populations through minimal competition and inbreeding that resulted from short residence times of stocked fish, as they were all captured and removed in the days after their release.

In contrast, angling for cyprinid fishes in the UK is primarily based on catch-and-release practises, thus stocked cyprinids are likely to persist in rivers, especially long lived (> 15 years) species such as *B. barbus* (Philippart 1987). Therefore, positive effects of

stocking can accrue, especially if the angling pressure remains stable post-stocking, providing less chance for wild species to be caught (Baer et al. 2007). Another positive example of stocking is evident in England, where across the non-indigenous range of *B. barbus* there has been the successful establishment of hatchery-originated populations that provide considerable benefits for catch-release fisheries, with no risk to the genetic integrity of other fishes due to no other *Barbus* species being present (Antognazza et al. 2016). Nevertheless, Satake and Araki (2012) concluded that stocking in indigenous ranges of a certain species cannot enhance fish abundance in natural systems without replacing the native gene pool, thus affecting genetic diversity of native communities in the longer-term. This was confirmed for *B. barbus* populations in the UK where most of genetic diversity has been lost across the majority of its indigenous range as a result of stocking hatchery reared fish using broodstock from a single catchment (Antognazza et al. 2016).

Numerous ecological and genetic risks are attributed to stocking practices with the possibility that the desired benefits are not delivered (Aprahamian et al. 2004; Satake and Araki 2012; Von Lindern and Mosler 2014). Genetic risks include loss of genetic variation (Hansen 2002; Ruzzante et al. 2004; Eby et al. 2006; Thaulow et al. 2013; Antognazza et al. 2016) and reduced fitness of wild populations following inbreeding and introgression (Hansen 2002, Ruzzante et al. 2004). However, these effects will vary, being dependent on the management strategies applied as well as the population structure of the wild and stocked fishes (Hansen 2002). Some of the ecological risks include increased top-down control, altered food web structure through displacement of native species (Holmlund and Hammer 2004; Ruzzante et al. 2004; Eby et al. 2006; Kopp et al. 2009), and reduced species richness in lower trophic levels or increased species richness in higher trophic levels (Eby et al. 2006). Trophic efficiency and

biogeochemical cycles are likely to be affected, with ecosystem resilience potentially reduced (Schindler et al. 2001; Holmlund and Hammer 2004; Eby et al. 2006). Numerous other parameters will influence the possible detrimental effects of stocking, such as habitat heterogeneity, prey availability, species characteristics and life history, and ontogenetic diet shifts (Eby et al. 2006). Therefore, numerous systems that satisfy some of the parameters mentioned could buffer various ecosystem effects from the impacts of fish stocking programmes.

Despite the detrimental effects reported, monitoring the efficiency or the necessity of stocking is rarely completed (Champigneulle and Cachera 2003; Baer and Brinker 2010; Von Lindern and Mosler 2014). In some study systems, there might be little long-term benefit from stocking, especially if introduced fish disperse fairly quickly from the initial stocking location (Champigneulle and Cachera 2003; Baer and Brinker 2010). Therefore, benefits from stocking can be short-term only, with possible unknown consequences in the long-term, emphasising the necessity of proper assessment and monitoring of implemented stocking programmes (Holmlund and Hammer 2004).

When new populations establish following an introduction or stocking event then niche-based competition theory predicts that where there is dietary overlap between species that is sufficient to result in competition, then the subordinate competitors will shift to alternative food resources, reducing their trophic niche but promoting their coexistence through partitioning (Sepulveda et al. 2012). Therefore, niche partitioning represents a facilitating mechanism enabling species coexistence through segregation at trophic, spatial or temporal levels (Ross 1986; Yick et al. 2011; Schulze et al. 2012; Jackson and Britton 2014; Juncos et al. 2015), with trophic segregation occurring more often in fish assemblages (Ross 1986). Alternatively, morphological differences between the species

might facilitate their coexistence through inter-specific functional differences (Yick et al.2011; Juncos et al. 2015). Trophic niche partitioning can also be observed at the population level, where intra-population habitat differences can occur in generalist predators, with substantial niche reduction through individual specialization (Quevedo et al. 2009), arising from factors including predation and parasitism (Britton and Andreou 2016). The effects of introducing a new species will also depend on the degree of diet specialization of the introduced species and receiving communities (Schulze et al. 2012; Juncos et al. 2015).

Stocked large-bodied species can also alter food webs through direct (competition and predation) and indirect (trophic cascades) impacts, inducing new biological interactions with extant fishes (Kopp et al 2009; Cucherousset et al. 2012). However, relatively limited work has been completed on the effects of stocking cyprinids into wild fish communities (Aprahamian et al. 2004; Bolland et al. 2009), despite this being commonplace in European recreational fisheries, especially for those species of large body sizes and high sporting qualities (Hickley and Chare 2004), such as *B. barbus* (Britton and Pegg 2011). Studies on stocking cyprinid fishes into rivers have reported high loss rates of the stocked fish (Aprahamian et al. 2004), with high dispersal rates due to acclimatization to the new environment, with limited knowledge on these dispersal patterns (Bolland et al. 2009). Consequently, stocking these fish might represent poor value for money with low cost-benefit returns (Aprahamian et al. 2004).

1.5.2 Habitat restoration

Freshwater systems are influenced by a variety of anthropogenic stressors that can diminish their natural resilience to present and future perturbations and, therefore, could affect the provision of numerous ecosystem services (Pander and Geist 2013). Due to

severe degradation of freshwater ecosystems globally, there is high demand for reversing the negative anthropogenic effects through improvements of physical habitats that would elicit the desirable ecological response (Bond and Lake 2003a; Lepori et al. 2005; Pander and Geist 2013). Therefore, river restoration methods have been widely applied for almost 30 years (Pander and Geist 2013), especially in Europe and America (Kondolf et al. 2007). They represent valuable tools in mitigating numerous anthropogenic impacts and sustaining ecosystem resilience, especially when coupled with other stressors, such as climate change (Giller 2005; Lepori et al. 2005; Palmer et al. 2007).

The aim of restoration ecology is to produce well-designed, often species-specific restoration projects that improve the quality of freshwater habitat (Pretty et al. 2003; Giller 2005; Lepori et al. 2005; Palmer et al. 2007). Few studies robustly quantify habitat conditions pre-treatment (Pander and Geist 2013), despite this being a key stage in river restoration. Numerous freshwater restoration projects lack the specific reasoning and necessity of mitigation efforts, with the target goal often omitted (Giller 2005; Palmer et al. 2007). Also, documentation on the restoration outcomes is often missing or not communicated to the public and due to a lack of monitoring, understanding of the factors that underpin successful restoration projects remains rudimentary (Giller 2005; Lepori et al. 2005; Palmer et al. 2007; Pander and Geist 2013). Therefore, assessing restoration success through implementation of monitoring programmes could be beneficial, particularly when applied at a holistic level to combine effects on target-species with bio-indicators that integrate community approaches (Pander and Geist 2013).

The transport and deposition of fine sediment within freshwater systems can affect aquatic habitat and biota respectively (Wood and Armitage 1997; Kemp et al. 2011). The nature and composition of fine fluvial sediment will vary between systems and, therefore, as functions of catchment geology, climate, hydrology and land use management (Wood and Armitage 1997; Kemp et al. 2011). The latter mainly covers agriculture, forestry and associated activities that can substantially increase fine sediment loadings into systems (Wood and Armitage 1997; Sutherland et al. 2002; Hendry et al. 2003; Curry and MacNeil 2004; Jensen et al. 2009; Kemp et al. 2011).

Detrimental effects of fine sediments on freshwater systems include reductions in primary production through increased turbidity, abrasion of macrophytes, smothering and removal of periphyton, as well as reduction of available habitat for benthic organisms (Wood and Armitage 1997; Kemp et al. 2011). The negative effects of high deposition of fine sediments on fish communities include changes in species abundance and diversity, as well as altered community composition through physiological and ecological responses (Wood and Armitage 1997; Louhi et al. 2008; Kemp et al. 2011). Fish can be directly affected by high levels of fine sediments through increased stress levels or physical damage to their organs, as well as indirectly through changes in habitat quality and quantity, which is especially profound during the spawning period when these sediments clog spawning gravels (Wood and Armitage 1997; Louhi et al. 2008; Kemp et al. 2011).

Small amounts of fine sediment (‘fines’) in spawning gravels can thus have detrimental effects on egg survival (Cocchiglia et al. 2012; Stopps et al. 2012) and hatching success (Meyer 2003; Sear et al. 2016), which is dependent on duration of exposure to fine sediment (Cocchiglia et al. 2012; Chapman et al. 2014). Fines can block inter-gravel

pores and chorion macropores, which impacts upon egg survival by inhibiting oxygen permeation and metabolic waste removal across the egg membrane (Greig et al. 2005a, Greig et al. 2005b; Greig et al. 2007; Louhi et al. 2008, Kemp et al. 2011; Sear et al. 2014). When coupled with low intra-gravel flow and high amounts of organic matter affecting oxygen concentration in the hyporheic zone, gravel bed siltation can result in low egg survival rates (Curry and MacNeil 2004; Greig et al. 2005a, Greig et al. 2005b; Greig et al. 2007; Jensen et al. 2009; Kemp et al. 2011; Cocchiglia et al. 2012; Stopps et al. 2012; Pulg et al. 2013; Utz et al. 2013; Sear et al. 2014). Even where eggs do survive, issues such as premature larvae emergence with related morphological constraints, or even inhibition of the emergence process via entombment, can occur (Meyer 2003; Sternecker and Geist 2010; Kemp et al. 2011; Franssen et al. 2012; Sear et al. 2016).

Most studies relating spawning habitat quality to fish recruitment have focused on salmonid species (e.g. Argent and Flebbe 1999; Soulsby et al. 2001; Meyer 2003; Curry and MacNeil 2004; Rubin et al. 2004; Greig et al. 2005a, Greig et al. 2005b; Greig et al. 2007; Louhi et al. 2008; Meyer et al. 2008; Jensen et al. 2009; Sternecker and Geist 2010; Kemp et al. 2011; Cocchiglia et al. 2012; Stopps et al. 2012; Pulg et al. 2013; Sternecker et al. 2013a; Utz et al. 2013 Sear et al. 2014). Here, prerequisites for suitable salmonid spawning habitats include appropriate depth, water velocity, substrate size and oxygen concentration in the hyporheic zone (Louhi et al. 2008), with the amount of fine sediment in gravel beds and interstitial oxygen concentration representing the most important factor in determining habitat suitability for egg survival and larval emergence (Argent and Flebbe 1999; Soulsby et al. 2001; Meyer 2003; Curry and MacNeil 2004; Meyer et al. 2008; Jensen et al. 2009; Kemp et al. 2011; Cocchiglia et al. 2012; Franssen et al. 2012; Stopps et al. 2012; Sternecker et al. 2013a; Chapman et al. 2014;

Sear et al. 2014). Salmonid egg-to-emergence survival is dependent on the entombment and asphyxiation processes, where fine sediment will physically block macrospores, inhibiting larval emergence from the sediment and influencing interstitial flow velocity and oxygen permeability (Greig et al. 2005a,b, 2007; Franssen et al. 2012; Sear et al. 2014). Greig et al. (2005a) suggested that measures of oxygen concentration and flux, and interstitial flow, could be used to assess potential rates of embryonic survival, which will vary as a function of site-specific conditions. This is supported by Curry and MacNeil (2004), who confirmed low survival to emergence due to high deposition of fine sediment, which was enhanced in areas with discharging ground water, assuming asphyxiation was the main cause of mortality rather than entombment. However, some studies report entombment processes as the main factor influencing egg-to-emergence survival success, thus high oxygen flux through increased flow velocity cannot necessarily mitigate high embryo mortality in fines-rich sediments, emphasizing the potential importance of fine sediment removal from freshwater systems (Franssen et al. 2012; Sternecker et al. 2013a).

Non-salmonid fishes can also be affected by high input of fine sediment into spawning gravels (Kemp et al. 2011). Phytophilic species can be affected indirectly through impacts of fine sediment on macrophyte growth or directly through adherence of fine particles to egg surface. However, gravel-spawning species (such as *B. barbus*, bullhead *Cottus gobio*, shads *Alosa* spp., and river lamprey *Lampetra fluviatilis*) are more susceptible to impacts of high levels of fine sediment, as they accumulate their eggs below surface particles, within interstices between grains, and during the late spring/ early summer period that tends to coincide with high temperatures and reduced water flows, at least compared to salmonid fishes (Sutherland et al. 2002; Kemp et al. 2011).

There is an increasing effort in Europe to enhance the habitat structural component of fishery enhancement works rather than remain reliant on stocking enhancement schemes (e.g. Champigneulle and Cachera 2003; Merz and Setka 2004; Arlinghaus and Mehner 2005). Mitigation of degraded spawning habitat is performed in several ways, including gravel augmentation, spawning bed enhancement and installation of various hydraulic structures such as flow deflectors (Hendry et al. 2003; Wheaton et al. 2004a; Wheaton et al. 2004b). Spawning bed enhancement through gravel addition and gravel cleaning represents suitable mitigation strategy in improving spawning habitats of lithophilic fish species (Merz and Setka 2004; Pulg et al. 2013; Utz et al. 2013; Sternecker et al. 2013b; Beechie et al. 2015; Pander et al. 2015), although improvements might be time-limited, ranging from several months (Rubin et al. 2004; Meyer et al. 2008; Pander et al. 2015) to several years (Merz et al. 2006; Pulg et al. 2013). In systems with numerous blockages to river connectivity that limit fish dispersal to more suitable areas, enhancing habitat quantity can be as beneficial as improving habitat quality (Pulg et al. 2013; Beechie et al. 2015).

Notwithstanding, it has been argued that local habitat restoration can have profound negative effects on downstream habitats, as well as creating patches with greater susceptibility for fine sediment accumulation (Kemp et al. 2011; Sternecker et al. 2013b). Therefore, habitat restoration on a larger spatial scale could provide a better management strategy for sustainable recreational fisheries, particularly in highly degraded rivers (Wood and Armitage 1997; Arlinghaus et al. 2002; Hendry et al. 2003; Kemp et al. 201; Sternecker et al. 2013b). Local restoration projects are, however, less time- and resource-consuming, and could be beneficial when considering local constraints to effective outcomes of mitigation efforts (Bond and Lake 2003a; Tambosi et al. 2014). They can also incorporate strong post-completion monitoring programmes,

leading to improved knowledge on how larger stretches of river might respond to enhancement schemes (Wheaton et al. 2004a). Appropriate target-species oriented restoration evaluations should include relevant bio-indicators, such as egg and larvae survival rates, as these are highly suitable for detecting the outcomes of habitat improvements, particularly when related to physicochemical alterations of the surrounding habitats (Pander and Geist 2013).

In the successful restoration of spawning habitats, it is also necessary to consider grain size distribution, as this can also determine the efficacy of egg deposition into redds, rates of exchange of gases through egg membranes, fry emergence, and gravel stability (Rubin et al. 2004), as well as oxygen concentration and flux, and interstitial flow velocity (Greig et al. 2005a; Greig et al. 2005b; Greig et al. 2007). Thus, the development of appropriate habitat restoration techniques can potentially increase fish population viability whilst also preserving the genetic integrity of wild populations, rather than relying on stocking programmes that are potentially unsustainable.

1.6 Research aims and objectives

The overall aim of this Ph.D. research is to develop new understandings of the efficacy of methods to mitigate long-term environmental changes in lowland rivers on river fish communities. It uses *B. barbus* as the focal species and the River Great Ouse as the focal river. The work develops novel insights into the ecological interactions of *B. barbus* with other fishes in their communities that will underpin their responses to the mitigation strategies. Correspondingly, the research objectives are:

O1. Through application of stable isotope analysis to historical fish scale samples, identify the trophic interactions of *B. barbus* with other fishes in their communities over three rivers, with a focus on chub *Squalius cephalus*, given that the two fishes

are relatively omnivorous across their length ranges and both species have the potential to attain relatively large and similar body sizes (> 500 mm);

O2. Using stable isotope analysis, assess the contribution to the diet of *B. barbus* of allochthonous inputs into four lowland rivers, particularly pelletized fishmeal introduced as bait by anglers, and identify how these compare to the dietary contributions of natural food resources and the non-native signal crayfish *Pacifastacus leniusculus;*

O3. Identify how releases of hatchery-reared *B. barbus* into fish communities affect the trophic ecology of extant fish communities, especially *S. cephalus* populations, and whether detrimental ecological impacts are measurable following stocking events, such as reduced trophic niche breadths and decreases in somatic growth rates;

O4. Characterize *B. barbus* spawning habitats in the River Great Ouse by assessing physicochemical properties of hyporheic layer with special emphasis on fine sediment content in river substratum and oxygen and ammonia concentrations in the interstitial water;

O5. Quantify how the habitat improvement method of the removal of fine sediments from spawning gravels via 'gravel jetting' affects measures of spawning gravel quality (e.g. reductions of fine sediments and oxygen concentrations through the gravel) and how jetting will potentially influence the subsequent spawning success of *B. barbus*; and

O6. Quantify threshold levels of fine sediment (sand content) that will detrimentally impact upon spawning success of *B. barbus* by observing egg-to-emergence survival and timing of emergence.

1.7 Thesis structure

The subsequent structure of the thesis is:

Chapter 2: Utility of fish scales from stock assessment surveys in stable isotope analysis for initial assessments of trophic relationships in riverine fishes (Objective 1).

Chapter 3: Diet composition of *Barbus barbus* in four rivers in England: importance of angling baits and invasive crayfish as trophic subsidies (Objective 2).

Chapter 4: Trophic ecology of stocked European barbel *Barbus barbus* and resident cyprinid fishes: consistency in niche partitioning over time, space and body sizes (Objective 3).

Chapter 5: Characteristics of *Barbus barbus* spawning substrata: a case study of the River Great Ouse (Objective 4).

Chapter 6: Effects of gravel jetting on the physicochemical habitat characteristics of spawning gravels of *Barbus barbus* (Objective 5).

Chapter 7: *Ex-situ* assessment of variable sand contents in spawning substrates on egg-to-emergence survival rates and timing of emergence of *Barbus barbus* (Objective 6).

Chapter 8: Discussion.

Chapter 9: References.

Chapter 10: Appendices.

As such, there is no separate Materials and Methods section.

2. Utility of fish scales from stock assessment surveys in stable isotope analysis for initial assessments of trophic relationships in riverine fishes

This chapter has been published in full in:

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2.1 Summary

The utility of using fish scales collected during historical stock assessment exercises to assess the trophic relationships of riverine fishes using their stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ was tested using three riverine fish communities in England (Rivers Great Ouse, Ivel and Goyt). In each river, European barbel *B. barbus* was an important species, with other cyprinid species, including chub *S. cephalus*, present. Stable isotope analysis was completed using relatively small sample sizes per species (<11) from fish samples collected in 2001, 2005 and 2006 when up to 5 scales were collected from each fish. The calculation of standard ellipse areas (as a measure of trophic niche size) revealed that relative to other fishes, *B. barbus* occupied high trophic positions, with minimal overlap in their trophic niche with other species, especially *S. cephalus*. As the analysed fish samples comprised of species of different length ranges, and length has strong ontogenetic consequences for fish diet composition, then generalized linear models were developed in which length was the covariate; model outputs included length-adjusted mean $\delta^{13}C$ and $\delta^{15}N$ for each species. In each fish community, significant differences in $\delta^{13}C$ and $\delta^{15}N$ were apparent between *B. barbus* and *S. cephalus*, but were less apparent between *B. barbus* and other fishes. Thus, whilst the utility of using fish scales from historical stock assessments in stable isotope analyses can be limited due to the differing length ranges of the sampled fishes, they can have utility in identifying trophic differences between species when methods, such as stomach contents analysis, are unavailable.

2.2 Introduction

Archives of biological samples are increasingly being used to provide new insights into ecological relationships, such as preserved samples being used for analysing trophic relationships and freshwater food web structure using the stable isotopes of ^{15}N and ^{13}C (Syväranta et al. 2008). This includes the use of fish scales (Hutchinson and Trueman 2006), with archived scales having been applied to, for example, assessment of the response of roach *Rutilus rutilus* to reduced eutrophication (Grey et al. 2009), and the trophic consequences of non-native fishes, including topmouth gudgeon *Pseudorasbora parva* in the UK (Jackson et al. 2013) and small mouth bass *Micropterus dolomieu* in North America (Galster et al. 2012).

Scales are often collected as part of stock assessment exercises for freshwater fish where they are used to assess the age structure, growth rates and recruitment of the sampled fishes (Britton 2007). They are increasingly being collected in European freshwaters as part of the Water Framework Directive, where the ecological status of freshwater fish communities should be assessed using metrics including age structure (Noble et al. 2007a). Scales tend to be collected for this purpose rather than other calcified body structures as their collection is non-destructive and they can provide reliable age estimates (Britton 2007). Here, an assessment is made of the utility of using scales that were collected for age structure analysis within stock assessment exercises for subsequent investigations into the trophic relationships of the sampled fish using stable isotope analysis. This is done in relation to assessing the mean isotope values, relative trophic positions and trophic niche breadth of three riverine populations of *B. barbus* against the other fishes in the sampled fish communities. Stable isotope analysis is increasingly used for providing long-term dietary perspectives in fish and is often used in preference to more traditional methods, such as stomach contents analysis, as it

requires smaller sample sizes and can use material whose collection is non-destructive (Grey et al. 2009). *Barbus barbus* is a large-bodied cyprinid fish (individuals are captured in rivers in England to over 800 mm; Britton et al. 2013) that are encountered in many European rivers as either an indigenous or non-indigenous species (Bianco and Ketmaier 2001; Britton and Pegg 2011). They tend to be present within fish communities dominated by smaller cyprinid species and many populations are enhanced through stocking (Britton and Pegg 2011). Despite their popularity as an angling species and the regularity of their stocking, there is limited knowledge on how they integrate into freshwater fish communities, such as their feeding interactions with other species.

Consequently, the study objectives were to: (i) use stable isotope data derived from fish scales to test for differences in trophic niche size, the extent of trophic niche overlap, and differences in mean $\delta^{13}C$ and $\delta^{15}N$ between *B. barbus* and other fish species within three riverine fish communities; and (ii) assess these outputs in the context of the utility of using scales collected during stock assessments as a method to complete initial assessments of trophic relationships when alternative methods, such as stomach contents analysis, are not available or are unsuitable. From the perspective of the Ph.D., developing understandings of how scales can be utilized for analyzing the trophic relationships of river fish communities is important for subsequent work in Chapters 3 and 4.

2.3 Materials and methods

The approximate locations and sampling dates of the fish communities used are provided in Table 1. All of the *B. barbus* analysed in Site 3 were of hatchery origin; in Site 1 and 2, whilst *B. barbus* had previously been stocked regularly, it was not possible to ascertain if the fish utilized in analyses were of wild or

hatchery origin. Irrespective, their ages and sizes indicated they had been present in the river for over three years (Table 2). These scales had been collected during fish stock assessments completed by the Environment Agency of England and Wales using electric fishing, and were available to the research through these archived scales being available to the Ph.D. candidate's supervisor.

During the stock assessments, sampled fish were identified to species, measured (fork length, nearest mm) and between 3 and 5 scale samples removed. The fish were then returned to the water. The scales were then stored in an archive room in dry conditions of stable temperature (15 to 18 ^{o}C) until they were retrieved and used in the stable isotope analysis. Given the size (> 500 mm; Table 2) and age (> 10 years; Britton et al. 2013) of some of the fish in samples, only material from the very outer portions of scales were used in stable isotope analyses, i.e. material produced through very recent growth (Hutchinson and Trueman, 2006). All samples were analysed at the Cornell Isotope Laboratory (Cornell University, New York, USA), where they were ground into a homogenous powder, with approximately 0.5 mg of this powder weighed out into a tin cup, with the actual weight recorded using a Satorius MC5 microbalance. The nitrogen and carbon isotopes were then analysed, using a Thermo Delta V Advantage Isotope Ratio Mass Spectrometer. The outputs were values of δ^{13}C (energy source indicator) and δ^{15}N (trophic level indicator) for each individual fish. As their basal resource isotope data were unavailable (i.e. their putative food resources), the actual diet composition of the fishes could not be completed and this represents the first limitation of using archived material to analyse trophic relationships in this manner.

The stable isotope data were then applied to two main analytical methods. The first was to use the δ^{15}N and δ^{13}C data from the individual fish of each species in each

community to calculate standard ellipse areas (SEA_C; the subscript 'c' indicates that a small sample size correction was used as sample sizes were < 11 fish per species and river) in the package 'Stable Isotope Analysis in R' (SIAR; Jackson et al. 2011). These standard ellipse areas are a bivariate measure of the distribution of individuals in trophic space; each ellipse encloses ~ 40% of the data and, therefore, represents the core dietary niche of that species in that river (Jackson et al. 2011; Jackson et al. 2012). Then, the extent to which the standard ellipse areas of each fish species overlapped in trophic space with *B. barbus* was assessed; the higher the proportion of the area that overlapped, the more the fishes were exploiting similar food items. The second method was to use the stable isotope data within generalized linear models (GLMs) in which fish length was a covariate, as proposed and used by Galster et al. (2012) in assessments of the trophic ecology of invasive *M. dolomieu* in North America using archived material. These GLMs were also used because of the issue that the diet composition of fishes (and so their stable isotope values) tends to change as fish length increases due to ontogenetic diet shifts, and the lengths used for each species in each fish community were variable (Table 2). The model outputs were the mean adjusted (for length) $\delta^{15}N$ and $\delta^{13}C$ values for each species in each community, and the significance of the differences in their means with other species according to pairwise comparisons with Bonferroni adjustment for multiple comparisons.

2.4 Results

The application of standard ellipse areas to the stable isotope data derived from these species revealed considerable variation in the standard ellipse areas of the species within and between the rivers (Table 1, 2) but with *B. barbus* generally occupying distinct areas of trophic space that had minimal overlap with any other species, especially *S. cephalus* (Table 1; Figure 4). The highest overlap was with perch *Perca fluviatilis* in the

River Ivel (29 %; Table 2), where values of $\delta^{15}N$ indicated both species occupied relatively high trophic positions (Figure 4).

Use of these stable isotope data in generalized linear models in which fish length was the covariate revealed that length had significant effects on $\delta^{13}C$ in the River Ivel (Wald $\chi^2 = 11.29$, $P < 0.001$) and on both $\delta^{13}C$ and $\delta^{15}N$ in the River Goyt ($\delta^{13}C$: Wald $\chi^2 = 6.23$, $P = 0.01$; $\delta^{15}N$: Wald $\chi^2 = 4.22$, $P = 0.04$). The effect of fish length on both $\delta^{13}C$ and $\delta^{15}N$ in the Great Ouse was, however, not significant ($\delta^{13}C$: Wald $\chi^2 = 0.11$, $P = 0.74$; $\delta^{15}N$: Wald $\chi^2 = 0.06$, $P = 0.81$). By contrast, the effect of species on $\delta^{13}C$ and $\delta^{15}N$ in all rivers was highly significant (Wald $\chi^2 > 12.52$ in all cases, $P < 0.001$ in all cases). The consequent length-adjusted mean $\delta^{13}C$ and $\delta^{15}N$ data for each river indicated that the stable isotope data for *B. barbus* were still significantly different to *S. cephalus* in all rivers (Table 3; Figure 5). For the other species in the communities, however, significant differences in their mean adjusted $\delta^{13}C$ and $\delta^{15}N$ values were less apparent, with no significant differences between *B. barbus* and *Leuciscus leuciscus* and *R. rutilus* in the Great Ouse (Table 3; Figure 5).

Table 1 Details of the sampling locations used in the study, where I: *Barbus barbus* indigenous range, NI: *Barbus barbus* non-indigenous range.

Site number	**River**	**Approximate location**	**Range (I/ NI)**	**Date sampled**
1	Ivel	52°06'N, 0°16'W	I	August 2006
2	Great Ouse	52°11'N, 0°36'W	I	August 2001
3	Goyt	53°24'N, 2°07'W	NI	September 2005

Table 2 Species, sample size, fork length range and mean fork length (± SE) of the fish communities and their standard ellipse area as SEAc (as a measure of trophic niche width with a correction applied for small sample size) and the extent to which *B. barbus* trophic niche overlaps (%) with other fish species in the community (chub *Squalius cephalus*, dace *Leucisicus leuciscus*, common bream *Abramis brama*, perch *Perca fluviatilis*, roach *Rutilus rutilus*, and grayling *Thymallus thymallus*).

Site	**Species**	**n**	**Length range (mm)**	**Mean length (mm)**	**SEA_c**	**% Overlap**
Ivel (1)	*B. barbus*	11	250 - 785	513 ± 60	4.44	
	S. cephalus	10	205 - 560	361 ± 34	2.33	< 0.01
	L. leuciscus	6	135 - 200	171 ± 9	1.43	< 0.01
	A. brama	6	380 - 490	448 ± 15	0.57	7
	P. fluviatilis	6	200 - 340	302 ± 23	2.63	29
Great Ouse (2)	*B. barbus*	6	188 - 643	400 ± 59	0.60	
	S. cephalus	9	88 - 343	238 ± 32	4.63	< 0.01
	L. leuciscus	6	93 - 203	160 ± 18	7.83	13
	R. rutilus	6	128 - 238	195 ± 20	4.39	2
Goyt (3)	*B. barbus*	6	365 - 413	401 ± 7	0.31	
	S. cephalus	8	211 - 393	318 ± 19	0.84	< 0.01
	T. thymallus	6	203 - 261	231 ± 9	0.85	0

Table 3 Pairwise comparisons with Bonferroni adjustment for multiple comparisons of the mean adjusted values of $\delta^{13}C$ and $\delta^{15}N$ (± SE) for *Barbus barbus* and the other species in the fish communities and where the difference between the mean values according to the pairwise comparisons is significant at * $P < 0.05$ and ** $P < 0.01$.

			Pairwise comparisons	
Site	**Species**		**$\delta^{13}C$**	**$\delta^{15}N$**
Ivel (1)	*B. barbus*	*S. cephalus*	-2.50 ± 0.39**	5.74 ± 0.52**
		L. leuciscus	-0.91 ± 0.55	2.19 ± 0.74*
		A. brama	-1.69 ± 0.41**	1.25 ± 0.55
		P. fluviatilis	-1.81 ± 0.46**	0.96 ± 0.63
Great Ouse (2)	*B. barbus*	*S. cephalus*	-2.05 ± 0.75*	2.64 ± 0.97*
		L. leuciscus	-2.15 ± 0.93	2.22 ± 1.20
		R. rutilus	0.92 ± 0.87	0.34 ± 1.13
Goyt (3)	*B. barbus*	*S. cephalus*	-0.87 ± 0.21**	2.54 ± 0.40*
		T. thymallus	-2.04 ± 0.34**	1.52 ± 0.66

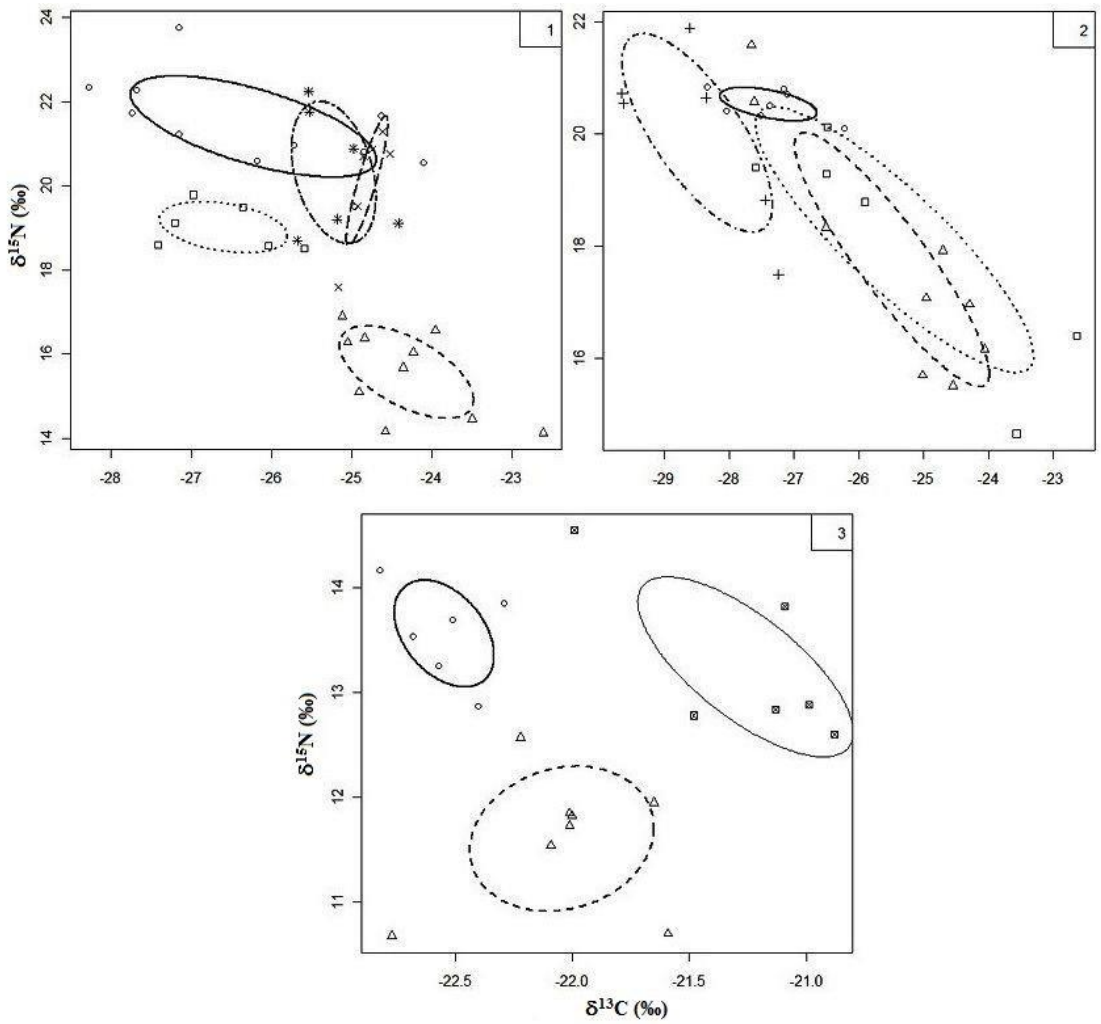

Figure 4 Stable isotope bi-plots for each site, where data points represent individual *Barbus barbus* (○), *Squalius cephalus* (Δ), *Leuciscus leuciscus* (□), *Rutilus rutilus* (+), *Abramis brama* (×), *Perca fluviatilis* (*) and *Thymallus thymallus* (⊗). Lines enclose the standard ellipse areas for each species at each site; *B. barbus* (solid), *S. cephalus* (dash), *L. leuciscus* (dot), *R. rutilus* (dash-dot), *A. brama* (long dash), *P. fluviatilis* (two size dash) and *T. thymallus* (non-bold solid). Note the different scales on the axes.

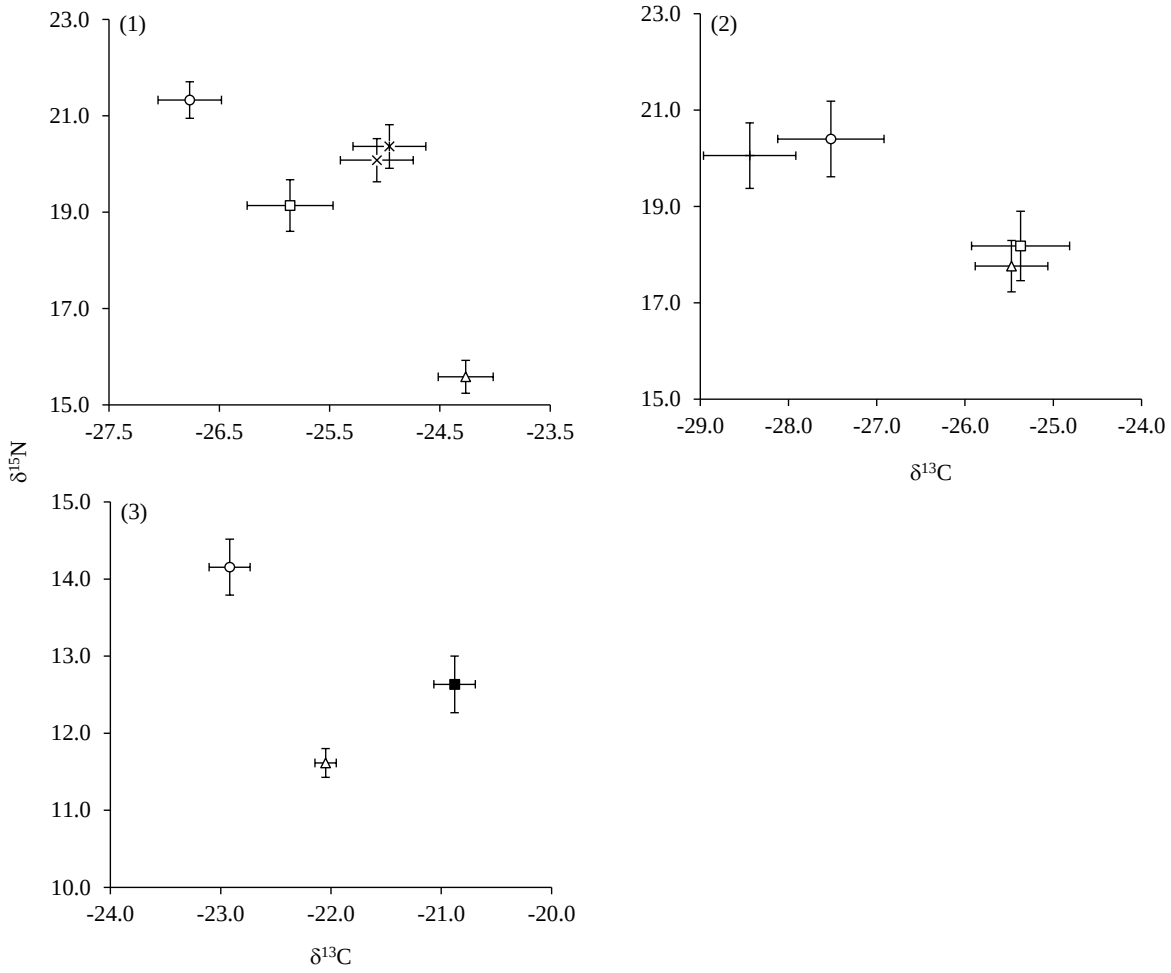

Figure 5 Stable isotope bi-plots showing the mean adjusted values of $\delta^{13}C$ and $\delta^{15}N$ (± SE) for each site and species, where *Barbus barbus* (○), *Squalius cephalus* (Δ), *Leuciscus leuciscus* (□), *Rutilus rutilus* (+), *Abramis brama* (×), *Perca fluviatilis* (*) and *Thymallus thymallus* (■). Note the different scales on the axes.

2.5 Discussion

The use of archived scale material derived from historical fish stock assessment programmes was used successfully to determine aspects of the trophic relationships of the analysed fishes, with the two data analytical methods providing some important ecological insights. The use of the standard ellipse areas indicated two main aspects relating to the trophic ecology of *B. barbus* and the other fishes in the communities. Firstly, the trophic positions of *B. barbus* compared to other fishes in the community tended to be relatively high, particularly when compared with *S. cephalus*, despite literature suggesting that *S. cephalus* is often a facultative piscivore (e.g. Mann 1976). Secondly, the extent of overlap in the standard ellipse areas between *B. barbus* and the other fishes in each community was low, generally < 1 %, and was only > 20 % with *P. fluviatilis* in the River Ivel. All perch were relatively large (mean length 302 mm; Table 2), sizes at which they tend to be facultative piscivores (Haakana et al. 2007).

The outputs of the standard ellipse area calculations thus suggested that the trophic niche of *B. barbus* was generally distinct from the other fishes in their communities, with their feeding on items that were relatively high in the food web including, potentially, other fishes, although their actual diet composition must remain speculative as it could not be assessed further. These patterns might be explained by the fish communities being composed of a range of species of different functional guilds (Noble et al. 2007b) that resulted in strong habitat and resource partitioning, and thus different patterns of resource acquisition and so low overlaps in trophic niches. Nevertheless, the length ranges of the analysed fish also varied with species, with *B. barbus* being considerably larger than the other species present. Given that in fishes, diet composition changes with ontogeny, such as the taking of larger food items as gape size increases (Hyslop 1980; Mittelbach 1981), then these outputs might just be indicative of

analysing data using fish that differ in their body lengths. Thus, drawing conclusions on patterns of resource sharing - and, potentially, competition between the species - is difficult with these data outputs. This highlights a key issue of using scales from stock assessments (or, indeed, those collected in any sampling of a fish community) for subsequent trophic analyses, i.e. the scales available for stable isotope analysis might not always be necessarily representative of the size ranges of all the fishes in the community, limiting their utility for comparative studies on their trophic ecology using metrics such as standard ellipse areas. Wherever possible, the size ranges of the fishes being compared need to be similar to avoid such confounding effects and thus this is an important consideration for work completed in Chapter 3 and 4.

Given this potential shortcoming in the application of standard ellipse areas to stable isotope data from fish populations of different length ranges, the GLMs were used as they enabled fish length to be used as a covariate. Thus, they enabled assessment of whether the mean adjusted values of $\delta^{13}C$ and $\delta^{15}N$ were significantly different between species when the effects of length were controlled. Outputs indicated that the stable isotope data for *B. barbus* was still significantly different to *S. cephalus* in all rivers, although in the other species in the communities, significant differences in their mean adjusted $\delta^{13}C$ and $\delta^{15}N$ values were less apparent. Thus, whilst standard ellipse areas provide a quantitative assessment of trophic niche size (Jackson et al. 2011, 2012), their usefulness was limited here due to inherent issues arising from differences in fish length that introduced a confounding factor on the analysis of fish diet composition (Hyslop 1980; Mittelbach 1981). Whilst this was overcome in the GLMs by using length as the covariate, these outputs could only be assessed in terms of whether differences in the length-adjusted mean $\delta^{13}C$ and $\delta^{15}N$ were significantly different between the species. Nevertheless, the consistency in the GLM and SEAc outputs for *B. barbus* and *S.*

cephalus across the three fish communities indicated significant differences in their trophic positions in the food web. Correspondingly, this output can be used as an initial finding that forms the basis of designing subsequent studies to assess how the trophic relationships between these larger riverine fishes develop over time and space, such as whether the apparent resource partitioning occurs as a consequence of initial resource sharing, for example, after a stocking event of *B. barbus* (Taylor et al. 2004).

In conclusion, fish stock assessment exercises provide archived material, such as fish scales, which can be used subsequently for initial assessments of fish trophic relationships. Indeed, fish scales generally should provide material for stable isotope analysis that can be collected in a non-destructive manner, a contrast to dorsal muscle (Busst et al. 2015; Busst and Britton 2016). Whilst analytical techniques, such as standard ellipse areas, provide quantitative insights into the trophic niches of the fishes, an issue highlighted here was that fish stock assessment surveys are often comprised of samples that are not necessarily representative of the actual length composition of the fish populations and, thus, interpretations of differences in, and overlaps between, trophic niches of the fishes can be unreliable. Although these can be partly overcome through multivariate statistical tests, their outputs are limited to highlighting the significance of differences in adjusted mean stable isotope values per species. Diet composition also cannot be estimated due to the absence of data on their putative foods. Correspondingly, Chapter 3 explores the diet composition of *B. barbus* in four rivers in England to identify their typical dietary components, given their trophic differences identified in this chapter. This work will use scales as the tissue of choice for the stable isotope analysis given their successful use, but will use these in conjunction with samples of *B. barbus* putative foods from each river, and utilise fish of similar size wherever possible.

3. Diet composition of *Barbus barbus* in four rivers in England: importance of angling baits and invasive crayfish as trophic subsidies

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3.1 Summary

Invasive species and anthropogenic sources of allochthonous trophic subsidies can have substantial ecological consequences for freshwater ecosystems, including modifying the diet of consumers and altering food web structure. Here, the diet composition of *B. barbus*, an omnivore, was assessed in four rivers in England, with emphasis on the contributions of invasive signal crayfish *P. leniusculus* and pelletized fish-meal ('pellets'). Pellets are often used in large quantities by river anglers and thus could provide an important trophic subsidy, not only to the fish but also indirectly via *P. leniusculus*. Carbon and nitrogen stable isotopes were used to estimate the proportion of diet assimilated from natural sources, *P. leniusculus* and pellets by *B. barbus* of lengths between 420 and 800 mm. Pellets generally made a large contribution to the overall biomass of *B. barbus* (up to 59 % of population diet) and in the two rivers where they were present, *P. leniusculus* were also an important resource (up to 30 % of population diet). The proportion derived from macro-invertebrates (excluding *P. leniusculus*) was substantially lower. Stable isotope mixing models further demonstrated considerable intraspecific variability in *B. barbus* diet within the rivers, with pellets comprising up to 79 % of the biomass of individual *B. barbus* in rivers where *P. leniusculus* was absent. Where present, *P. leniusculus* effectively replaced and thus reduced the contribution of pellets to individual fish diet. Thus, isotopic evidence from three of the four rivers indicates that *B. barbus* populations are heavily reliant (>50%) upon angler-introduced baits that act as an important allochthonous subsidy and will also prey upon invasive *P. leniusculus* where they are present. The feeding of *B. barbus* on these subsidies might thus help explain some of the trophic niche patterns observed in the Results of Chapter 2.

3.2 Introduction

Environmental disturbances caused by human activities, such as deforestation, invasive species and over-exploitation of fisheries, are impacting upon food web structure and ecosystem functioning (Petchey et al. 1999; Harmon et al. 2009). These disturbances alter the supply of resources and, therefore, often cause changes in the diet of resident species (Harmon et al. 2009). Evidence suggests that food webs in freshwater ecosystems shift under the influence of invasion and fishery activities through inputs of novel resources (Vander Zanden et al. 1999; Britton et al. 2010; Jackson et al. 2012). Fishery activities associated with angling and aquaculture can magnify the input of allochthonous resources to freshwater ecosystems via the introduction of energy rich foods, such as pelletized fishmeal, and introductions of invasive species; both of which can supplement the diet of native species (Grey et al. 2004; Jackson et al. 2013). Whilst inputs of allochthonous resources enhance the *in situ* productivity of freshwater systems and increase food web stability (Jones et al. 1998; Jefferies 2000), when inputs become excessive, the food web is often modified across numerous trophic levels through alterations of food web connectivity and bottom-up or top-down control (Jefferies 2000; Marzcak et al. 2007). This can lead to shifts in the diet composition of consumers as they become increasingly reliant on the allochthonous resource as a trophic subsidy (Marcarelli et al. 2011; Sato and Watanabe 2013).

Trophic subsidies that originate from fishery activities can provide recipient aquatic communities with alternative food resources that are energy rich and highly nutritious (Grey et al 2004; Arlinghaus and Niesar 2005; Fernandez-Jover et al. 2011a; Fernandez-Jover et al. 2011b), such as pelletized fishmeal that are usually high in protein (from fishmeal) and lipid (from fish oil) (Naylor et al. 2000). Whilst it was recently estimated that the global annual production of fishmeal pellets was 3.7 million tonnes (Tacon and

Metian 2008), only a small proportion of this production is used directly as bait for recreational angling. Nevertheless, pellets are increasingly being used by freshwater anglers in Europe as both an attractant and hook-bait to target fish of the Cyprinidae family, such as *C. carpio* and *B. barbus* (Jackson et al. 2013). Moreover, the quantities used can be substantial, with the amount of bait used annually per angler in Germany estimated at 7.3 kg (Arlinghaus and Niesar 2005). Given that these pellets were originally designed for feeding carnivorous fish in aquaculture to maximise their growth through the input of an energy rich resource that is relatively easy to assimilate (Naylor et al. 2000), then this at least partially explains their effective use within freshwater angling for a range of omnivorous and carnivorous species.

Aquatic ecosystems are also vulnerable to species invasions, especially those that are already disturbed through human activities (MacDougall and Turkington 2005). These invasive species, when present in sufficient abundance, can act as novel autochthonous resources for native species, resulting in shifts in food web structure (Vander-Zanden et al. 1999; Coulas et al. 1998; Ellis et al. 2011). Moreover, invasive species often create novel trophic pathways, acting as both consumers and resources with, for example, invasive crayfish consuming both plant and animal material (Jackson et al. 2014) and providing an abundant food resource for many taxa (e.g. Beja 1996; Correia 2001; Tablado et al. 2010). In many European countries, invasive crayfish species have been widely introduced, with the signal crayfish (*P. leniusculus*) usually being the most abundant (Kouba et al. 2014), including in the UK (Jackson and Grey 2013).

Consequently, the aim of this Chapter was to assess how angling baits and invasive crayfish influenced the diet of freshwater fish in riverine environments, using *B. barbus* as the model species. They were studied in four English rivers in which they are the

main target species for the majority of the anglers practising catch-and-release; of these rivers, invasive *P. leniusculus* had well-established populations in two but were absent from the other two. As Grey et al. (2004) established that the predominantly marine-derived material of pellets makes them isotopically distinct in freshwater food webs, the specific objective was to assess the relative dietary contribution of fishmeal pellets and *P. leniusculus* to *B. barbus* compared to that from native and naturally available species. *Barbus barbus* is indigenous in some English rivers but non-indigenous in others, and is popular with many anglers due to its sporting qualities and relative ease of capture (Britton and Pegg 2011). An omnivore that is occasionally piscivorous (Kottlelat and Freyhof 2007), it is regularly fished for using relatively large quantities of fishmeal pellets (often >1 kg per angler per day; personal observation).

3.3 Materials and methods

The four study rivers were the Rivers Teme (52°19.40' N; 2°28.50' W), Hampshire Avon (50°54.38' N; 1°47.30' W), Kennet (51°25.32' N; 1°05.11' W) and Lee (51°48.40' N; 0°14.29' W). On all of these rivers, angling was permitted throughout the coarse angling open season (between June 16th and March 14th), with the majority of angling activity focused between June and September. *Pacifastacus leniusculus* was present in the Kennet and Lee, but not the Teme and Hampshire Avon. Following work in Chapter 2, the stable isotope analysis of fish scales was used as a non-destructive method to assess the diet of *B. barbus* in preference to using muscle tissue or gut contents analysis (GCA). This was because the sampling sites were all recreational fisheries that practised catch-and-release angling and thus destructive sampling was not possible. Stable isotope analysis reveals food web structure and trophic linkages through the naturally occurring ratios of ^{15}N:^{14}N and ^{13}C:^{12}C (Grey 2006). The carbon ratios reflect the consumer diet with typical enrichment of 0 to 1 ‰ whereas nitrogen ratios show greater enrichment of

2 to 4 ‰ from resource to consumer (i.e. indicate trophic position) (Post, 2002; McCutchan et al. 2003).

Samples of *B. barbus* were captured from each river during August and September 2012 by angling and as coordinated by the Ph.D. supervisor for the purposes of work in this thesis. Following capture, each fish was measured (fork length, nearest 5 mm) and between 3 and 5 scales were removed from between the base of the dorsal fin and the lateral line. These were transferred to paper envelopes and rapidly dried to maintain their condition. Concomitantly, samples of the angler bait were taken for subsequent analyses. To obtain samples of the putative food resources of *B. barbus* from each river, kick-sampling was used in September 2012 to provide representative samples of the macro-invertebrate communities. In all rivers, this also provided samples of small fishes, primarily 3-spined stickleback *Gasterosteus aculeatus*, minnow *Phoxinus phoxinus* and *C. gobio* (subsequently referred to as 'small fishes'). In the Rivers Kennet and Lee, kick sampling was also used to sample *P. leniusculus*.

For stable isotope analysis, replicate samples of the putative food resources of *B. barbus* were used (n = 3 to 10 per resource). Given the size (> 400 mm; Table 4) and likely age (> 10 years; Britton et al. 2013) of all of the *B. barbus* in the samples, only material from the very outer portions of scales were used in analyses, i.e. material outside of the last annulus that was produced through growth in 2012 rather than earlier in life (Hutchinson and Trueman 2006). Prior to analysis, all samples were ground using an agate pestle and mortar and 0.5 mg was weighed into 6 x 4 mm tin cups using an ultra-microbalance (UMX2 Automated-S, Mettler Toledog). Carbon and nitrogen isotopic analysis was carried out at Queen Mary, University of London, in December 2012 using an elemental analyser (Flash EA, 1112 series, Thermo-Finnigan) coupled to a

continuous flow isotope ratio mass spectrometer (Finnigan MAT DeltaPlus, Thermo-Finnigan). Ratios of ^{13}C:^{12}C and ^{15}N:^{14}N are expressed in per mille (‰) using the delta notation (δ). Secondary standards (sucrose for carbon; ammonium sulphate for nitrogen) with known relation to international standards (Pee Dee Belemnite for carbon; nitrogen in air for nitrogen) were used as reference materials. Cyclohaxonone-2, 4-dinitrophemylhydrazone or urea was used as an internal standard and repeat analyses resulted in typical precision of <0.1 ‰ for carbon and <0.3 ‰ for nitrogen.

Prior to the data analysis, the stable isotope data from the *B. barbus* scales were converted to values for dorsal muscle, as muscle stable isotope values reflect that of the diet of individual fish most closely (Pinnegar and Polunin 1999; Grey et al. 2009). Consequently, samples of scales and dorsal muscle from 20 *B. barbus* of 150 to 250 mm that were available from an unconnected and completed study (Pegg and Britton 2011) and that had been raised on a standardised diet of consistent isotopic composition were analysed; the offset between scale and muscle was determined by simple subtraction. Material only on the scale edge was used in the analyses (as per the fish used in the main study) and provided muscle values of -1.8 ± 0.49‰ for $\delta^{13}C$, and +0.6 ± 0.35‰ for $\delta^{15}N$ relative to scale values. Fish length was tested against $\delta^{13}C$ and $\delta^{15}N$ in each river using linear regression to identity any ontogenetic influences. Bayesian mixing models were then used to determine the relative contribution of each resource to the diet of each *B. barbus* population and individual (Jackson et al. 2011). The individual analysis was used to assess individual variation in diet choice. Models were run using the SIAR package in the R computing program (Parnell et al. 2010; R Core Development Team 2013). As excessive putative food resources can cause the model to underperform, the data for resources with similar isotope values were combined *a priori*, whilst respecting the taxon and functional affiliation of the individual species

(Phillips et al. 2005). Accordingly, the resources were pooled into the following groups at each site where available: fish pellets, small fish, Arthropoda (*Gammarus pulex*, Hydropsychidae, Simuliidae spp. and Ephemeroptera spp. that were present in all the rivers) and *P. leniusculus*. To correct for isotopic fractionation between resources and consumers, 3.4 ‰ (±0.98 ‰) was used for $\delta^{15}N$ and 0.39 ‰ (±1.3 ‰) for $\delta^{13}C$ (Post 2002).

Anecdotal evidence from anglers encountered during the study revealed concerns over *P. leniusculus* consuming angler baits, particularly pellets. To test for this, a further mixing model was run substituting *P. leniusculus* as the consumer, and inserting values for pellets, small fish, leaf litter and arthropods as resources. The fractionation factors used were as already described for *B. barbus*.

3.4 Results

The lengths of the *B. barbus* captured from the rivers spanned from 420 to 800 mm (Table 4). There was considerable variability in the $\delta^{13}C$ of individual *B. barbus* in the rivers, with this less apparent for $\delta^{15}N$ (Figure 6). Small fishes and *P. leniusculus* tended to be very similar in $\delta^{13}C$, with *P. leniusculus* ^{15}N-depleted by 1-2‰, whilst values for fishmeal pellets were clearly isotopically distinct compared to any other resource (Figure 6). The influence of fish length on $\delta^{13}C$ and $\delta^{15}N$ was not significant in all rivers ($\delta^{13}C$: Teme $R^2 = 0.50$, $F_{1,8} = 2.31$, $P = 0.17$; Kennet $R^2 = 0.01$, $F_{1,7} = 0.01$, $P = 0.94$; Lee $R^2 = 0.03$, $F_{1,8} = 3.84$, $P = 0.09$; Avon $R^2 = 0.05$, $F_{1,17} = 0.82$, $P = 0.38$; $\delta^{15}N$: Teme $R^2 = 0.10$, $F_{1,8} = 0.77$, $P = 0.54$; Kennet $R^2 = 0.01$, $F_{1,7} = 0.05$, $P = 0.81$; Lee $R^2 = 0.35$, $F_{1,8} = 4.38$, $P = 0.07$; Avon $R^2 = 0.20$, $F_{1,17} = 4.15$, $P = 0.06$).

Fishmeal pellets generally made a substantial contribution to the overall biomass of *B. barbus* (mean value range: 23 - 59 %) and were the most important resource in the Hampshire Avon and Kennet (Figure 7). Where *P. leniusculus* was present (Lee and Kennet), it was also an important resource in *B. barbus* diet (mean values: 30 and 20 %; Figure 7). The dietary contribution of the other food resources varied between rivers, with Arthropoda generally representing the least important food source (Figure 7).

Data from individual *B. barbus* per river also suggested that in the rivers where *P. leniusculus* was absent, there were relatively high proportions of pellets in the diet, ranging from 35 to 72 % in the Avon and 20 to 79 % in the Teme (Table 5). Where *P. leniusculus* was present, the contribution of pellets varied substantially between rivers, ranging from 22 to 77 % in the Kennet, and 8 to 41 % in the Lee (Table 5). In the Lee, pellets contributed less than 30% to the diet of most fish (7 of 9 fish) and *P. leniusculus* was an additional important resource, contributing 22 to 31%. The proportion of pellets in the diet of *B. barbus* varied considerably between individuals in the Rivers Teme and Kennet (as indicated by high standard deviations; Table 5), suggesting a degree of individual specialisation. In contrast, the proportion of crayfish in the diet of *B. barbus* (when available) varied little between individuals (as indicated by low standard deviations; Table 5). Small fish were also important resources in all four rivers, contributing up to 50 % to fish diet especially in the absence of crayfish (Table 5).

The mixing model run with crayfish as the consumer revealed that, contrary to anecdotal reports, the pellets were of relatively low dietary importance to *P. leniusculus*, with a mean contribution of 6 % in the Kennet and 12 % in the Lee.

Table 4 Sample size, fork length range, mean fork length (± SE) and mean stable isotope values (± SE) of *B. barbus* at each site.

Site	**River**	**n**	**Length range (mm)**	**Mean length (mm)**	**Mean $\delta^{13}C$ (‰)**	**Mean $\delta^{15}N$ (‰)**
1	Avon	19	590 – 800	680 ± 16	-27.28 ± 0.31	11.51 ± 0.29
2	Teme	9	470 - 650	556 ± 24	-25.52 ± 0.67	11.81 ± 0.32
3	Kennet	9	550 - 710	631 ± 19	-25.02 ± 0.78	11.34 ± 0.31
4	Lee	9	420 - 600	534 ± 18	-27.23 ± 0.48	18.16 ± 0.49

Table 5 Intra-population variations in estimated diet of *B. barbus* at each site, indicating minimal, maximal and mean (± SE) contribution of each source, and where crayfish are exclusively *P. leniusculus*.

Site	**River**	**Source**	**Min (%)**	**Max (%)**	**Mean (%)**
1	Avon	Pellet	34.6	71.6	52.0 ± 2.4
		Small fish	11.5	43.6	23.9 ± 2.5
		Arthropoda	12.1	44.4	24.1 ± 2.1
2	Teme	Pellet	20.0	79.5	50.5 ± 7.1
		Small fish	11.9	49.8	29.1 ± 4.3
		Arthropoda	8.1	33.1	20.4 ± 2.9
3	Kennet	Pellet	21.7	77.2	55.8 ± 7.8
		Small fish	8.1	30.3	16.0 ± 3.0
		Crayfish	8.7	27.6	16.3 ± 2.7
		Arthropoda	6.1	21.1	12.0 ± 2.1
4	Lee	Pellet	7.9	40.6	22.2 ± 4.1
		Small fish	20.4	32.8	29.0 ± 1.6
		Crayfish	21.9	30.9	28.8 ± 1.1
		Arthropoda	12.2	30.2	20.1 ± 2.0

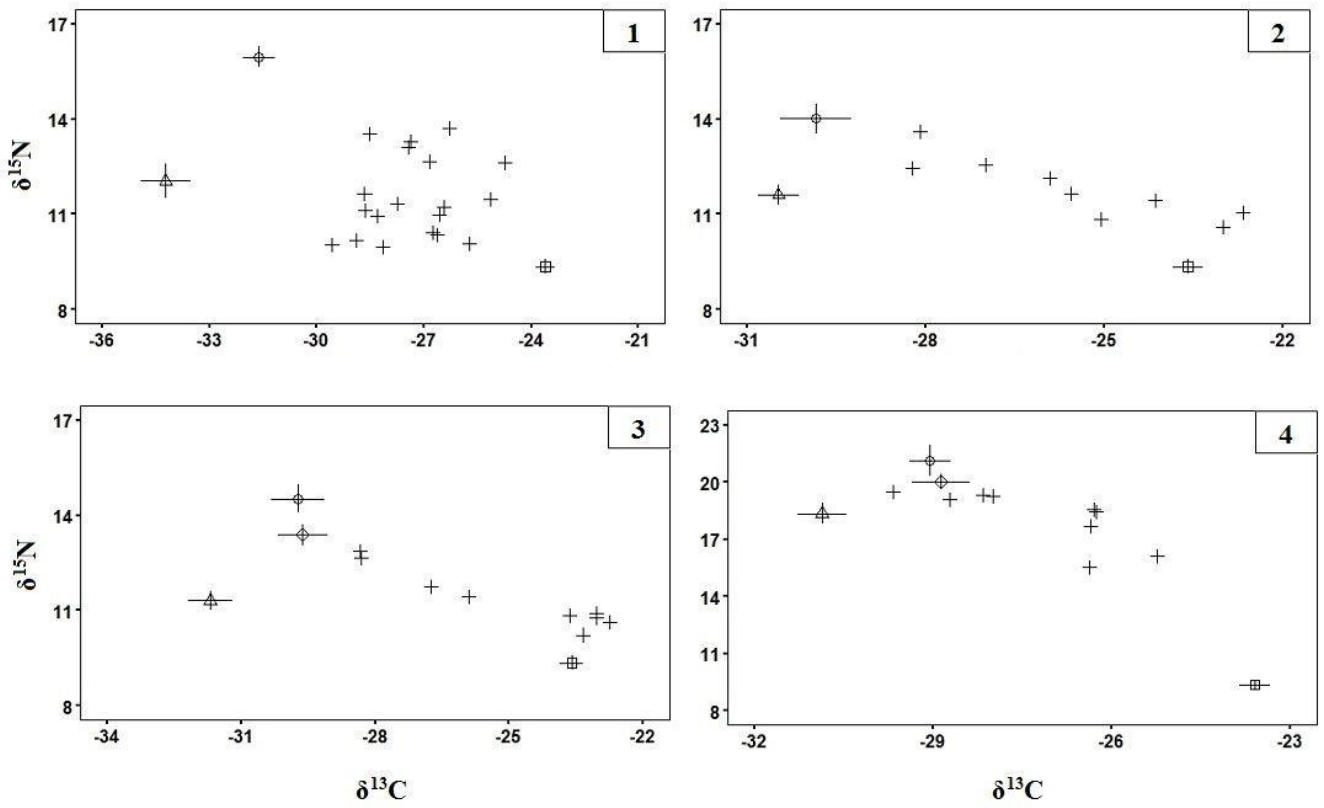

Figure 6 Stable isotope bi-plots for each site, showing individual *B. barbus* muscle isotope values (pluses) and mean (± SE) values of potential food sources (corrected for isotopic fractionation); pellet (square), small fish (circle), crayfish (triangle point down), Arthropoda (triangle point up). Note the different scales on the axes. The number in the right hand top corner of each plot denotes the river (*cf.* Table 4)

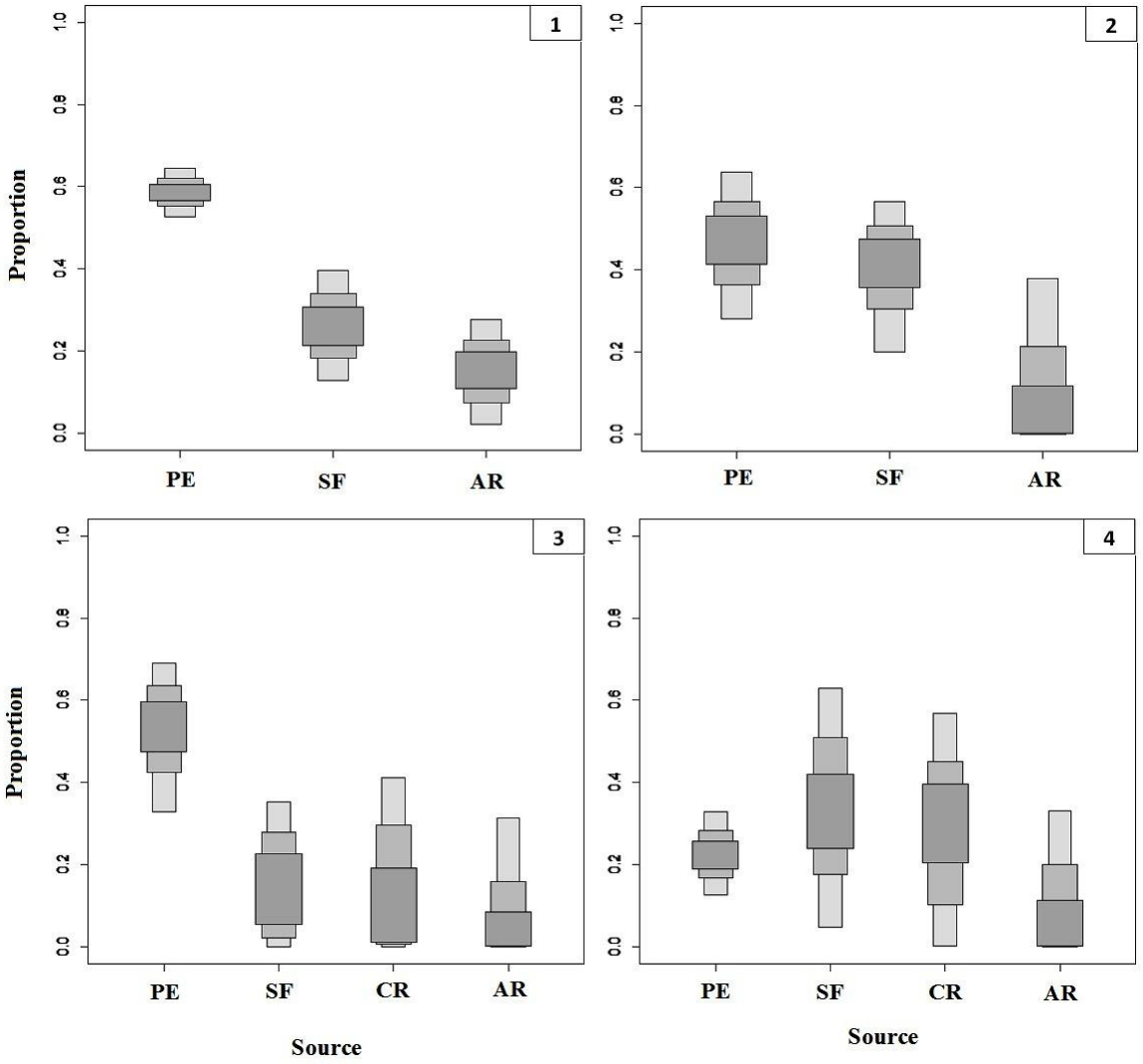

Figure 7 Boxplots for each site, showing estimated contribution of different carbon sources (PE, pellet; SF, small fish; AR, Arthropoda; CR, crayfish) to the diet of *B. barbus*; dark grey box represents the 50% of the data, posterior light grey box 75% of the data and the outer light grey box 95% of the data. The number in the right hand top corner of each plot denotes the river (*cf.* Table 4).

3.5 Discussion

In Chapter 2, the trophic relationships of *B. barbus* and other fishes in the community were assessed by stable isotope analysis and revealed that *B. barbus* occupied distinct isotopic space compared to other fishes. However, the nature of the samples analysed meant that the diet composition of *B. barbus* could not be assessed. This was overcome in this chapter by application of both *B. barbus* scales and their putative food resources to stable isotope analysis and associated data analytical tools. These analyses, on four *B. barbus* populations, indicated their diet was strongly reliant on introduced fishmeal pellets as a food resource. In the Rivers Kennet, Teme and Hampshire Avon, analyses from individual fish revealed that pellets comprised up to 79% of assimilated resources. The River Lee differed in that the highest contribution of pellets to the diet of an individual fish was estimated at only 41 %. Here, other items in the diet, especially invasive *P. leniusculus* and small fish, were important dietary resources. Perhaps surprisingly, the models estimated that Arthropods were the least important of the natural dietary resources included in the models.

Although the study was based on single collections of material sampled towards the start of autumn 2012, the use of stable isotope analysis provided a temporally integrated assessment of *B. barbus* diet that reflected their assimilated food items in the preceding months (Grey 2006). The influence of pellets on *B. barbus* diet might have decreased had samples been taken following the winter period, given that angling activities tend to be focused in summer on the study rivers. However, *B. barbus* growth rates, movement and activities peak in summer and almost cease when winter temperatures fall close to the species' thermal limit (4 ^{o}C; Baras 1995a, b). Consequently, their food intake and muscle turnover would be likely to be very low in winter, emphasising that sampling following the summer was the optimal period for the study (Perga and Gerdeaux 2005).

For similar reasons, it is likely that estimates of the contribution of crayfish to *B. barbus* biomass is also near to the annual maximum, as crayfish are less active over winter and hence probably less available as a prey resource. Ideally, including control rivers where pellets were not used would have made the study more robust, allowing identification of the isotopic niche of *B. barbus* without such an allochthonous resource; this, however, represents a major challenge in many English rivers given that *B. barbus* is a highly attractive target species for anglers and the use of pellets is now ubiquitous.

Fishmeal pellets used by anglers were thus an important allochthonous trophic subsidy for these *B. barbus* populations. There are, however, few studies that have dealt with how subsidies such as these are incorporated into food webs and what their relative importance is at the population and community level. Notwithstanding, Grey et al. (2004) revealed that in Esthwaite Water, England, approximately 65 % of *Daphnia* spp. and over 80 % of roach *R. rutilus* body carbon was ultimately derived from pellet material originating from an *in situ* fish farm. Other studies on the fate of pelletized feeds from aquaculture have shown their integration into the food web of the surrounding environment (Fernandez-Jover et al. 2011a; Fernandez-Jover et al. 2011b; Demétrio et al. 2012). Jackson et al. (2013) revealed that the growth, density and fitness of the invasive fish, *P. parva,* was enhanced in pond mesocosms that received trophic subsidies in the form of small fishmeal pellets, with this often being an indirect mechanism as the elevated nutrient concentrations that occurred as a result of pellet introduction had the effect of increasing rates of algal standing stocks. Whilst the quantity of fishmeal pellets that were introduced into each river was not quantified, for comparative purposes it has been estimated that recreational anglers in Germany introduced a total of 24,000 tonnes of angling bait into freshwater fisheries in 2004 (Arlinghaus and Niesar 2005). This was not only believed to represent a significant

trophic subsidy for the fish, but also elevated nutrient concentrations in the water and subsequently impacting adversely on water chemistry (Niesar et al. 2004, Arlinghaus and Niesar 2005, Lewin et al. 2006).

Invasive crayfish have been shown to have numerous negative effects on local fish communities through predation on small benthic fish and eggs (Guan and Wiles 1997; Thomas and Taylor 2013), competition for food and shelter (Guan and Wiles 1997; Bobeldyk and Lamberti 2010), and alteration of habitat by burrowing activities (Guan and Wiles 1997). Other studies on invasive crayfish have highlighted their importance as a food resource for many predatory fish (Blake and Hart 1995; Garvey et al. 2003; Hein et al. 2006; Nyström et al. 2006; Jackson et al. 2012), which could potentially help in controlling the invasive crayfish abundance (Hein et al. 2006; Nyström et al. 2006). The results indicate that invasive crayfish represent an important food source for adult *B. barbus*, even in the presence of an abundant allochthonous food resource such as fishmeal pellets. Although it was estimated that both the crayfish and pellets were important dietary resources, it was not determined whether their presence in *B. barbus* diet provided any benefits to the fish or indeed the wider population, such as in improved condition and increased somatic growth rates and fitness. However, fish-meal pellets are manufactured to be highly nutritious compared with many other food resources and, in other studies, have resulted in enhanced fish growth rates (Naylor et al. 2000; Jackson et al. 2013) and so it can be speculated that similar advantages might have been provided to *B. barbus* here. When the mixing models were being developed, it was apparent that the presence of *P. leniusculus* and 'small fish' in analyses tended to reduce the model performance due to their isotopic similarity. However, even with the small fish in the analyses, it was evident that *P. leniusculus* was an important food

source in fish diet, as suggested by Nyström et al. (2006), who showed their high contribution to fish diet in streams and lakes in Sweden.

Classical dietary studies have reported that *B. barbus* is omnivorous, eating benthic invertebrates (Cherghou et al. 2002; Piria et al. 2005, Corse et al. 2010) and small fish (Kottlelat and Freyhof 2007), with algae also present in their diet (Cherghou et al. 2002, Piria et al. 2005). The stable isotope data revealed a different story with little evidence that benthic macro-invertebrates (excluding *P. leniusculus*) were as important to diet when compared to fishmeal pellets and *P. leniusculus*. It may be that the majority of fish which were sampled by angling for this study were 'conditioned' to feeding upon high quality angling baits simply by the sheer volume of bait introduced and thus favoured those over other more natural diets. Nevertheless, the results tend to support Cherghou et al. (2002), who observed high dietary plasticity in *B. barbus* populations depending on the available prey items. In the Rivers Teme and Kennet there was also high intraspecific variability in the use of fishmeal pellets, with certain individuals clearly specializing on pellets as a principal food source. This plasticity could play an important role in different environments with diverse population dynamics, where resources might vary in their quantity and quality, enabling individual *B. barbus* to shift diet according to prey availability.

In addition, *B. barbus* used in the study were all relatively large and had smaller individuals been available for analysis, particularly those less than 200 mm, then it is likely that much higher proportions of macro-invertebrates would have been estimated in their diets due to their more limited gape-size. This could be an important aspect relating to hatchery reared, stocked *B. barbus*, as these tend to be < 200 mm in body size (Antognazza et al. 2016). This is explored further in Chapter 4, where the trophic

relationships of hatchery-reared, stocked *B. barbus* are explored in relation to fishes of similar trophic guilds and, following Chapter 2, of similar body size.

4. Trophic ecology of stocked European barbel *Barbus barbus* and resident cyprinid fishes: consistency in niche partitioning over time, space and body sizes

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4.1 Summary

Hatchery-reared fish are commonly stocked into freshwaters to enhance recreational angling. As these fishes are often of high trophic position and attain relatively large sizes, they potentially interact with functionally similar resident fishes and modify food web structure. Hatchery-reared *B. barbus* are frequently stocked to enhance riverine cyprinid fish communities in Europe; these fish can survive for over 20 years and exceed 8 kg. Here, their trophic consequences for resident fish communities were tested using co-habitation studies, mainly involving *S. cephalus*, a similarly large-bodied, omnivorous and long-lived species. These studies were completed over three spatial scales: pond mesocosms, two streams and three lowland rivers, and used stable isotope analysis. Experiments in mesocosms over 100 days revealed rapid formation of dietary specialisations and discrete trophic niches in juvenile *B. barbus* and *S. cephalus*. This niche partitioning between the species was also apparent in the streams over two years. In the lowland rivers, where fish were mature individuals within established populations, this pattern was also generally apparent in fishes of much larger body sizes. Thus, the stocking of these hatchery-reared fish only incurred minor consequences for the trophic ecology of resident fish, with strong patterns of trophic niche partitioning and diet specialisation. Application of these results to decision-making frameworks should enable managers to make objective decisions on whether cyprinid fish should be stocked into lowland rivers according to ecological risk.

4.2 Introduction

The release (stocking) of hatchery-reared fish into freshwater fisheries remains a widespread management technique used around the world to enhance recreational angling (Cowx 1994; Hunt et al. 2014). It can involve the supplementary stocking of extant species as well as the introduction of non-indigenous species (Hickley and Chare 2004; Horreo et al. 2015; Antognazza et al. 2016). It is often completed in preference to alternative options to enhance fish communities, such as habitat management (Arlinghaus and Mehner 2005). Given their attraction to anglers through their sporting qualities, stocked fish are often species that will grow to relatively large sizes and thus are species with high trophic positions in food webs (Holmland and Hammer 2004; Fujitani et al. 2016), such as apex predators (Eby et al. 2006). Correspondingly, stocked fishes can influence the natural functioning of ecosystems through, for example, increasing species richness at higher trophic levels, invoking top-down control processes and cascades, and altering food-web linkages and complexity (Radomski and Goeman, 1995; Eby et al. 2006). Whilst the benefits delivered by fish stocking tend to be social and recreational (Arlinghaus et al. 2014), the activity also promotes the eventual replacement of wild fish by hatchery-descended fish that have not been exposed to the same selection pressures (Van Poorten et al. 2011; Le Cam et al. 2015; Love Stowell et al. 2015).

Where the stocking or introduction of fish is into an ecosystem where the resources are not fully exploited, the released individuals could occupy vacant dietary niches that would facilitate their integration into the community by minimising competition with extant fishes (Shea and Chesson 2002; Jackson and Britton 2014; Tran et al. 2015). However, given that stocking often involves the enhancement of population sizes of extant species to increase

angler catch rates (Cowx 1994; Arlinghaus et al. 2014), then it could lead to increased intra- and inter-competition for food resources (Vehanen et al. 2009). The niche variation hypothesis then predicts that the consequence would be populations becoming less generalized in their diet (Van Valen 1965), resulting in reductions in population trophic niche breadths following stocking (Human and Gordon, 1996; Thomson, 2004; Olsson et al. 2009). In contrast, increased competition for resources can also result in populations having larger trophic niches that enable species and individuals to maintain their energy requirements through switching to more general diets (Svanback and Bolnick 2007).

Species of the Salmonidae family are stocked regularly into recreational fisheries and this is reflected in a large literature base in relation to both their benefits to angling and their impacts on wild stocks (e.g. Hansen 2002; Champigneulle and Cachera 2003; Ruzzante et al. 2004; Baer and Brinker 2010; Larsen et al. 2015). Stocking salmonids often involves the release of fish for put-and-take angling, with the majority of the fish captured soon after stocking, limiting their long-term impacts due to relatively short residence times (Baer et al. 2007). Where these fishes do survive in the wild, then their life spans are usually relatively short, limiting their persistence, although ecological and genetic consequences can still accrue (Simon and Townsend 2002; Le Cam et al. 2015). In European recreational fisheries, stocking of species of the Cyprinidae family is also commonplace, especially those of larger body sizes such as *B. barbus* and common carp *C. carpio* (Britton et al. 2010; Britton and Pegg 2011; Antognazza et al. 2016). These fish are often released at relatively small sizes after being hatchery-reared, especially *B. barbus* (Britton and Pegg 2011). Following their release, they can remain in the vicinity of the stocking location (Bolland et al. 2008) and ultimately some do integrate into communities (Bolland et al.

2009), including genetically (Antognazza et al. 2016). Stocked individuals can then persist for at least 20 years (Britton et al. 2013), providing considerable benefits to catch-and-release recreational angling (Britton and Pegg 2011; Antognazza et al. 2016). The combination of their persistence and their exploitation involving catch-and-release practises means their subsequent trophic consequences for resident fishes might be prolonged, if not permanent.

In European rivers, *B. barbus* are stocked regularly in areas covering both their indigenous and non-indigenous ranges (Antognazza et al. 2016). Their riverine populations in Great Britain are regularly enhanced with hatchery-reared fish of between 15 and 25 cm (age 1+ and 2+ years) to either enhance indigenous populations or provide new catch-and-release angling opportunities in rivers in the non-indigenous range (Wheeler and Jordan 1990). Whilst there is now some knowledge on the genetic outcomes of these stocking activities (Antognazza et al. 2016), these is little knowledge on their ecological outcomes, despite their omnivory, potential for long life spans and individuals attaining weights in excess of 8 kg (Britton & Pegg 2011; Britton et al. 2013). Consequently, the ecological consequences of *B. barbus* stocking for extant fish communities was investigated here through determining their trophic relationships with extant fishes and the consequences for somatic growth rates. Work was completed over three spatial scales that increased in ecological complexity and enabled testing of data using fish over the entire length range of the species: experimental pond mesocosms (juvenile fish), side channels of a lowland river (juvenile fish) and lowland rivers (mature fish). Due to the propensity of *B. barbus* for attaining large body sizes and their functional traits that favour feeding on the benthos, then assessments of their trophic consequences was primarily through co-habitation experiments

and field studies involving *S. cephalus*, a similarly large-bodied, omnivorous and long-lived species (e.g. Mann 1976) that was also used in Chapter 2. These two species occur in sympatry across both the indigenous and non-indigenous range of *B. barbus* in Great Britain. The hypothesis tested was that following a stocking event, *B. barbus* share food resources with *S. cephalus*, resulting in increased diet specialisation as per the niche variation hypothesis, and decreased somatic growth rates.

4.3 Materials and methods

4.3.1 Pond mesocosm experiment

The pond mesocosm experiment involved *B. barbus* and *S. cephalus* in allopatric and sympatric contexts. All experimental procedures were completed after ethical review and under protocols and procedures within the UK Home Office project licence 70/8063.

The pond mesocosm experiment tested the outcomes for the trophic niches and somatic growth rates of both fishes between their allopatric and sympatric contexts with natural food resources available. The experiment comprised of three treatments, both species in allopatry (n = 10), and then a final treatment where they were present in sympatry (n = 5 + 5), with three replicates of each treatment. The rationale was that their fundamental niche size and position would be estimated in allopatry and compared to their realised niche when the species were present in sympatry. Following the results of Chapter 2, fish starting lengths were similar between all treatments, being 60 to 88 mm.

Each mesocosm comprised of an independent enclosure situated within one larger natural pond (30 x 12 m; 1 m depth; Figure 8). The rationale for the use of enclosures was that they

provided uniform habitats across the treatments and replicates in which the fish would be exposed to same prey fauna. As these prey were all located within the larger pond then their stable isotope values would be similar. Thus, any differences in the stable isotope data of the fishes would be the result of their dietary interactions within the treatments, not due to inherent variability in the stable isotope values of their prey. The enclosures comprised of aluminium frames of 1.66 m (length) x 1.05 m (width) x 1.2 m (height) that were enclosed within a net of 7 mm square mesh that prevented fish movements in and out of the enclosure, but allowed the movement of water and invertebrates (Figure 8). The enclosures were located randomly across the larger pond, with spacing of at least 0.5 m between them to ensure they provided enclosed and independent habitats for each replicate and that were identical at the commencement of the experiment. Anti-predator netting (15 mm mesh) was placed over the top of all enclosures. The enclosures were sufficiently heavy that their remained stationary throughout the experimental period without moving and without needing to be tied down. The height of the enclosures meant they settled on the substrate, with macrophytes able to grow within each of them (mainly *Elodea* spp.)

Figure 8 Fish enclosures in the mesocosm experiment.

The experiment commenced in May 2014 and ran for 100 days, providing sufficient time for fish dorsal muscle to reach isotopic equilibrium and thus for the stable isotope values of their tissues to be representative of their diet composition in the mesocosms (Jackson et al. 2013; Busst and Britton 2016). The mean water temperature during the experiment was 18.2 ± 0.3 ºC, measured using a temperature logger in the centre of the pond that recorded temperature hourly (TinyTag TGP-4017). The enclosures were placed into the ponds 7 days prior to the start of the experiment and prior to their release, all fish were measured (fork length, nearest mm). On day 100, each enclosure was removed from the ponds, the fish removed, euthanized (anaesthetic overdose, MS-222) and placed on ice. At the same time, samples of macro-invertebrates were taken from each enclosure via sorting through the remaining pond substrate and macrophytes. These were mainly Chironomid larvae, but also included *Gammarus pulex*, *Asellus aquaticus* and corixids.

In the laboratory, the fish were re-measured and a sample of dorsal muscle was taken for stable isotope analysis. Their growth rates were calculated as incremental length (IL), determined from $(L_{t+1} - L_t) / {}_t$, where L_t = initial starting lengths, L_{t+1} = total end lengths and ${}_t$ = number of days. The macro-invertebrate samples were sorted to species, enabling three samples per species to be prepared for stable isotope analysis. A random selection of fish dorsal muscle samples (n = 15 to 18 per species and treatment; minimum number of samples per replicate = 4) was then also selected for stable isotope analysis. All of these samples were then dried at 60°C for 24 hours, ground and weighed, and analysed at the Cornell Isotope Laboratory, New York, USA for their stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ that were expressed as isotope ratios per mille (‰). The analytical process was as outlined in Section 2.3. For initial analyses, $\delta^{15}N$ data were transformed to trophic position (TP), using

the equation $TPi = [(\delta^{15}N_i - \delta^{15}N_{base})/3.4]+2$, where TP_i is the trophic position of the individual fish, $\delta^{15}N_i$ is the isotopic ratio of that fish, $\delta^{15}N_{base}$ is the isotopic ratio of the primary consumers (macro-invertebrates), 3.4 is the fractionation between trophic levels and 2 is the trophic position of the baseline organism (Post 2002).

The stable isotope data were initially used in linear mixed models to assess differences between the species, and their allopatric and sympatric treatments. Species were entered into models according to their treatments so, for example, *B. barbus* was present in models as (1) allopatric *B. barbus*, and (2) in sympatry with *S. cephalus*. The dependent (response) variable was $\delta^{13}C$ or $\delta^{15}N$ and each model was fitted with mesocosm number as a random effect on the intercept. This was to prevent inflation of the residual degrees of freedom that would occur had each individual fish been used as a true replicate (Tran et al. 2015). The differences in the stable isotope values by species and treatment were determined using estimated marginal means and pairwise comparisons with Tukey correction for multiple comparisons. Tukey was used here in preference to Bonferroni adjustment, as it is a less conservative method for pairwise comparisons (Bretz et al. 2011; Howell 2012). A similar linear mixed model approach was also used to test for differences in the initial fish lengths between the species and their treatments, and to assess differences in IL between treatments per species at the end of the experiment, linear model was used.

The stable isotope data were then used to calculate the trophic niche sizes of both species per treatment using the metric 'standard ellipse area' (SEA_c; the subscript 'c' indicates a small sample size correction). These calculations were completed in the SIAR package (Jackson et al. 2011) in the R computing program (R Development Core Team 2011). The

data from each mesocosm were combined for each treatment, as there were no differences between their isotopic baselines due to the enclosures being placed in the same pond. SEA_c is a bivariate measure of the distribution of individuals in their trophic space, with the models used enclosing 60 % of the data. Thus, SEA_c represented the core dietary niche of that population (hereafter referred to as the trophic niche) (Jackson et al. 2011; Jackson et al. 2012). Where SEA_c overlapped between the sympatric fishes within a treatment then the area and percentage of *B. barbus* overlap with *S. cephalus* was also calculated to indicate the extent of actual resource sharing. In addition, this overlap was also calculated for each combination of species in their allopatric contexts in order to demonstrate their potential niche overlap and enable comparison with their realised niche overlap in sympatry. These comparisons were possible due to the similarity of the habitats and prey items within the enclosures, the result of their placement within one larger pond.

4.3.2 Side channels of a lowland river

Work on assessing the trophic consequences of stocking *B. barbus* for *S. cephalus* and other extant fishes was then completed in two side channels of the River Great Ouse, the Houghton Stream (52.328607,-0.116417; Figures 9, 11) and the St. Ives Chub Stream (52.321542,-0.072521; Figures 10, 11). The source of both streams was an outflowing connection from the main River Great Ouse. They both then flowed for approximately 1500 m before re-joining the main river. At either end of the streams, the Great Ouse was canalized with highly regulated flows.

Given the low probability of recapturing individually marked fish in these wild situations then growth assessments were not included, with the focus on only assessing the trophic

interactions between extant fishes and stocked *B. barbus*. Whilst *B. barbus* is indigenous to the Great Ouse catchment (Antognazza et al. 2016), the two side channels were located at least 30 km downstream of the reaches where *B. barbus* populations were prevalent. However, their flow regimes, habitats and substrates were all perceived to be suitable for *B. barbus* and the Environment Agency, the responsible authority for inland fisheries in England, was seeking to establish *B. barbus* populations in these streams that already had a fish assemblage present that was dominated by cyprinid species, with *S. cephalus* dominant by biomass. The Chub Stream was approximately 1300 m in length, with a mean width of 6 m and depths to 1.5 m, and the Houghton Stream was approximately 1000 m in length with a mean width of 10 m and depths to 2 m. At either end of both streams, the Great Ouse is a large, impounded river with very low flows, and thus represents a very poor habitat for juvenile *B. barbus* (Noble et al. 2007a). The hatchery-reared *B. barbus* were released in December 2013, with 500 individuals released into each stream, all of lengths 100 to 150 mm and age 1+. A subsequent release of 1000 fish was then also completed in December 2014. Electric fishing surveys were conducted in July to August 2014 and June to September 2015. This involved fishing all the major habitats, with all captured fish identified to species, measured (fork length, nearest mm) and between 3 and 5 scales removed prior to their release back into the streams. Concomitantly, macro-invertebrate samples were collected.

The trophic relationships of the fishes from each sampling occasion were assessed using stable isotope analysis and corrected values by converting data to TP and C_{corr}. There were two differences from the methods used for the mesocosm experiment. Firstly, for the fishes, stable isotope data was derived from scales rather than dorsal muscle, as this provides a

non-lethal method of tissue collection and thus the fish could be released back into these catch-and-release fisheries (Busst et al. 2015; Busst and Britton 2016; Chapter 2). As it is only the outer proportion of scales that reflect the recent growth of the fish and thus their recent isotopic values, then in all cases only the very outer edge of the scales were removed and analysed (Grey et al. 2009; Chapter 2, 3). Secondly, to account for differences in the isotopic baseline between years in the streams, the stable isotope data were corrected. The $\delta^{15}N$ data were transformed to trophic position (TP) as previously described; $\delta^{13}C$ was corrected according to: $\delta^{13}Ccorr = \delta^{13}C_i - \delta^{13}C_{meaninv}/CR_{inv}$, where $\delta^{13}C_{corr}$ is the corrected carbon isotope ratio of the individual fish, $\delta^{13}C_i$ is the uncorrected isotope ratio of that fish, $\delta^{13}C_{meaninv}$ is the mean invertebrate isotope ratio (the 'baseline' invertebrates) and CR_{inv} is the invertebrate carbon range ($\delta^{13}Cmax - \delta^{13}Cmin$; Olsson et al. 2009). The stable isotope metrics of SEAc for each species and the extent of their overlap with *B. barbus* were then calculated as per the mesocosm experiment. Wherever possible, only fishes of similar lengths were compared for their trophic niche sizes and overlap to prevent confounds relating to ontogenetic shifts in diet.

Figure 9 Sampling site at Houghton stream.

Figure 10 Sampling site at Chub stream.

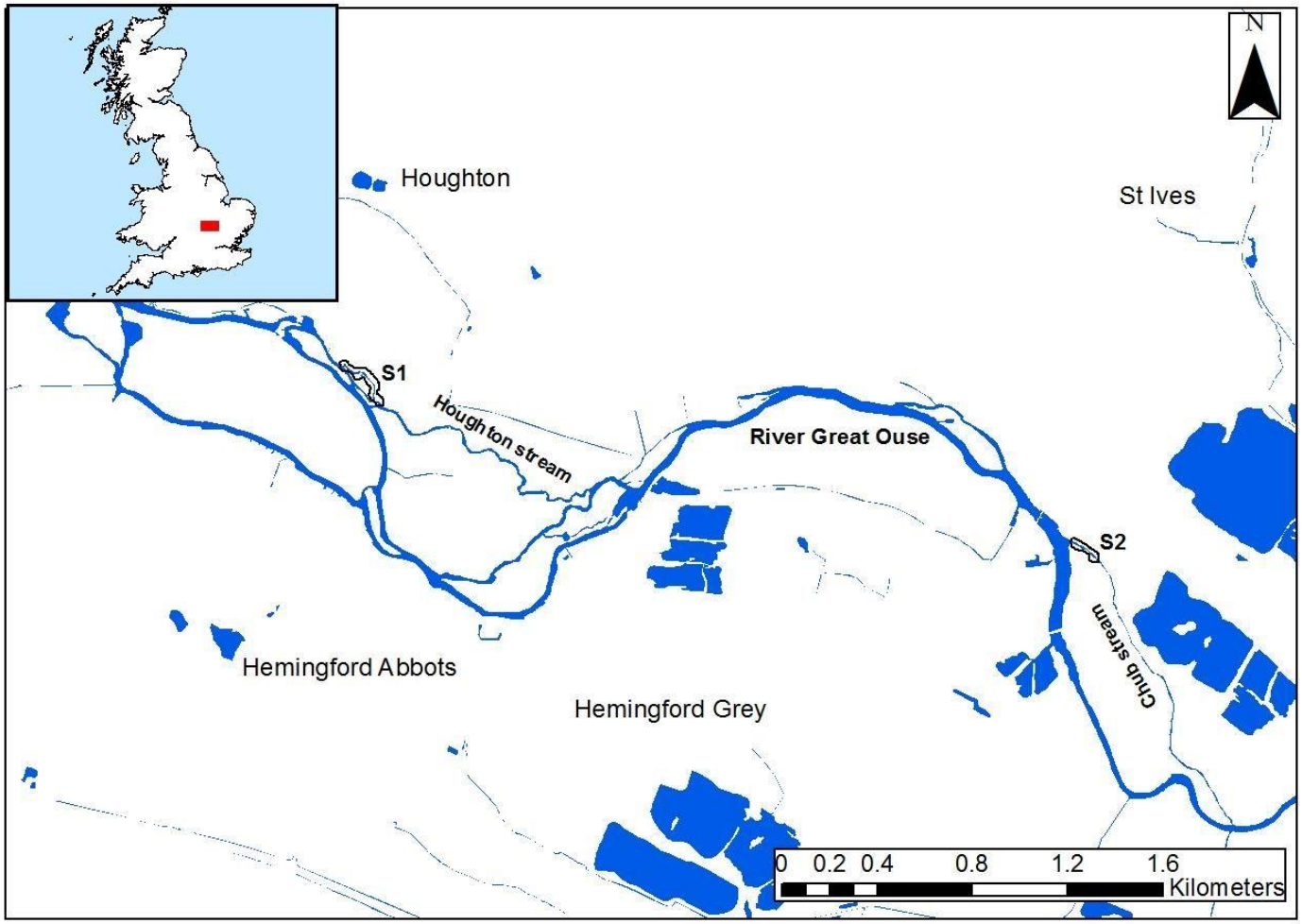

Figure 11 Streams. Inset: approximate locations in the UK where S1 represents Houghton stream and S2 Chub stream's study area (Ordnance survey 2005; Ordnance survey 2015b).

4.3.3 Lowland rivers

This final step was to assess the trophic niche breadths and overlaps of *B. barbus* and *S. cephalus* in large lowland rivers to determine whether patterns observed at smaller spatial scales in the mesocosm experiment and river channels were also apparent in more complex communities. Three rivers were used, two sections of the River Great Ouse (the focal river; Section 5.3.1), the River Lea and River Avon. All rivers have received regular stockings of hatchery-reared *B. barbus* in recent years. The Lea and Great Ouse also have indigenous populations but the Avon population is non-indigenous but established for over 100 years (Antognazza et al. 2016).

The two sites on the Great Ouse were at Newport Pagnell (Site 1: 52.088232,-0.714125; Figures 12, 14) and Odell (Site 2: 52.209929,-0.584748; Figures 13, 14). Both sites were a reach of river of approximately 100 m in length and up to 20 m wide, and comprising a large pool-riffle habitat. The site on the River Lea was at Batford (51.821735,-0.337205, Figure 15), with the sampled area being approximately 100 m in length, with widths to 12 m and the habitat comprising smooth flowing glides of up to 2.5 m depth. Both of these rivers were sampled by electric fishing from a boat in July 2014. The data collected was as described for the side channels, although an invertebrate baseline was unable to be collected from the River Lea. At the two Great Ouse sites, crayfish traps were also set (10 traps for 24 hours) to collect samples of invasive signal crayfish *P. leniusculus.* For the River Avon, samples were collected from Ellingham (50.874070,-1.804103; Figure 16) using angling, with an invertebrate baseline collected by kick-sampling. In all cases, the sizes of fish sampled from these sites were considerably larger than those used experimentally and in the side channels, with smaller fish not captured by either sampling

method. At all sites, fish lengths were recorded (fork length, nearest mm) and scale samples taken, with these scales used in the stable isotope analysis. As the stable isotope metrics of trophic niche size (as SEAc) and trophic overlap were being compared between the *B. barbus* and *S. cephalus* within each site then there was no requirement to correct the data, with all stable isotope analyses as per the mesocosm experiment.

Figure 12 Sampling site at Newport Pagnell, River Great Ouse.

Figure 13 Sampling site at Odell, River Great Ouse.

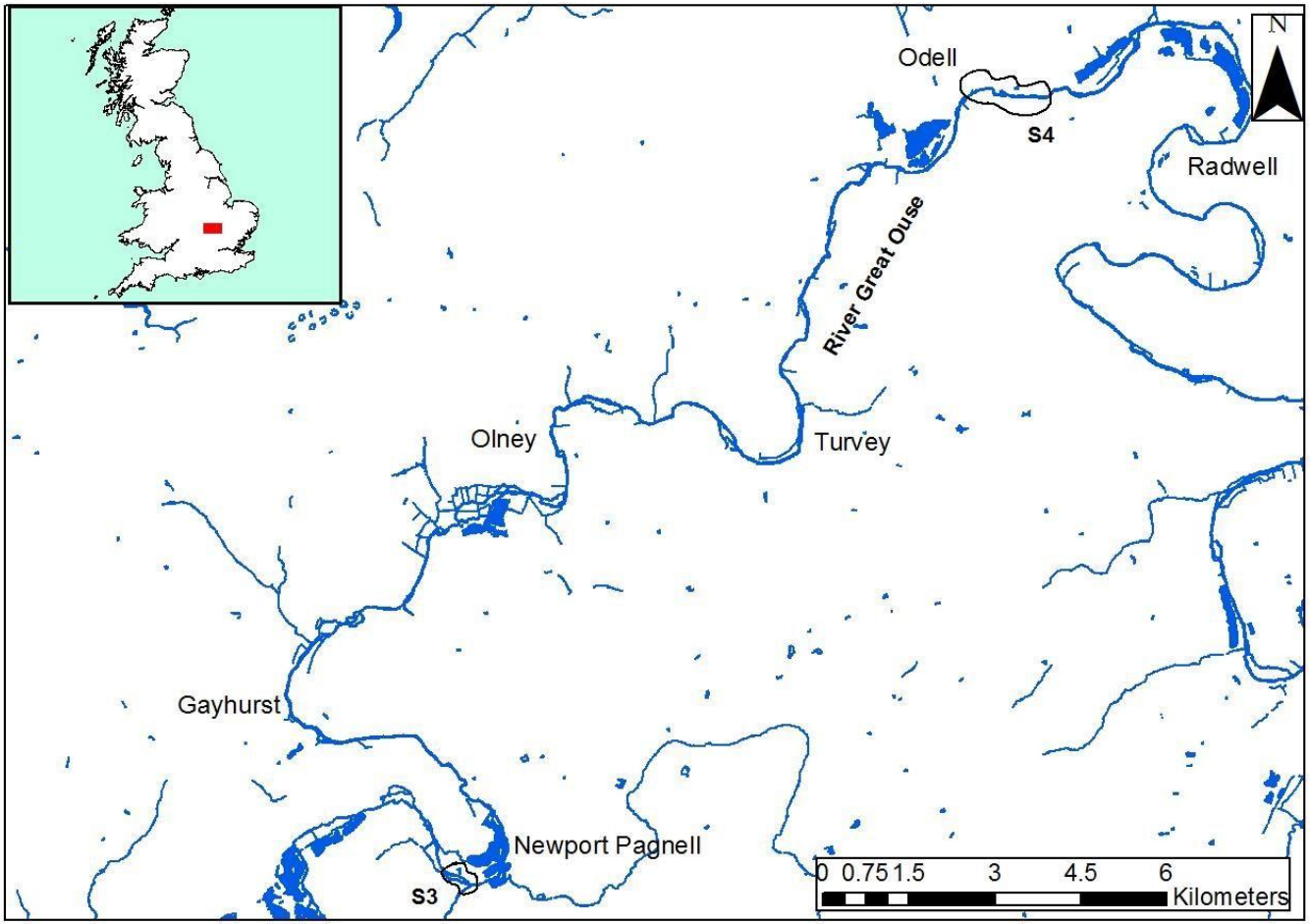

Figure 14 Lowland rivers: Inset: approximate location in the UK where S3 represents Newport Pagnell's study area and S4 Odell's study area (Ordnance survey 2005; Ordnance survey, 2015c).

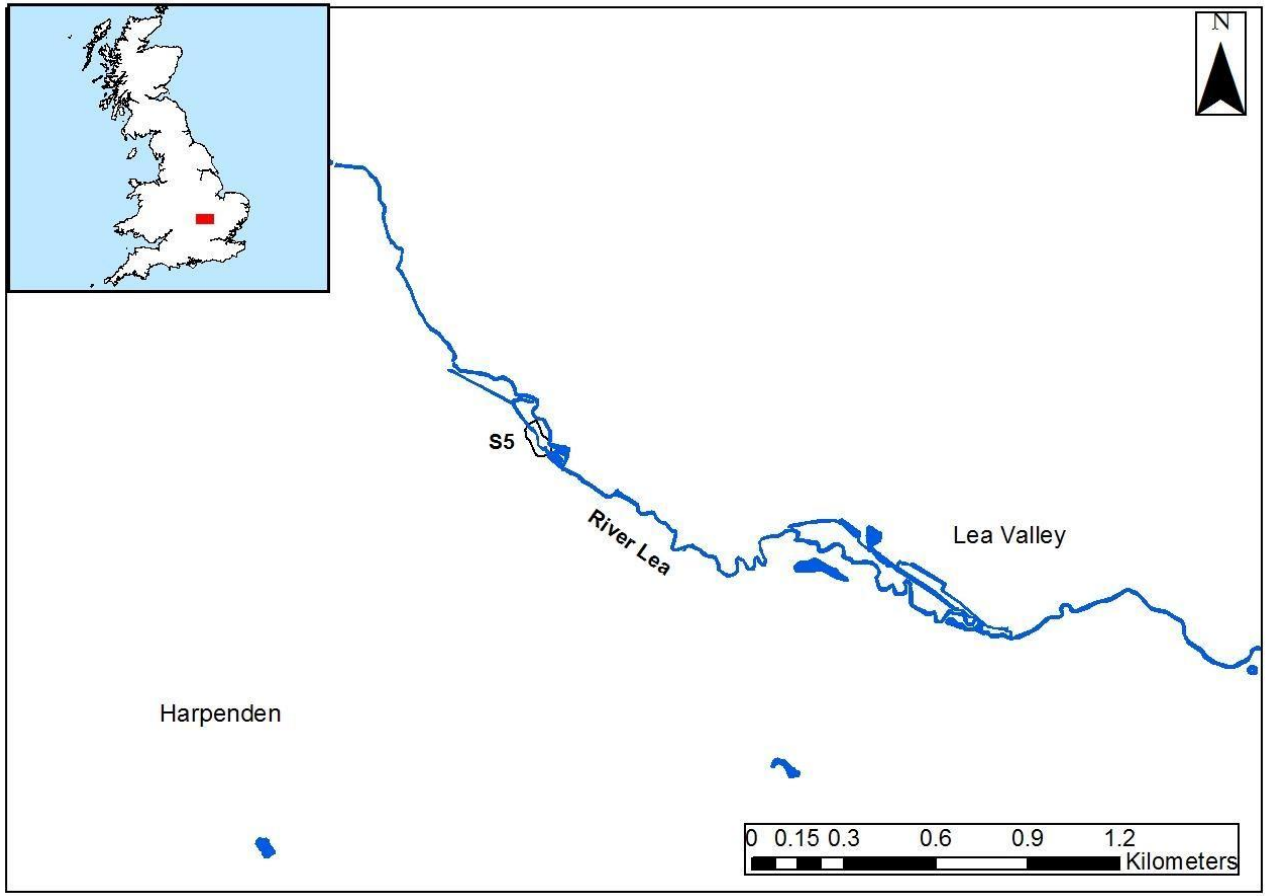

Figure 15 Lowland rivers: Inset: approximate location in the UK where S5 represents River Lea's study area (Ordnance survey 2005; Ordnance survey, 2015d).

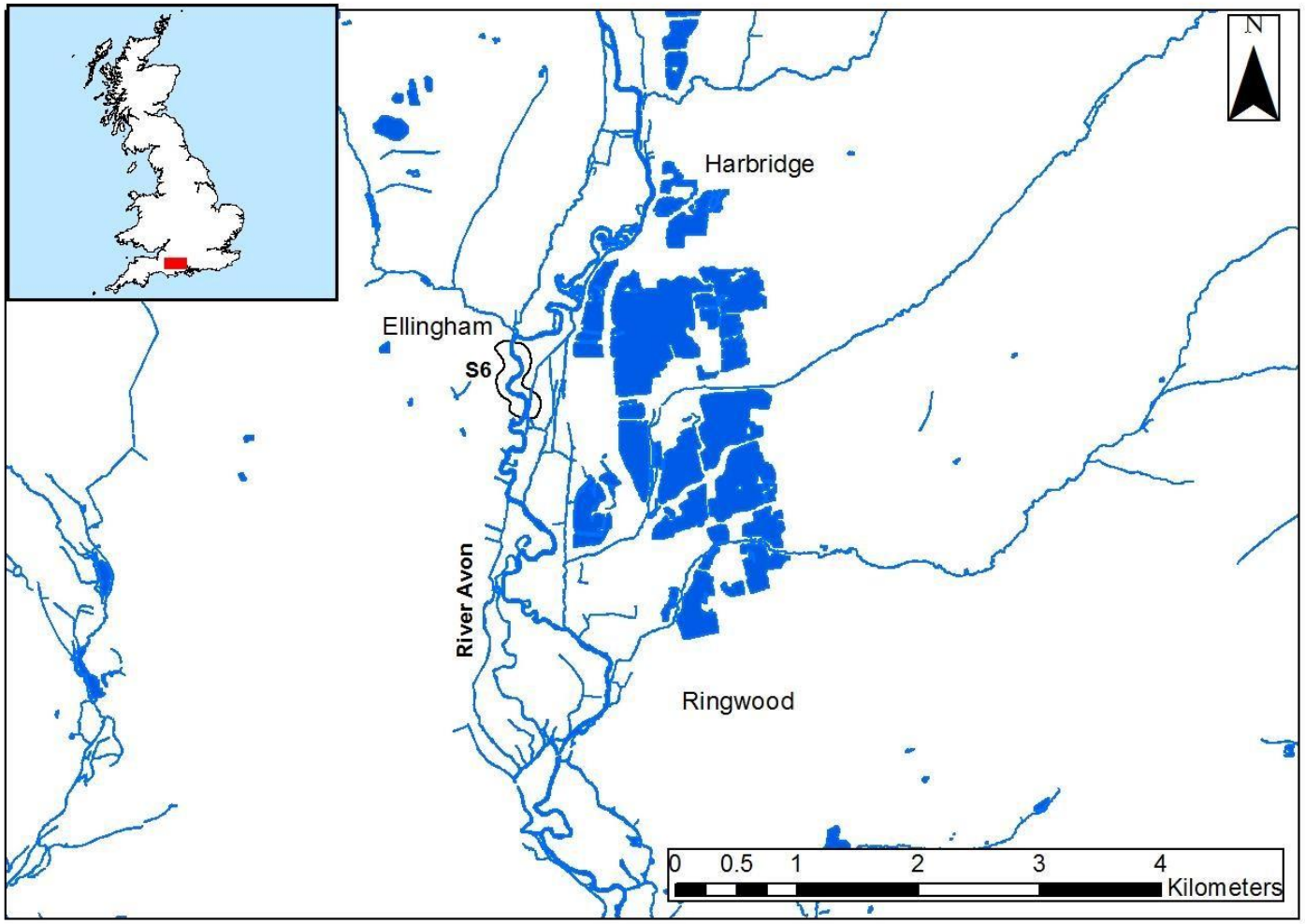

Figure 16 Lowland rivers: Inset: approximate location in the UK where S6 represents River Avon's study area (Ordnance survey 2005; Ordnance survey 2015e).

4.4 Results

4.4.1 Pond mesocosm experiment

There were no significant differences in the starting length ranges of the fish between treatments (linear mixed model, $P > 0.05$; Table 6). At the end of the experiment, 95 % of the fish introduced into the enclosures were recovered. The LMEM testing for differences in the final lengths of these fishes revealed that the overall model was significant ($P < 0.01$), with pairwise comparisons indicating the significant differences were only between *B. barbus* and *S. cephalus*, irrespective of the treatment ($P < 0.05$ in allopatry and $P < 0.01$ in sympatry). There were no significant differences in the final lengths of the species between the allopatric and sympatric contexts ($P > 0.05$; Table 6). When converted to IL, the 95 % confidence range for *B. barbus* in allopatry was 0.98 to 1.10 mm d^{-1} and in sympatry 0.98 to 1.09 mm d^{-1}. For *S. cephalus*, this was 1.01 to 1.17 mm d^{-1} in allopatry and 1.02 to 1.17 mm d^{-1} in sympatry, indicating no significant differences in growth rates in each species between treatments.

The influence of species and treatment on the stable isotope data was significant for both $\delta^{13}C$ and $\delta^{15}N$ ($P < 0.01$ in all cases; Table 7). For $\delta^{13}C$, significant differences between the species were evident between their allopatric contexts and when they were in sympatry ($P < 0.01$, Tables 6, 7); *S. cephalus* was depleted in $\delta^{13}C$ compared to *B. barbus*. For $\delta^{15}N$, when analysed as trophic position, there was a significant difference between the species in allopatry ($P < 0.01$). There was no significant difference in TP between the species in sympatry ($P > 0.05$; Tables 6, 7). Regarding SEAc, both species had larger trophic niches in allopatry than in sympatry, with no overlap between them in both contexts (Table 6; Figure 17). Additionally, *B. barbus* had a considerably larger trophic niche than *S. cephalus* in both allopatry and sympatry (Table 6).

Table 6 Number of fishes analysed for stable isotopes, the mean starting fork lengths (as estimated marginal means from the linear mixed model) and mean incremental lengths (IL; as estimated marginal means from the generalized linear model), mean δ13C, mean $\delta^{15}N$, trophic position (TP) and trophic niche size (as standard ellipse area corrected for small sample size, SEA_c) of *Barbus barbus* and *Squalius cephalus* at the conclusion of the second pond mesocosm experiment and the extent to which *B. barbus* trophic niche overlapped (%) with *S. cephalus*. Error around the mean represents standard error.

Species	**Treatment**	**n**	**Mean starting length (mm)**	**Mean IL (mm d^{-1})**	**Mean $\delta^{13}C$ (‰)**	**Mean $\delta^{15}N$ (‰)**	**Mean TP (‰)**	**SEA_C ($‰^2$)**	**Overlap (%)**
B. barbus	Allopatry	18	77.6 ± 0.96	0.34 ± 0.03	-28.2 ± 0.20	11.2 ± 0.05	2.79 ± 0.02	0.56	
	Sympatry	15	77.5 ± 1.31	0.41 ± 0.03	-29.1 ± 0.11	10.8 ± 0.05	2.68 ± 0.02	0.31	0
S. cephalus	Allopatry	17	73.9 ± 1.22	0.45 ± 0.05	-30.3 ± 0.19	10.7 ± 0.05	2.66 ± 0.02	0.54	
	Sympatry	15	76.1 ± 1.60	0.50 ± 0.01	-30.7 ± 0.14	10.8 ± 0.03	2.68 ± 0.01	0.21	0

Table 7 Outputs and significance of the final linear mixed models testing the differences in mean $\delta^{13}C$ and trophic position (TP) between the species across the mesocosm experiment, where mesocosm was the random effect on the intercept. Mean differences are from estimated marginal means (difference significant at * $P < 0.05$ and ** $P < 0.01$).

Final model structure (and result):

$\delta^{13}C$ ~ species x experimental treatment (AIC = 141.8; log likelihood = -64.9; $P < 0.01$)

Trophic position x species x experimental treatment (AIC = - 178.9; log likelihood = 95.4; $P < 0.01$)

Pairwise comparison		**Mean difference in $\delta^{13}C$**	**Mean difference in TP**
Allopatric *B. barbus*	Allopatric *S. cephalus*	2.12 ± 0.36, $P < 0.01$**	0.13 ±0.03, $P < 0.01$**
	Sympatric with *S. cephalus*	0.85 ± 0.36, $P > 0.05$	0.11 ± 0.03, $P < 0.05$*
Allopatric *S. cephalus*	Sympatric with *B. barbus*	0.36 ± 0.36, $P > 0.05$	0.02 ± 0.03, $P > 0.05$
B. barbus in sympatry with *S. cephalus*		1.63 ± 0.23, $P < 0.01$**	0.004 ± 0.02, $P > 0.05$

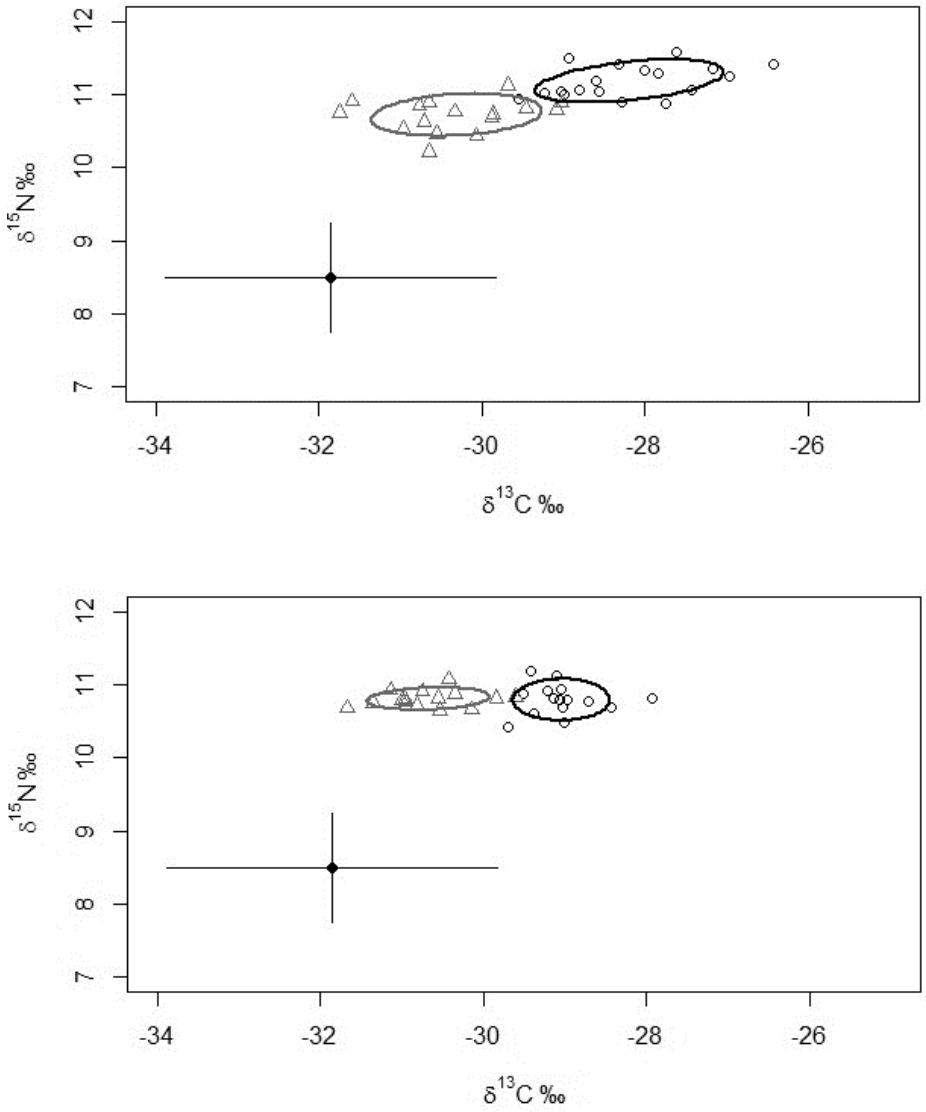

Figure 17 Stable isotope bi-plots for the mesocosm experiment, where (○) *B. barbus* individuals, (Δ) *S. cephalus* individuals and (●) mean (± SE) values of putative macro-invertebrate food resources. Solid lines enclose the standard ellipse areas for each species, where black: *B. barbus*, dark grey: *S. cephalus*. Top: species in allopatry; Bottom: species in sympatry.

4.4.2 Side channels of a lowland river

Across the surveys of both side channels, three species were used, *B. barbus*, *S. cephalus* and dace *L. leuciscus* (Table 8). Whilst the fish were considerably larger than used in the mesocosm experiments, mean lengths per species were all between 151 and 217 mm (Tables 6, 8). Sample sizes tended to be small, especially for *B. barbus*, where only 10 stocked fish were captured in subsequent sampling in the Houghton Stream and 19 in the Chub Stream (Table 9). Although there was some temporal variability in the stable isotope data in each stream, there was a general pattern of minimal trophic overlap between stocked *B. barbus* and the resident *S. cephalus* and *L. leuciscus* (< 1 %) in both years following their initial introductions especially (Table 8; Figures 18, 19), with this particularly apparent in samples collected in 2015.

Table 8 Date of sampling, species, sample sizes, mean fork lengths, mean $\delta^{13}C$ and mean $\delta^{15}N$ of fish and their trophic niche size (SEAc*; values obtained from data corrected for baseline variations across treatments.) and the extent to which *Barbus barbus* trophic niche overlaps (%) with other fish species in the community (*Squalius cephalus* and *Leuciscus leuciscus*), at (a) Chub stream and (b) Houghton stream. Error around the mean is standard error.

(a)

Date	**Species**	**n**	**Mean length (mm)**	**Mean $\delta^{13}C$ (‰)**	**Mean $\delta^{15}N$ (‰)**	**SEAc (‰^{2})***	**Overlap (%)**
June	*B. barbus*	7	209.9 ± 9.9	-27.1 ± 0.3	16.2 ± 0.2	0.06	
2014	*S.cephalus*	7	217.4 ± 5.7	-26.4 ± 0.3	14.7 ± 0.3	0.11	< 0.01
	L. leuciscus	7	203.1 ± 2.6	-28.1 ± 0.4	17.0 ± 0.3	0.24	0.40
June	*B. barbus*	8	151.1 ± 6.5	-22.3 ± 0.9	13.3 ± 0.8	1.66	
2015	*S.cephalus*	8	153.6 ± 8.0	-26.4 ± 0.4	16.6 ± 0.4	0.90	0
	L. leuciscus	8	152.6 ± 9.6	-27.9 ± 0.2	17.1 ± 0.3	0.44	0
Sept 2015	*B. barbus*	4	212 ± 20.9	-27.5 ± 0.1	18.6 ± 0.4	0.16	
	S. cephalus	6	209.2 ±15.3	-26.9 ± 0.1	17.8 ± 0.5	0.30	0
	L. leuciscus	6	184.8 ± 6.6	-28.2 ± 0.1	18.4 ± 0.3	0.31	0
(b)							
June	*B. barbus*	4	185.3 ± 9.2	-28.2 ± 0.4	17.1 ± 0.5	0.12	
2014	*S. cephalus*	6	194.8 ± 6.2	-27.3 ± 1.0	16.0 ± 0.8	1.07	0.58
	L. leuciscus	6	191.7 ± 3.9	-28.7 ± 0.1	17.9 ± 0.1	0.05	0.17
June	*B. barbus*	6	159.0 ± 8.8	-22.8 ± 0.3	13.4 ± 0.4	0.77	
2015	*S.cephalus*	5	198.4 ±23.7	-27.5 ± 0.2	17.7 ± 0.3	0.28	0
	L. leuciscus	6	161.7 ±15.1	-28.4 ± 0.5	17.8 ± 0.1	0.20	0

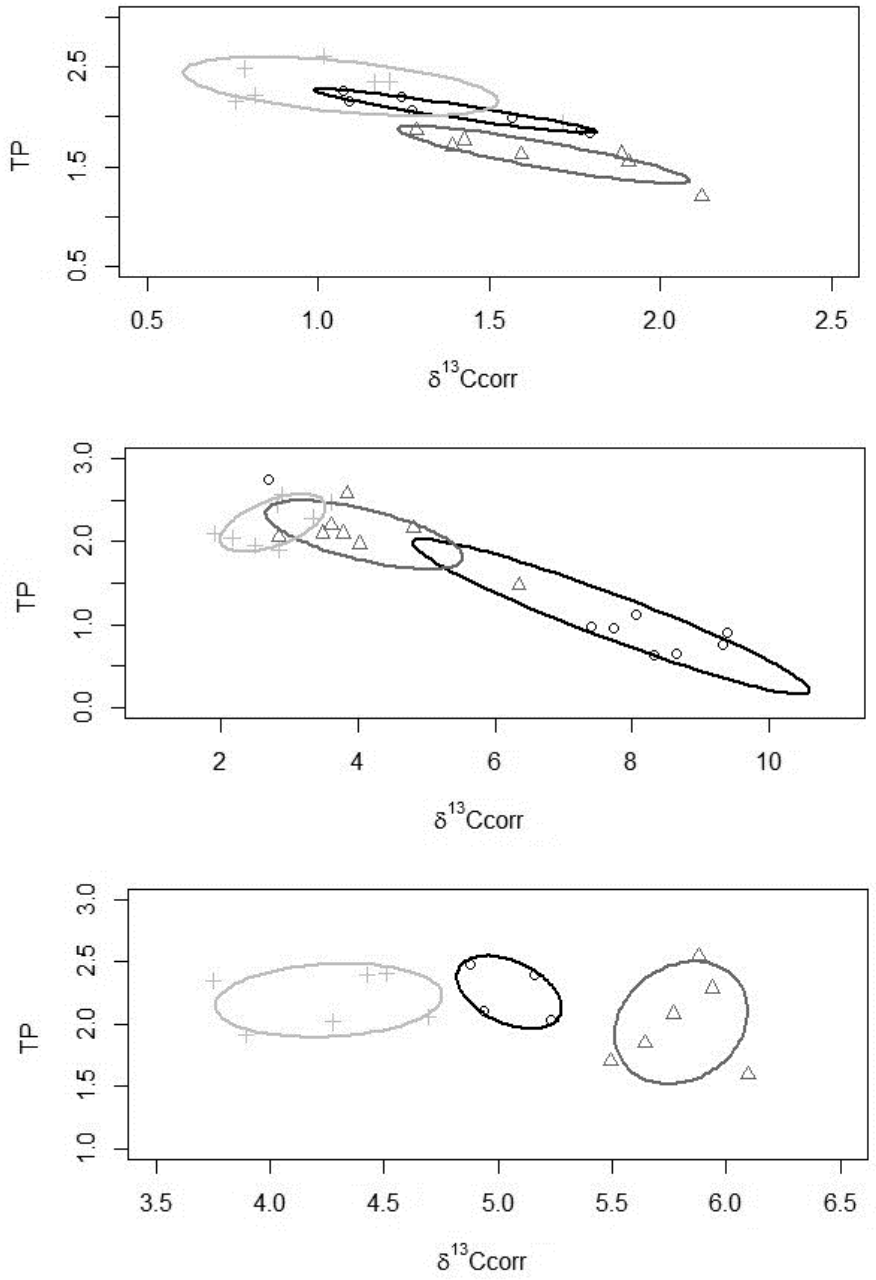

Figure 18 Stable isotope bi-plots for the Chub stream where (○) *Barbus Barbus* individuals, (Δ) *Squalius cephalus* individuals and (+) *Leuciscus leuciscus* individuals. Solid lines enclose the standard ellipse areas for each species, where black: *B. barbus*, dark grey: *S. cephalus*, light grey: *L. leuciscus*. Note the different scales on the axes. Top: June/August 2014; Middle: June 2015; Bottom: September 2015.

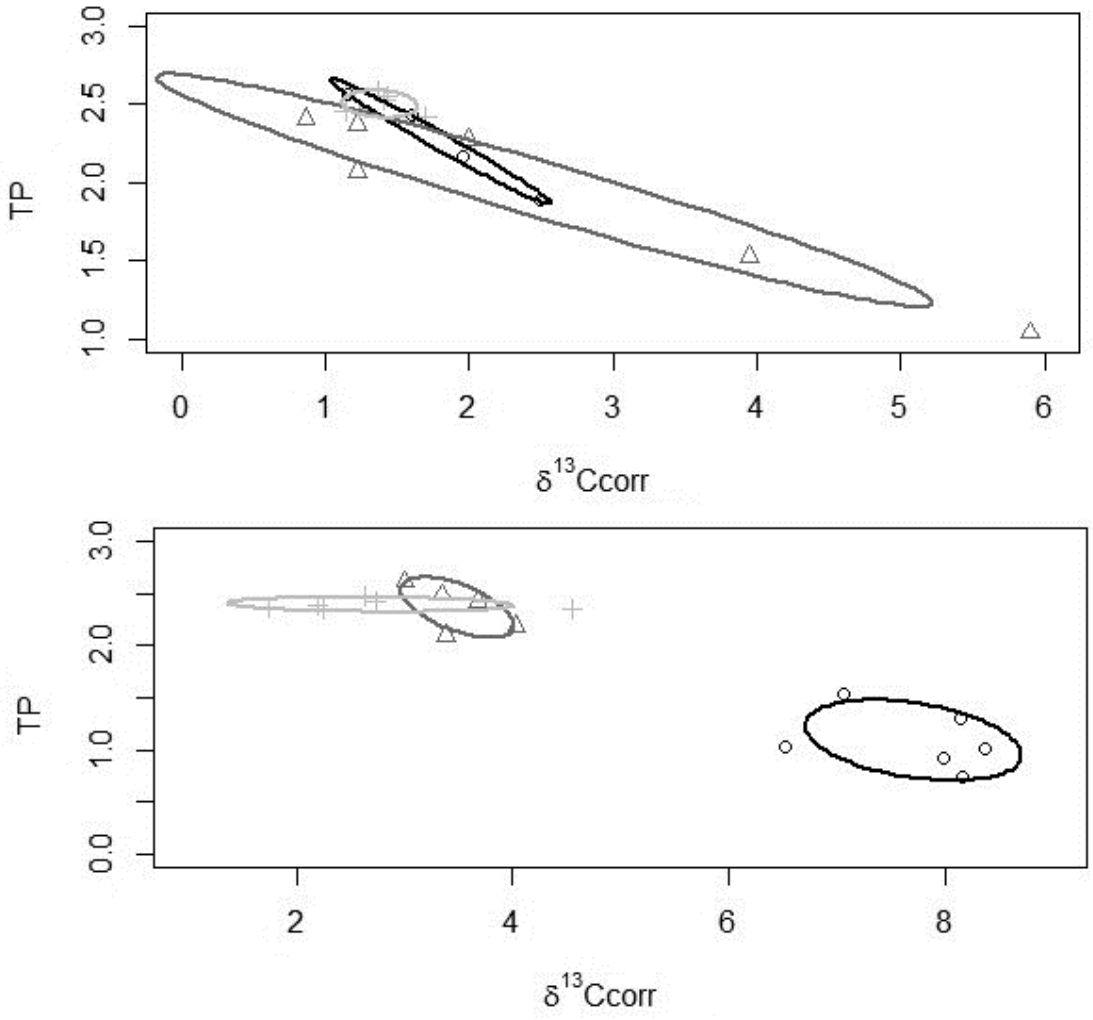

Figure 19 Stable isotope bi-plots for the Houghton stream where (○) *Barbus barbus* individuals, (Δ) *Squalius cephalus* individuals and (+) *Leuciscus leuciscus* individuals. Solid lines enclose the standard ellipse areas for each species, where black: *B. barbus*, dark grey: *S. cephalus*, light grey: *L. leuciscus*. Note the different scales on the axes. Top: June/August 2014; Bottom: June 2015.

4.4.3 Lowland rivers

The fish sampled across the three rivers tended to be the largest used in the study, with some *B. barbus* present in samples > 600 mm (Table 9). In the River Lea, two size classes of *B. barbus* and *S. cephalus* were present and so were analysed separately (due to the results from Chapter 2). As with the mesocosm experiment and the side channels, the extent of the trophic overlap of *B. barbus* with other cyprinid species was minimal, including across both size ranges in the River Lea, indicating this was consistent over spatial scales and fish size structure (Table 9; Figures 20, 21).

Table 9 Species, sample sizes, mean fork lengths, mean $\delta^{13}C$ and mean $\delta^{15}N$ of sampled fish, their trophic niche breadth (SEA_c) and the extent to which *Barbus barbus* trophic niche overlaps (%) with other sampled fishes (*Squalius. cephalus* and *Leuciscus leuciscus*). Error around the mean is standard error.

Site	**Species**	**n**	**Mean length (mm)**	**Mean $\delta^{13}C$ (‰)**	**Mean $\delta^{15}N$ (‰)**	**SEAc (‰^{2})**	**Overlap (%)**
Site 1, Great Ouse	*B. barbus*	7	162.6 ± 44.9	-29.1 ± 0.2	20 ± 0.5	2.54	
	S.cephalus	6	290.2 ± 70.4	-26.5 ± 0.3	20.3 ± 0.8	4.85	0
	L. leuciscus	5	138.4 ± 19.8	-27.0 ± 0.6	18.0 ± 0.8	3.60	< 0.01
Site 2, Great Ouse	*B. barbus*	6	252.5 ± 8.4	-27.6 ± 0.2	17.0 ± 0.2	0.79	
	S. cephalus	6	346.0 ± 39.6	-25.6 ± 0.2	16.9 ± 0.7	2.32	0
	L. leuciscus	6	167.7 ± 1.9	-26.0 ± 0.3	15.0 ± 0.5	3.16	0
Lea (> 400 mm)	*B. barbus*	10	415.1 ± 3.9	-24.3 ± 0.1	16.3 ± 0.5	2.21	
	S. cephalus	9	415.3 ± 3.8	-25.7 ± 0.1	14.2 ± 0.4	3.87	< 0.01
Lea (< 250 mm)	*B. barbus*	10	225.5 ± 4.6	-27.0 ± 0.3	19.4 ± 0.3	1.29	
	S. cephalus	10	213.9 ± 4.2	-27.0 ± 0.3	16.4 ± 0.4	1.02	0
Avon	*B. barbus*	6	586.7 ±13.8	-25.8 ± 0.4	11.2 ± 0.4	3.87	
	S. cephalus	6	531.7 ± 7.0	-22.9 ± 0.6	11.9 ± 0.3	3.38	0

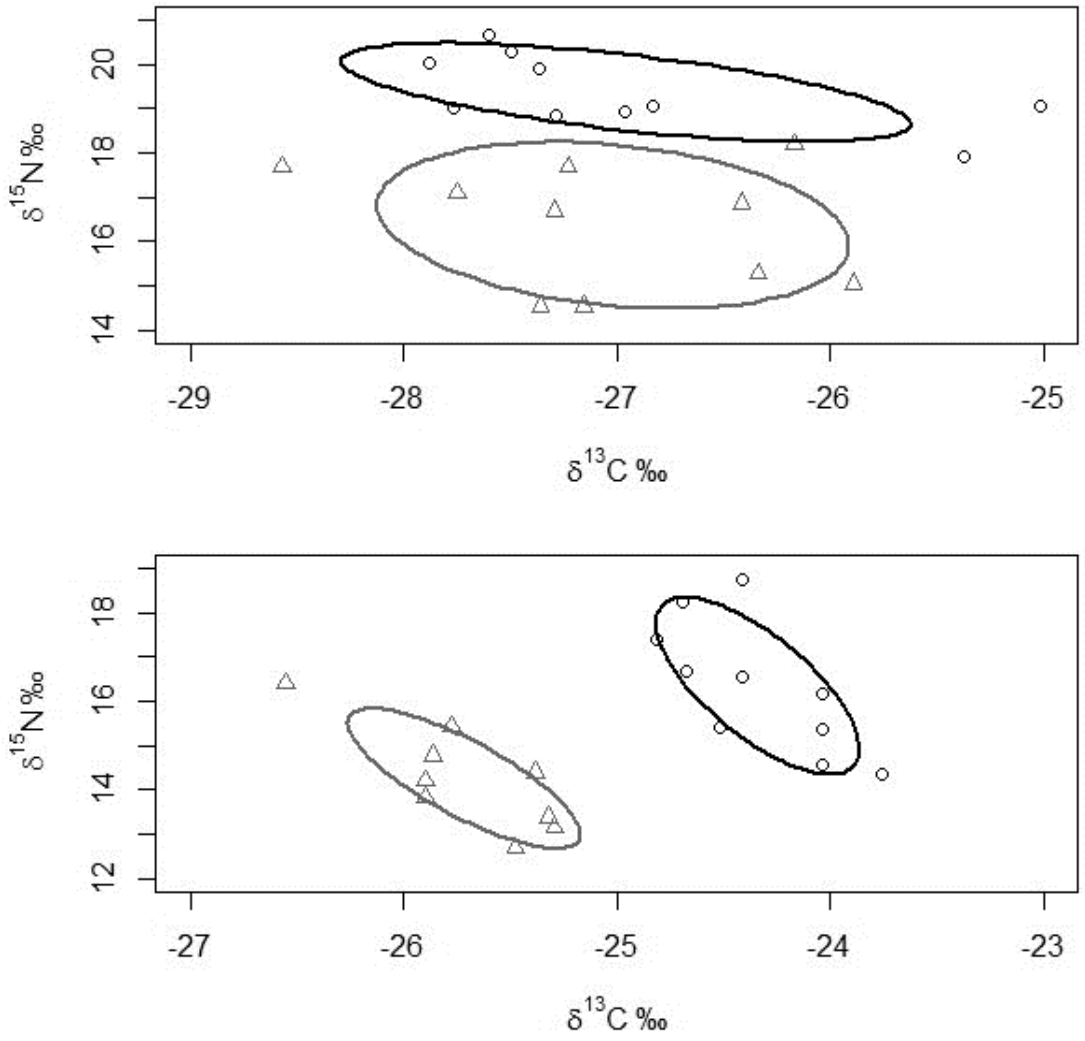

Figure 20 Stable isotope bi-plots for the River Lea where (○) *B. Barbus* individuals, (Δ) *S. cephalus* individuals. Solid lines enclose the standard ellipse areas for each species, where black: *B. barbus*, dark grey: *S. cephalus*. Note differences in scales on all axes. Top: all fish between 186 and 237 mm; Bottom: all fish between 400 and 435 mm.

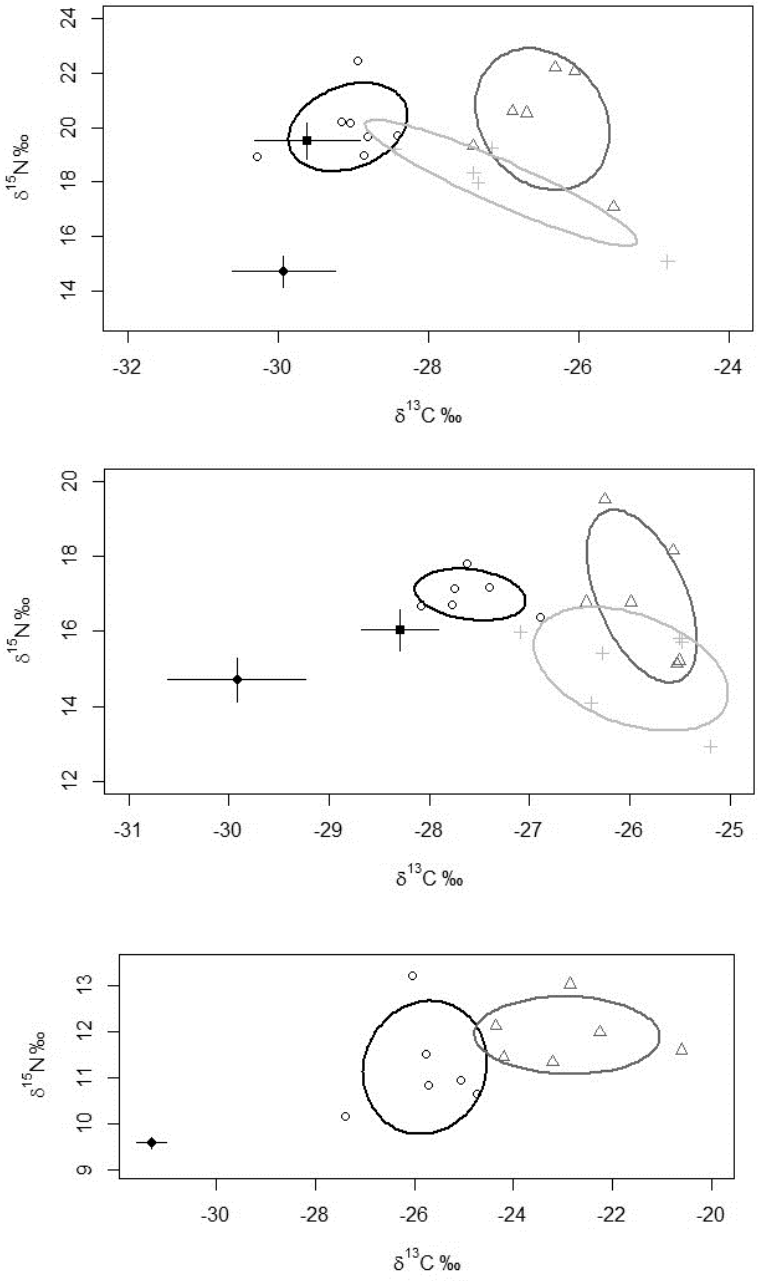

Figure 21 Stable isotope bi-plots for the Site 1 (Top) and 2 (Middle) on the Great Ouse, and the River Avon (Bottom), where (○) *Barbus barbus* individuals, (Δ) *Squalius cephalus* individuals and (+) *Leuciscus leuciscus* individuals with mean (± SE) values of putative food sources: macroinvertebrates (●) and signal crayfish (■). Solid lines enclose the standard ellipse areas for each species, where black: *B. barbus*, dark grey: *S. cephalus*, light grey: *L. leuciscus*. Note the different scales on the axes.

4.5 Discussion

Experimental and field evidence suggested that there was substantial divergence in the trophic niches of sympatric *B. barbus* and *S. cephalus*, with no evidence for resource sharing or inter-specific competition, with this consistent with the initial results of Chapter 2. This pattern was apparent over a 100 day period in the mesocosm enclosures and over a two year post-stocking period in the side channels. Moreover, when the trophic niches of these fishes were assessed at larger spatial scales in lowland rivers, this divergence was also apparent in groups of fishes of much larger body sizes, including across two distinct size ranges in the River Lea and in relatively large fishes in the River Avon. In addition, where there was data available for other fishes in the community, such as *L. leuciscus*, this pattern of trophic niche divergence with *B. barbus* was still evident.

The outputs of the allopatric treatment in the mesocosm experiment suggested that *B. barbus* rapidly established a trophic niche that was divergent from allopatric *S. cephalus*, suggesting that there would be no sharing of food resources when the species were in sympatry. When the species were in sympatry, their actual trophic niches did remain separated. However, their niche breadths were reduced in sympatry, indicating some individual specialisation (Araújo et al. 2011). This result was consistent with both the prediction and the niche variation hypothesis that predicts populations become less generalized in more competitive environments (Van Valen 1965; Human and Gordon, 1996; Olsson et al. 2009). Similar patterns of trophic niche divergence and partitioning have been detected when non-native fishes that have been introduced into similar environments. For example, the trophic niche divergence between the small, invasive fish topmouth gudgeon *P. parva* with extant species, including carp *C. carpio*, facilitates their co-existence (Jackson and Britton 2013; Tran et al. 2015).

These trophic niche outputs were also important in the context of the growth rates of the fishes. In the mesocosm experiment, the growth rates of both fishes were similar between their allopatric and sympatric treatments, despite their reduced realised trophic niche sizes. This suggests that when the fishes have access to less limiting and more abundant natural food resources, their trophic niche divergence and specialisations maintained their energetic requirements sufficiently to enable them to grow at rates that were not significantly different between the allopatric and sympatric contexts. This was contrary to the testable hypothesis that suggested increased dietary specialisation would result in decreased growth rates. This was also an important outcome given the difficulty of measuring differences in growth rates in more wild situations, where there is a wide range of abiotic factors that cause temporal and individual variability in fish growth rates (Beardsley and Britton 2012; Liu et al. 2015).

Introduced and stocked salmonid fishes often cause detrimental impacts for native salmonids. Predation by introduced lake trout (*Salvelinus namaycush*) can limit the distribution of bull trout (*Salvelinus confluentus*) (Donald and Alger 1993) and cause population declines of cutthroat trout (*Onchorhynchus clarki*) (Ruzycki et al. 2003). Their stocking can cause trophic cascades (Tronstad et al. 2010) that influence predator–prey interactions in surrounding terrestrial ecosystems (Middleton et al. 2013). For *B. barbus*, however, there was minimal evidence to suggest that their ecological interactions resulted in any substantial alteration in the trophic ecology of *S. cephalus*. It is acknowledged that the approach used within this study were relatively simple, focusing primarily on the trophic interactions of *B. barbus* with *S. cephalus*. This was to ensure that the inter-specific comparisons were being made for functionally similar fishes that grew to relatively similar body sizes and that live for similar long life spans (Britton 2007). This could, however, have resulted in some over-simplification of the

outcomes of their stocking into more complex fish communities. However, there is also no evidence of *B. barbus* sharing a trophic niche space with fishes such as *L. leuciscus*, roach *R. rutilus* and graying *T. thymallus*, both here and from other studies (e.g. Bašić and Britton 2015).

The design of the experimental and field studies meant that regular assessment of the trophic niches of the fishes in each system was not possible. Logistical constraints limited the number of treatments that could be included within the mesocosm experiment. This meant that fish numbers, i.e. density, was maintained across the experimental treatments. This was important to ensure that comparisons could be made in trophic niche sizes between species and the allopatric and sympatric contexts, as the numbers of fish involved were consistent. However, the partitioning of trophic niches between species can be related to competition for food resources and predation (Nilsson 1967) and thus patterns can change as the population abundances of the species increase (Spurgeon et al. 2014). Although the patterns of partitioning were strong in the mesocosms and were detected in the field studies, it is acknowledged that the incorporation of more complexity into the experimental designs, such as including treatments that increased fish abundance or also used fish of contrasting body sizes, might have provided greater insights. Moreover, the focus here was on the trophic relationships of the fishes, yet the impacts of stocked and invasive fishes can include other ecological issues, including habitat disturbances (Gozlan et al. 2010). Indeed, *B. barbus* act as 'zoogeomorphic agents' in rivers, as their foraging activities reduce bed material stability, increase bedload transport, and impact microtopographic roughness and sediment structure (Pledger et al. 2014, 2015). Thus, their release into rivers where populations are not currently present could have considerable effects on the substrate. By extension, their foraging activities could also impact aspects of the macro-

invertebrate communities, although again this was unable to be tested here. In addition, whilst stable isotope data can provide a powerful tool to determine trophic interactions, they are only a proxy for this. Studies that compare the diet of fishes across methods such as stable isotope analysis and stomach contents analysis often show some differences in their results (e.g. Hamidan et al. 2015). Consequently, studies that rely solely on stable isotope analysis should be evaluated with some caution (Locke et al. 2013).

The application of these data to fish stocking strategies suggest that in addition to considering the survival and establishment of the fish and their genetic introgression into extant populations, some consideration of their ecological interactions are required. Although this has been well established for native and non-native salmonid fishes (e.g. Simon and Townsend 2002), there remains a paucity of knowledge for other fishes stocked into alternative habitats such as lowland rivers. Here, the results here and from Chapter 2 suggested that, fundamentally *B. barbus* occupy a trophic niche that is distinct from some other cyprinid fishes, although their presence could result in certain diet specialization. It should be noted, however, that the stocking exercises here involved relatively low numbers of fish released on single occasions as oppose to high number of fish often dispensed during stocking (Aprahamian et al. 2004). Likewise, although the studied population in the River Avon was non-indigenous, it had been present for over 100 years and was thus well established (Antognazza et al. 2016). Consequently, these data might be a poor surrogate for the trophic interactions and consequences that might have occurred in rivers such as the Severn in western England where, following the translocation of approximately 500 fish in the 1950s, the species invaded large areas of the catchment (Wheeler and Jordan 1990), substantially altering the composition of the fish community and angler catches (Hunt 1974; Hunt and Jones

1975; North and Hickley 1989). There were also no other species of the *Barbus* genus in the rivers studied that would have been more functionally similar to *B. barbus* than *S. cephalus*. In addition, where the rivers have an indigenous *B. barbus* population, the release of hatchery-reared individuals from other river catchments can result in genetic introgression and a loss of genetic integrity (Antognazza et al. 2016).

Consequently, whilst the application of the outputs of this study suggests that there are relatively minor ecological consequences from a management activity that can provide recreational and socio-economic benefits (Britton and Pegg 2011), it is recommended that stocking policies take cognisance of a wide range of abiotic, ecological and genetic issues before commencing. These should include determining the factors why the fish stocking is required, i.e. the identification of current constraints on the fish community (Cowx 1994), and whether habitat restoration and rehabilitation would be a more appropriate management tool (Pretty et al. 2003). Only then should ecological and genetic considerations be applied to the decision of why, when and how to stock the fishes.

In summary, this chapter focusing on the trophic consequences of stocking of *B. barbus* using hatchery-reared fish detected negligible impacts on other fishes, such as *S. cephalus*. However, the results of Antognazza et al. (2016), indicating a loss of genetic integrity in stocked *B. barbus* populations, which allied with the low recapture rates of stocked fish in the side channels, suggests that enhancement stocking in this manner is of limited utility for enhancing the *B. barbus* population of the River Great Ouse. Therefore, subsequent chapters will switch focus to the physical habitats for *B. barbus* provided by this river, with focus on determining spawning habitat conditions and their potential consequences for spawning success and recruitment.

5. Characteristics of *B. barbus* spawning substrata: a case study of the River Great Ouse

5.1 Summary

Habitat availability and suitability dictate fish species viability in freshwater systems. This is particularly profound in lithophilic fishes that are dependent on the hyporheic zone during early development. Thus, substrate conditions and water quality in the hyporheic layer impact egg-to-emergence survival and larval development, with fines content and oxygen concentration particularly important. With most work done on Salmonid fishes, there is minimal information on other lithophiles, especially those that spawn during higher temperatures and lower flows. Therefore, the conditions of spawning substrates of the lithophilic spawner *B. barbus* were assessed in the River Great Ouse, England, through assessment of 13 riffles in the upper river in summer 2014 and 2015. Surface and subsurface substrates were assessed and expressed using common parameters (D5, D50 and D95 percentiles, mean, sorting, skewness and kurtosis), with emphasis on fine sediment contents. The extent of each riffle was also assessed, coupled with depth and flow measures under conditions similar to those at the time of *B. barbus* spawning. Additionally, surface and hyporheic water conditions were assessed at three depths (10, 20 and 30 cm). Results indicated that spawning habitats were generally shallow (< 0.5 m), with fast flows and high oxygen content in surface and hyporheic layers. Surface substrates were well sorted with low concentrations of fine sediment and were not too coarse for redd excavation. However, subsurface sediments were characterized by high levels of fine sediments, particularly sand, indicating a potential detrimental impact on *B. barbus* egg-to-emergence survival and

larval emergence. The potential implications of these fine subsurface sediments are discussed and then explored in subsequent chapters.

5.2 Introduction

Regardless of life stage, habitat availability and suitability are considered the two most important factors determining populations' viability in freshwater systems (Kondolf 2000; Bond and Lake 2003). Fish are important indicators of ecosystem health (Karr 1991; Directive 2000/60/EC) and are strongly influenced by habitat quality, particularly during early development when mobility is restricted (Balon 1975; Cunjak et al. 1998; Noble et al. 2007). As different fish species display different reproductive strategies, they have been associated with disparate ecological guilds, primarily in respect to the habitats they utilise during spawning (Balon 1975; Aart and Nienhuis 2003; Noble et al. 2007). Amongst these reproductive guilds, lithophils are recognized as particularly sensitive to habitat degradation (Balon 1975), hence their importance within river habitat assessment metrics, such as the European Fish Index (Pont el al. 2006; Noble et al. 2007; Pont et al. 2007). The majority of fish-based metrics are calculated using data on presence/ absence and abundance, with only semi-quantitative assessments of surface water quality and river geomorphology. However, numerous abiotic and biotic factors are known to affect selection of suitable spawning grounds by certain lithophilic fish (Montgomery et al. 1999; Buffington et al. 2004), ranging from microhabitat characteristics (e.g. depth, velocity, grain size and inter-gravel flow) that are determined by channel-specific geomorphology and hydraulics (Lisle 1989; Montgomery et al. 1999; Moir et al. 2004; Cienciala and Hassan 2013) to competitive abilities (Montgomery et al. 1999). These properties are often precluded or modelled inappropriately within habitat assessment metrics (Guisan and Thuiller 2005).

As suitable spawning habitat is a prerequisite for successful reproduction, activities or events that degrade spawning substrata are likely to have negative implications for species viability. For example, lithophils and their habitats are profoundly influenced by anthropogenic inputs of fine sediments into freshwater systems, particularly via intensive agriculture and forestry (Wood and Armitage 1997; Jensen et al. 2009; Kemp et al. 2011), with a body of work focusing mainly on salmonids (Kemp et al. 2011). For example, fine sediment deposition within spawning and nursery grounds can reduce egg-to-emergence survival and affect larvae emergence and development (e.g. Levasseur et al. 2006; Kemp et al. 2011; Sear et al. 2014; Sear et al. 2016). Specifically, high concentrations of fines can consolidate the bed and so prevent fish from digging redds (Zeh and Dönni 1994), reduce interstitial flows and oxygen permeability to the egg pocket and within spawning gravels respectively (Soluslby et al. 2001; Greig et al. 2007; Pulg et al. 2013), and lead to the death of embryos (Greig et al. 2005a, Greig et al. 2005b; Sear et al. 2014). Furthermore, excessive sedimentation can inhibit or delay larval emergence and deform larvae during development (Kemp et al. 2011; Franssen et al. 2012; Sear et al. 2016).

Some lithophilic, non-salmonid species require spawning conditions that are similar to those utilised by salmonids. It is therefore reasonable to assume that these species might also be affected by sediment composition, specifically fines content, particularly because many species spawn in summer when flow conditions are generally low and temperatures and rates of sedimentation tend to be high. Some relevant species in Europe include *C. gobio*, *A. alosa*, *Alosa fallax*, *Lampetra spp*. and *B. barbus* (Kemp et al. 2011). Considering the high ecological and socio-economic value of *B. barbus* in Britain, several studies have focused on species distribution, habitat use and ecology, including the impacts of habitat fragmentation, pollution and parasites on the viability

of in-situ populations (Britton and Pegg 2011). However, the role of suitable spawning habitats and the importance of habitat restoration schemes on their reproductive success have not been investigated. Consequently, it is reasonable to assume that species that spawn when temperatures and sedimentation rates are high are likely more susceptible to the adverse effects of degraded spawning substrates than winter-spawning salmonids (Sternecker et al. 2014). Albeit the period which is spent in the gravel will be considerably shorter for *B. barbus* due to more rapid development (Wijmans 2007; Kemp et al. 2011), which could mitigate some of the deleterious effects of fines rich substrates.

The aim of this chapter was to thus explore the habitat characteristics of *B. barbus* spawning substrates, with special emphasis on the nature and availability of spawning grounds within the River Great Ouse, England. By using *B. barbus* as the focal species, the habitats of other rheophilic species, including *S. cephalus* (Balon 1975; Pinder 1997; Arlinghaus and Wolter 2003), are also assessed. Objectives (O) were to determine, within the River Great Ouse, the:

(O1) typical river-bed sediment characteristics (surface and subsurface sediment composition: D5, D50, D84, D95, mean, sorting, skewness and kurtosis; % of fine sediment and organic matter) of substrates which *B. barbus* utilises for spawning;
(O2) typical hydraulics conditions (water depth, channel width, riffle width and length, 0.6 depth velocity, near-bed velocity, river bed and water surface slope, reach mean shear stress, critical shear stress, mobility ratio and Reynolds number) of flows which *B. barbus* utilises for spawning; and

(O3) typical water quality conditions in *B. barbus* spawning substrates (temperature, dissolved oxygen concentration, pH, conductivity, and concentration of ammonium in the surface and hyporheic water layers).

5.3 Materials and methods

5.3.1 River Great Ouse

The River Great Ouse drains an estimated catchment area of 8600 km^2 (Pinder et al. 1997). This area represents one of the largest watersheds in England (Pinder et al. 1997). The river's source is in central England, from where it flows through the towns of Buckingham, Milton Keynes, Bedford and Huntingdon, finishing in the Wash at King's Lynn (Pinder et al. 1997). In a UK context, mean annual rainfall is relatively low (< 0.63 cm y^{-1}; Pinder et al. 1997) and the Ouse is predominately fed by groundwater sources (Neal et al. 2000).

The River Great Ouse has undergone flow regulations for almost 1000 years, with a peak in regulation during the 17^h century, when the main drainage of the fens occurred, resulting in production of arable land (Pinder et al. 1997). This was coupled with substantial river regulation for flood defence purposes, particularly below Bedford (Garner 2010), rendering the Great Ouse amongst the most regulated rivers in the UK (Pinder et al. 1997; Pinder 1997; Garner 2010). It is also one of the most degraded river systems due to agricultural inputs, particularly nitrates (Neal et al. 2000). The Great Ouse basin has a population of approximately 1,600,000 people. However, population density is relatively low with rural areas dominating. Consequently, agriculture is the main type of land use, including but not limited to wheat, sugar beet, barley and oats production. Major towns such as Bedford, Cambridge and Milton Keynes have minor

industries, such as production of farm implements, brewing and brick industry (Neal et al. 2000). The mean river discharge can be low particularly during summer and where fields are subject to intensive farming, which could have detrimental impacts upon the aquatic ecosystem (Neal et al. 2000).

In terms of fish fauna, the Great Ouse was once a system of high biological diversity, hence its popularity amongst recreational anglers, although this has diminished over time due to poor catch returns (Copp 1990; Pinder 1997; Pinder et al. 1997). Historically reported species have either completely disappeared, such as the pelagic spawner burbot *Lota lota,* or have had their abundance and distribution reduced, as is the case for many rheophilic and limnophilic cyprinids (e.g. *B. barbus*, common bream *Abramis brama*, bleak *Alburnus alburnus*; Copp 1990; Pinder 1997). Present day fish communities are dominated by a low number of generalist species, particularly *R. rutilus,* which are present in high numbers (Copp 1990; Pinder 1997; Garner 2010). Extensive river regulations and their impacts upon aquatic habitats are considered responsible for river-wide decreases in fish fauna (Copp 1990; Pinder 1997; Garner 2010).

5.3.2 Sampling areas and data collection

In the summer of 2014 and 2015 (August and September), following the completion of *B. barbus* spawning activities, 13 spawning riffles in the upper section of the River Great Ouse were sampled to quantify the size distributions of surface and subsurface sediments (Figures 22). Additionally, at six historically known *B. barbus* spawning sites (see Table 11), physicochemical properties of surface and hyporheic water were determined at three depths (10, 20 and 30 cm), which cover the range of depths at which salmonids and cyprinids lay their eggs (Van den Berghe and Gross 1984; Lisle 1989,

Montgomery et al. 1996; Wijmans 2007). Work was conducted under summertime baseflow conditions for ecological relevance, with conditions similar to those at the time of *B. barbus* spawning (Young et al. 1989; Wijmans 2007; Denic and Geist 2015).

Study sites were selected using a strict set of criteria; they needed to be able to be waded under baseflow conditions and be either natural spawning sites of *B. barbus* which previous work confirmed (*cf.* Twine 2013), or representative of spawning sites described within literature (Baras 1992; Banarescu and Bogutskaya 2003; Wijmans 2007), in terms of hydraulic and substrate conditions.

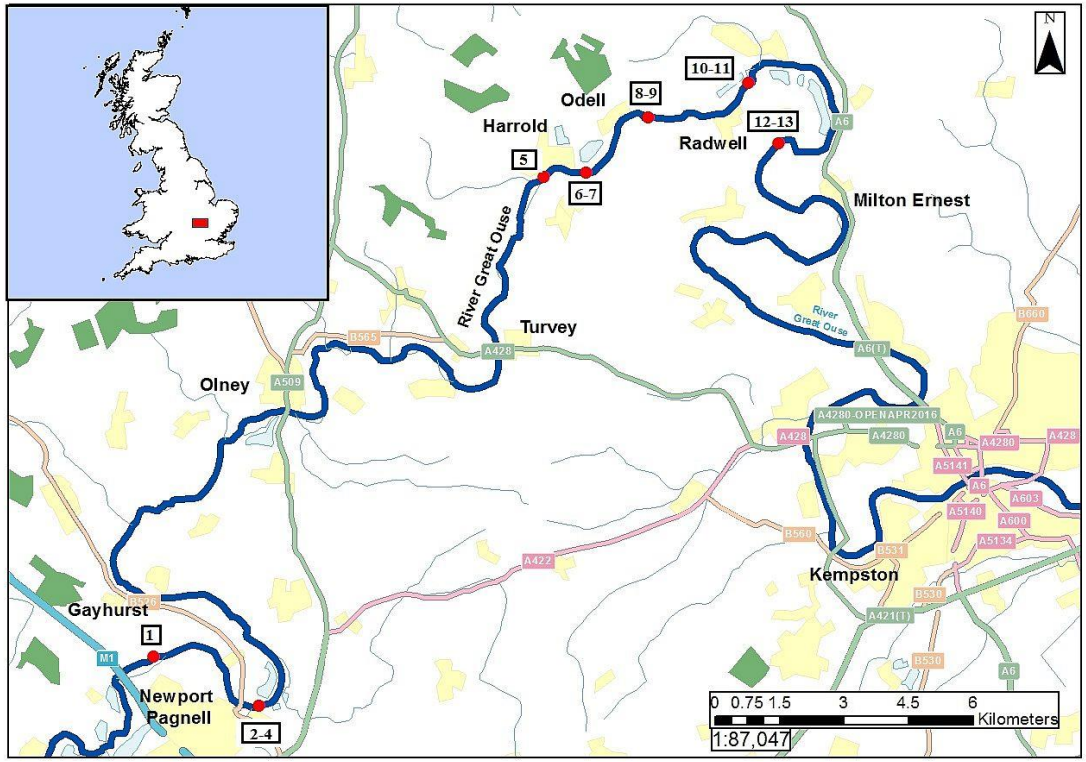

Figure 22 Approximate location of the study section in the UK, highlighting sampled riffles between Newport Pagnell and Bedford. Labelled red dots correspond to the locations of the following sites: 1 – Gayhurst, 2 – U/S Newport Pagnell 2, 3 – U/S Newport Pagnell 1, 4 – D/S Newport Pagnell, 5 - Harrold weir, 6 - U/S Harrold bridge 7 - D/S Harrold bridge, 8- U/S Odell, 9 - D/S Odell, 10 - U/S Pinchmill island, 11 - D/S Pinchmill island, l2 - Radwell viaduct, 13 - Radwell bridge (Ordnance survey 2005; Ordnance survey 2015a).

5.3.3 Sampling methodology

Four key metrics were measured during the sampling at each site, namely surface sediment composition, subsurface sediment composition, hydraulic conditions and water quality. The rationale for measuring each of these is provided in Table 10.

Table 10 Summary and rationale of the data collected during the study with relevant literature.

Metric	**Rationale**	**References**
Surface sediment composition	Describes quality of spawning substrate for redd building and egg survival and larval emergence (shallow spawners).	Kondolf and Wolman 1993; Kondolf 2000; Bunte and Abt 2001; Riebe et al. 2014
Subsurface sediment composition	Describes quality of spawning substrate for egg survival and larval emergence.	Bunte and Abt 2001; Meyer 2003; Lapointe et al. 2005; Bryce et al. 2010; Franssen et al. 2014; Sear et al. 2016
Hydraulics conditions	Describes flow conditions for spawning, egg survival and larval emergence.	Kondolf and Wolman 1993; Montgomery et al. 1996; Kondolf 2000; Lapointe et al. 2000; Louhi et al. 2008 Buffington et al. 2004
Water quality	Background chemical parameters affecting all fish life-stages.	Soluslby et al. 2001; Greig et al. 2007; Louhi et al. 2008; Pulg et al. 2013

Surface and subsurface sediment characteristics

Samples of subsurface sediments were collected at each of the sites using a McNeil sampler (coring tube diameter and depth were 16 and 26 cm, respectively) and a Koski plunger (Figure 23). This method was selected to obtain sediment samples that adequately represented subsurface grain size distributions and reduce potential for bias towards finer and coarser grain size fractions (Bunte and Abt 2001). This was completed under the assumption that the diameter of the coring tube was sufficiently large as to capture all grain sizes at each of the sites. Also, access at some sites was restricted meaning the use of other coring methods such as freeze coring were not appropriate (Bunte and Abt 2001). Surface grain-size distributions were determined at each site via a 400-count Wolman sample (Rice and Church 1996; Figure 24).

During field sampling in 2014, 10 sediment cores were collected at random across each of the 6 spawning riffles. This was to ensure representative samples of subsurface sediments were collected (Table 11), in line with Church et al. (1987) adjusted sample-mass equation (1). Subsurface data were then used to calculate the mean maximum grain size ($Dmax = D95$) across all sites (44.50 ± 15.76; n = 10 cores per 6 sites), which was used to estimate the minimum mass of sediment required to obtain a representative sample. According to Church et al. (1987) adjusted sample-mass equation (1); for particles with Dmax > 32 mm and mean maximum gran size in the study area, an estimated mass of 80 kg was calculated, implying samples exceeding this value were representative. In general, total dried sub-aerial mass of sediment collected at each site exceeded the threshold value or was close to it (1 core = 7.59 ± 1.23 kg of sediment * 10 samples = 63.60 – 88.20 kg/site). Therefore, samples collected in 2014 were representative of the study area. This also allowed for an assessment of spatial variability across sites within the study reach, allowing for collection of less samples in

2015 which was necessary due to time and resource restraints. Consequently, in 2015, only five sediment cores were collected per site (1 core (V=0.0052 m^3) = 7.70 ± 0.89 kg of sediment * 5 samples = 34.05 to 42.95 kg/site) (Table 11).

$$m(s)=2.9*Dmax-47.56 \quad (1)$$

Subsurface sediment samples from each site were dried and sieved into whole phi size fractions (0.062 to 45 mm) using an electronic sieve shaker and sieve stacks (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). The sub-aerial mass of sediment within each discrete size fraction was then weighed. Clasts > 45 mm were measured using a gravelometer (Figure 25) and placed within one of three categories (64, 90 and 180 mm), depending on their size. Sediment masses were then determined for each discrete size fraction.

During Wolman counts, grains were sampled along 10 flow-parallel transects that were distributed across spawning riffles at each of the sites. Along each of the 10 flow-parallel transects, 40 grains were selected at random and their B-axis measured using a gravelometer (Figure 24), such that 400 grains were measured per site (Table 11). Grains were measured and graded using the following size classes; <2, 2.8, 4, 5.6, 8, 11, 16, 22.6, 32, 45, 64, 90, 128, 180, and > 180 mm.

Figure 23 Process of collecting subsurface sediment samples using a Mcneil sampler and Koski plunger.

Figure 24 Assessing surface grain sizes using a gravelometer.

Table 11 Summary on the collected data at each site per sampling methodology. Site reference is per Figure 22.

	Sediment		**Water**		**Hydraulics**	
Site reference	Wolman count	McNeil samples	Surface samples	Hyporheic samples	Depth measure	Velocity measures
1	1 x 400 count	5	/	/	16	16
2	1 x 400 count	10	/	9	16	16
3	1 x 400 count	5	/	/	24	24
4	1 x 400 count	10	/	27	16	16
5	1 x 400 count	10	9	27	16	16
6	1 x 400 count	10	9	27	16	16
7	1 x 400 count	10	9	27	16	16
8	1 x 400 count	10	9	27	16	16
9	1 x 400 count	5	/	/	16	16
10	1 x 400 count	5	/	/	16	16
11	1 x 400 count	5	/	/	16	16
12	1 x 400 count	5	/	/	16	16
13	1 x 400 count	5	/	/	16	16

Hydraulic conditions

At each of the 13 sites, flow velocity measures were obtained using a Valeport Open Channel Flow Meter (Model 801) that took mean values over 60 s. Measurements were made at 16 points per site (Table 11). These 16 points were located at 4 equidistantly spaced points across 4 flow-parallel transects that were distributed across each riffle width. At each of the 16 locations, velocity measurements were made at two depths; near bed (approximately 1-2 cm from bed) and at 0.6 depth (distance from water

surface; Table 11). Simultaneously, a single depth measurement was taken at each of the 16 points using a metre rule (Table 11). In addition, width measurements of the wetted channel and the surveyed barform were made at each of the 4 parallel transects using a 30-metre-long tape measure (Table 11). At each of the sites, a length measurement was made and a Leica dumpy level was used for a single site measurement of bed and surface water slopes (Table 11; Figure 25).

Figure 25 Measuring bed and water surface slope using dumpy level and staff.

Water chemistry

Water samples were collected from the hyporheic zone at six sites (D/S Newport Pagnell, U/S Newport Pagnell 2, Harrold weir, U/S Harrold, D/S Harrold and U/S Odell; Figure 23). Hyporheic water samples were collected across three depths, 10, 20 and 30 cm, which correspond to the depths at which lithophilic spawners typically lay their eggs (Van den Berghe and Gross 1984; Crisp and Carling 1989; Lisle 1989; Montgomery et al. 1996). These samples were taken using a hand-operated water pump

attached to a 1 L bottle (Figure 26) and a set of tubes of varying lengths corresponding to the three sampling depths (Figure 27). At each sampling point, three tubes corresponding to 10, 20 and 30 cm depth were inserted into the river bed (Figure 27). During sample collection, one person held the pump inflow tube within the hyporheic tube which had been buried within the substrate, whilst another pumped water into the sample bottle. Upon extraction, in each case, the first 1 L water sample was discarded to avoid sample contamination. At sites D/S Newport Pagnell, Harrold weir, U/S Harrold bridge, D/S Harrold bridge and U/S Odell, 9 hyporheic water samples were extracted per depth. At site U/S Newport Pagnell 2, only 3 samples per depth were able to be collected due to time constraints (Table 11). In addition, 9 surface water samples were collected at 4 of the 6 sites (36 in total; Table 11), allowing for comparisons between hyporheic and surface water conditions and an assessment of possible groundwater impacts on the hyporheic layer (Bowerman et al. 2014). Temperature, dissolved oxygen concentration and saturation (mg l^{-1}; %), pH and conductivity were measured on-site using Hanna probes. Additional water samples (6 per depth, per site) were collected and transported back to the laboratory in cool boxes. Upon arrival, samples were frozen for further assessment of total nitrogen ammonia (mg l^{-1}) and unionized nitrogen ammonia (mg l^{-1}) content, using the colorimetric method (Bower and Holm-Hanse 1980; Le and Boyd 2012).

Figure 26 Using a hand-operated water pump to extract hyporheic water samples.

Figure 27 Set of tubes (10, 20 and 30 cm in length), used to extract water from within the hyporheic zone.

5.3.4 Data analysis

Surface sediment characteristics

Cumulative distribution curves were used to extract percentiles D5, D50 and D95, as well as D10, D25, D75 and D90 required for the calculation of mean (2), sorting (3), skewness (4) and kurtosis (5) metrics, in line with Trask's (1932) graphic mixed approach (Bunte and Abt 2001).

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

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

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

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

In addition, D84 was extracted from cumulative distribution curves to calculate the threshold particle size (DT) that fish of a certain length could potentially move (Riebe et al. 2014). The Riebe et al. (2014) threshold particle size DT (mm), fractional coverage by moving particles (Fm (mm)), spawning capacity (Nredds) and reproductive capacity (Neggs) of a certain area was calculated using equations (6), (7), (8) and (9), where L is mean fish fork length, A is redd area (10) and E is fecundity (11). Mean fish fork length (L= 615.90 ± 23.28 mm; ± SE) was obtained from a telemetry study by Twine (2013) on *B. barbus* and its use of spawning habitats within the upper part of the River Great Ouse. There are several limitations of this model. First, it was built for redd-building American salmonid fishes rather than UK cyprinids. Second, the model precludes the effects of 1) fines and scour depth, 2) the reproductive success of females, and 3) conditions within the hyporheic layer, on spawning (Riebe et al. 2014). However, it still

provides valuable data on the coarseness of the river bed and potential limitation for redd-building process, which is then comparable to other studies.

$$DT = 115 * (L/600)^{0.62} \quad (6)$$

$$Fm = (1 + e^{-1.702*\left(\frac{\log\left(\frac{DT}{D50}\right)}{\log\left(\frac{D84}{D50}\right)}\right)^{-1}}) \quad (7)$$

$$Nredds = FM/A \quad (8)$$

$$Neggs = (Fm * E)/A \quad (9)$$

$$A = 3.3 * (L/600)^{2.3} \quad (10)$$

$$E = 8.1L - 1450 \quad (11)$$

Reach-mean values (± SE) were then calculated across 13 sites.

Subsurface sediment characteristics

Percentiles and statistical parameters were extracted as described above, with the exceptions of D84 values and the related threshold particle size, which specifically relates to surface sediments (Riebe et al. 2014). Additionally, the percentage of fine particles was calculated for each subsurface sample, by focusing on: 1) diameter ≤ 2 mm, 2) 0.063 mm < diameter ≤ 2 mm and 3) diameter ≤ 0.063 mm.

In 2015, a 10 g subsample of fine sediment (≤ 2 mm) was taken from each dried and sieved core sample and processed to determine organic matter content using loss on ignition (LOI). Each subsample was dried in an oven for 24 hours at 100 °C and transferred to a glass dessicator jar before being weighed to measure the pre-ignition mass (m_{pre}). Samples were then placed in the furnace for 3 hours at 550 °C. After the three-hour period, samples were transferred back to the glass desiccator jar until cool

before being weighed to calculate the post-ignition mass (m_{post}). The percentage of organic matter in each sample was determined using the equation (12).

$$\% \text{ organic matter} = \left(\frac{\text{mpre}-\text{mpost}}{\text{mpre}}\right) * 100 \quad (12)$$

Reach-mean values (± SE) were then calculated using mean values from 13 sites.

Hydraulic conditions

Hydraulics conditions were quantified at each of the 13 sites. Reach mean shear stress during baseflow conditions was estimated using equation (13), where τo is reach mean shear stress (N/m^2), ρw is water density (ρw = 998.2 kg/m^3), g is acceleration due to gravity (g = 9.81 m/s^2), R is hydraulic radius (m; equation 14, where A is cross sectional area of the river and P is wetted perimeter) and S was assumed equal to water surface slope (Montgomery et al. 1996; Lapointe et al. 2000). To calculate the critical shear stress necessary to entrain surface sediments, the equation (15) was used, where τc is critical shear stress (N/m^2), 0.035 is the estimated Shields parameter typical for mixed gravel streams, ρw is water density (ρw = 998.2 kg/m^3), ρs is sediment density (ρs = 2650 kg/m^3), g is acceleration due to gravity (g = 9.81 m/s^2) and D50 is the surface median grain size (m) (Montgomery et al. 1996). Mobility ratio was estimated as the ratio between reach mean shear stress and critical shear stress (Lapointe et al. 2000). Reynolds number (Re) was estimated using the equation (16), where v is the mean velocity (at 0.6 depth; m/s), R is hydraulic radius (m) and υ is water kinematic viscosity (10^{-6} m^2/s). Slope (S, %) was calculated using the equation (17), where Avertical is the height at point A (m), Bvertical is the height at point B and ABhorizontal is the horizontal distance between point A and point B (m) (Bariweni and Abowei 2011).

$$\tau o = \rho w * g * R * S \quad (13)$$

$$R = A/P \quad (14)$$

$$\tau c = 0.035 * (\rho s - \rho w) * g * D50) \quad (15)$$

$$Re = (vR)/\upsilon) \quad (16)$$

$$\% S = \left(\frac{Avertical - Bvertical}{ABhorizontal}\right) * 100 \quad (17)$$

All measured/ calculated parameters were then averaged across the 13 sites to get reach-mean values. Additionally, discharge values were obtained from the local gauge station in Newport Pagnell. A mean value was calculated for the 2014/2015 sampling period (August – September).

Water chemistry

The majority of water chemistry tests were performed on-site as they are temperature sensitive (dissolved oxygen concentration, dissolved oxygen saturation, pH and conductivity). Tests for total nitrogen ammonia (TAN) and unionized nitrogen ammonia (NH_3) concentrations were completed in the laboratories at Bournemouth University where in most cases, 6 replicates per depth per site were prepared for analysis. However, due to time constraints during sample preparation, some samples were processed in lower numbers (see Appendix B; Tables 4 to 7).

In the laboratory analysis, prior to any treatment, samples were left to defrost for 12 hours to reach room temperature. Samples were then filtered using syringe-driven filter units, each with a Millipore membrane of 33 mm to remove unwanted particulates. The

indophenol blue method is the standard method for determining total nitrogen ammonia in freshwater systems. However, the salicylate method was used here, as it utilises less hazardous chemicals with overall higher precision and accuracy, relative to the indophenol blue method (Bower and Holm-Hanse 1980; Le and Boyd 2012). A Varian Cary 50 Probe UV-variable spectrophotometer was used to determine total nitrogen ammonia concentration with absorbance set at 640 nm.

The salicylate method for determining total nitrogen ammonia in freshwater systems is presented in Appendix A, as taken from Le and Boyd (2012).

Variations in ammonia concentrations were expressed as total nitrogen ammonia concentration (TAN; mg l^{-1}) that was a direct result of colorimetric analysis. However, total ammonia in water is presented in ionized and unionized form, with the unionized form particularly toxic for aquatic biota (Daniels et al. 1987; De LG Solbé and Shurben 1989; Eddy 2005; Finn 2007). Equilibrium between the two forms will mainly depend on pH and temperature with an increase in unionized form as a function of elevated pH and temperature (Eddy 2005). Therefore, for samples collected during the 2014 field campaign, unionized nitrogen ammonia concentrations were calculated according to mean pH and temperature conditions at each of the depths and sites, using the method by Emerson et al. (1975).

For all metrics, across all depths, site means (n = 6) derived from within-site measurements were averaged to give reach-mean values (see Table 15). However, the potential effect of depth on each of the metrics was assessed in R 3.3.2 using raw data in mixed models (package lme4) to account for the random effect of site and to correct for spatial dependence of samples. Most data (temperature, conductivity, pH, oxygen

concentration and oxygen saturation) were normally distributed (assessed using car and MASS packages in R 3.2.2 to determine best fit probability distribution), except for total nitrogen ammonia and unionized nitrogen ammonia concentrations. Therefore, a generalized linear mixed effect model with Laplace approximation (family: Gaussian; link: log) was used to analyse ammonia concentrations as a function of depth. The other parameters were analysed using a linear mixed effect model. The model parameters in the latter were estimated using restricted maximum likelihood to account for crossed random effects, small sample size and unbalanced design. Following a significant effect of depth on each of the metrics, pairwise comparisons of covariate adjusted means were performed in R 3.3.2 using least-squares means with Tukey adjustment for *P* values for multiple comparisons.

5.4 Results

The mean values of important parameters for the river are presented here, with the site-specific data from which these values are derived available in Appendix B.

5.4.1 Surface sediment characteristics

Results from the 400-grain Wolman samples reveal that, generally, surface sediments within the study reach were relatively coarse (D50 = 19.32 ± 0.85 mm; Mean = 21.36 ± 2.23 mm; Table 12; Figure 28) and moderately well sorted (sorting = 0.66 ± 0.02; Table 12; Figure 28). Grain-size distributions were nearly symmetrical (skewness = 1.01 ± 0.05; Table 12; Figure 28) and leptokurtic (kurtosis = 0.24 ± 0.01; Table 12; Figure 28). Mean D5 and D95 were 5.35 ± 1.45 mm and 54.40 ± 8.05 mm, respectively Table 12; Figure 28).

For fish with a mean length of 615.9 ± 23.3 mm (Twine 2013), the estimated threshold surface particle size was 116.88 mm and percentage cover of movable particles across study sites was high (Fm = 0.99 ± 0.01). Furthermore, spawning capacity (Nredds/m) and reproductive potential estimates were 0.28 ± 0.002 and 999.04 ± 8.28 respectively. This would mean that in general, spawning sites in the upper part of the River Great Ouse could accommodate 10.81 ± 1.93 redds with approximately 38,252.60 ± 6,840.49 eggs, assuming other factors were suitable.

Table 12 Characteristics of surface sediments from the River Great Ouse. Values derive from 400-count Wolman samples. Reach-mean values from 2014/2015 (n = 13, ± SE).

Metric	Value
D5 (mm)	5.35 ± 0.85
D50 (mm)	19.32 ± 1.45
D84 (mm)	36.72 ± 4.97
D95 (mm)	54.40 ± 8.05
Mean (mm)	21.36 ± 2.23
Sorting	0.66 ± 0.02
Skewness	1.01 ± 0.05
Kurtosis	0.24 ± 0.01
Fine sediment (%)	1.95 ± 0.38

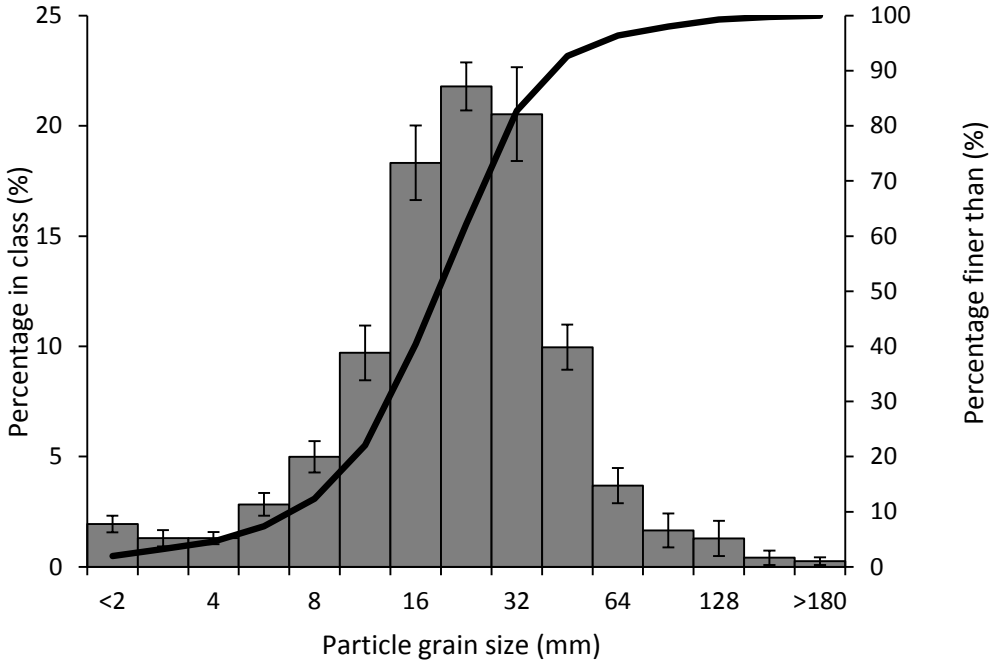

Figure 28 Surface grain size distribution of River Great Ouse bed material, derived from 400-count Wolman samples. Reach-mean values (n = 13 ± SE). The line represents cumulative distribution curve.

5.4.2 Subsurface sediment characteristics

Subsurface sediments were medium-course in nature (D50 = 11.66 ± 1.19 mm; Mean = 13.51 ± 0.98 mm; Table 13; Figure 29) and poorly sorted (sorting = 0.38 ± 0.02; Table 13; Figure 29). Grain-size distributions were strongly positively skewed towards coarser grain sizes (skewness = 0.62 ± 0.04; Table 13; Figure 29) and mean D5 and D95 were 0.48 ± 0.04 mm and 48.35 ± 3.65 mm respectively (Table 13; Figure 29).

Regarding subsurface samples, fine sediment concentrations varied between sites (from 12.49 % to 37.92 %) and were above 20 % in general (21.76 ± 2.07 %; Table 13; Figure 29), with sand dominating in each sample (21.59 ± 2.04 %; Table 13; Figure 29). Organic matter content was consistently low across all sites (2.37 ± 0.14 %; Table 13).

Table 13 Characteristics of subsurface sediments from the River Great Ouse. Values derive from 10 McNeil samples per site. Reach-mean values from 2014/2015 (n = 13, ± SE).

Metric	Value
D5 (mm)	0.48 ± 0.04
D50 (mm)	11.66 ± 1.19
D95 (mm)	48.35 ± 3.65
Mean (mm)	13.51 ± 0.98
Sorting	0.38 ± 0.02
Skewness	0.62 ± 0.04
Kurtosis	0.29 ± 0.02
Fine sediment (%)	21.76 ± 2.07
Sand (%)	21.59 ± 2.04
Silt (%)	0.17 ± 0.04
Organic matter content (%)	2.37 ± 0.14

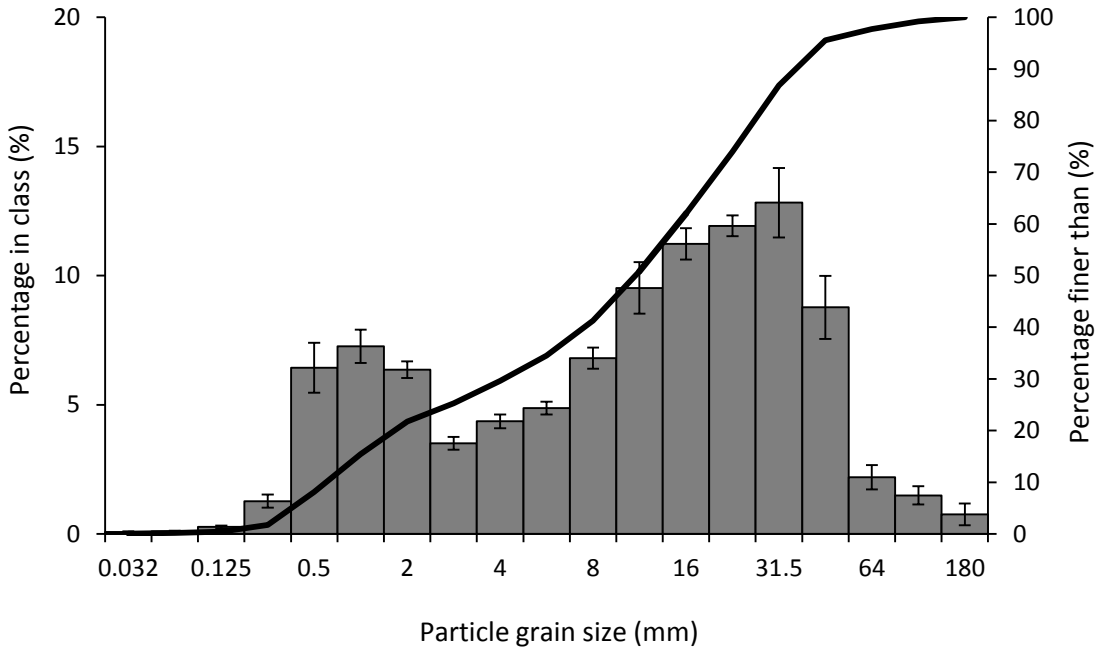

Figure 29 Subsurface grain size distribution of River Great Ouse bed material, derived from 10 McNeil samples per site. Reach-mean values (n = 13, ± SE). The line represents cumulative distribution curve.

5.4.3 Hydraulic conditions

Riffle dimensions varied between locations. Generally, sites were small (mean width = 4.70 ± 0.52 m; mean length = 7.84 ± 0.68 m; Table 14) and maintained flows that were turbulent (Reynolds number = 139 043.87 ± 22 236.17; Table 14), relatively fast (near-bed velocity = 0.36 ± 0.03 m/s; 0.60 depth velocity = 0.54 ± 0.05 m/s; Table 14) and shallow (0.26 ± 0.03 m, Table 14). Gentle bed and water surface slopes (bed slope = 1.43 ± 0.56 %; water surface slope = 0.28 ± 0.05 %; Table 14) were observed whilst the mean discharge for the study period was 1.34 ± 0.07 m^3/s (Table 14). Reach mean shear stress (6.82 ± 1.50 N/m^2; Table 14) was relatively low compared to estimated critical shear stress (10.96 ± 0.82 N/m^2; Table 14). Accordingly, the mobility ratio was low (0.65 ± 0.51; Table 14).

Table 14 Hydraulic characteristics from the River Great Ouse. Reach-mean values from 2014/2015 (n = 13, ± SE).

Metric	Value
Wetted width (m)	13.47 ± 1.09
Site width (m)	4.70 ± 0.52
Site length (m)	7.84 ± 0.68
Bed slope (%)	1.43 ± 0.56
Water surface slope (%)	0.28 ± 0.05
Flow depth (m)	0.26 ± 0.03
Near-bed velocity (m/s)	0.36 ± 0.03
0.6 depth velocity (m/s)	0.54 ± 0.05
Reach mean shear stress (N/m^2)	6.82 ± 1.50
Critical shear stress (N/m^2)	10.96 ± 0.82
Mobility ratio	0.65 ± 0.14
Reynolds number	139 043.87 ± 22 236.17
Discharge (m^3/s)	1.34 ± 0.07

5.4.4 Water chemistry

In the period of data collection, the study reach maintained a mean surface temperature of 16.99 ± 0.38 °C, conductivity of 809.19 ± 38.99 µS/l, pH of 8.09 ± 0.05, dissolved oxygen content of 6.28 ± 0.44 mg/l and oxygen saturation of 70.88 ± 4.76 % (Table 15). In general, total nitrogen ammonia concentration was 0.38 ± 0.11 mg/l within the water column (Table 15). There were no significant differences in water temperature (χ^2 (3) = 4.14, $P > 0.05$), water conductivity (χ^2 (3) =3.72, $P > 0.05$), pH (χ^2 (3) = 4.12, $P > 0.05$), total nitrogen ammonia (χ^2 (3) = 0.68, $P > 0.05$) or unionized ammonia concentrations (χ^2 (3) = 2.09, $P > 0.05$) between depths (Tables 15, 16). However, dissolved oxygen (χ^2

(3) = 18.50, p < 0.01) and oxygen saturation (χ^2 (3) = 17.38, p < 0.01) varied significantly as a function of depth (Tables 15, 16).

Pairwise comparisons revealed that dissolved oxygen concentrations were significantly different between the surface water and hyporheic layer(s). Specifically, differences were found at 20 cm (LMEM, $P < 0.01$; Table 16) and 30 cm (LMEM, $P < 0.01$; Table 16) depths, with no significant difference between the surface layer and hyporheic layer at 10 cm depth (LMEM, $P > 0.05$; Table 16). Oxygen saturation also varied significantly between the surface layer and hyporheic layer at 20 cm (LMEM, $P < 0.01$; Table 16) and 30 cm (LMEM, $P < 0.01$; Table 16), with no alteration between surface layer and hyporheic layer at 10 cm depth (t (LMEM, $P > 0.05$; Table 16). In general, oxygen concentration and saturation reduced with depth (Table 15). Regarding differences in water conditions between hyporheic layers, dissolved oxygen and oxygen saturation did not vary significantly between 10 and 20 cm (LMEM, $P > 0.05$), 10 and 30 cm (LMEM, $P > 0.05$), and 20 and 30 cm (LMEM, $P > 0.05$) (Table 16).

Table 15 Characteristics of surface and hyporheic water from the River Great Ouse. Values derive from 9 samples per depth and site. Reach-mean values from 2014 (n = 6, ± SE).

Water quality parameters	**Surface**	**Hyporheic**		
		10 cm	**20 cm**	**30 cm**
Temperature (°C)	16.99 ± 0.38	17.01 ± 0.20	16.87 ± 0.17	16.77 ± 0.17
Conductivity (µS/l)	809.19 ± 38.99	764.63 ± 36.53	766.67 ± 36.17	771.72 ± 34.59
pH	8.09 ± 0.05	7.65 ± 0.28	7.76 ± 0.23	7.63 ± 0.27
Dissolved oxygen (mg/l)	6.28 ± 0.44	5.69 ± 0.28	5.25 ± 0.35	5.15 ± 0.29
Dissolved oxygen (% sat)	70.88 ± 4.76	67.10 ± 2.18	62.74 ± 2.45	61.78 ± 1.72
Total nitrogen ammonia (TAN; mg/l)	0.38 ± 0.11	0.45 ± 0.11	0.42 ± 0.11	0.35 ± 0.14
Unionized ammonia (NH_3; mg/l)	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.004	0.01 ± 0.004

Table 16 Outputs from linear and generalized linear mixed models testing for differences in water parameters between depths across sites, where site and sample were random effects on the intercept. Mean differences are from estimated least-square means (difference significant at * $P < 0.05$ and ** $P < 0.01$).

Final models:

Temperature ~ depth + (1|Site) + (1|Sample) (AIC = 106.61; log likelihood = - 46.31; $P > 0.05$)

Conductivity ~ depth + (1|Site) + (1|Sample) (family – Gaussian (link-log); penalized quasilikelihood; AIC = NA; log likelihood = NA; $P > 0.05$)

pH ~ depth + (1|Site) + (1|Sample) (family – Gaussian (link-log); penalized quasilikelihood; AIC = NA; log likelihood = NA; $P > 0.05$)

Oxygen~ depth + (1|Site) + (1|Sample) (AIC = 458.66; log likelihood = - 222.33; $P < 0.01$)

Oxygen saturation~ depth + (1|Site) + (1|Sample) (AIC = 1273.3; log likelihood = - 629.64; $P < 0.01$)

TAN ~ depth + (1|Site) + (1|Sample) (family – Gaussian (link-log); Laplace approximation, AIC = 106.9; log likelihood = -46.4; $P > P > 0.05$)

NH_3 ~ depth + (1|Site) + (1|Sample) (family – Gaussian (link-log); Laplace approximation, AIC = -782.6; log likelihood = 398.3; ; $P > P > 0.05$)

Pairwise comparison		**Mean difference in Dissolved oxygen (mg/l)**	**Mean difference in oxygen saturation (%)**
Surface water	10 cm	0.61 ± 0.25, $P > 0.05$	5.47 ± 2.34, $P > 0.05$
	20 cm	1.10 ± 0.25, < 0.01**	10.21 ± 2.34 , $P < 0.01$*
	30 cm	1.20 ± 0.25, < 0.01**	11.22 ± 2.34, $P < 0.01$*
10 cm	20 cm	0.48 ± 0.22, $P > 0.05$	4.73 ± 2.10, $P > 0.05$
	30 cm	0.58 ± 0.22, $P > 0.05$	5.75 ± 2.10, $P > 0.05$
20 cm	30 cm	0.10 ± 0.22, $P > 0.05$	1.02 ± 2.10, $P > 0.05$

5.5 Discussion

The spawning grounds of *B. barbus* in the River Great Ouse maintained shallow and fast flows that were characterised by high oxygen concentrations. These findings broadly supported those from previous studies on *B. barbus* spawning grounds across various rivers (*cf.* Wijmans 2007; Kemp et al. 2011; Turnpenny Horsfield Associates 2012; Pledger 2014), as well as in previous work on the River Great Ouse (Twine 2013). Surface water temperature, conductivity and pH were likewise in accordance with published literature on optimal spawning conditions (Baras 1995; Wijmans 2007). Levels of total nitrogen ammonia indicated high ecological status of surface water (TAG 2008), whilst unionized nitrogen ammonia concentration in the surface layer corresponded with reported optimal conditions required for salmonid development (EPA 2001; Eddy 2005; Finn 2007).

Temperature, conductivity, pH, total nitrogen ammonia and unionized nitrogen ammonia in general, did not vary significantly as a function of depth within the hyporheic zone. Oxygen concentration was, however, significantly reduced in the hyporheic layer compared to surface water, indicating a potential influence of subsurface fines on hyporheic exchange (Kemp et al. 2011; Sear et al. 2014; Sear et al. 2016). However, as levels of dissolved oxygen were similar between subsurface layers, a possible influence of groundwater on subsurface layer was suggested (Malcolm et al. 2003 and 2004; Youngson et al. 2004; Sear et al. 2014), particularly as the Great Ouse is predominately a groundwater-fed river (Neal et al. 2000). Nevertheless, mean temperature, oxygen concentration, pH and conductivity in all subsurface layers (Table 15) remained optimal for *B. barbus* spawning (Wijmans 2007). Total nitrogen ammonia concentration in the first 10 cm was higher than previously reported levels in the study

system by Twine (2013). However, the previous study focused on trends in ammonia through time with fewer sites and replicates per site and depth and therefore neglected to quantify the degree of variation as a function of location (both within the river and at a single site) and depth. Despite this, total nitrogen ammonia levels in this study, in all subsurface layers, remained below 0.5 mg/l generally, indicating good ecological status of interstitial water (TAG 2008). Additionally, levels of unionized ammonia in all subsurface layers were in general below 0.02 mg/l, a value reported optimal for *B. barbus* larvae (Policar et al. 2007, 2010, 2011) and salmonid development (EPA 2001; Eddy 2005; Finn 2007).

Surface sediments mainly consisted of relatively coarse and well sorted gravels that were suitable for redd excavation. Generally, subsurface sediments were in contrast to surface substrates. In general, subsurface sediments consisted of moderately coarse and poorly sorted gravels. The concentration of fine sediment was generally high, with a particularly high sand content. However, organic matter content was low, in line with findings from Twine (2013). Mean D5 was lower in subsurface sediments than in surface sediments due to enhanced fine sediment transport from the surface during lower flows than required to mobilize subsurface sediments (Kondolf 2000). Despite calculations supporting positive skewness in the subsurface sediment, Figure 29 suggested a bimodal distribution, with peaks at both distribution tails. Mean D95, representing the coarse tail of the distribution curve, was similar between both surface and subsurface sediments. Surface and subsurface grain size distributions were both better sorted in the central part of distribution and therefore, leptokurtic.

Most studies on *B. barbus* spawning habitats have either focused on surface sediment conditions (e.g. Baras 1992; Banarescu and Bogutskaya 2003; Pledger 2014) or were

qualitative in nature (e.g. Twine 2013). Therefore, this work represents the first quantitative assessment of known *B. barbus* spawning habitats that includes robust assessments of substrate (surface and subsurface), hydraulics and water chemistry within a European river. Assessing subsurface substratum is crucial for river habitat management, as sediments are important for aquatic organisms and river purification (Boulton et al. 1998; Sternecker at al. 2013). For example, a decline in the quality of the subsurface zone can affect macro-invertebrates and the development of lithophilic fish species, whose reproductive cycle is dependent on this zone (Boulton et al. 1998; Sternecker at al. 2013). Content of fine sediment (e.g. < 2 mm diameter, generally known as 'fines') is a useful proxy for substrate suitability and different particles sizes are acknowledged to influence different life stages in different ways (Kondolf 2000; Chapter 7). For example, particles above 1 mm (1-10 mm) can prevent larval emergence (Kondolf 2000, Louhi et al. 2008) while particles below 0.125 mm, even in low proportions (e.g. 1.5 %), affect oxygen uptake by developing embryos (Louhi et al. 2008).

As there are no studies linking the early development of *B. barbus* to habitat quality and, specifically, fines content, published literature on other lithophilic species can be used to hypothesise about the potential effects of sediment composition on *B. barbus* recruitment within the River Great Ouse. Numerous studies report variable fine sediment thresholds that affect salmonid egg-to-emergence survival and larval emergence (Table 17). For example, Kondolf (2000) generalises that content of particles with diameter less than 1 mm should be below 14 % for successful incubation (more than 50 % emergence). Field studies across 12 states in the USA have revealed aquatic vertebrates are detrimentally impacted by levels > 5 % for silt (≤ 0.06 mm) and 13 % for sand content (0.06 - 2 mm) (Bryce et al. 2010). Bowerman et al. (2014) found that

grain sizes < 6.4 mm affected salmonid egg-to-emergence survival which dropped to 40 % in concentrations above 20 % and 30 % in artificial and natural redds respectively. Therefore, as the study area had generally above 20 % fines in the subsurface layer (0.06 - 2 mm), it is reasonable to assume that fines content might be negatively impacting upon lithophilic species. Despite silt content was low in general (0.17 ± 0.04 %), the high amount of sand present (21.59 ± 2.04 %) could trap silt inside the egg pocket during incubation, potentially damaging embryos and larvae (Lapointe et al. 2005; Levasseur et al. 2006; Sear et al. 2016). Also, whilst potential spawning riffles are relatively common within the area, the majority of them are relatively small (mean width = 4.7 ± 0.52 m; mean length = 7.48 ± 0.68 m) and disconnected by several migration barriers, especially weirs (Twine 2013). A lack of suitable spawning habitat and indeed, presence of migration barriers could be having significant detrimental impacts on fish populations within the surveyed stretch of river.

Table 17 Comparison between reported literature on grain sizes negatively affecting early development of salmonids and the results for the River Great Ouse as reported in this chapter. The threshold values vary across the studies and represent grain sizes above which survival to emergence or timing of emergence was significantly affected (increased mortality or premature emergence) in relation to control conditions. In bold are values from this study that exceed the reported thresholds.

Grain diameter	**Reported threshold value**	**Measured variables in relation to threshold values**	**References**	**This study (mean ± SE)**
< 0.063 mm	> 0.5 % (if sand (0.063 – 2 mm) > 10 %)	Mean egg-to-emergence survival	Lapointe et al. 2004	0.17 ± 0.04 %
	> 0.3 – 0.4%	Mean survival to pre-eyed and eyed stage	Julien and Bergeron 2006	
	< 1.5 % (usually < 0.5 %)	Mean egg-to-emergence survival	Louhi et al. 2011	
	> 9 %	Mean egg-to-emergence survival Timing of emergence (premature)	Franssen et al. 2012	
	> 3 %	Mean egg-to-emergence survival Timing of emergence (premature) Larvae condition	Sear et al. 2016	

Grain diameter	Reported threshold value	Measured variables in relation to threshold values	References	This study (mean ± SE)
< 0.125 mm	> 0.2 %	Mean survival to hatching	Levasseur et al. 2006	**0.44 ± 0.10 %**
≤ 1 mm	> 15 %	Mean egg-to-emergence survival	O'Connor and Andrew 1998	**15.40 ± 1.85 %**
	> 12 -14 %	Less than 50 % emergence	Kondolf 2000	
0.06 - 2 mm	> 13 %	Mean survival to hatching	Bryce et al. 2010	**21.59 ± 2.04 %**
≤ 2 mm	> 20 %	Mean egg-to-emergence survival	Soulsby et al. 2001	**21.76 ± 2.07 %**
	> 22 %	Mean egg-to-emergence survival	Franssen et al. 2012	
< 4 mm	> 25 %	Timing of emergence (premature)	Fudge et al. 2008	**29.62 ± 2.30**
< 6.4 mm	> 20 – 30 %	Mean egg-to-emergence survival	Bowerman et al. 2014	**34.49 ± 2.37 %**

Oxygen dynamics are important during fish reproduction (Michel et al. 2014) and should be considered during assessments of spawning habitat quality. Oxygen consumption rises with the temperature and the stage of the embryo. Therefore, just before emergence, the concentration of oxygen should be above 7 mg l^{-1} compared to early stages when it can drop to 1 mg/l (Louhi et al. 2008). The optimal oxygen concentration during *B. barbus* development was reported above 5 mg l^{-1}, with lethal concentration at 2.1 mg l^{-1} after 24 hours (Wijmans 2007). In general, all hyporheic

layers maintained oxygen levels just above 5 mg l^{-1}, indicating oxygen was not a limiting factor in the Great Ouse during the study period. However, at some locations, oxygen levels dropped below 5 mg/l, which was particularly pronounced in deeper layers (20 and 30 cm) at several locations. This could still be suitable conditions for shallow spawners, such as *B. barbus*, at least in terms of oxygen conditions. However, at Harrold weir, oxygen deficiency was spread equally across all depths, indicating the importance of local hyporheic conditions on oxygen dynamics, which will vary as a function of fines content (Sear et al. 2014, 2016), interstitial flow velocity (Greig et al. 2005a; Franssen et al. 2012, 2014), temperature (Kemp et al. 2011), presence of organic matter (Kemp et al. 2011; Sear et al. 2014, 2016) and influence of groundwater (Malcolm et al. 2003, 2004; Sear et al. 2014).

Reported levels of toxic unionized ammonia during salmonid development vary across studies. Eggs appear to be more resilient than larvae (Burkhalter and Kaya 1977; Daniels et al. 1987), with lethal concentrations between 0.05 and 0.80 mg l^{-1} (Thurston et al.1978, 1986; Daniels et al. 1987). In aquaculture systems, suggested levels of unionized ammonia are below 0.05 mg l^{-1}, whilst TAN concentrations should be below 1.0 mg l^{-1} for long-term exposure of fish to ammonia (Timmons et al. 2002). However, reported detrimental levels of unionized ammonia in freshwater systems lie between 0.068 to 2 mg l^{-1} (96 hours, LD50) with UK standards for salmonids estimated at 0.02 mg l^{-1} (EPA 2001; Eddy 2005 and Finn 2007). Even though cyprinids seem to be more resilient to low ammonia concentrations (0.35 to 2.00 mg/l of unionized ammonia), laboratory experiments with *B. barbus* larvae report utilisation of water with unionized ammonia levels just under 0.02 mg l^{-1} (Policar et al. 2007, 2010 and 2011). Therefore, both total ammonia nitrogen and unionized ammonia nitrogen levels in this study were below reported detrimental levels, indicating adequate status of interstitial water during

B. barbus development. However, excessive amounts of fine sediment in the study area can trap ammonia excreted during early development, which can add to ammonia already present within the hyporheic zone, potentially increasing concentrations above optimal reported levels (Wood and Armitage 1997; Kemp et al. 2011).

Findings presented here support and extend previous research pertaining to *B. barbus* ecology. Specifically, this study pertains to the first quantitative investigation into the spawning habitats of *B. barbus*, particularly regarding subsurface sediment and hyporheic water properties, in an anthropogenically disturbed, lowland river. Nevertheless, transferability of these findings to other systems is difficult due to geomorphological heterogeneity of river catchments (Wenger and Olden 2012; Choi et al. 2015; Huang and Frimpong 2016), indicating the need for work at broader spatial scales. Also, comparing these data with those from the literature on other lithophilic species gives some insight into the potential negative impacts of fine sediment accrual on *B. barbus* recruitment within the study river. However, presented results cannot be definitive unless species-specific responses to various habitat factors are assessed under controlled conditions. Work on the influences of different environmental pressures on *B. barbus* egg incubation and larvae development is therefore required, so that findings can be used as benchmarks against which river managers can compare habitat conditions within their systems to establish if they are suitable for spawning, rendering natural recruitment viable.

Consequently, as sand content of River Great Ouse spawning substrates is of potential concern for *B. barbus* early development, the following two chapters will focus on the: 1) role of substrate management in improving habitat conditions *in-situ* (Chapter 6); and

2) impact of increasing sand content on egg-to-emergence survival and emergence timing of *B. barbus ex-situ* (Chapter 7).

6. Effects of gravel jetting on the physicochemical habitat characteristics of spawning gravels of *Barbus barbus*

6.1 Summary

Anthropogenic inputs of fine sediments can detrimentally impact freshwater environments, with lithophilic fish especially affected by the accrual of fine sediment within spawning habitats. To increase spawning habitat quality a variety of restoration methods are used, including "cleaning" existing gravels *in-situ* via gravel jetting. Whilst this is performed regularly in the River Great Ouse, its benefits have not been quantified. Here, an *in-situ* experiment at two spatial scales, riffle and patch, determined the magnitude and persistence of the impact of gravel jetting on surface and subsurface substrate conditions. At the riffle scale, comparison of surface grain size in the pre- and post-jetting period indicated that gravel jetting had a profound impact, removing fines from the bed, and resulting in coarser and better sorted sediments. Similar patterns were observed at the smaller patch scale, with the exception that sorting was not significantly altered. At the riffle scale, jetting did not impact the composition of subsurface spawning gravels, other than reducing the amount of fines in the river bed. Jetting at the patch scale did not show any improvements for the subsurface layer, potentially due to effects of scale and/ or site choice. None of the observed changes in surface or subsurface sediments persisted longer than a year at the riffle scale, while the patch scale experiment implied longevity of jetting effects to be less than 3 months in most cases. This suggests that catchment scale management via changes in agricultural and livestock practices could be more suitable than gravel jetting in dealing with excessive sedimentation in freshwater systems.

6.2 Introduction

In-stream degradation of river functional habitats are amongst the most-studied of all forms of freshwater degradation (Morandi et al. 2014), with substrate degradation associated with global declines in freshwater biodiversity (Hancock 2002). The importance of river substrata includes its provision as functional habitat for the development of many taxa (Palmer et al. 1997; Hancock 2002; Geist 2011; Sternecker at al. 2013a). Therefore, any processes and activities that impact its composition, such as inputs of fine sediment (≤ 2 mm; 'fines'), could negatively impact riverine biota (Wood and Armitage 1997; Dudgeon et al. 2006; Kemp et al. 2011). For example, egg incubation, reproductive success and recruitment of lithophilic fishes can be influenced by fine sediment ingress via altered composition of spawning gravels (Kemp et al. 2011). Fines content in spawning gravels can alter interstitial flows and oxygen permeation, metabolic waste removal and larval emergence (e.g. Kemp et al. 2011; Pattison et al. 2015; Sear et al. 2016). Consequently, river restoration methods often focus on reducing fines content in spawning gravels to enhance fish populations (Wood and Armitage 1997; Bernhardt et al. 2005; Giller 2005).

Attempts to reduce the fines content of spawning gravels uses a variety of methods including gravel augmentation, placement of in-stream structures (e.g. woody debris, boulders etc.) and gravel cleaning (Wheaton et al. 2004a; Wheaton et al. 2004b). Despite wide application, mitigation projects are inhibited by practitioners not utilising robust scientific approaches, with frequent omission of specific objectives, post-monitoring evaluations and landscape processes within experimental designs (Bond and Lake 2003; Wheaton et al. 2004a; Wheaton et al. 2004b). Most studies also report on small-scale (patch and barform), localised projects that lack a temporal component (Palmer et al. 2010; Pander and Geist 2013). Equally, some projects lack pre-restoration

assessments, a component crucial to understanding the longevity of effects through time and/or space (Wheaton et al. 2004a; Wheaton et al. 2004b; Morandi et al. 2014). Thus, studies that utilise robust experimental designs are integral for understanding the factors that contribute to successful restoration (Palmer et al. 2007).

Gravel jetting as a technique to remove fines from gravels and provide enhanced spawning gravels for fish has been widely applied in British rivers (Hendry et al. 2003). Despite this, only two studies exist reporting impacts of gravel jetting on spawning substrates (Shackle et al. 1999; Twine 2013). Both studies found gravel jetting decreased percentage fines within subsurface sediments (Shackle et al. 1999; Twine 2013). However, both lacked replication and temporal perspectives, limiting their utility. Whilst gravel jetting might improve local spawning substrate conditions, the process could potentially have negative consequences for downstream habitats and their biota through the release of fine sediments (Kemp et al. 2011; Sternecker et al. 2013b). Also, gravel jetting loosens fluvial substrates, removing stabilising sediment structures, with this possibly reducing critical entrainment thresholds and increasing bed mobility under ambient and high flows, with potential implications for egg-to-emergence survival (Buffington et al. 2004; Hassan et al. 2015). Shackle et al. (1999) observed increased rates of sedimentation downstream of restored areas, but failed to quantify some potential negative impacts of different gravel cleaning methods on downstream habitats. Other studies have reported increased sedimentation downstream of restoration works (Sternecker et al. 2013b; Pander et al. 2015). Specifically, sediment accrual was observed in close proximity to restored sections and it could be assumed that this would create problems for downstream habitats by, for example, causing siltation of gravels.

Consequently, this chapter utilised an experimental approach under field conditions to quantify the effects of gravel jetting on fish spawning grounds. Given the paucity of knowledge on gravel improvement schemes for non-salmonid fishes generally (Kemp et al. 2011), the model river was the middle reaches of the Great Ouse, Eastern England, where the gravels are utilised for spawning by the lithophilic European barbel *B. barbus* and chub *S. cephalus*. Migratory salmonid fishes cannot access these gravels due to negligible longitudinal connectivity due to flood management schemes in the lower river. The experiment was completed at two spatial scales; riffle (approximately 25 to 100 m^2) and patch (approximately 0.25 m^2, i.e. small areas within a riffle). The objectives (O) were to:

(O1) Determine changes in sediment by gravel jetting, by measuring:

(a) surface sediment composition at riffle and patch scales;

(b) subsurface sediment composition at riffle and patch scales and percentage of organic matter at patch scale;

(c) longevity of gravel jetting effects: composition of surface and subsurface sediments (above mentioned metrics) after 12 months (riffle scale) and after 3 and 9 months (patch scale);

(d) quantity and composition of sediment washed from the bed during patch-scale jetting; and

(O2) Determine changes in hyporheic water conditions by gravel jetting, by measuring dissolved oxygen, total nitrogen ammonia and unionized nitrogen ammonia concetrations at the riffle scale (three depths; 10, 20 and 30 cm).

6.3 Materials and methods

6.3.1 Gravel jetting (riffle and patch scales)

Following the *B. barbus* spawning period, the experimental work at the riffle (2014) and patch (2015) scales was conducted on spawning sites that are known to have been used in recent years following observations by anglers, the Environment Agency and Twine (2013) (Figure 30). Five sites were sampled in 2014 at the riffle scale, utilising a before-after experimental design, hence samples of surface and subsurface sediments and hyporheic water were taken pre- and post-gravel jetting (Figure 31a). Three sites were assessed in 2015 at the patch scale using a BACI design that utilised separate patches as control and jetted areas (Figure 31b). This allowed for the separation of restoration actions from natural processes during the post-restoration period. All work was conducted under summertime base-flow conditions so that it was ecologically relevant to *B. barbus* (Wijmans 2007; Chapter 5).

All five sites at the riffle scale were jetted for different lengths of time depending on their size, but effort per unit area was consistently applied across all sites. Specifically, the largest riffles (U/S Newport Pagnell 2 and D/S Newport Pagnell; approximately 100 m^2) were jetted for approximately 180 minutes, the riffle at Harrold weir was approximately 50 m^2 and jetted for 90 minutes and the 25 m^2 riffles at U/S Harrold bridge and U/S Odell were each jetted for 45 minutes. For consistency, each site was jetted by three operators, who started at the upstream end of the riffle and worked downstream (Figure 32). During the experimental period, no flood events were recorded (Figure 33) and in each case, pre- and post-treatment sampling was conducted 7 days pre- and 1 day post-gravel jetting, respectively (Figure 31a). It was thus assumed that measured differences between pre- and post-jetting conditions were a direct result of gravel jetting.

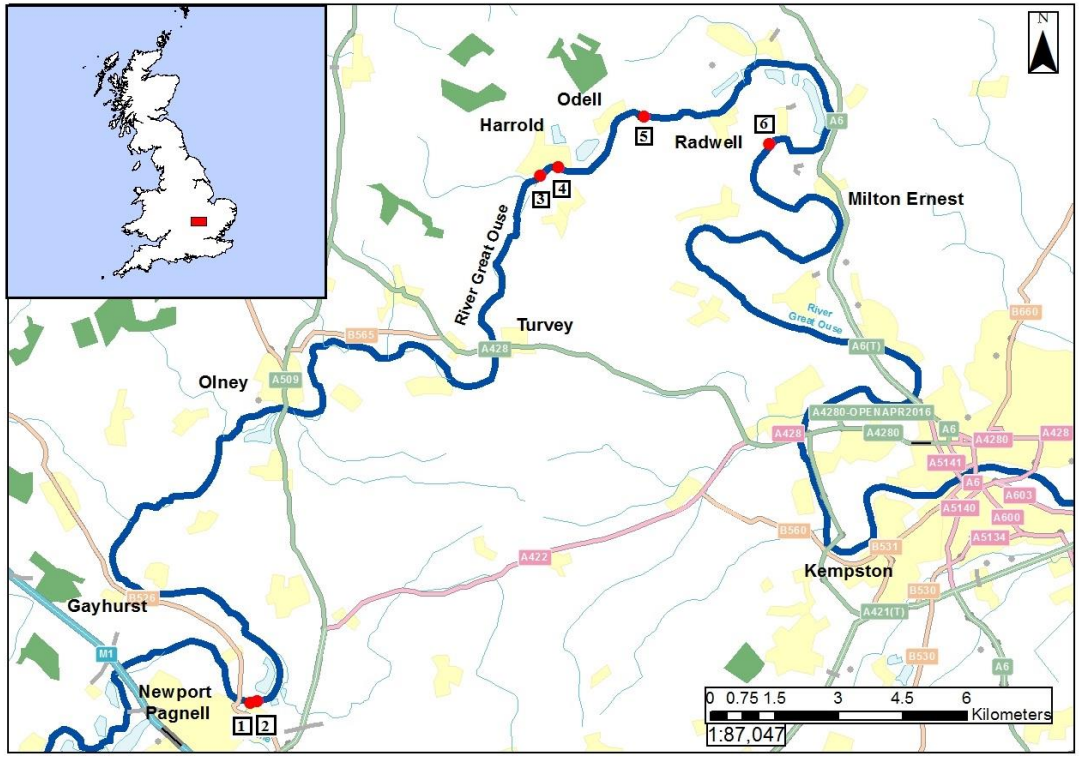

Figure 30 Approximate location of the study section in the UK, highlighting sampled riffles in the River Great Ouse between Newport Pagnell and Bedford. Labelled red dots correspond to the locations of the following sites: 1 - U/S Newport Pagnell 2, 2 - D/S Newport Pagnell, 3 – Harrold weir, 4 – U/S Harrold bridge, 5 - U/S Odell, 6 - Radwell bridge, sampled during riffle-scale (n = 5; sites 1-5; riffle scale) and patch-scale (n = 3; sites 3, 5 and 6; patch scale) experiments (Ordnance survey 2005; Ordnance survey 2015a).

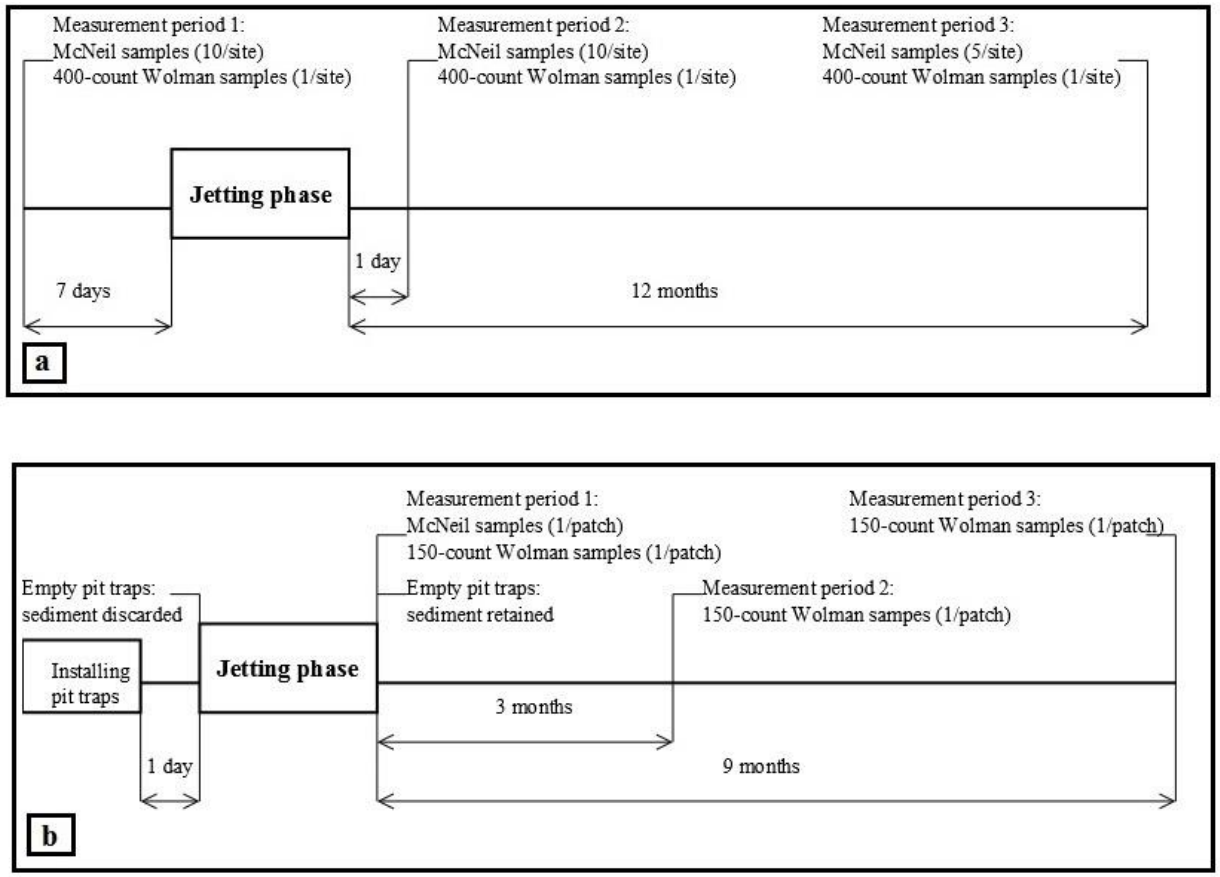

Figure 31 Schematic diagram presenting the experimental procedure for: a) riffle-scale experiment and b) patch-scale experiment.

Figure 32 Gravel jetting at the riffle scale.

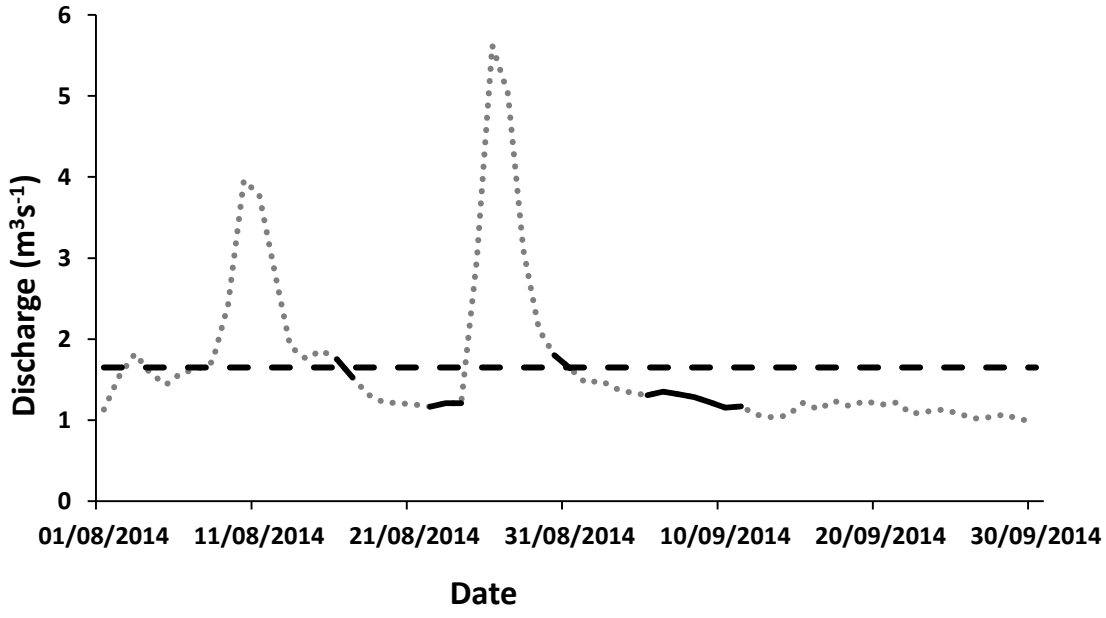

Figure 33 Daily-mean gauged flow (m^3 s^{-1}) at Newport Pagnell in August and September 2014 (grey dot line) obtained from the EA. The daily-mean flow for August and September 2014 black (dash line) and daily-mean flow values (black lines), pertaining to days when sampling was conducted are also presented (riffle scale).

The effects of gravel jetting at the patch scale were assessed at three riffles to achieve an adequate degree of replication. The three riffles were selected on the basis of their similarities in flow velocity, depth and surface sediment properties, which had been investigated and quantified in Chapter 5 (sites Harrold weir, U/S Odell and Radwell). The 3 patches at each of the riffles were selected to allow minimum distance of 3 m apart in each direction, hence ensuring spatial independence. On each selected riffle, the experimental design incorporated the selection of three downstream patches that were exposed to jetting for a fixed period of time and three upstream patches (controls) that were not jetted (patch size: 0.5 x 0.5 m; Figures 31b, 34). Therefore, each riffle consisted of 3 treatment and 3 control patches, hence 9 treatment and 9 control patches were used in total. Control patches were always located upstream of jetted patches to ensure no impact of gravel jetting on controls (Figure 34). Each treatment patch was jetted for 15 minutes by a single operator.

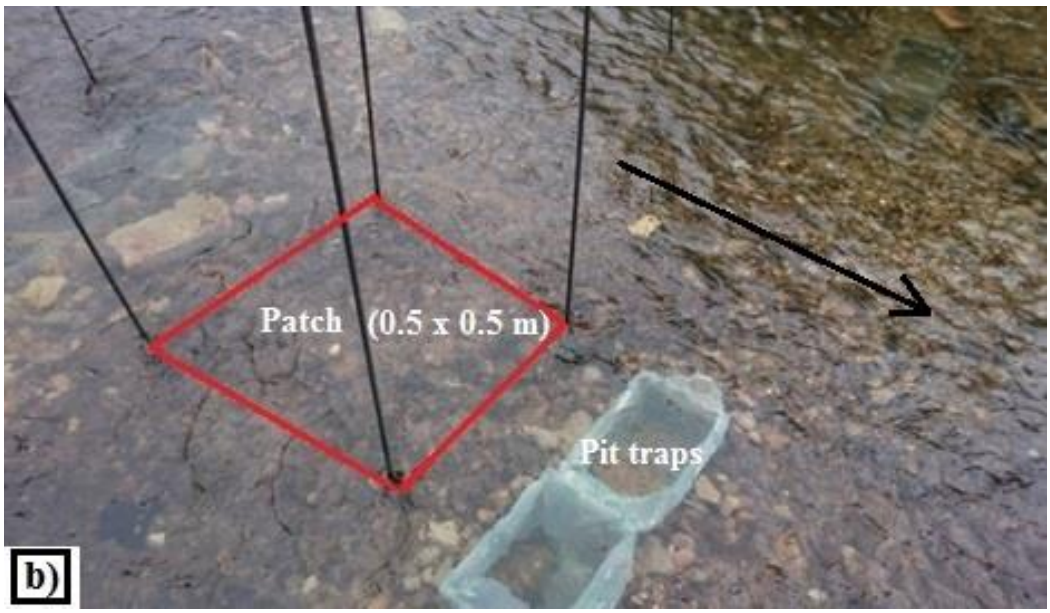

Figure 34 Experimental setup during patch scale assessments with a) patches and pit traps at Harrold weir, where red areas represent patches and black areas represent pit traps and b) detailed view of the experimental patch and position of pit traps at Harrold weir. The arrow represent the direction of water flow.

Whilst gravel jetting might improve local spawning substrate conditions, the process could potentially have negative consequences for downstream habitats and biota due to the release of fine sediment from spawning gravels (Kemp et al. 2011; Sternecker et al. 2013b). Also, gravel jetting loosens and restructures fluvial substrates, with this possibly reducing critical entrainment thresholds and increasing bed mobility under ambient and high flows, which could have implications for egg-to-emergence survival (Wilcock and McArdell 1997; Wood and Armitage 1997; Powell 1998; Buffington et al. 2004; Hassan et al. 2015). Therefore, two bedload slot samplers were buried downstream of each of the patches 24 hours prior to gravel jetting to capture sediment washed from the bed during jetting (patch scale) (Figure 35). Slot samplers were emptied before jetting and any sediment collected in the traps during jetting was retained for further analysis (Figure 35).

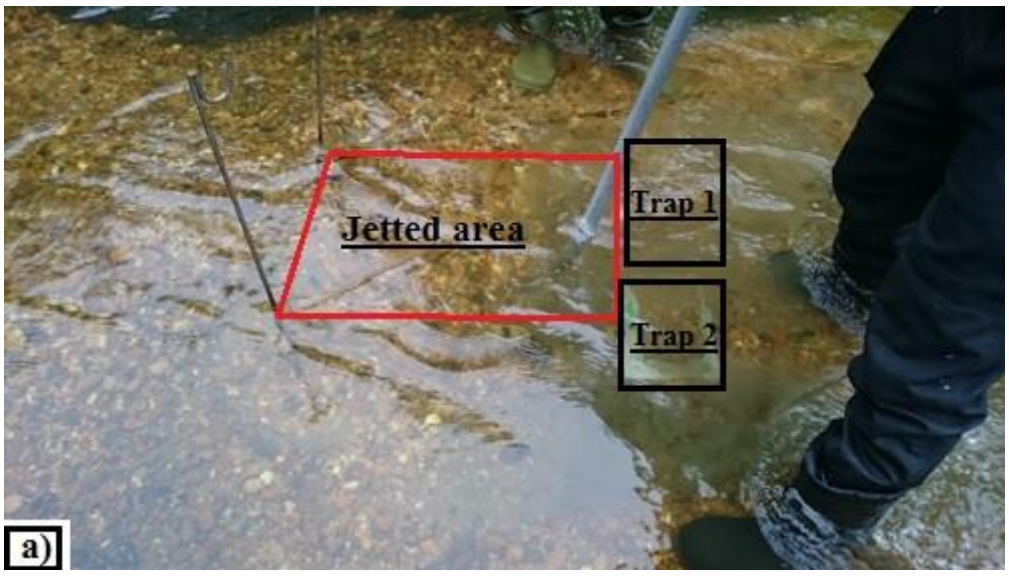

Figure 35 Mobile sediment during jetting which was transported: a) as bedload and collected in pit traps and b) in suspension under ambient flows.

6.3.2 Impact of gravel jetting on surface and subsurface sediment composition and mobility at different temporal scales

For sampling at both the riffle and patch scales, subsurface and surface sediment samples were collected using a McNeil sampler and a gravelometer respectively, as per protocols outlined in Section 5.3.3. From these surface and subsurface samples, D5,

D50, D95 percentiles and mean, sorting, skewness and kurtosis were obtained. Additionally, for subsurface sediment samples, sand and silt content and percentage of organic matter (patch scale) were also determined.

At the riffle scale, to complete O1a and b, subsurface and surface sediment samples were collected at each site pre- and post-gravel jetting, such that 10 subsurface samples and a single 400-count Wolman were obtained per site, pre- and post-jetting (Table 18; Figure 33a). Additionally, to investigate the persistence of gravel jetting effects at the riffle scale (O1c), measurements of surface and subsurface sediment characteristics were made at four sites (Table 18) approximately 12 months after, during summer 2015. However, due to time constraints, only 5 McNeil samples were collected from each riffle in 2015 (Table 18; Figure 31a).

At the patch scale (O1a, b), 1 subsurface sample and a 150-count Wolman sample were collected post-jetting from each of the treatment and control patches (Table 19; Figure 31b). Longevity of jetting at the patch scale (O1c) was monitored only in surface sediments, as no subsurface changes had been detected following jetting at the riffle scale (*cf.* Results). Control and jetted patches were monitored after 3 and 9 months (Table 19; Figure 31b) by collecting a 150-count Wolman sample from each of the treatment and control patches. Note that monitoring the patches 6 months after jetting was not possible due to high flows and so dangerous working conditions. The experiment was terminated after 9 months as results from these samples already showed no significant differences between pre- and post-jetting conditions for any of the metrics (*cf.* Results).

Additionally, data derived from bedload samples collected downstream of treatment and control patches during gravel jetting (patch scale; O1d) were compared to identify the influence of jetting on sediment mobility (Figures 31b, 35). Sediment metrics extracted from these samples were the same as for subsurface sediments at the patch scale as described above. Also, data pertaining to the total mass of sediment washed from the bed during jetting were extrapolated to the riffle scale to provide an estimation of the total mass of sediment removed from each riffle.

Table 18 Summary on data collected at riffle scale, including replicate number, sample size and methods used at different temporal scales. Site reference is as per Figure 30.

	Pre-jetting			**Post-jetting 1 (after 24 hours)**			**Post-jetting 2 (after 1 year)**	
Site reference	**Wolman count**	**McNeil sample**	**Hyporheic water samples/depth**	**Wolman count**	**McNeil sample**	**Hyporheic water samples/depth**	**Wolman count**	**McNeil sample**
1	1 x 400 count	10	3	1 x 400 count	10	9	n/a	n/a
2	1 x 400 count	10	9	1 x 400 count	10	9	1 x 400 count	5
3	1 x 400 count	10	9	1 x 400 count	10	9	1 x 400 count	5
4	1 x 400 count	10	9	1 x 400 count	10	9	1 x 400 count	5
5	1 x 400 count	10	9	1 x 400 count	10	9	1 x 400 count	5

Table 19 Summary on data collected at patch scale, including replicate number, sample size and methods used at different temporal scales. Wolman count is expressed as number of counts per patch and McNeil sample is expressed as number of bulk samples per patch. Site reference is as per Figure 30.

		Post jetting 1 (+24 h)				Post jetting 2 (+3 months)		Post jetting 3 (+9 months)	
		Control patch		Jetting patch		Control patch	Jetting patch	Control patch	Jetting patch
Site reference	**Number of patches**	**Wolman count**	**McNeil sample**	**Wolman count**	**McNeil sample**	**Wolman count**	**Wolman count**	**Wolman count**	**Wolman count**
3	3	1 x 150	1	1 x 150	1	1 x 150	1 x 150	1 x 150	1 x 150
5	3	1 x 150	1	1 x 150	1	1 x 150	1 x 150	1 x 150	1 x 150
6	3	1 x 150	1	1 x 150	1	1 x 150	1×150^{-1}	1 x 150	1 x 150

6.3.3 Impacts of gravel jetting on hyporheic water conditions at the riffle scale

At the riffle scale, water samples were collected from the hyporheic zone and surface layer at each of the sites, pre- and post-jetting, with the latter completed after 24 hours (Table 18; Figure 31a). In most cases, 9 replicates were collected at each depth (10, 20, 30 cm) for both pre- and post-jetting. The exception was U/S Newport Pagnell 2, where only 3 samples per depth were taken due to time constraints in the field. The hyporheic water samples were collected using the same equipment and protocol outlined in Section 5.2.2. The majority of parameters were assessed on-site (temperature, pH, conductivity, dissolved oxygen concentration and dissolved oxygen saturation). However, total nitrogen ammonia (TAN) and unionized nitrogen ammonia (NH_3) were analysed subsequently in the laboratory (Section 5.2.3). In most cases, 6 replicates per site, depth and treatment were used for the ammonia analysis. However, in some cases, due to time constraints during analyses, replicate numbers ranged between 2 and 5 (Appendix B; Tables 4 to 7 and Appendix C; Tables 10 to 13).

The water quality parameters of oxygen and ammonia concentration are the only chemical parameters presented in the Results section to show the effect of gravel jetting, as these are generally considered the most important water quality factors for fish egg development and larvae survival (Daniels et al. 1987; de LG Solbé and Shurben 1989; Wood and Armitage 1997; Greig et al. 2005a, Greig et al. 2005b; Greig et al. 2007, Kemp et al. 2011). However, the complete data set is presented in Appendices B (Tables 4 to 7) and C (Tables 10 to 13).

6.3.4 Data analysis

At the riffle scale, the potential effects of jetting on each of the metrics for surface and subsurface sediment and hyporheic water were assessed using linear (LMM) and

generalized linear mixed (GLMM) models (package lme4; R 3.3.2). This enabled accounting for the random effect of site for surface and subsurface sediment and hyporheic water data and to correct for spatial dependence of samples in subsurface sediment and hyporheic water data. Prior to any analysis, data were tested for normality using car and MASS packages in R 3.2.2 to determine best fit probability distributions. In the case of normally distributed residuals, model parameters were estimated using restricted maximum likelihood in linear mixed models to account for crossed random effects, small sample sizes and unbalanced design. In the case of log normal distribution, data were analysed using the flexible penalised quasi-likelihood method (family-Gaussian; link-log) that is suitable for over-dispersed data, crossed random effects and unbalanced design. However, where the mean of the response variable was below 5, the estimate was biased (Bolker et al. 2009; Bates 2010). In these cases, a Laplace approximation (family-Gaussian; link-log) was used, as it can handle the mean of response variable below 5 as well as up to 3 random effects (Bolker et al. 2009; Bates 2010). To test for differences in the proportions of sand and silt at the riffle scale, Laplace approximation with binomial logistic regression models (family-binomial; link-logit) was used, with weight argument specified as the total amount of sediment analysed for each sample.

At the patch scale, data were analysed using linear models (LM) and generalized linear models (GLM), as no spatial dependency was assumed between patches and so they could be treated as single experimental units. However, in cases of data over-dispersion (if the residual deviance is much larger than degrees of freedom), each sample was used as random effect on the intercept in mixed models. To test for differences in the proportions of sand, silt and organic matter at the patch scale, generalized linear models (family-binomial; link-logit) was used, with weight argument specified as the total

amount of sediment analysed for each sample. Additionally, to determine the effect of treatment in time at the patch scale, linear mixed effect models were used with repeated measure as a random effect on the intercept to account for the temporal dependency of data.

At both spatial scales, where significant effects of treatment on each metric was detected, pairwise comparisons of covariate adjusted means were then performed using least-squares means with Tukey adjustment for *P* values for multiple comparisons (riffle and patch scales). However, when determining the effect of treatment in time at the patch scale, comparisons of covariate adjusted means were performed using least-squares with Dunnett adjustment for *P* values for multiple independent comparisons of treatments with the control. Dunnett adjustment was used here in preference to Tukey and Bonferonni (used in previous chapters), as it provides the most powerful adjustment for multiple comparisons of treatments versus a common control group (Bretz et al. 2011; Howell 2012).

6.4 Results

Only the most important results are presented here. However, the raw data set can be found in Appendices B and C.

6.4.1 Impacts of gravel jetting on surface sediment composition at different temporal scales

At the riffle scale, gravel jetting had a significant impact on the D5 (LMM; $P < 0.01$), D95 (GLMM; $P < 0.01$), mean (LMM; $P < 0.01$) and degree of sediment sorting (LMM; $P < 0.01$) (Table 20a). As a function of gravel jetting, mean D5, D50 and D95 values for surface sediments increased significantly (Table 20b; Figure 36). These data

and a significant increase in the mean grain size indicate a coarsening of the sediment surface (Table 20b; Figure 36). Even though sediments were already well sorted prior to jetting, sediment sorting increased significantly through jetting (Table 20b; Figure 36). However, kurtosis (LMM; $P > 0.05$) and skewness (LMM; $P > 0.05$) did not differ significantly (Table 20a). Specifically, sediments derived before and after jetting phase maintained nearly symmetrical and leptokurtic grain size distributions that were better sorted in the central part (Figure 36). There were no significant differences in any of the surface percentiles when comparing conditions before and 12 months after the jetting phase (Table 20b; Figure 36). A similar pattern was apparent for mean, sorting, skewness and kurtosis values, with no significant differences found between before-jetting and 12 months after jetting (Figure 36).

Table 20 Outputs from linear mixed models testing for differences at the riffle scale in surface sediment parameters: a) final models; and b) pairwise comparisons; where: 1) pre- and 24 hours post-jetting; and 2) pre- and 12 month post-jetting. Site was specified as a random effect on the intercept. Mean differences are from estimated least-square means (difference significant at * $P < 0.05$ and ** $P < 0.01$).

a)

Final models

D5 ~ Treatment + (1|Site) (AIC = 71.20; log likelihood = - 30.60; $P < 0.05$)*

D50 ~ Treatment + (1|Site) (AIC = 61.22; log likelihood = - 24.01; $P < 0.01$**)

D95 ~ Treatment + (1|Site) (family – Gaussian (link-log); penalized quasilikelihood; AIC = NA; log likelihood = NA; $P < 0.01$**)

Mean ~ Treatment + (1|Site) (AIC = 56.31; log likelihood = - 23.15; $P < 0.01$**)

Sorting ~ Treatment + (1|Site) (AIC = -47.26; log likelihood = 28.63; $P < 0.01$**)

Skewness ~ Treatment + (1|Site) (AIC = - 35.42; log likelihood = 22.71; $P > 0.05$)

Kurtosis ~ Treatment + (1|Site) (AIC = -62.93; log likelihood = 36.46; $P > 0.05$)

b)

Metric	**Mean difference**	
	1	**2**
D5	- 3.28 ± 1.15, $P < 0.05^{*}$	- 0.11 ± 1.26, $P > 0.05$
D50	- 7.24 ± 0.64, $P < 0.01^{**}$	- 0.60 ± 0.69, $P > 0.05$
D95	- 0.12 ± 0.04, $P < 0.05^{*}$	0.02 ± 0.05, $P > 0.05$
Mean	- 6.66 ± 0.62, $P < 0.01^{**}$	- 0.56 ± 0.67, $P > 0.05$
Sorting	- 0.06 ± 0.02, $P < 0.05^{*}$	0.01 ± 0.02, $P > 0.05$

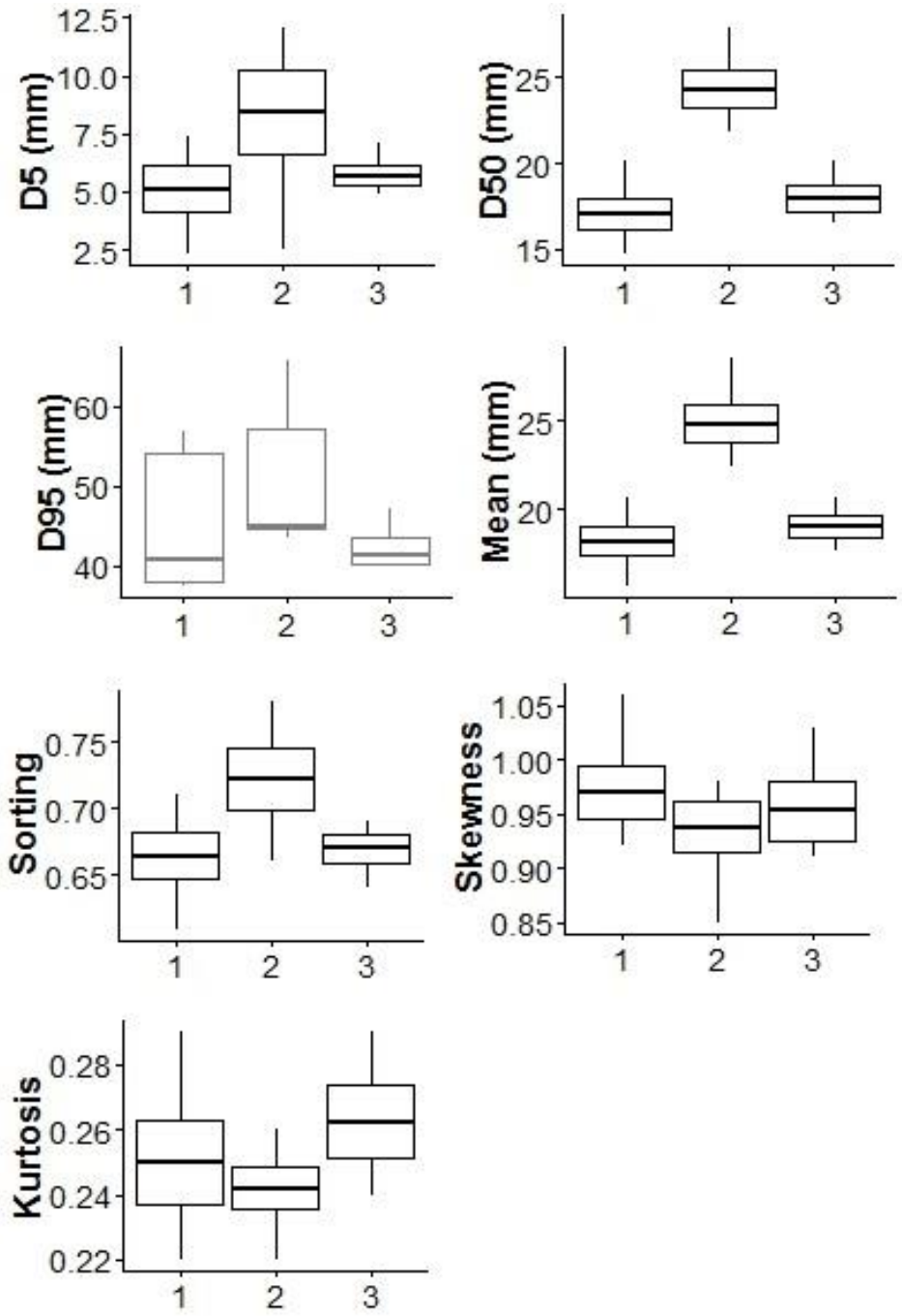

Figure 36 Surface percentiles and statistical parameters pre-jetting (1), 24 hours post-jetting (2) and 12 months post-jetting (3) at the riffle scale. Black horizontal lines represent mean (bold black line) and standard error; vertical lines represent max and min values. Grey horizontal lines represent 25, 50 (median – bold grey line) and 75 percentiles; vertical lines represent 95% confidence interval of the median.

At the patch scale, there was a significant effect of treatment and time interaction on D5 (GLMM; $P < 0.01$), D50 (LMM; $P < 0.01$), D95 (GLMM; $P < 0.05$), mean (LMM; $P < 0.01$) and sorting parameters (LMM; $P < 0.01$) (Table 21a). Compared to control patches, the sediments of jetted patches one hour after jetting had significantly higher D5, D50, D95 and mean values, although differences in sorting, skewness and kurtosis values were not significantly different (Table 21b; Figure 37). After 3 months, only the *D*5 significantly differed from the control patches, while other percentiles showed no significant differences between control and treated patches (Table 21b; Figure 37). Furthermore, none of the percentiles or statistical parameters were significantly different after 9 months when comparing data derived from control and treatment patches (Figure 37).

Table 21 Outputs from linear mixed models testing for differences in surface sediment parameters between control and jetted patches: a) final models; and b) pairwise comparisons; where: 1) 1 hour post-jetting; 2) 3 months post-jetting; and 3) 9 months post-jetting . Each repeated sample was specified as a random effect on the intercept. Mean differences are from estimated least-square means (difference significant at * $P < 0.05$ and ** $P < 0.01$).

a)

Final models:

D5 ~ Treatment x Time + (1|Sample) (family – Gaussian (link-log); Laplace approximation, AIC = -251.84; log likelihood = - 117.92; $P < 0.01$**)

D50 ~ Treatment x Time + (1|Sample) (AIC = 303.31; log likelihood = - 143.66; $P < 0.01$**)

D95 ~ Treatment x Time + (1|Sample) (family – Gaussian (link-log); penalized quasilikelihood; AIC = NA; log likelihood = NA; $P < 0.05$*)

Mean ~ Treatment x Time + (1|Sample) (AIC = 301.99; log likelihood = - 143.00; $P < 0.01$**)

Sorting ~ Treatment x Time + (1|Sample) (AIC = -142.47; log likelihood = 79.14; $P < 0.01$**)

Skewness ~ Treatment x Time + (1|Sample) (AIC = -68.92; log likelihood = 42.46; $P > 0.05$)

Kurtosis ~ Treatment x Time + (1|Sample) (AIC = -220.28; log likelihood = 118.14; $P > 0.05$)

b)

Metric	**Mean difference**		
	1	**2**	**3**
D5	- 0.45 ± 0.13, $P < 0.01$**	- 0.81 ± 0.32, $P < 0.05$*	- 0.41 ± 0.61, $P > 0.05$
D50	- 4.23 ± 1.89, $P < 0.05$*	- 0.78 ± 1.89, $P > 0.05$	- 0.002 ± 1.89, $P > 0.05$
D95	- 0.20 ± 0.06, $P < 0.01$**	- 0.01 ± 0.09, $P > 0.05$	0.03 ± 0.08, $P > 0.05$
Mean	- 5.15 ± 1.77, $P < 0.01$**	- 0.97 ± 1.77, $P > 0.05$	- 0.16 ± 1.77, $P > 0.05$
Sorting	- 0.03 ± 0.03, $P > 0.05$	- 0.02 ± 0.03, $P > 0.05$	- 0.03 ± 0.03, $P > 0.05$

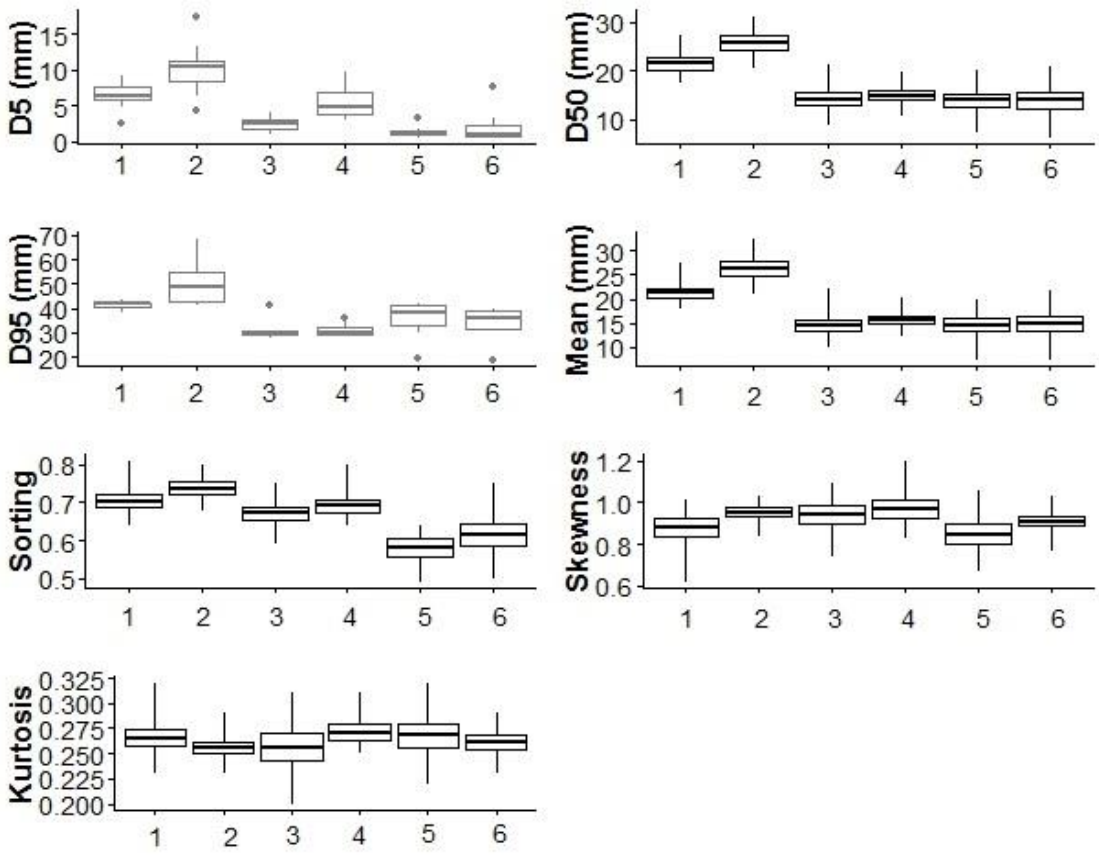

Figure 37 Surface percentiles and statistical parameters 1 hour post-jetting (1 - control, 2-treatment), 3 months post-jetting (3-control, 4-treatment) and 9 months post-jetting (5-control, 6-treatment). Black horizontal lines represent mean (bold black line) and standard error; vertical lines represent max and min values. Grey horizontal lines represent 25, 50 (median – bold grey line) and 75 percentiles; vertical lines represent 95% confidence interval of the median.

6.4.2. Impact of gravel jetting on subsurface sediment composition at different temporal scales

At the riffle scale, gravel jetting only significantly affected the subsurface *D*5 (GLMM; $P < 0.05$), sand (GLMM; $P < 0.05$) and silt (GLMM; $P < 0.01$) contents (Table 22a). In contrast, *D*50 (LMM; $P > 0.05$), *D*95 (GLMM; $P > 0.05$) mean (LMM; $P > 0.05$), sorting (LMM; $P > 0.05$), skewness (LMM; $P > 0.05$) and kurtosis (LMM; $P > 0.05$) values were not significantly altered by gravel jetting (Table 22a; Figure 38). Riffle-scale assessments of substrate condition 24 hours after the jetting phase indicated an increase in the *D5* and decreases in subsurface sand and silt contents (Table 22b; Figure 38). The longevity of this impact was short-lived, with conditions at 12 months being not significantly different to pre-jetting conditions (Table 22b; Figure 38).

Table 22 Outputs from linear mixed models testing for differences at the riffle scale in subsurface sediment parameters: a) final models; and b) pairwise comparisons, where: 1) pre- and 24 hours post-jetting; and 2) pre- and 12 month post-jetting. Site and sample were random effects on the intercept. Mean differences are from estimated least-square means (difference significant at * $P < 0.05$ and ** $P < 0.01$).

a)

Final models:

D5 ~ Treatment + (1|Site) + (1|Sample) (family – Gaussian (link-log); Laplace approximation, AIC = - 14.0; log likelihood = 13.0; $P < 0.05$*)

D50 ~ Treatment + (1|Site) + (1|Sample) (AIC = 656.84; log likelihood = - 322.42; $P > 0.05$)

D95 ~ Treatment + (1|Site) + (1|Sample) (family – Gaussian (link-log); penalized quasilikelihood; AIC = NA; log likelihood = NA; $P > 0.05$)

Mean ~ Treatment + (1|Site) + (1|Sample) (AIC = 612.23; log likelihood = - 300.12; $P > 0.05$)

Sorting ~ Treatment + (1|Site) + (1|Sample) (AIC = 226.74; log likelihood = 119.37; $P > 0.05$)

Skewness ~ Treatment + (1|Site) + (1|Sample) (AIC = 149.03; log likelihood = - 68.52; $P > 0.05$)

Kurtosis ~ Treatment + (1|Site) + (1|Sample) (AIC =- 529.82; log likelihood = 270.91; $P > 0.05$)

Sand content ~ Treatment + (1|Site) + (1|Sample) + (1|Sample_ID) (family – binomial (link-logit); AIC = 1930.60; log likelihood = -959.30; $P < 0.05$*)

Silt content ~ Treatment + (1|Site) + (1|Sample) + (1|Sample_ID) (family – binomial (link-logit); AIC = 782.30; log likelihood = -385.10; $P < 0.01$**)

b)

Metric	Mean difference	
	1	**2**
D5	-0.32 ± 0.11, $P < 0.01$**	0.21 ± 0.13, $P > 0.05$
Sand content	0.43 ± 0.16, $P < 0.05$*	0.28 ± 0.18, $P > 0.05$
Silt content	0.73 ± 0.15, $P < 0.01$**	0.33 ± 0.18, $P > 0.05$

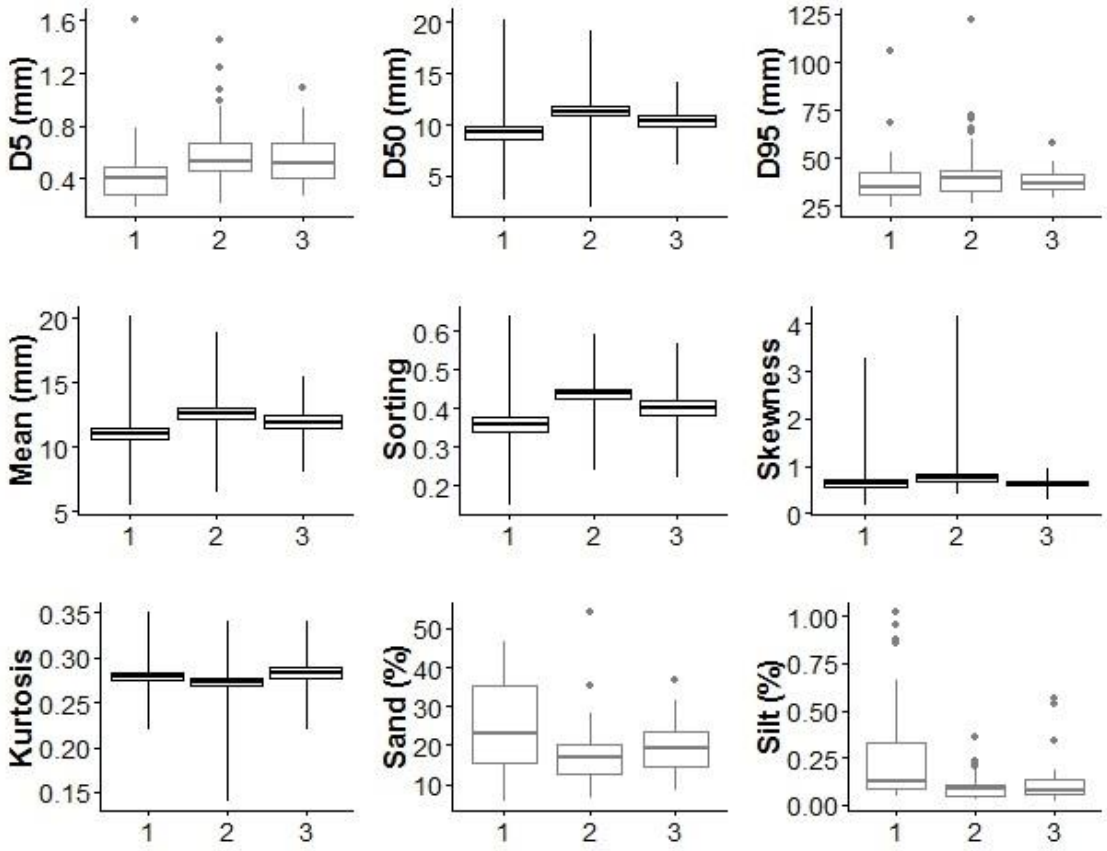

Figure 38 Subsurface percentiles and statistical parameters pre-jetting (1), 24 hours post-jetting (2) and 12 months post-jetting (3) at the riffle scale. Black horizontal lines represent mean (bold black line) and standard error; vertical lines represent max and min values. Grey horizontal lines represent 25, 50 (median – bold grey line) and 75 percentiles; vertical lines represent 95% confidence interval of the median.

At the patch scale, gravel jetting did not significantly impact upon subsurface sediment composition, indicating no difference between treatment and control patches 1 hour after the jetting phase (Table 23; Figure 39).

Table 23 Outputs from linear and mixed linear models testing for differences in subsurface sediment parameters between control and jetted patches 1 hour post-jetting. Mean differences are from estimated least-square means (difference significant at * $P < 0.05$ and ** $P < 0.01$).

Final models:

D5 ~ Treatment (family – Gaussian (link-log); $\chi^2 = 1.93$; $P > 0.05$)

D50 ~ Treatment (F (16) = 0.67; $R^2 = 0.04$; $P > 0.05$)

D95 ~ Treatment + (1|Sample_ID) (family – Gaussian (link-log); penalized quasilikelihood; AIC = NA; log likelihood = NA; $P > 0.05$)

Mean ~ Treatment (F (16) = 1.11; $R^2 = 0.06$; $P > 0.05$)

Sorting ~ Treatment (F (16) = 3.89; $R^2 = 0.20$; $P > 0.05$)

Skewness ~ Treatment (F (16) = 3.76; $R^2 = 0.19$; $P > 0.05$)

Kurtosis ~ Treatment (F (16) = 4.02; $R^2 = 0.20$; $P > 0.05$)

Sand content ~ Treatment + (1|Sample_ID) (family – binomial (link-logit); AIC = 280.70; log likelihood = -137.40; $P > 0.05$)

Silt content ~ Treatment + (1|Sample_ID) (family – binomial (link-logit); AIC = 113.40; log likelihood = -53.70; $P > 0.05$)

Organic matter content ~ Treatment (family – binomial (link-logit); $\chi^2 = 2.20$; $P > 0.05$)

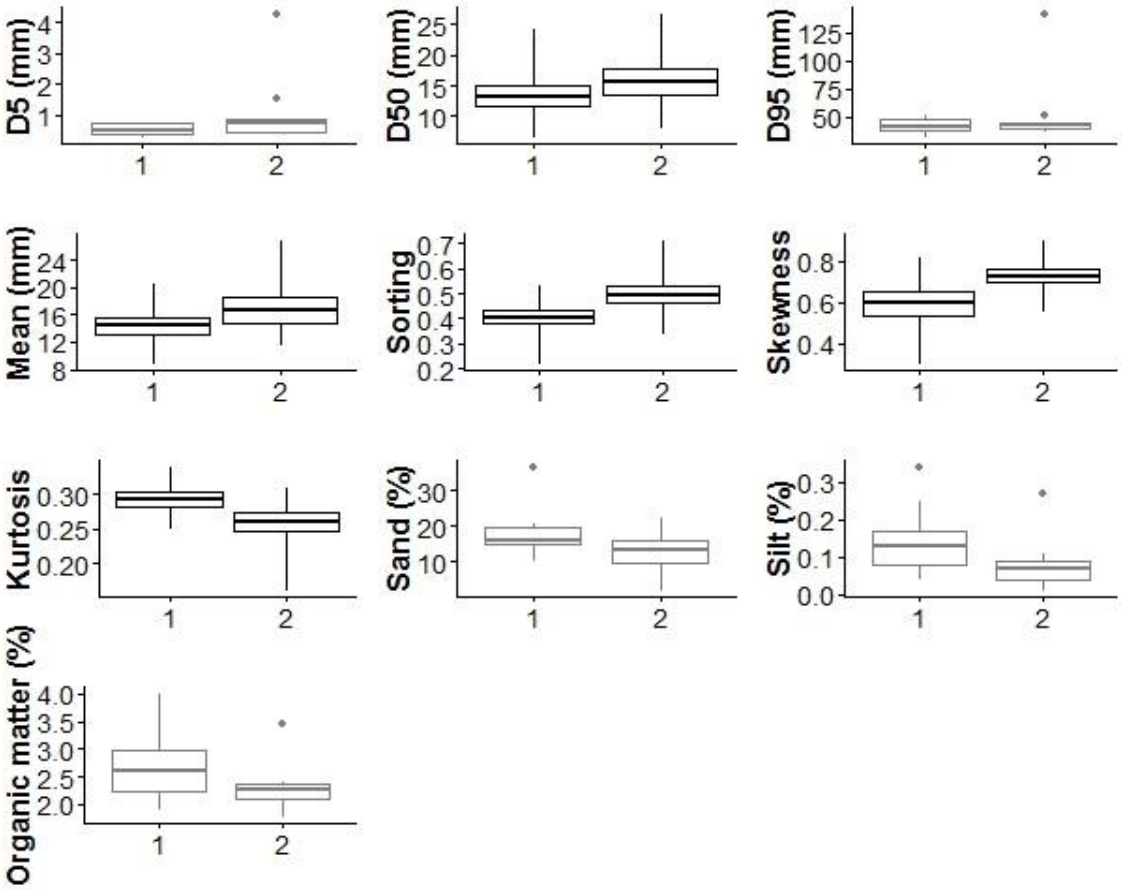

Figure 39 Subsurface percentiles, statistical parameters and sand, silt and organic matter content 1 hour post-jetting at the control (1) and jetted pacthes (2). Black horizontal lines represent mean (bold black line) and standard error; vertical lines represent max and min values. Grey horizontal lines represent 25, 50 (median – bold grey line) and 75 percentiles; vertical lines represent 95% confidence interval of the median.

6.4.3. Impact of gravel jetting on subsurface sediment composition and mobility at the patch scale

Data derived from bedload samples collected downstream of treatment and control patches during the patch scale component of the experiment revealed that gravel jetting had a significant impact on sediment mobility. The mean mass of displaced sediment from each patch was 7.04 ± 2.37 kg, with no mobility observed under control conditions (Table 24). When this was extrapolated to the mean riffle scale, estimations were close to 1 tonne of mobilized sediment (945.76 ± 284.51 kg; Table 24). In general, mobile sediments predominately consisted of poorly sorted gravels and sand, with leptokurtic distributions that were strongly skewed towards finer grain sizes (Table 24). The majority of mobile sediment was sand (60.31 ± 2.91 %; Table 24).

Table 24 Quantity and composition of mobile sediment, washed from the bed during patch-scale jetting. Patch mean values (n = 9; ± SE).

Metric	Value
D5 (mm)	0.29 ± 0.02
D50 (mm)	1.68 ± 0.47
D95 (mm)	30.05 ± 8.33
Mean (mm)	3.51 ± 0.41
Sorting	0.36 ± 0.03
Skewness	1.72 ± 0.36
Kurtosis	0.17 ± 0.02
Sand (%)	60.31 ± 2.91
Silt (%)	0.14 ± 0.02
Organic matter content (%)	1.59 ± 0.20
Amount of sediment/patch (kg)	7.04 ± 2.37
Riffle size (m^2)	34.45 ± 5.59
Amount of sediment/site (kg)	945.76 ± 284.51

6.4.4 Effects of gravel jetting on water chemistry at the riffle scale

Gravel jetting did not significantly impact upon dissolved oxygen at 10 (LMM; $P > 0.05$), 20 (LMM; $P > 0.05$) or 30 cm (LMM; $P > 0.05$) depths when comparing pre- and post-jetting conditions (Table 25; Figure 40). Total nitrogen ammonia (TAN) (Table 25; Figure 40) and unionized nitrogen ammonia (NH_3) (Table 25; Figure 40) concentrations were also not significanly altered by gravel jetting at depths of 10 (TAN: GLMM; $P > 0.05$; NH_3: GLMM; $P > 0.05$) 20 (TAN: GLMM; $P > 0.05$; NH_3: GLMM; $P > 0.05$) and 30 cm (TAN: GLMM; $P > 0.05$; NH_3: GLMM; $P > 0.05$).

Table 25 Outputs from linear mixed models testing for differences at the riffle scale in hyporheic water parameters pre- and 24 hours post-gravel jetting: a) Final models; and b) pairwise comparisons. Site and sample were random effects on the intercept. Mean differences are from estimated least-square means (difference significant at * $P < 0.05$ and ** $P < 0.01$).

a)

Final models:

O_2 (10) ~ Treatment + (1|Site) + (1|Sample) (AIC = 148.56; log likelihood = - 69.28; $P > 0.05$)

O_2 (20) ~ Treatment + (1|Site) + (1|Sample) (AIC = 250.29; log likelihood = - 120.15; $P > 0.05$)

O_2 (30) ~ Treatment + (1|Site) + (1|Sample) (AIC = 260.44; log likelihood = - 125.22; $P > 0.05$)

TAN (10) ~ Treatment + (1|Site) + (1|Sample) (family – Gaussian (link-log); Laplace approximation, AIC = 48.9; log likelihood = -19.4; $P > 0.05$)

TAN (20) ~ Treatment + (1|Site) + (1|Sample) (family – Gaussian (link-log); Laplace approximation, AIC = 51.9; log likelihood = -20.9; $P > 0.05$)

TAN (30) ~ Treatment + (1|Site) + (1|Sample) (family – Gaussian (link-log); Laplace approximation, AIC = 45.7; log likelihood = -17.8; $P > 0.05$)

NH_3 (10) ~ Treatment + (1|Site) + (1|Sample) (family – Gaussian (link-log); Laplace approximation, AIC = -345.7; log likelihood = 177.8; $P > 0.05$)

NH_3 (20) ~ Treatment + (1|Site) + (1|Sample) (family – Gaussian (link-log); Laplace approximation, AIC = -337.8; log likelihood = 173.9; $P > 0.05$)

NH_3 (30) ~ Treatment + (1|Site) + (1|Sample) (family – Gaussian (link-log); Laplace approximation, AIC = -327.5; log likelihood = 168.8; $P > 0.05$)

b)

Metric	Depth (cm)	Mean difference (pre- vs post-jetting)
O_2 (mg/l)	10	-0.01 ± 0.11, $P > 0.05$
	20	-0.17 ± 0.22, $P > 0.05$
	30	0.06 ± 0.23, $P > 0.05$
TAN (mg/l)	10	0.59 ± 0.72, $P > 0.05$
	20	0.73 ± 0.61, $P > 0.05$
	30	0.40 ± 0.81, $P > 0.05$
NH_3 (mg/l)	10	-0.10 ± 1.22, $P > 0.05$
	20	0.55 ± 0.98, $P > 0.05$
	30	0.05 ± 0.86, $P > 0.05$

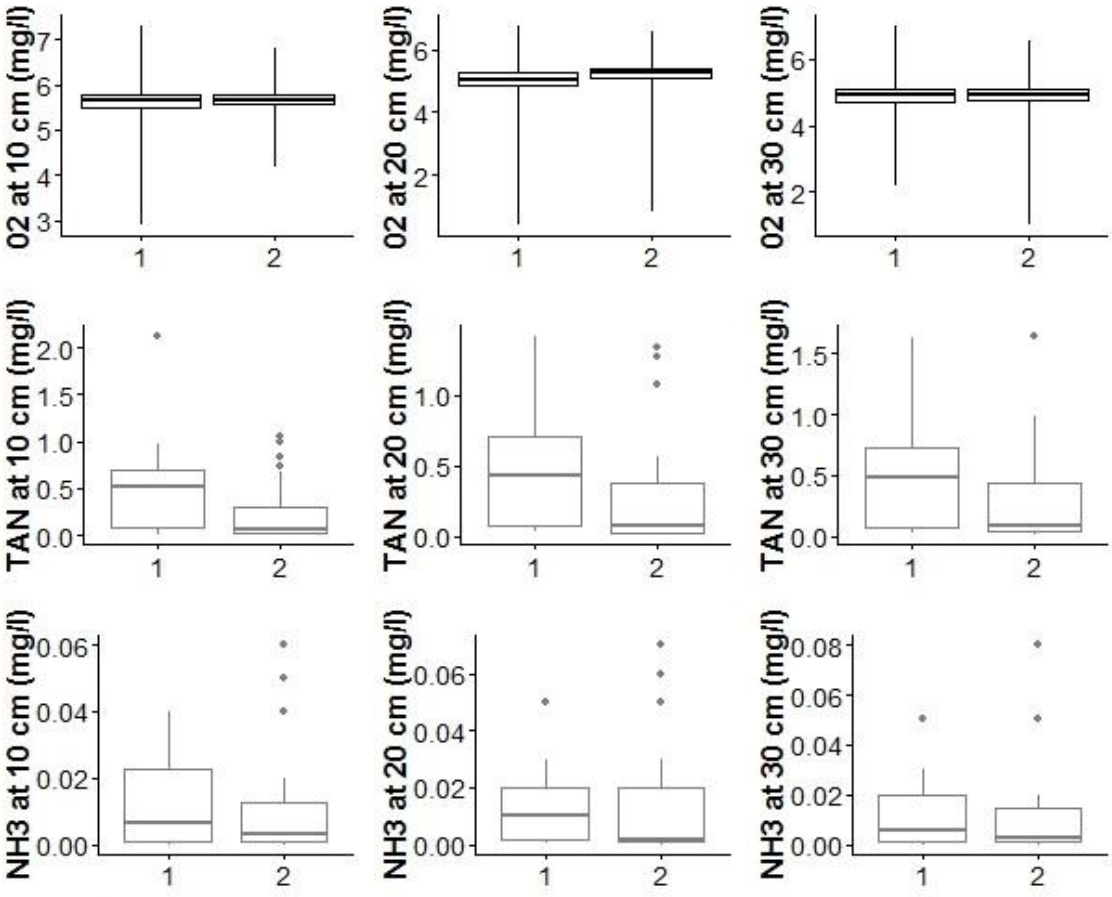

Figure 40 Hyporheic water parameters: dissolved oxygen concentration (O_2), total nitrogen ammonia (TAN) and unionized nitrogen ammonia (NH_3) at 10, 20 and 30 cm depth pre-jetting (1) and 24 hours post-jetting (2) at the riffle scale. Black horizontal lines represent mean (bold black line) and standard error; vertical lines represent max and min values. Grey horizontal lines represent 25, 50 (median – bold grey line) and 75 percentiles; vertical lines represent 95% confidence interval of the median.

6.5 Discussion

Results from this *in-situ* experiment revealed that gravel jetting had a significant impact on the composition of fluvial surface gravels, but with negligible changes to subsurface sediment characteristics at both patch and riffle scales. These quantified effects of gravel jetting at both scales diminished during the 12-month period, with no significant differences for surface percentiles and statistical parameters detected after 3 months at the patch scale in most cases. Significant quantities of fine sediments, largely consisting of sand, were purged from the bed during jetting and transported downstream and deposited by the flow. It is reasonable to assume that fine sedimentation in downstream locations would have negative consequences for the biota through changes in the size distribution of sediments. Equally, due to the loss of fines from jetted areas, changes to the size distribution and mobility of sediments within these areas are to be expected, which could potentially influence scour depths and hence, egg-to-emergence survival of lithophilic fish. Additionally, gravel jetting had no significant impact on water quality parameters within the hyporheic zone at any of the sampled depths at the riffle scale.

Analysis of surface grain size distributions during both riffle and patch scale assessments indicated that gravel jetting had a profound impact on the composition of surface spawning gravels by removing fines from bed, resulting in coarser, better sorted sediments. All percentiles increased significantly following gravel patch- and riffle-scale jetting, indicating sediments were made coarser by gravel jetting. This was also confirmed by an increase in the mean grain size following jetting. Even though sediments were already well sorted prior to gravel jetting, sediment sorting increased significantly following jetting at the riffle scale. However, at the patch scale, sediments within control and treatment patches were well sorted, with no quantifiable differences in their condition. Kurtosis and skewness were not significantly altered by gravel jetting

at the riffle or patch scale, with sediments maintaining nearly symmetrical and leptokurtic grain size distributions.

The analysis of subsurface grain size distributions at riffle and patch scales indicated that gravel jetting did not have such a profound effect on the composition of subsurface spawning gravels. D50 and D95 values did increase as a result of gravel jetting at patch and riffle scales, but the change was not significant, indicating that sediments did not become significantly coarser through jetting. This was emphasised further by no significant change in the mean grain size by gravel jetting at both scales. Also, sediments remained poorly sorted at each of the scales. Other statistical parameters remained unchanged by jetting with values implying positively skewed and leptokurtic grain size distributions. Fines content was the only parameter influenced by gravel jetting at the riffle scale. This was indicated by significant increases in the D5 percentile and decreases in sand and silt content. Despite jetting, the amount of sand remained above the previously reported 15% threshold, above which salmonids are detrimentally affected during early development in the substrate (O'Connor and Andrew 1998; Kondolf 2000).

Jetting at the patch scale did not influence the composition of surface gravels. There are two possible reasons for this. First, the condition of the river bed at each site varied significantly between the two components which could have impacted upon quantitative data. For example, the different sites used during the riffle based work each maintained substrates with high sand/ silt contents (range = 18.03 ± 10.51 % - 37.30 ± 4.86 % and 0.10 ± 0.03 % - 0.62 ± 0.25 % for sand and silt, respectively) whereas substrates at sites utilised during the patch-scale component had in general lower silt and sand contents (range = 14.38 ± 3.48 % to 23.41 ± 12.14 % and 0.10 ± 0.07 % to 0.17 ± 0.15 % for

sand and silt, respectively). High standard deviations prior to gravel jetting at the riffle scale further support these findings, as these imply high variability between sites during the riffle work. Differences in pre-jetting substrate conditions could possibly be due to differences in local habitat properties and/or histories of gravel jetting which will vary between sites and indeed, components. Pander et al. (2015) confirmed the importance of local site dynamics in shaping natural grain size distributions that would therefore impact upon the efficacy of investigated restoration techniques. Significant decreases in subsurface fines content at the riffle scale were evident at locations with high amounts of fines prior to any treatment (above 30 %). However, sites with lower amounts of fines prior to jetting showed less change post-treatment. Therefore, it is reasonable to assume that more effort is required to remove fines from sites maintaining fines-poor relative to fines-rich sediments, a finding which could have implications for where and how it is used. For example, collected data could suggest that jetting is more effective for sediments with a high fines component but it could also indicate that gravel jetting effects vary as a function of scale. Cleaning at larger scales could promote more flow through the river bed leading to more improvements in comparison to patch-scale jetting. Furthermore, despite the cleaning of riffles vs. patches being more expensive and labour-intensive, it does correspond to a scale that is more relevant for the target organism (Milner et al. 2003; Palm et al. 2007; Schmutz et al. 2016).

Measured changes in the surface and subsurface sediments at the riffle scale persisted less than a year, in line with findings from previous studies (Rubin et al. 2004; Meyer et al. 2008; Pander et al. 2015). However, as none of the investigated techniques was gravel jetting, this study is the first to quantify the longevity of effects on surface and subsurface sediment composition. Upon closer inspection of patch-scale data, it is evident that after only 3 months, sediment conditions regarding most percentiles and

statistical parameters within both control and treatment patches were similar. With regard to control patches, increases in fine sediment and decreases in coarser fractions were observed after only 3 months and changes persisted for an additional 6 months. Jetted patches followed the same pattern, regardless of temporary decreases in fine sediment fractions immediately after gravel jetting. This was expected after 3 months as the low rainfall and low river levels favour fine sediment deposition (Kondolf 2000; Levasseur et al. 2006). However, it was predicted that fines content would decrease for jetted and control patches following precipitation and high flows that could flush fines from spawning gravels. Despite this, after additional 6 months, infiltration of fines increased further, potentially due to increased run-off, which can lead to an increase in deposition rates as a function of increased turbidity and so sediment availability (Acornley and Sear 1999). Also, flushing the gravels may stimulate mixing of the surface and subsurface layers, hence increasing the propensity for fines to migrate through the sediment mixture (Franssen et al. 2014).

The amount of sediment removed from the bed during patch-scale jetting was significant (mean amount per patch: 7.04 ± 4.09 kg) and almost double that released during substratum raking, a method previously reported as the most effective fine sediment removal method (Pander et al. 2015). Extrapolation of patch-scale results suggests that in general, almost 1 tonne of sediment per site, consisting mainly of sand (mean amount per site; 60.45 ± 5.03 %), would have been mobilised via jetting during the riffle-scale experiment. This could be beneficial for egg-to-emergence and larvae survival as high concentrations of sand within sediments can aid in trapping fines within the egg pocket (Levasseur et al. 2006; Pulg et al. 2013; Sear et al. 2016). However, substrata with high proportions of sand will lead to excessive amounts of sediment deposition just below treated areas that could have implications for downstream habitats

(Pander et al. 2015). Nevertheless, under base flow conditions, this sediment could be removed from the river to avoid any potential impacts of its removal from spawning substrates on downstream habitats (personal observations). Another method to avoid the effects on downstream habitats, particularly for substrates with high amounts of silt, would be to use a modified suction dredge which would suck fines from the river bed (Sepulveda et al. 2015). Whilst this experiment has quantified the effects of jetting on sediment composition, little is known about the effects of the method on critical entrainment thresholds and the stability of bed materials under subsequent high flows. Franssen et al. (2014) emphasised the importance of system-specific knowledge on discharge characteristics so that river managers can make informed decisions about how best to flush and retain specific grain size fractions from and within the bed respectively. Even though more research is needed to understand the implications of jetting for bed stability and bedload transport under ambient and high flow conditions, this study highlights the potential impacts on aquatic fauna and flora due to the removal of sediment during jetting, and its subsequent deposition in downstream locations.

Concentrations of dissolved oxygen, total nitrogen ammonia and unionized nitrogen ammonia in the hyporheic layer during the riffle work were not significantly affected by gravel jetting over all three investigated depths. Oxygen concentration should be above 5 mg/l for optimal *B. barbus* development (Wijmans 2007), which was the case pre- and post-gravel jetting. Similarly, unionized ammonia concentration remained below reported threshold levels of 0.02 mg l^{-1} (Policar et al. 2007; Policar et al. 2010; Policar et al. 2011) at both pre- and post-gravel jetting. Total nitrogen ammonia concentration decreased following gravel jetting, but the effect was not great enough to elicit a significant difference. Regardless, conditions were representative of a hyporheic zone of high ecological status (TAG 2008). Despite levels of ammonia remaining optimal,

presence of subsurface fines could reduce hyporheic flows, thereby inhibiting ammonia removal during *B. barbus* development, increasing its toxicity, particularly during high temperatures and pH levels (Wood and Armitage 1997; Kemp et al. 2011).

Gravel jetting has been reported as a successful tool for removing fine sediment from the river bed (Shackle et al. 1999; Twine 2013). However, small sample sizes and lack of post-treatment monitoring requires further assessment. Therefore, to the best of my knowledge, this has been the first attempt at determining the effect of gravel jetting on surface and subsurface sediment properties and hyporheic water quality at different spatial and temporal scales, with an appropriate scientific design including control, treatment and replication. Even though jetting had an effect on surface sediments during this study, subsurface grain size distributions were rarely altered, particularly at the patch scale. Additionally, hyporheic water quality was not impacted upon by gravel jetting. Consequently, benefits for biota dependent on the hyporheic zone are expected to be minor following gravel jetting, at least based on the quantitative evidence presented here.

Despite this, the results of this study suggest that gravel jetting could be beneficial for shallow spawners such as *B. barbus* if it is completed just prior to their spawning aggregations and activities. This could be a short term solution for rivers maintaining fines-rich bed sediments and limited spawning grounds that are disconnected by barriers. Nevertheless, gravel jetting can result in significant bedload transport, with potential to affect downstream habitats via increased deposition. Consequently, removal of high amount of binding sediment such as sand increases the scour depth in the treated areas, and thus also increases the mobility of the remaining sediment together with buried eggs during high flow conditions. Additionally, jetting activities prior to *B.*

barbus spawning season could coincide with spawning activities of other fish species, such as *L. leuciscus* (Maitland and Linsell 2006). Therefore, careful planning is required in order not to disturb other fish species while ensuring jetting is conducted no later than 3 months prior to *B. barbus* spawning season.

Consequently, as gravel jetting represents a short-term solution solely for surface substrates with potential negative implications, catchment scale management would be more suitable in dealing with excessive sedimentation in freshwater systems (Milan et al. 2000; Hendry et al. 2003; Pulg et al. 2013). Changes in the land management, addition of buffer zones, protection of river banks from erosion and livestock, enhancement of flow via removal of dams or using flow constrictors could be some of the solutions (Hendry et al. 2003). Also, ensuring adequate size of spawning grounds is important (Honea et al. 2009; Beechie et al. 2015) with assessment of habitat connectivity emphasized as the key factor for successful habitat restoration (Tambosi et al. 2014). Notwithstanding, more research is needed on the specific tolerances of *B. barbus* eggs and larvae to fine sediments under controlled conditions. This would then provide benchmark data for river managers to assess whether or not changes in sediment composition as a function of restoration attempts would benefit target species. Therefore, the next chapter (Chapter 7) explores tolerance of *B. barbus* eggs and larvae to sand content *ex-situ*, particularly survival to emergence and timing of emergence as a result of increased sand content in the substrates.

7. *Ex-situ* assessment of variable sand contents in spawning substrates on egg to emergence survival rates and timing of emergence of *Barbus barbus*

7.1 Summary

The spawning success of lithophilic fishes is strongly dependent upon the quality of the substratum during the egg incubation period. In particular, the proportion of fine sediments in the substratum can strongly dictate its suitability for eggs and larval development. As the main study river is characterized by high sand content (*cf.* Chapter 5) and low natural recruitment of the lithophilic spawner *B. barbus*, then an *ex-situ* experiment was conducted to test how sand content in spawning gravels might impact upon *B. barbus* egg survival and the timing of emergence. The experiment was completed in incubator boxes in a recirculating water filtration system, utilising a design that comprised of 4 treatments with variable sand content (10, 20, 30 and 40 % of substrate by mass) and a control (no sand); each was replicated six times. Each incubator box housed approximately 300 eggs that were buried 5 cm deep within the substrate. Physicochemical parameters were monitored during the experiment to ensure optimal and consistent abiotic conditions in surface and hyporheic water layers across all treatments and replicates. Emerged larvae were captured and counted on a daily basis until no further emergence was evident. Results indicated there were no effects of sand on the survival of eggs to larval emergence, with survival rates no higher than 80 % irrespective of sand contents. However, the timing of emergence was significantly affected by sand content, with increases in the rate of early emergence of larvae for the 30 and 40 % sand treatments, compared with the control and lower sand content treatments. If this is reflected in the wild then it could impact upon larval post-

emergence survival through increased risks of predation and downstream displacement, impacting recruitment success.

7.2 Introduction

The reproductive success of lithophilic fishes, such as *B. barbus*, is related to the environmental conditions experienced during the period of egg incubation and larval development that influences the timing of larval emergence from the spawning substrate (the process hereafter referred to as 'emergence') (Balon 1975; Kondolf 2000; Louhi et al. 2008). The optimal levels of environmental parameters vary with early development with, for example, oxygen consumption increasing with ontogeny (Louhi et al. 2008). The period of highest oxygen uptake tends to be just prior to emergence (Greig et al. 2007; Louhi et al. 2008). Thus, the ability of eggs and larvae to survive the period between spawning and emergence is reliant on there being sufficient oxygen levels to avoid asphyxiation (Malcolm et al. 2010; Kemp et al. 2011; Franssen et al. 2012).

The main factors affecting oxygen concentration within spawning substrates include the content of fine sediments (e.g. Kemp et al. 2011; Sear et al. 2014, 2016), interstitial flow velocity (Kemp et al. 2011; Pattison et al. 2015), temperature (Kemp et al. 2011; Sear et al. 2014), presence of organic matter (Kemp et al. 2011; Sear et al. 2014, 2016) and the influence of groundwater (Youngson et al. 2004; Sear et al. 2014). Fine sediments can directly block macrospores on the egg membrane and decrease oxygen uptake through the egg chorion (Greig et al. 2005a, b; Kemp et al. 2011). Fine sediments can also indirectly decrease oxygen levels within gravel substrates by blocking interstitial pores, resulting in decreased water flow and thus reduced oxygen delivery to eggs (e.g. Franssen et al. 2012; Sear et al. 2014). Consequently, the proportion of fine sediments in spawning substrates is a major factor influencing egg

and larval survival rates, and larval development and emergence (e.g. Jensen et al. 2009; Kemp et al. 2011; Chapman et al. 2014).

Following emergence from the egg, entombment of larvae in the substrate is a mechanism that impacts upon larval survival and timing of emergence (Kemp et al. 2011; Franssen et al. 2012; Sear et al. 2016). In addition to increased mortality, sub-lethal effects of entombment also occur (e.g. Franssen et al. 2012; Sear et al. 2016). These effects include premature emergence, where fine sediments block interstitial pores and so force larvae into open water to avoid asphyxiation (Sear et al. 2016). Indirect effects of entombment include decreased oxygen levels that can also cause premature emergence (Chapman et al. 2014; Sear et al. 2016). Alternatively, it can delay larval emergence due to the decreased oxygen levels that whilst sub-lethal, slow down metabolic and growth rates, slowing development (Chapman et al. 2014; Sear et al. 2016).

Where conditions are sub-optimal in the substrate, it remains unclear which environmental parameters actually trigger larval emergence. Moreover, whilst there might be some life history advantages in premature emergence, such as the claiming of feeding territories and avoiding starvation through switching earlier to feeding on exogenous foods (Sear et al. 2016), premature emergers are often smaller with a larger yolk sac, with these characteristics increasing their susceptibility to predation and downstream displacement (Franssen et al. 2012; Sear et al. 2016). By contrast, delayed emergence can assist predation avoidance, as the larvae are protected within the gravels, although this can also increase mortality risk as more time is spent in the sub-optimal environment (Sear et al. 2016). Some studies report no difference in proportion of emerged larvae as a response to increase in fine sediment content (Fudge et al. 2008;

Sternecker and Geist 2010). However, sediments with high fines concentrations tend to experience higher emergence rates due to unsuitable conditions that form in the hyporheic layer. Still, this slows down later on due to formation of sediment seals that directly inhibit emergence (Fudge et al. 2008; Sternecker and Geist 2010).

The majority of work on the role of sediment deposition in determining egg and larval survival and emergence rates has focused on salmonid fishes. However, it is likely that other lithophilic species, such as *B. barbus*, will be impacted by similar issues during spawning and the subsequent ontogenetic development of eggs and larvae, especially because - unlike salmonids - these periods tend to be in early summer and so often coincide with low river flows (Kemp et al. 2011). There are however, no data available on *B. barbus* egg and larval tolerance to fine sediments. Consequently, the aim of this chapter was to investigate, under *ex-situ* controlled conditions, how the composition of spawning sediments affected survival rates and timing of the emergence of larval *B. barbus*. In the Great Ouse study system, *B. barbus* spawning substrates are characterised by high sand content in the subsurface layer, especially medium and coarse sand (0.064 to 2 mm) (*cf.* Chapters 5, 6). Correspondingly, the experiments conducted here were based on determining the impact of different proportions of sand in spawning substrates for emergence. The objectives were to:

(O1) determine the impact of variable subsurface sand content on *B. barbus* egg-to-emergence survival rates; and

(O2) quantify how variable sand content in the subsurface impacts the timing of *B. barbus* larval emergence. It was predicted that as the proportion of subsurface sand content increases in the subsurface sediment, egg survival rates decrease and the timing of emergence increases due to entombment.

7.3 Materials and methods

7.3.1 Experimental setup

The experiment was designed to test differences in the number and timing of emerged larvae from a range of substrates composed of varying proportions of sand (0.064 to 2 mm) concentrations (0 to 40 %). Actual egg hatching success was not assessed, as *B. barbus* larvae are photophobic after hatching and so should remain in the sediment until yolk sac absorption (Balon 1975). The experiment was completed within 'incubator boxes' of dimensions 0.5 x 0.3 x 0.095 m that were filled with different combinations of sediment mixtures. These boxes were then located inside troughs containing water of low nutrient concentrations, such that each trough accommodated 6 boxes (replicates) (Table 26). Sediment mixtures were representative of grain size distributions encountered at known *B. barbus* spawning sites within the River Great Ouse (*cf.* Chapter 5). A control and four treatments were used. The control treatment only used grain sizes above 2.8 mm, whereas the four treatments contained different proportions of sand in substrates (10, 20, 30 and 40 % sand by mass; Table 26; Figure 41). The total mass of each sediment mixture within each incubator box was 14 kg.

Table 26 Grain size distributions used in the experimental control and treatments.

	Treatment				
Grain size (mm)	**Control (n=6)**	**10 % sand (n=6)**	**20 % sand (n=6)**	**30 % sand (n=6)**	**40 % sand (n=6)**
	%	%	%	%	%
0.13	0.00	0.13	0.27	0.40	0.53
0.25	0.00	0.58	1.17	1.75	2.33
0.50	0.00	3.21	6.41	9.62	12.83
1.00	0.00	3.19	6.39	9.58	12.77
2.00	0.00	2.88	5.77	8.65	11.54
2.80	5.17	4.65	4.13	3.62	3.10
4.00	7.30	6.57	5.84	5.11	4.38
5.60	7.84	7.06	6.27	5.49	4.70
8.00	11.32	10.18	9.05	7.92	6.79
11.20	14.45	13.01	11.56	10.12	8.67
16.00	18.43	16.59	14.75	12.90	11.06
22.40	17.48	15.73	13.98	12.23	10.49
31.50	18.02	16.21	14.41	12.61	10.81

Figure 41 Sediment mixtures used in the experiment starting from no fine sediment (control) on the far left continuing with increasing amount of fines to the right.

The boxes were set up for experimental use within a recirculating system in spring 2015 at an Environment Agency fish hatchery, Calverton Fish Farm, Nottinghamshire. The recirculating system consisted of 5 larger troughs (1.7 x 0.55 x 0.21 m) connected via main inflow (top) and outflow (bottom) pipes. (Figure 42). Additionally, there was another main transport pipe just above the inflow pipe. This set up is shown in Figures 42 to 45. The overall system consisted of 2 tanks of 500 L, situated at each end of the recirculating system. Water circulated in the system from the anterior tank, through the UV filter, through the troughs containing the spawning boxes, into the posterior tank where it passed through an electric heater to maintain its temperature. This heated water was then transported back to the anterior tank where it was filtered once more and then recirculated. Water flow was maintained in the system via a system of smaller pumps, valves and pipes as per Figures 44 and 45. This ensured the inflow into each spawning box was approximately 7 l/min as per Fudge et al. (2008) who used a similar experimental design for *Oncorhynchus mykiss*.

Flow velocity was also measured in each box just above the inflow outlet with an ADV velocity meter to ensure that similar conditions were present between replicates of the experiment. As the velocity of the inflowing water was constant across the troughs and thus the spawning boxes, the different substrate compositions (Table 26) influenced the extent of the interstitial flow velocity within each box. The excess water that overflowed the boxes was collected in the troughs and transported away via the outflow pipe. To ensure emerged larvae did not escape from each box with the overflowing water, fine mesh (1 mm) was placed on the outer edges of each box (Figure 44).

Figure 42 Recirculating system at Calverton fish farm.

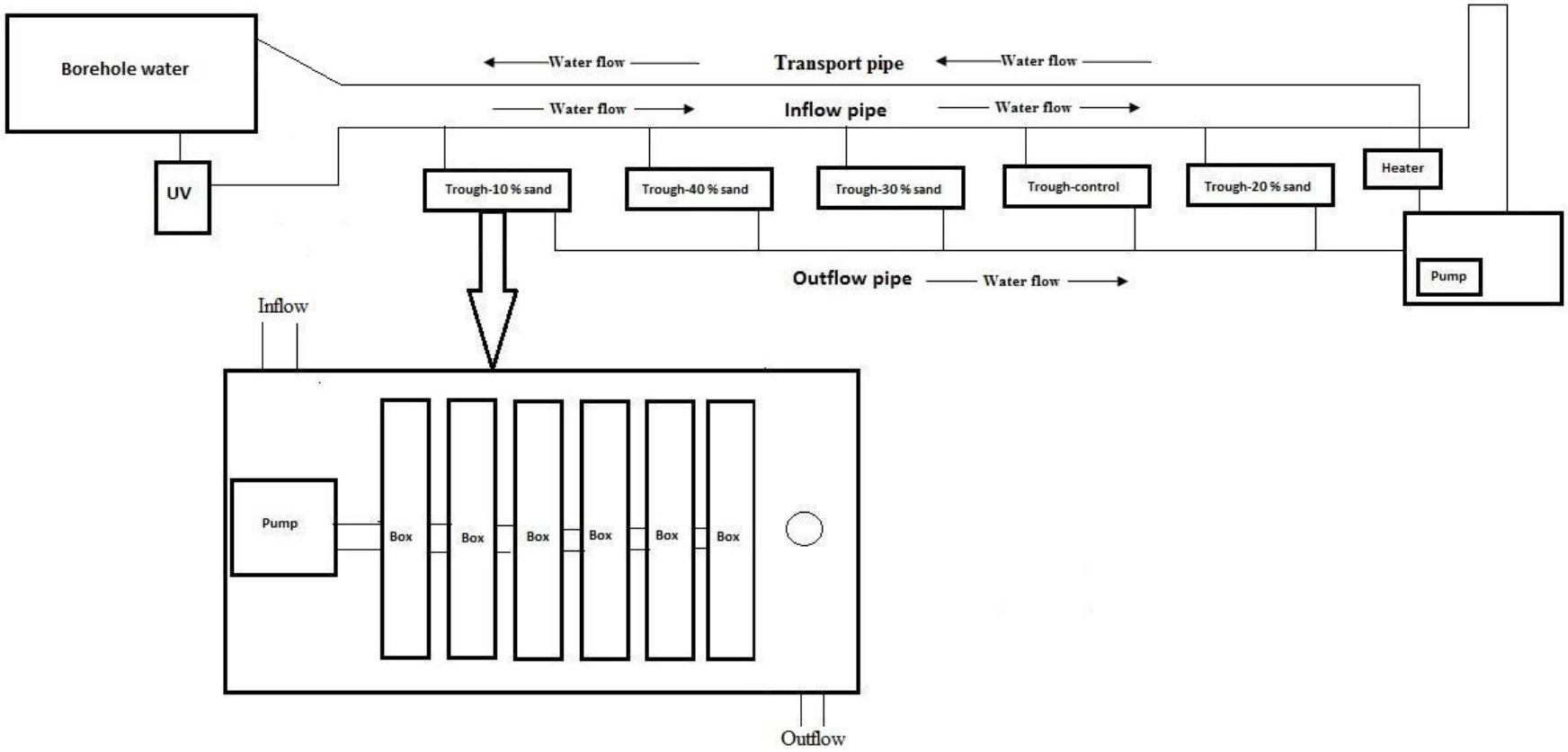

Figure 43 The set-up of the experimental design, showing the input of water from the borehole, its flow through the UV steriliser and heater, and its pumping through the system. The inset image shows the boxes within the troughs.

Figure 44 Transport of water to boxes inside each trough.

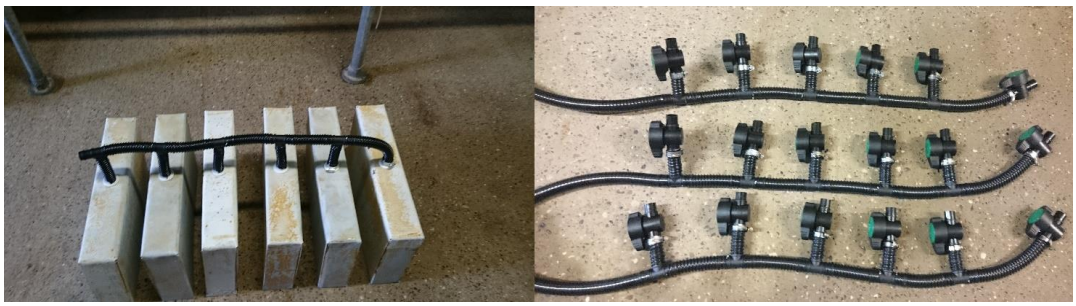

Figure 45 Tube connecting boxes inside each trough with valve gates.

Following the set-up of the system (Figures 42 to 45), it was allowed to run for 7 days to enable stabilization prior to adding *B. barbus* eggs. In this period, water velocity was measured once just above the substrate of each incubator box with an ADV velocity meter, with three replicate readings taken over one minute each, with mean values of the vertical component of velocity from each trough used as a proxy for interstitial water velocity at the start of the experiment. There were no significant differences in near-bed flow velocity between treatments (one-way ANOVA; $F_{4,25} = 1.03$, $P > 0.05$). Mean near-bed velocity was 0.01 ± 0.002 m/s, a value well above the minimal interstitial flow velocity associated with high salmon embryo survival ($4.17e^{-05}$ m/s; Greig et al. 2007; Franssen et al. 2012). Therefore, it was assumed that starting conditions within the hyporheic layer were similar and suitable across treatments with differences solely expressed in substrate composition.

7.3.2 *B. barbus* sampling, egg stripping and egg seeding in spawning boxes

On the morning May 23rd 2015, single boat electrofishing in the River Trent, Newark, captured 6 ovulating female and 6 male *B. barbus*, all of lengths above 450 mm (Figure 46). On the same day, carp pituitary extract (0.1 ml/kg), which contains gonadotropin, was administered to these individuals to initiate spawning in captivity (Figure 47a). This was repeated in the evening for females only. The fish were kept in hatchery tanks (2000 L) at 17 °C, and oxygen levels of approximately 8.5 mg/l and without feeding. The following day, the female fish were stripped of their eggs, with the eggs from one female (fork length: 690 mm; mass: 4.5 kg; Figure 47b) and sperm from 2 males (fork lengths: 490 and 530 mm; Figure 47c) mixed in a plastic bowl for approximately 10 minutes (Figure 47d). The fertilized eggs were then transferred to the experimental recirculating system (Figures 42 to 45), with approximately 300 eggs deposited inside each spawning box (mean value: 319 ± 33). The eggs were carefully deposited inside

boxes and covered with additional sediment, creating a 5 cm layer above the eggs. The exact number of eggs per box was determined using image analysis (in Image J; https://imagej.nih.gov/ij/) of the photos of the eggs prior to their deposition in the incubator boxes (Figure 48). These eggs were not counted manually due to high sensitivity of *B. barbus* eggs to handling (personal observation).

Figure 46 Electric fishing the River Trent for reproductive *B. barbus*.

Figure 47 Egg fertilization at Calverton fish farm displaying: a) hormone injection, b) female stripping, c) male stripping and d) egg and sperm mixture.

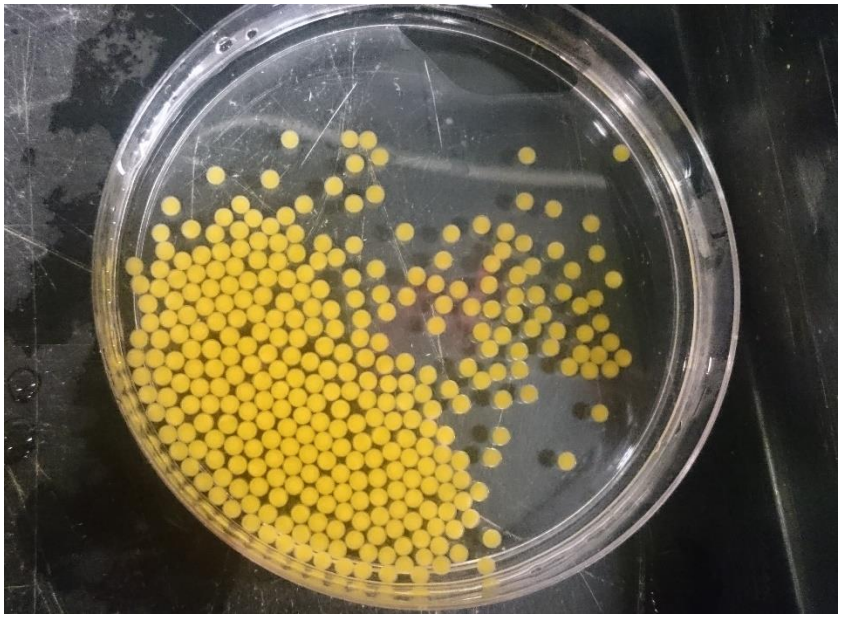

Figure 48 An example of the photo used in ImageJ for determining the number of eggs per box and treatment at the start of the experiment.

The eggs were all deposited on the morning of 24/05/15 and thus this marked the start of the experiment (6:00 am). The water temperature was set at 16 °C which was

increased to 17.5 °C five days later when hatching started, according to reported optimal temperatures and timing for eggs and larvae development (Wijmans 2007). Prior to hatching, some eggs drifted from the sediment and were removed immediately so that these eggs would not confound the outcome of the experiment, as eggs on the surface would not exhibit any potential impact of fine sediment accrual. Additionally, these eggs were counted so that the final number of eggs left in the subsurface was known to enable accurate estimates of egg to emergence survival. Table 27 shows the final egg count per box and per treatment at the start of the experiment. A 14 h light: 10 h dark photoperiod was utilized during the experiment (Policar et al. 2010; Policar et al. 2011). In addition, water temperature, pH, conductivity, dissolved oxygen and unionized ammonia concentration were monitored regularly (two to three times/day/treatment) using a YSI probe, ensuring these physical parameters remained constant and within optimal levels for *B. barbus* eggs and larvae (temperature: 16-20 °C; dissolved oxygen concertation: 8-9 mg/l, not abating below 5 mg/l; pH: 7.4-8.2; conductivity: 677-800 (Wijmans 2007); unionized ammonia concentration: $\leq$ 0.2 mg/l (Policar et al 2007, 2010, 2011)).

Table 27 Initial number of *B. barbus* eggs per box and treatment, adjusted for the number of eggs removed from the treatments that drfited from the sediment before hatching.

Box	**Number of eggs/treatment**				
	Control	**10 % sand**	**20 % sand**	**30 % sand**	**40 % sand**
1	226	452	253	258	174
2	215	384	245	290	221
3	292	273	333	243	348
4	308	324	282	269	144
5	309	257	349	256	240
6	330	427	304	210	224
Mean (± SE)	**280.0 ± 19.5**	**352.8 ± 33.0**	**294.3 ± 17.2**	**254.3 ± 11.0**	**225.2 ± 28.6**

Each egg box was inspected twice a day (morning and evening) for emerged larvae. Upon inspection, emerged larvae were captured with an aquarium net, transferred to a separate small tank of water and counted (Figure 49). Daily enumeration and removal of emergent larvae continued through the emergence period, and after 3 consecutive days of no emergence from any treatment, the experiment concluded.

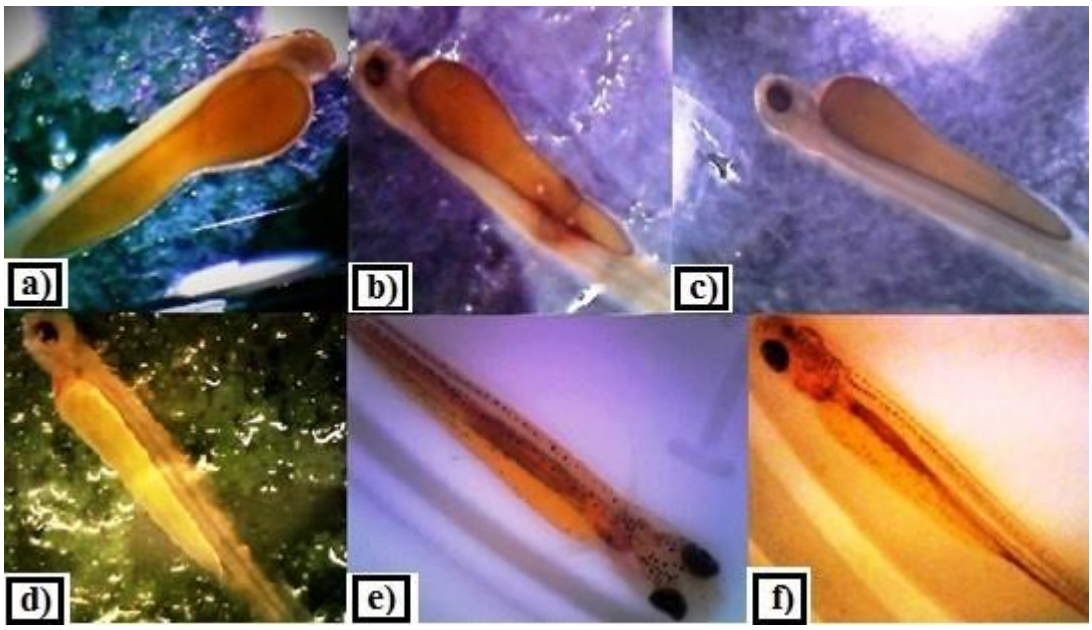

Figure 49 *B. barbus* larvae development where a) Day 2, b) Day 3, c) Day 4, d) Day 6, e) Day 8, f) Day 9.

7.3.3 Data analysis

The effect of the control and treatment on egg to emergence survival was assessed by calculating the proportion of eggs that resulted in an emerged larva in each replicate (as a value between 0 and 1). Differences in these proportions between the treatments were tested in the R package lme4. As the response variable was expressed as a proportion between 0 and 1, binomial logistic regression was performed using a generalized linear mixed effect model (family-binomial; link-logit). The proportion of eggs resulting in an emerged larva was the response variable, treatment was the fixed effect and each sample was fitted as a random effect to correct for over-dispersion. Model parameters were estimated using Laplace approximation due to data structure where the mean of the response variable was below 5. Also, weight argument was specified as the total number of eggs per box at the start of the experiment. Where a significant effect of treatment on egg to emergence survival was detected, differences in the proportions between control

and treatments were assessed by comparisons of covariate adjusted means with Dunnett adjustments for *P* values for multiple independent comparisons.

To test the effect of treatment on emergence time, a generalized linear mixed effect model was used, with the interaction between treatment and time used as a fixed effect. The response variable was the cumulative proportion of daily emerged larvae (each daily proportion value per box was added to previous available proportions to get total proportion of emerged larvae for a certain day and treatment). Hereafter, this is referred to as the proportion of daily emerged larvae. Each incubator box was assessed as a repeated random effect on the intercept to account for temporal dependency of data. Additionally, each observation (sample) was modelled as a random effect on the intercept to correct for over-dispersion. Model parameters were estimated using Laplace approximation (family-binomial; link-logit). Following significant effects of treatment and time interaction on proportion of emerged larvae, comparisons of covariate adjusted means were conducted using least-squares means with Dunnett adjustments for *P* values for multiple independent comparisons.

7.4 Results

7.4.1 Abiotic parameters and egg hatching times

During the experiment, mean water temperature across the troughs was 17.54 ± 0.11 °C, whilst dissolved oxygen was 8.25 ± 0.05 mg/l, oxygen saturation 86.04 ± 0.42 %, pH 8.04 ± 0.01, conductivity 738.38 ± 3.27 µS/cm and unionized ammonia 0.03 ± 0.001 mg/l. Therefore, all measured water quality parameters (see Appendix D; Table 19) were within reported optimal levels during *B. barbus* development (Wijmans 2007; Policar et al. 2010; Policar et al. 2011).

Emergent larvae were detected on the surface of the sediment in treatments with high sand content (30 and 40 %) from day 5 of the experiment. However, larvae were not sampled until they started swimming in the surface water, as their capture would have involved disturbing the sediments whilst the remaining eggs and larvae were still buried. Emergence to surface water column started 12 days after hatching in all treatments, and so the sampling of the emerged larvae commenced.

7.4.2. Proportion of eggs resulting in emerged larvae

The median proportion of larvae that survived to emergence was similar in all troughs (Table 28; Figure 50), with treatment not having a significant effect on egg to emergence survival (GLMM; $P > 0.05$; Table 29; Figure 50). In general, survival to emergence was never above 80 % regardless of the treatment (Table 28).

Table 28 Proportions (0 to 1) of egg to emergence survival per replicate and treatment; values in the final row are the median proportions of egg survival per treatment and interquartile range.

Proportion of eggs which survived to emergence				
Control	**10 % Sand**	**20 % Sand**	**30 % Sand**	**40 % Sand**
0.87	0.91	0.79	0.75	0.66
0.99	0.72	0.71	0.65	0.80
0.77	1.00	0.89	0.77	0.67
0.62	0.70	0.77	0.88	0.86
0.78	0.73	0.80	0.79	0.82
0.44	0.69	0.74	0.67	0.83
0.78 (0.19)	**0.73 (0.16)**	**0.78 (0.05)**	**0.76 (0.10)**	**0.81 (0.13)**

Table 29 Outputs from generalized linear mixed models testing: 1) differences in egg to emergence survival between treatments, where each sample was random effects on the intercept; 2) differences in cumulative proportion of daily emerged larvae between treatments, where each sample as well as temporal replicates were random effects on the intercept. Weight represent total number of eggs per box at start of the experiment. Mean differences are from estimated least-square means (difference significant at * $P < 0.05$ and ** $P < 0.01$).

Final models:

1. Egg to emergence survival ~ Treatment + (1|Sample), weights=Total number (family – binomial (link-logit); Laplace approximation, AIC = 316.60; log likelihood = -152.30; $P > 0.05$)

2. Total daily emergence ~ Time x Treatment + (1|Replicate) + (1|Sample), weight=Total number, (family – binomial (link-logit); Laplace approximation, AIC = 2706.90; log likelihood = -1301.50; $P < 0.01$)

Contrast	**z**	**Mean difference (± SE) between treatments in time**
Control, day 1 – 10 % sand, day 1	- 1.78	- 0.65 ± 0.37, $P > 0.05$
Control, day 1 – 20 % sand, day 1	- 4.16	- 1.52 ± 0.36, $P < 0.01$**
Control, day 1 – 30 % sand, day 1	- 7.06	- 2.57 ± 0.36, $P < 0.01$**
Control, day 1 – 40 % sand, day 1	- 8.18	- 2.98 ± 0.36, $P < 0.01$**
Control, day 2 – 10 % sand, day 2	- 2.59	- 0.94 ± 0.36, $P < 0.05$*
Control, day 2 – 20 % sand ,day 2	- 5.94	- 2.14 ± 0.36, $P < 0.01$**
Control, day 2 – 30 % sand, day 2	- 9.25	- 3.34 ± 0.36, $P < 0.01$**
Control, day 2 – 40 % sand, day 2	- 9.82	- 3.55 ± 0.36, $P < 0.01$**
Control, day 3 – 10 % sand, day 3	- 3.48	- 1.26 ± 0.36, $P < 0.01$**
Control, day 3 – 20 % sand ,day 3	- 6.44	- 2.32 ± 0.36, $P < 0.01$**
Control, day 3 – 30 % sand, day 3	- 10.50	- 3.79 ± 0.36, $P < 0.01$**
Control, day 3– 40 % sand, day 3	- 10.10	- 3.65 ± 0.36, $P < 0.01$**

Contrast	**z**	**Mean difference (± SE) between treatments in time**
Control, day 4 – 10 % sand, day 4	- 2.38	- 0.84 ± 0.35, $P > 0.05$
Control, day 4 – 20 % sand ,day 4	- 5.24	- 1.84 ± 0.35, $P < 0.01$**
Control, day 4 – 30 % sand, day 4	- 8.43	- 2.99 ± 0.35, $P < 0.01$**
Control, day 4 – 40 % sand, day 4	- 7.96	- 2.82 ± 0.35, $P < 0.01$**
Control, day 5 – 10 % sand, day 5	- 2.40	- 0.84 ± 0.35, $P > 0.05$
Control, day 5 – 20 % sand ,day 5	- 3.53	- 1.23 ± 0.35, $P < 0.01$**
Control, day 5 – 30 % sand, day 5	- 6.05	- 2.12 ± 0.35, $P < 0.01$**
Control, day 5 – 40 % sand, day 5	- 5.52	- 1.94 ± 0.35, $P < 0.01$**
Control, day 6 – 10 % sand, day 6	- 2.30	- 0.80 ± 0.35, $P > 0.05$
Control, day 6 – 20 % sand ,day 6	- 2.78	- 0.97 ± 0.35, $P < 0.05$*
Control, day 6 – 30 % sand, day 6	- 4.52	- 1.59 ± 0.35, $P < 0.01$**
Control, day 6 – 40 % sand, day 6	- 3.95	- 1.39 ± 0.35, $P < 0.01$**
Control, day 7 – 10 % sand, day 7	- 2.39	- 0.84 ± 0.35, $P > 0.05$
Control, day 7 – 20 % sand ,day 7	- 1.96	- 0.68 ± 0.35, $P > 0.05$
Control, day 7 – 30 % sand, day 7	- 3.40	- 1.20 ± 0.35, $P < 0.01$**
Control, day 7 – 40 % sand, day 7	- 2.99	- 1.05 ± 0.35, $P < 0.05$*
Control, day 8 – 10 % sand, day 8	- 1.15	- 0.40 ± 0.35, $P > 0.05$
Control, day 8 – 20 % sand ,day 8	- 0.75	- 0.26 ± 0.35, $P > 0.05$
Control, day 8 – 30 % sand, day 8	- 1.61	- 0.56 ± 0.35, $P > 0.05$
Control, day 8 – 40 % sand, day 8	- 1.24	- 0.44 ± 0.35, $P > 0.05$
Control, day 9 – 10 % sand, day 9	- 0.49	- 0.16 ± 0.35, $P > 0.05$
Control, day 9 – 20 % sand ,day 9	0.10	- 0.04 ± 0.35, $P > 0.05$
Control, day 9 – 30 % sand, day 9	- 0.21	- 0.07 ± 0.35, $P > 0.05$
Control, day 9 – 40 % sand, day 9	0.16	- 0.06 ± 0.35, $P > 0.05$
Control, day 10 – 10 % sand, day 10	- 0.97	- 0.34 ± 0.35, $P > 0.05$
Control, day 10 – 20 % sand ,day 10	- 0.17	- 0.06 ± 0.35, $P > 0.05$
Control, day 10 – 30 % sand, day 10	- 0.03	- 0.01 ± 0.35, $P > 0.05$
Control, day 10 – 40 % sand, day 10	0.32	- 0.11 ± 0.35, $P > 0.05$

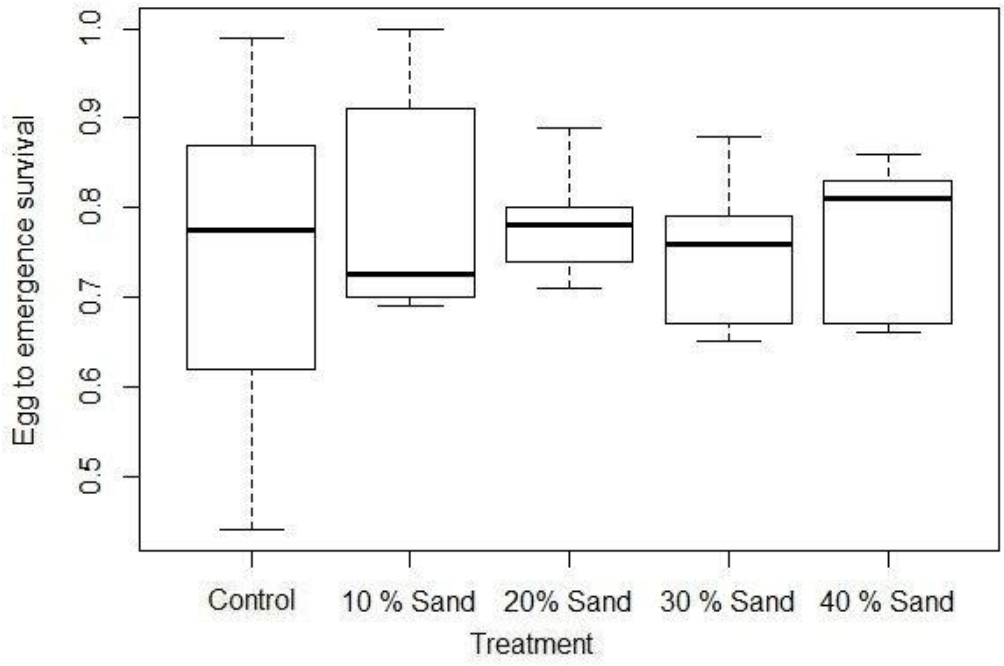

Figure 50 Proportion of eggs surviving to larval emergence, for each of the treatments. Horizontal lines represent the 10, 25, 50, 75 and 90 percentiles.

7.4.3 Timing of larval emergence

Regarding the proportion of emerged larvae between the treatments, the interaction of treatment and time had a significant effect (GLMM; $P < 0.01$; Table 29; Figure 51). Additional comparisons revealed significant differences between proportions of emerged larvae in the control and 10 % sand treatment during second, third and fourth day of the emergence ($P < 0.05$; Tables 29, 30; Figure 51). For differences between the control and 20 % sand treatment, the proportions of emerged larvae varied significantly during first 6 days, but the overall rate of emergence equalized thereafter (Tables 29, 30; Figure 51).

For the timing of larval emergence, treatments with the highest amount of fine sediment (30 and 40 %) differed significantly from the control, with a general pattern of more rapid emergence rates as the proportion of sand increased in the spawning sediment (Tables 29, 30). For example, the 30 % and 40 sand treatment differed significantly from the control in it having a higher proportion of emerged larvae during first 8 days (GLMM; $P < 0.05$; Table 29; Figure 51). In general, more than 50 % of larvae emerged from the 30 % and 40 % sand treatments in the first two days, whereas it took 4 days for more than 50 % of the larvae to emerge in the 20 % sand treatment, 6 days in the 10 % sand treatment and 7 days in the control (Table 29; Figure 51).

Table 30 Daily proportions of emerged larvae per treatment (Median and interquartile range).

Time/Treatment	Control	10 % Sand	20 % Sand	30 % Sand	40 % Sand
Day 1	0.06 (0.03)	0.10 (0.08)	0.23 (0.06)	0.41 (0.05)	0.46 (0.11)
Day 2	0.07 (0.03)	0.18 (0.11)	0.41 (0.14)	0.65 (0.07)	0.71 (0.07)
Day 3	0.07 (0.03)	0.20 (0.10)	0.45 (0.14)	0.75 (0.11)	0.72 (0.09)
Day 4	0.18 (0.07)	0.28 (0.14)	0.51 (0.17)	0.79 (0.10)	0.74 (0.09)
Day 5	0.29 (0.11)	0.41 (0.18)	0.55 (0.12)	0.80 (0.10)	0.74 (0.09)
Day 6	0.43 (0.15)	0.52 (0.19)	0.64 (0.07)	0.80 (0.12)	0.74 (0.09)
Day 7	0.51 (0.20)	0.59 (0.16)	0.66 (0.06)	0.81 (0.12)	0.75 (0.10)
Day 8	0.68 (0.22)	0.63 (0.20)	0.70 (0.07)	0.81 (0.12)	0.76 (0.10)
Day 9	0.75 (0.22)	0.66 (0.17)	0.74 (0.06)	0.81 (0.12)	0.76 (0.10)
Day 10	0.78 (0.19)	0.73 (0.16)	0.78 (0.05)	0.81 (0.13)	0.76 (0.10)

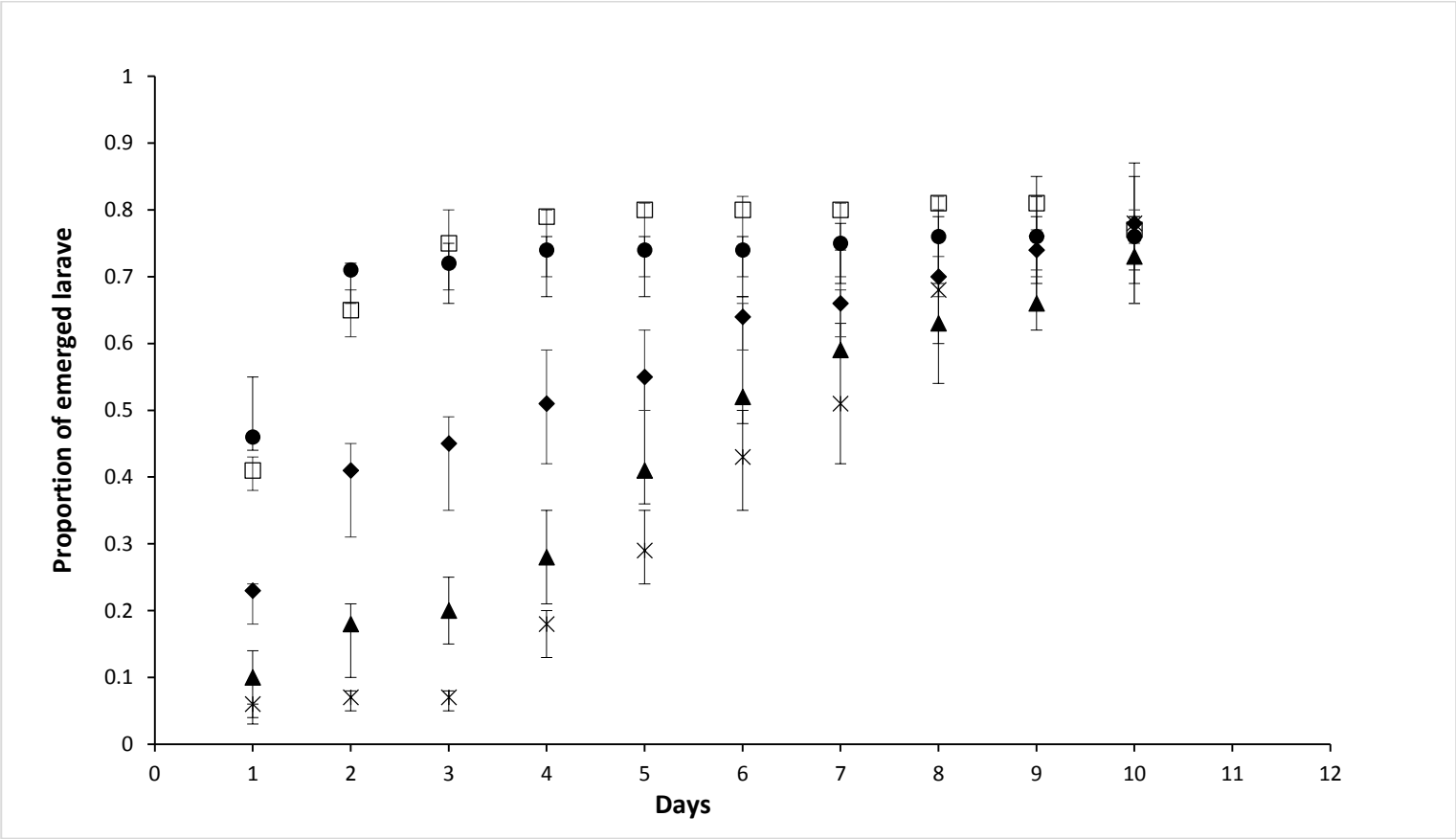

Figure 51 Cumulative daily emergence of *B. barbus* larvae between treatments. Dots represent median values with interquartile range (Q3-Q1), where (white square) control, (black square) 10 % sand, (grey triangle) 20 % sand, (white circle) 30 % sand and (black circle) 40 % sand.

7.5 Discussion

When compared to controls with no fines, treatments with variable amounts of sand content did not significantly impact upon *B. barbus* egg to emergence survival rates in the experiment, contrary to the prediction that increasing sand content in the substrata would have a negative effect. Importantly, following daily inspections of emergence rates per treatment, it was apparent that there were significant differences in the timing of larval emergence between treatments with high sand content compared to control conditions and relatively low sand content. This was particularly marked in treatments with the 30 % and 40 % sand treatments, where most larvae emerged during first 2 days as opposed to control conditions where it took 7 days to reach 50 % larval emergence. Thus, even though substrate composition did not significantly impact the proportions of eggs surviving to emergence, it did affect the timing of emergence, with the direction of this effect being contrary to the prediction and suggested entombment of larvae did not occur, at least in this initial period of emergence.

Analysis of the interaction between time and treatment revealed a significant effect on the proportion of larvae that emerged daily, where larvae from the control and 10 % sand treatment stayed in the sediment the longest. Emergence in high sand content (30 and 40 %) treatments reached 50 % during the first 2 days, which was likely to be due to either smaller voids between gravels which limit their size (Sear et al. 2016) or by low oxygen levels in sediments with high fine contents (Bowerman et al. 2014; Chapman et al. 2014; Sear et al. 2016). For example, Franssen et al. (2012) showed premature emergence of *Salvelinus fontinalis* as a response to increasing amounts of fine sediment (< 0.5 mm) under controlled conditions, with larvae maintaining smaller body sizes and weights and a greater yolk sack, due to their premature condition.

Similarly, premature larval emergence of *Salvelinus confluentus* with a larger yolk sac was evident *in situ*, as a result of high fines content (1 mm) of subsurface sediments (Bowerman et al. 2014).

Emergence to the water column in treatments with high sand content was initially fast in the experiment, with subsequent slower rates due to either entombment issues or due to emergence reaching its maximum in relation to the remaining larvae in the sediment. However, this was not evident in higher mortality rates, as emergence equalized between all treatments after 10 days. This is in accordance with Fudge et al (2008) who found no significant difference in *O. mykiss* larvae emergence or larvae condition between treatments with variable fine sediment content *in situ*. Emergence from sediments with high amount of fines exhibited higher rates initially due to unsuitable conditions in the hyporehic layer which gradually slowed down, possibly due to formation of sediment seals. Therefore, longer residence times in the substratum could be a good strategy in the wild to receive sufficient nourishment while avoiding predation and downstream drift until a size is reached where the individual is more competent at avoiding these in open water (Bowerman et al. 2014; Chapman et al. 2014; Sear et al. 2016). Laboratory experiments with variable fine sediment concentration and presence/absence of predators have revealed no significant effects of predators in high sediment treatments on the timing of emergence of *S. trutta* larvae as opposed to controls where larvae tended to postpone their emergence in predator presence (Louhi et al. 2011). Therefore, avoiding unsuitable conditions in the substratum was the prevalent mechanism to increase survival in that experiment.

In this experiment, surface water quality and flow were kept constant and optimal for early development of *B. barbus*. Therefore, the non-significant effect of the treatments on egg to emergence survival was a consequence of the increased proportion of sand not having a negative effect on survival. Nevertheless, even though hyporheic water quality and interstitial flow were assumed to be optimal at the beginning of the experiment, they were not monitored during the experiment. Thus, optimal conditions could have deteriorated in the hyporheic layer during the experiment as a result of indirect impacts of incremental sand content on interstitial water flow and hence the oxygen supply to the eggs. For these reasons, it was not possible to separate the direct effect of fines on egg chorion and emerged larvae from indirect effects on interstitial water flow and oxygen concentration.

This non-significant effect of variable sand content on egg to emergence survival of *B. barbus* detected in the experiment is in contrast to numerous studies that relate low emergence of lithopholic fish species to fine sediment content, sand in particular (e.g. Zimmerman and Lapointe 2005; Levasseur et al. 2006; Sear et al. 2016). This might be due to a number of factors. For example, *B. barbus* spawns during late spring in warmer conditions than salmonids, thus their incubation time is significantly shorter, often only one to two weeks depending on temperature (Wijmans 2007; Kemp et al. 2011). In contrast, salmonid eggs and larvae can spend four to six months in the gravel (Murray and McPhail 1987; Hendry et al. 1997; Malcolm et al. 2010). Salmonid eggs and larvae are also usually at comparatively greater depths in the substratum, leading to higher potential for entombment effects (Lisle 1989, Montgomery et al. 1996; Wijmans 2007). As incubation time affects the severity of fine sediment impacts on egg survival (Pattison et al. 2015), it was arguably not surprising that *B. barbus* was not affected

during such a short time period in the sediment. In addition, the experiment only assessed impact of sand, with little evidence of the strong relationship between sand content and survival to hatching of salmonids in some studies (Levasseur et al. 2006; Louhi et al. 2011). In these studies, silt content was reported as most detrimental to egg to hatching survival of salmonids due to blockage of egg chorion pores disabling exchange of vital gasses (Meyer 2003; Greig et al. 2005a; Sear et al. 2016), especially in sites with high sand content (Lappointe et al. 2004). However, some studies show detrimental effects of sand seals through decreases in water flow and oxygen delivery (Pattison et al. 2013; Patisson et al. 2015), as well as through blocking larvae emergence during post hatching development (Levasseur et al. 2006; Fudge et al. 2008; Sternecker and Geist 2010). This entombment process was not evident in the *B. barbus* described experiment here.

Transferring outcomes of this *ex-situ* experiment to the *in-situ* conditions of the River Great Ouse, where the mean sand composition of spawning substrates is >20 % (Section 5.4.2), suggests that these sand concentrations could be causing premature larval emergence in the river. Indeed, several other studies have reported high potential impact of premature emergence on larval survival due to their smaller body sizes and larger yolk sac that inhibits their ability to avoid predators and be displaced downstream (Franssen et al. 2012; Chapman et al. 2014; Sear et al. 2016). This could at least partially explain the low natural recruitment of *B. barbus* in the area despite adults being observed spawning on some gravels on an annual basis (Twine 2013). Moreover, the river suffers high abundances of invasive signal crayfish (*cf.* Chapter 4) that that could predate upon prematurely emerged larvae (Peay et al. 2009). However, egg survival and larval emergence can also be affected by other factors, such as temperature,

presence of high amount of organic matter, high silt content and influence of groundwater (Sear et al. 2014) that were not assessed in this experiment. Content of organic matter in the Great Ouse was generally low (Chapter 5 and 6) and was assumed to be less likely to represent a crucial factor affecting early development of lithophilic fish in the study area. Additionally, silt content was also generally low, but as sand content was high (Chapter 5 and 6), then its detrimental impact could be even more severe, particularly for egg to hatching survival (Lappointe et al. 2004). As the Great Ouse is primarily a groundwater fed river (Neal et al. 2000) then this could impact egg to emergence survival rates due to the provision of water with low oxygen levels (Malcolm et al. 2004; Youngson et al. 2004). When coupled with high temperatures, the high fines content could have an even greater negative impact on egg to emergence survival rates of *B. barbus in-situ*.

Despite some issues with the experimental design, this experiment represents a valid benchmark for further work with some adjustments required during experimental set up. This would include utilization of incubator boxes with two compartments for hatched and emerged larvae for easier daily inspection as well to ensure no escape of eggs to the surface. Also, an experiment investigating impact of silt content on egg to emergence survival is suggested due to detrimental effects already reported for other species. Monitoring interstitial water flow and oxygen levels in hyporheic layer would be beneficial in separating direct and indirect effects of increasing fine sediment in the subsurface layer. Additionally, *in-situ* experimentation would be useful to examine those effects under more complex, natural conditions, and could utilise incubator boxes and/or artificial redds (Dumas and Marty 2006; Pander et al. 2009). Nevertheless, this experiment observed some clear patterns regarding negative effect of sand content on

larvae emergence time that could play an important role in the wild, especially due to the presence of additional risks including predation and downstream displacement.

8. Discussion

8.1 Overview

The detrimental effects for fish communities arising from anthropogenic alterations of freshwater systems emphasize the importance of initiating appropriate restoration and mitigation techniques that then require robust evaluation and monitoring. This should include long-term studies, which would integrate different spatial scales of increasing complexity to represent natural systems (Fausch et al. 2002), and focus on communities rather than single species (Lindenmayer et al. 2007). However, often there are insufficient resources to support large scale community studies, hence the need for alternative approaches (Karr 1981). Using an approach based on indicator species that incorporates different life stages, in conjunction with other metrics focused on communities (Lindenmayer et al. 2007), as well as physical habitat metrics, could provide a more holistic understanding of the studied environment (Fausch et al. 2002). Thus, novel approaches, particularly if they are non-destructive, are required in studies on restoration ecology, ideally using combinations of *ex-situ* and *in-situ* experiments (Fausch et al. 2002). With the focal species of the research, *B. barbus,* encountering poor angling returns in the main study river, the Great Ouse, the emphasis here was on developing knowledge on their ecology and interactions, and how physical manipulation of river habitat could benefit these fish through a combination of *in-situ* and *ex-situ* studies.

Restoration methods that are used to increase habitat suitability and restore depleted fish stocks generally follow two strands. Firstly, fish restocking (predominately with juveniles) is commonplace and considered a viable but costly method of replenishing

depleted populations (Vilizzi et al. 2006). Secondly, habitat restoration methods (e.g. installation of fish passes, gravel jetting and use of flow deflectors) are utilised to reduce the influence of or remove the stressors that detrimentally impact upon native populations (Wheaton et al. 2004a; Wheaton et al. 2004b). However, despite gravel jetting being applied frequently as a habitat restoration method, few quantitative studies exist on its effectiveness (Shackle et al. 1999, Twine 2013). Additionally, for the majority of fishes, knowledge of the specific requirements and thus their tolerances to environmental pressures, such as fine sediments, remains limited (Kemp et al. 2011).

Consequently, following two initial chapters assessing the trophic interactions and diet composition of *B. barbus* populations, the efficacy of enhancing their wild populations with hatchery-reared stocked fish was tested. The following chapters then quantified the spawning substrates characteristics of *B. barbus* in the River Great Ouse and their effects on egg survival and timing of larval emergence. Effectiveness of gravel jetting in enhancing spawning substrates was also assed.

In Chapter 2, the utility of using historical scale data for stable isotope analysis within studies to investigate the trophic relations of riverine fish communities was assessed, including communities where stocking has been practised. Outputs revealed no trophic overlap of *B. barbus* with other species in the community, particularly in relation to *S. cephalus*, a functional analogue. The trophic position of *B. barbus* was also higher than other species in the communities. Nevertheless, as the study utilized fish species of various length ranges, lacked data on their putative food resources and a pre-stocking assessment of trophic niche size and position of each species, then further evaluation was unable to be completed. As a result, in Chapter 3, a more detailed study was

completed on *B. barbus* diet in English rivers. It revealed that their diet is heavily modified by current angling practises that involve the frequent use of marine fishmeal within 'pellets' used as bait. In a community context, this might reduce trophic interactions of stocked *B. barbus* with other species or might result in their trophic niche convergence with other species, depending on how other species utilise this allochthonous resource. This had implications for the work developed in Chapter 4, where the potential effects of stocking *B. barbus* on other fishes was assessed.

In Chapter 4, the initial experimental part of the study revealed that stocked *B. barbus* co-existed with the *S. cephalus* through their occupancy of a discrete trophic niche, probably as a result of their functional differences resulting in habitat and/ or dietary partitioning. As food abundance, ontogeny and seasonal changes in natural environments could affect the inter-specific trophic interactions, it was then necessary to evaluate trophic interactions in the natural environment with pre- and post-stocking assessment of adjacent fish communities. Therefore, this was assessed using data from two streams and three larger rivers, revealing strong patterns of trophic partitioning of the hatchery-reared *B. barbus* following stocking, with no evidence of long-term competitive interactions with other fish species. These results suggested that stocking with *B. barbus* has negligible impact upon extant fishes, at least from a trophic perspective. Nevertheless, long-term declines of low catch returns and the potential for low survival of stocked cyprinid fish in general, imply that a more sustainable method for mitigating the impacts of environmental pressures on fish populations and communities is required in the River Great Ouse.

This work commenced in Chapter 5, where the condition of Great Ouse *B. barbus* spawning grounds was assessed according to their surface and subsurface substrate properties, hydraulic characteristics, and surface and hyporheic water parameters. In general, surface sediment properties, and hydraulic and surface water characteristics in the upper part of the river maintained optimal values according to previous studies on *B. barbus* spawning habitats (Wijmans 2007; Twine 2013; Pledger 2014). However, subsurface sediment parameters suggested spawning gravels would be unsuitable for the successful incubation of salmonid fishes, due to high concentrations of fines within the substratum. Additionally, hyporheic water parameters varied substantially across sites, indicating the importance of local hydraulic conditions in determining habitat suitability for early development of lithophils.

Following the work showing that sand concentrations in the subsurface sediments of the gravels were of potential concern, Chapter 6 analysed the efficacy of gravel jetting in improving spawning habitats of *B. barbus* via alteration of surface and subsurface substrates and hyporheic water properties. Surface grain size distributions were significantly altered by gravel jetting at both riffle and patch scales, with reductions in the percentage of fines observed. The median grain size was significantly affected, indicating potential complex impacts of gravel jetting on bed mobility and downstream habitats. This was confirmed at the patch scale, where around 7 kg of sediment was mobilized by gravel jetting from each patch, which equates to almost 1 tonne of sediment when extrapolated to the riffle scale. Impacts of jetting on subsurface substrates were, however, negligible, particularly at the patch scale, where no change was evident. Additionally, at the riffle scale, all surface and subsurface substrate parameters reverted back to pre-jetting values within 12 months. Furthermore, most

surface substrate parameters at the patch scale, apart from D5, remained similar between jetted and control patches only 3 months later. Finally, hyporheic water conditions (oxygen, total nitrogen ammonia and unionized ammonia concentrations) were not significantly affected by gravel jetting.

Finally, in Chapter 7, the implications of the results of Chapter 5 and 6 on *B. barbus* egg to emergence survival and emergence timing were assessed via *ex-situ* experimentation. Following Chapter 5, there was focus on how high sand contents in spawning gravels impacted fish spawning metrics. Increases in the sand content of spawning sediments did not significantly impact upon egg to emergence survival. However, timing of emergence was affected, with significantly earlier emergence measured in treatments with high sand content, particularly 30 and 40 %.

The wider implications of the results of these data chapters are now discussed.

8.2 Applied ecology and management of *Barbus barbus* in lowland rivers

8.2.1 Trophic ecology of *B. barbus*

Stable isotope analysis was used as a tool for investigating trophic ecology of *B. barbus.* Despite studies focusing solely on stable isotopes analysis being criticized as often being inconclusive (Locke et al. 2013), they are capable of providing time-integrated measures of species interactions, with possibilities of using cross stream comparisons of food web structure (Rybczynski et al. 2008). Additionally, use of metrics based on standard ellipse areas allowed for comparison across systems with relatively small sample sizes that could be collected non-destructively, as well as incorporation of natural variability within systems by using a Bayesian inference technique (Jackson et

al. 2011). Hence, the emphasis here was on determining diet preferences of a large-bodied fish in lowland rivers, with focus on trophic interactions with native communities, particularly those with similar body sizes and functional traits, such as *S. cephalus.*

Across Chapters 2 and 4, it was revealed that riverine *B. barbus* occupy a distinct trophic niche from other fishes in their communities, with the pond enclosure work of Chapter 4 revealing that this trophic partitioning develops relatively quickly following the release of the fish. Work in Chapter 3 and 4 was assisted by the results of Chapter 2 highlighting that whilst scales can be used within stable isotope studies on fish, care is needed in relation to making comparisons between fishes of different sizes due to the strong influence of fish length on diet composition (Hyslop 1980; Mittelbach 1981). Chapter 3 then revealed that both angler baits and invasive crayfish can act as strong trophic subsidies for *B. barbus* and thus potentially plays a role in their habitat partitioning with other fishes in their community. Other studies have also indicated that marine fishmeal from fishery and aquaculture activities can provide alternative food sources in freshwater systems, leading to substantial modifications to fish diet due to their high availability (Grey et al. 2004; Fernandez-Jover et al. 2011; Demétrio et al. 2012). Additionally, introductions of invasive species can lead to their prevalence in the extant communities and so can also represent additional and valuable trophic subsidies in freshwater systems (Grey et al. 2004; Jackson et al. 2013). However, relatively few studies have focused on how these two allochthonous sources are utilised together within freshwaters (Fernandez-Jover et al. 2011; Jackson et al. 2013). Where crayfish were absent, up to 79 % of the assimilated resources of *B. barbus* represented marine fishmeal, but with this generally reducing when crayfish were also present. The

relatively high intra-population variability in *B. barbus* diet was also shown by Cherghou et al. (2002). Overall, the consumption by *B. barbus* of pelletized fishmeal in rivers as a trophic subsidy from anglers could be a mechanism involved in the patterns of trophic niche partitioning observed in the wild sites of Chapters 2 and 4, indicating the potential for *B. barbus* niche specialization when there is high availability of allochthonous resources.

The utilization of high amounts of pellets as a food resource by *B. barbus* could also have implications for their natural behaviour during foraging. This is because *B. barbus* represents a potentially important zoogeomorhological agent in freshwater systems, with the species possibly impacting upon sediment mobility and structure *in-situ* (Pledger et al. 2014, 2015). Hence, angling activities and presence of pellets in non-natural feeding habitats could alter regularity and severity of *B. barbus* impact on natural feeding habitats and thus potentially alter sediment dynamics, although it was beyond the scope of this study to investigate this further.

8.2.2 Ecological consequences of stocking *B. barbus*

Stocking fish into freshwater systems remains a popular and easy method of enhancing recreational fisheries (Cowx 1994, Aprahamian et al. 2004; Eby et al. 2006; Von Lindern and Mosler 2014), particularly with species of the Salmonidae family (Cowx 1994; Eby et al. 2006; Baer and Brinker 2010). While stocking provides benefits for recreational fisheries (Aprahamian et al. 2004; Satake and Araki 2012; Arlinghaus et al. 2014), there are genetic and ecological risks which also need consideration. If the stocked fish are from outside of the basin, then genetic impacts potentially include loss of genetic diversity of the local population (Satake and Araki 2012; Antognazza et al.

2016). Ecological consequences of stocking are related to competitive abilities of stocked fishes, as stocked species tend to be preselected by anglers due to their large body sizes and high sporting qualities (Holmland and Hammer 2004; Eby et al. 2006; Fujitani et al. 2016). For example, introduced and stocked salmonid fishes often impact upon natural ecosystems functioning via top-down control, as a result of increased species richness at higher trophic levels (Radomski and Goeman, 1995; Eby et al. 2006). This can, for example, disrupt trophic connections between aquatic and terrestrial food webs, potentially contributing to declines of native consumers in riparian habitats (Finlay and Vrendenburg 2007).

Correspondingly, Chapter 4 explored the impact of stocking *B. barbus* across different spatial and temporal scales, including various life stages. This was to ensure that stocked *B. barbus* is not detrimentally impacting upon other fish species, but also to determine if it is capable of establishing a discrete niche within the existing food web. Given the nature of the experimental and field studies, where there was minimal intervention to measure trophic interactions prior to collecting final samples to avoid disrupting the development of the trophic niches, the absence of overlaps in trophic niches of *B. barbus* and *S. cephalus* might relate to a number of factors that can only really be speculated at present. Firstly, whilst the species are both bentho-pelagic (Froese and Pauly 2014), the functional morphology of *B. barbus* favours benthic feeding, whilst *S. cephalus* arguably have greater flexibility in their feeding, thus providing considerable scope for partitioning between the species (Noble et al. 2007b; Knickle and Rose 2014). Also, in comparison to *S. cephalus*, *B. barbus* is strongly a crepuscular species, particularly during the summer (Lucas and Batley 1996; Britton and Pegg 2011). Secondly, exploitation of resources by *B. barbus* that were not already

utilized by other species could facilitate their coexistence (Shea and Chesson 2002). Finally, and conversely, the stocking of *B. barbus* into these communities and their occupancy of a discrete trophic niche could have resulted in initial competitive interactions with sympatric *S. cephalus*, with subsequent trophic niche partitioning to then avoid inter-specific antagonistic relationship (Bašić and Britton 2015).

Nevertheless, the outputs of the allopatric treatment from the mesocosm experiment in regard to comparing allopatric trophic niche sizes and positions suggested that *B. barbus* rapidly established a trophic niche and positions that were completely divergent from *S. cephalus*. Furthermore, this suggests that there would be no sharing of food resources when the species were in sympatry, facilitating the integration of stocked *B. barbus* into the fish community by preventing competition with extant fishes (Shea and Chesson 2002). Indeed, the sympatric treatment revealed that the realised trophic niches of the two species remained divergent. There were, however, reductions in the sizes of the realised niches of both species, with some adjustment in their position in isotopic space, indicating a certain level of individual specialisation (Araújo et al. 2011), with this consistent with the niche variation hypothesis (Van Valen 1965; Human and Gordon, 1996; Thomson, 2004; Olsson et al. 2009). Niche-based competition theory predicts trophic shifts of subordinate competitors when in sympatry with a dominant competitor with similar life traits (Van Valen 1965). This generally results in constriction of the trophic niche of the subordinate competitor (Human and Gordon, 1996; Thomson, 2004; Olsson et al. 2009) through diet specialization (Van Valen 1965), facilitating species coexistence (Sepulveda et al. 2012). Whilst niche constriction was indeed evident for sympatric *B. barbus* and *S. cephalus*, their somatic growth rates were not significantly different between their allopatric and sympatric contexts,

suggesting that dietary specialisation did not affect their ability to meet their energy requirements.

Experimental approaches in ecology can be ambiguous, as patterns measured under controlled conditions might not necessarily correspond with those that develop over prolonged time periods in wild systems of greater complexity and stochasticity (Korsu et al. 2009; Spivak et al. 2011; Tran et al. 2015). Nevertheless, mesocosm experiments can facilitate the understanding of the processes, in more contained and controlled settings, which underpin more complex situations in wild systems and thus can help explain temporal and/ or spatial patterns within field data (e.g. Spivak et al. 2011; Tran et al. 2015). There is also increasing evidence that such experimental approaches provide results that are consistent with more natural and complex systems, but with the added benefit of their completion at greater replication (Tran et al. 2015). Here, the outputs from the pond mesocosm experiment, where there were strong patterns of trophic niche divergence between *B. barbus* and *S. cephalus*, were highly consistent with those measured in more wild situations, whether they were fish of slightly larger body size in river side channels or considerably larger in the lowland rivers. Correspondingly, it can be argued that the development of the trophic niches measured in the two fishes in the mesocosm was a strong representation of the trophic niche alterations that develop following the stocking and establishment of *B. barbus*. However, it needs to be acknowledged that the experiment and stocking exercise were completed in simplistic systems using relatively low abundances of fish, whereas wild fish communities are composed of multiple, interacting species, and stocking often involves the sudden release of a high number of fish (Aprahamian et al. 2004). Also, an outstanding issue is how applicable *B. barbus* and *S. cephalus* are as a model species for

studying the trophic consequences of stocking non-salmonid fishes more generally. However, given their similar body sizes, life-spans and functional traits, they have high utility for transferring knowledge to other study species and systems, particularly when used in conjunction with studies on smaller-bodied introduced cyprinid fishes, such as *P. parva*, that also experienced similar ecological processes as a result of introductions (Jackson and Britton 2013, 2014; Tran et al. 2015).

With low ecological risks present, benefits to catch and release fisheries in terms of increased fish returns, as well as the genetic implications of stocking, need to be considered for *B. barbus* in the river Great Ouse. Even though no tracking studies were completed as a part of this project, monitoring small streams for two years following *B. barbus* stocking revealed low recapture rates, with the same pattern observed in the main river. Additionally, a recent genetic study on *B. barbus* in the rivers in Britain revealed that stocking *B. barbus* in its indigenous range can present a risk to the genetic integrity of its populations (Antognazza et al. 2016). Consequently, shifting to habitat restoration in fisheries management could be more sustainable long term while preserving genetic material of wild fish populations.

8.2.3 Early development of *B. barbus* and the role of incubation environment

Extensive literature on salmonid fishes report a common trend of decrease in egg survival and premature or postponed larval emergence mainly as a result of high content of fines and low oxygen concentration in the hyporheic layer (e.g. Meyer 2003; Meyer et al. 2008; Kemp et al. 2011; Franssen et al. 2012; Sear et al. 2014; Sear et al. 2016). Consequently, Chapter 5 focused on quantitative and qualitative assessments of *B. barbus* spawning riffles in the Great Ouse River. While the number of assessed riffles

(13) in the 30 km barbel zone in the Great Ouse River (see Figure 3) might represent a sufficient quantity of spawning grounds, their restricted size and fragmentation represent additional pressures for spawning *B. barbus* (Tambosi et al 2014). Furthermore, qualitative assessment of riffles detected they had high concentration of fine sediment in their subsurface layer, particularly sand, with overall mean concentrations above 20 % and were up to 47 % at several sites. These concentrations have been reported detrimental for salmonid egg to emergence survival and larval emergence in several studies (Soulsby et al. 2001; Fudge et al. 2008; Bryce et al. 2010).

Experimental assessment of sand accrual on egg to emergence survival and timing of emergence of *B. barbus* in Chapter 7 revealed no impact upon egg to emergence survival. This could be at least in part due to much shorter incubation times for *B. barbus* compared to salmonids (Balon 1975; Kemp et al. 2011), but also an absence of silt particles inside the substratum, which have been reported as the most detrimental of grain sizes for egg to hatching survival of lithophilic fish species (Levasseur et al. 2006; Louhi et al. 2008; Cocchiglia et al. 2012; Sear et al. 2016). However, timing of *B. barbus* emergence was significantly affected, with more than 50 % of larvae emerging from the gravel into the surface water in the two days after the start of larval emergence in the 30 and 40 % sand treatments. In comparison, it took around 7 days for more than 50 % of larvae to emerge from the control incubation boxes. Even though larval condition was not assessed, it could be assumed that stage of their development at the emergence differed across treatments due to the timing differences, with this potentially having implications for post-emergence survival (Franssen et al. 2012; Bowerman et al. 2014; Chapman et al. 2014; Sear et al. 2016).

While surface sediment properties and surface water parameters were not limiting factors for *B. barbus* spawning activities (Wijmans 2007) in the Great Ouse River, hyporheic water properties varied across riffles. In particular, oxygen (0.4 to 7.3 mg l^{-1}) and total ammonia nitrogen (0.01 to 2.3 mg l^{-1}) concentrations deviated substantially, indicating suboptimal conditions for early development of *B. barbus* at some locations (Wijmans 2007; TAG 2008). This could be related to several reasons including but not limited to fines content in the substratum, organic matter content and the influence of groundwater (Kemp et al. 2011; Franssen et al. 2012, 2014; Sear et al. 2014). However, the experiment in Chapter 7 could not verify impacts of fine sediment on hyporheic water conditions, hence larval emergence, as it was not possible to monitor hyporheic water quality during the experiment.

8.2.4 Impacts of restoration on spawning substrates

Increasing concentrations of fine sediments within fish spawning grounds means there is a requirement for spawning habitat enhancements to minimise or mitigate the detrimental impacts. These enhancements are mainly accomplished through small localized projects that include, but are not limited to, the addition of substrates, improvement of the existing substrates or adding flow deflectors to improve hyporheic habitats during early development (Hendry et al. 2003; Wheaton et al. 2004a; Wheaton et al. 2004b). With different enhancement methods utilised across studies, it is important to evaluate their effectiveness through adequate pre- and post-restoration assessments, including use of representative sample sizes and post monitoring periods (Giller 2005; Pander and Geist 2013; Morandi et al. 2014).

Despite presence of extensive literature on impacts of gravel addition, flow deflectors and some form of gravel improvement on fish spawning habitats (e.g. Merz and Setka 2004; Sarriquet et al. 2007; Meyer et al. 2008 Pulg et al. 2013; Pander et al. 2015), current knowledge on the effects of gravel jetting remains limited, despite its frequent application within British rivers (Hendry et al. 2003). Additionally, with only two previous studies available (cf. Shackle et al. 1999; Twine 2013) that lacked adequate quantitative assessments and post-monitoring stages, then a more robust evaluation of the effects of gravel jetting on fish spawning substrates was required here. This was completed in Chapter 6 using *in-situ* studies on substrates where *B. barbus* are known to spawn, with an additional *ex-situ* experiment completed in Chapter 7 to measure specific tolerances of *B. barbus* to fine sediments. Hence, by using both *in-situ* and *ex-situ* studies, and by including both physical and biological components into the metrics, then considerable insights into the efficacy of gravel jetting were acquired. Even though using a specific focal species, such as *B. barbus*, is sometimes criticized in ecological studies (Kar 1981), in restoration projects it can be beneficial to be species-specific, as different organisms will respond differently to restoration activities and so might require disparate actions (Pander and Geist 2013; Mueller et al. 2014). Also, to ensure that community-scale benefits are achieved, tolerances of different species to fine sediment/low oxygen concentrations would be required (Mueller et al. 2014), which is not feasible in most cases.

Consequently, using *B. barbus* as the focal species for evaluating spawning substrates enhancement more generally could be beneficial for several reasons. Firstly, *B. barbus* has been experiencing low recapture rates in the Great Ouse River, with their early life stages suggested as a bottleneck in their population viability (Twine 2013). Due to *B.*

barbus being a good indicator of good ecological status (Britton and Pegg 2011), a potentially powerful zoogeomorphological agent (Pledger et al. 2014, 2015), and their fisheries having relatively high economical values (Britton and Pegg 2011), there is high incentive for their populations to be sustainable in the longer term. Secondly, with high levels of fine sediment confirmed in the study area (Chapter 5), and with no data available on the impacts on fine sediment on lithophilic fish other than salmonids, there is a pressing requirement for further assessments on non-salmonid fishes. Finally, in restoring *B. barbus* spawning areas, other fish species might benefit as well regarding their spawning (e.g. *S. cephalus*; Balon 1975; Pinder 1997; Arlinghaus and Wolter 2003) and feeding habitats through, for example, increased macro-invertebrate abundances (Merz and Chan 2005; Mueller et al. 2014).

Thus, work was completed that explored the efficiency of gravel jetting on surface and subsurface substrates and hyporheic water conditions in the Great Ouse River at various spatial and temporal scales. Two spatial scales (patch and riffle) were used to enable the spatial extent of change to be detected and to determine which of the two approaches produced 'high quality' spawning gravels more efficiently. Importantly, the study detected a low impact of gravel jetting on the hyporheic layer, in regard to substrate and water properties, particularly in the subsurface layer at the patch scale. At the riffle scale, a significant decrease in fines content in the subsurface sediment was detected, indicating that jetting is only suitable for either enhancing larger areas or sites where it has already been determined that they contain large volumes of fine sediment (> 30 %). However, in this study, jetting never reduced the sand content below 17 %, which could still influence timing of emergence of *B. barbus* according to the outcomes of Chapter 7. Additionally, improvements persisted for only short period (< one year at riffle

scale), which is consistent with other studies on substrate restoration (Rubin et al. 2004; Meyer et al. 2008; Pander et al. 2015).

Nevertheless, as the surface substrates were altered by gravel jetting, this could be beneficial for shallow spawners such as *B. barbus* (personal observations). With benefits at the patch scale detected to last less than 3 months for most surface parameters, it would mean, however, that jetting activities would need to be planned just prior to spawning period of *B. barbus* with annual repetitions. Additionally, due to significant impact of gravel jetting on sediment mobility, this could have further implications for the structure of the remaining substrate and subsequent sediment dynamics, especially during higher flows (Wilcock and McArdell 1997; Powell 1998; Buffington et al. 2004). In particular, this could influence scour depth (Montgomery el al. 1996; Montgomery et al. 1999), hence inducing further risks for shallow spawning lithophils. Furthermore, sediment released during jetting was composed mostly of fine particles (60.31 ± 2.91 %) that are then deposited downstream, altering the structure and mobility of sediments within those as well.

Another issue to address is the applicability of these outcomes for studying impacts of gravel jetting more generally. Transferability of these data to high energy systems might be difficult, due to different geomorphological conditions and thence, interactions between sediments and water flow (Choi et al. 2015; Huang and Frimpong 2016). However, the results could be extrapolated to other low energy rivers, where gravel jetting would now appear to not be the most suitable method for enhancing spawning substrates. In contrast to salmonid fishes, there remains some potential for applying gravel jetting to *B. barbus* spawning habitat enhancement, but only if it is done just

prior to spawning and without disturbing other spawning fishes. Nevertheless, the lack of improvement in the spawning gravels for sustained periods, allied to potential negative impacts on sediment mobility and downstream habitats, emphasises that it is not a method that can mitigate sedimentation impacts easily and merely highlights the need for catchment scale management.

8.3 Conclusions and future directions

8.3.1 Main findings

The main findings of this Ph.D. research were that:

- There is a strong pattern of *B. barbus* trophic niche partitioning with other fish species in their communities, particularly regarding functionally similar *S. cephalus*; this was consistently observed across several spatial and temporal scales, and with increasing complexity and body sizes.
- While trophic niche partitioning could be a result of competitive interactions between *B. barbus* and other fish species, experimental assessment confirmed no overlap in trophic niches of functionally similar *B. barbus* and *S. cephalus* in allopatry or sympatry.
- One of the mechanisms that could explain some of the above patterns is strong diet plasticity of *B. barbus*, which was observed across 4 rivers through substantial intra-population diet specialization. This was particularly the case for fishmeal pellets that, in general, comprised a high proportion of the diet of *B. barbus*, but were partly substituted by signal crayfish at sites with invasive populations.

- Spawning grounds of *B. barbus* in the River Great Ouse are substantially impacted by the accrual of fine sediment, particularly sand, which has negative implications for *B. barbus* timing of emergence, with earlier emergence observed in high sand content treatments (particularly 30 and 40 %).
- Gravel jetting as a spawning substrate enhancement technique only improves surface sediments and has negative implications for sediment structure and mobility at treated areas and downstream habitats. Additionally, with only short term benefits reported, it would need to be repeated each year and always just prior to the commencement of the *B. barbus* spawning season. In addition, gravel jetting did not prove to be a suitable technique for enhancement of subsurface sediment or hyporheic water conditions, with only minor effects of low duration measured.

Much of the research completed in this study represents some initial steps in developing understandings of the relationships between *B. barbus* spawning habitats characteristics, their early development and subsequent recruitment. Additionally, this was the first insight into the ecological consequences of stocking large cyprinids (other than *C. carpio*), which is often neglected during management practices. Hence, some of the outcomes are still inconclusive and thus there remains a strong incentive for follow-up work. Apart from Chapter 5, which represented a descriptive overview of the studied sites that was required as the basis for Chapter 6 and 7, all other chapters raised several questions. Therefore, the following sub-section will focus on developing these in relation to future work.

8.3.2 Future work

Trophic ecology of B. barbus

While some knowledge was gained regarding *B. barbus* trophic ecology and interactions with other fish species in the community, there is still a requirement to validate the outcomes of stable isotope studies with gut content analysis where possible (Locke et al. 2013; Hamidan et al. 2015). Only then can the results on *B. barbus* diet be highly conclusive. Additionally, whilst adult *B. barbus* are highly reliant on pellets in their diet, this is less likely in smaller individuals and thus identification of their ontogenetic switch to feeding on fishmeal pellets is important ecologically. Furthermore, understanding whether there are physiological issues for *B. barbus* from consuming pellets could be important, given the high lipid levels of some of these pellets. Work could investigate *B. barbus* growth and body condition (e.g. body fat, intestinal histology) via using different types of pellets commercially available and different feeding regimes (Refstie et al. 2001; Mundheim et al. 2004). This could be done in controlled conditions, in systems similar to mesocosms previously used for stocking experiment. Additional assessment of potential impacts of angling pressure could then be conducted in natural systems by separating reaches that are heavily fished from those with minor angling pressure. This could enable further insight into importance of pellets in the diet of *B. barbus* but also determine impact of pellets on angling catching rates by assessing if fish heavily feeding on pellets are more susceptible to catching with potential implications for their welfare.

Stocking with B. barbus

This study indicated that stocking low number of *B. barbus* as a fisheries management strategy to recover its populations and benefit catch and release angling is fairly safe

from the ecological perspective at least. However, stocking exercises usually include high number of fish species being released on several occasions (Aprahamian et al. 2004) in contrast to this study with small sample sizes in the experimental and wild stocking assessments. Hence, *ex-situ* experiments that include higher number of fish species with larger sample sizes, could provide more insight into complex interactions present in natural systems with benefits of controlled conditions. Also, it would be important to determine if similar patterns of niche divergence between stocked *B. barbus* and *S. cephalus* are repeated in the systems with more limited food supplies. With the benefits of stocking in terms of angling recaptures were not quantified, there would be high utility for assessing long-term contributions to angler catches, as well as completing tracking studies to determine the fate of the stocked individuals.

Improving the survival of hatchery-reared *B. barbus* in the wild could identify how the hatchery environments influences their natural behaviours. By increasing the complexity of rearing conditions, such as mimicking natural habitats rather than rearing fish in aquaculture tanks and/ or earthen ponds. As environmental enrichment can enable more natural selection in hatchery-reared salmonids, this is then expected to increase their fitness later in life (Roberts et al. 2014; Stirngwell et al. 2014). Consequently, *B. barbus* reared in a complex hatchery environment and those from control hatcheries could be monitored via telemetry and angling catches following their stocking into natural habitats to determine potential differences in their survival, growth rates and habitat utilization.

Early development of B. barbus

As one of this study's outcomes suggested a potential impact of high sand content on larval emergence *ex-situ*, more work should be devoted to further exploring early development of *B. barbus*. Firstly, this would involve extensive redd examinations in the study system, but also other river systems, in order to determine typical redd characteristics, specifically egg burial depths. There is a paucity of knowledge on *B. barbus* spawning behaviour, particularly related to redd construction, and thus this suggests further *ex-situ* work is needed. These would involve novel assessments, including impact of silt content on *B. barbus* survival and timing of emergence and the sub-lethal effects of fine sediment accrual on larval condition. This could be conducted in incubation boxes with separate compartments for eggs and larvae, to separate egg to hatching survival and egg to emergence survival, while controlling for potential drift and evasion of eggs and larvae (Dumas and Marty 2006; Pander et al. 2009). Additionally, assessment of egg survival and larvae emergence should be repeated *in-situ* in similar incubation boxes to validate the outcomes of *ex-situ* experiments in more complex natural systems (Walling et al. 2003; Pander and Geist 2013).

Habitat restoration

Loss of genetic integrity represents a risk for wild *B. barbus* populations (Antognazza et al. 2016), indicating that fish stocking as a mitigation tool needs to be reconsidered, particularly when stocking into the *B. barbus* indigenous range and using only single catchment as a source of genetic material (Antognazza et al. 2016). Hence, more effort should be devoted to habitat restoration and its implications for fisheries management. With potential negative implications of gravel jetting strongly emphasized in this study, further research is required on impacts of gravel jetting on sediment mobility, during

high flows in particular. This could be done by measuring changes in critical shear stress as a function of gravel jetting. However, as gravel jetting only provides short terms benefits, catchment scale management has been suggested. Hence, the first step would be to determine the sources of fine sediment in the whole catchment and their overall contribution to fine sediment flux in the studied area. This could be done via a fingerprinting technique, which is a valuable tool in determining key sources of fine sediment in freshwater systems (Walling et al. 2003; Collins and Walling 2007a, b). Additionally, as the quantity of spawning substrates is also an issue for lithophilic species (Tambosi et al. 2014), particularly in the Great Ouse River, more tracking studies are required to determine potential connectivity restrictions in the area.

8.3.3 Management recommendations

Consequently, while stocking hatchery reared *B. barbus* was not detrimental for extant fish, there remains limited evidence that it benefits the sustainability of their population in the Great Ouse and indeed has potential to impact their genetic integrity (Antognazza et al. 2016). Thus, until more research is available on stocking success of fish from enriched hatchery environments, stocking ought to be replaced with more sustainable approaches for mitigating population level impacts. These include restoration of specific habitats important for different *B. barbus* life stages, including spawning and nursery habitats. Whilst localised restoration projects often provide benefits for local fish communities, they usually only represent short term solutions. Hence, catchment scale management is advised. First step here would involve determination of main sources of fine sediment in the catchment, with mitigation planning and further actions dependent on this. For example, if the source is mainly agricultural, changes in land use would be advised. However, if fine sediment is mostly derived from the river banks, this would

then involve small-scale projects related to protection of river banks, including but not limited to fencing to limit cattle access and stabilization of the river banks through tree plantations. Additionally, more work is required to determine connectivity restrictions in the studied area with incentive to remove some of the barriers for spawning fish. As this would have implications for spawning substrates in the studied reach as a result of flow and depth alterations, further modelling of habitat changes are suggested prior to the removal of any barrier. In the meantime, creation of artificial redds (incubation boxes) could provide further benefits and potentially protection from fine sediment accrual during incubation period, particularly if coupled with the installation of flow deflectors which could increase flows across the riffles.

9. References

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10. Appendices

10.1 Appendix A

Reagents:

Salicylate catalyst solution. Dissolve 440 g sodium salicylate ($C_7H_5NaO_3$) and 0.28 g sodium nitroprusside ($Na_2[Fe(CN)_5NO]\cdot 2H_2O$) in 1000 ml analytical grade water. Store in a brown glass bottle at 5^o C for up to 3 months.

Alkaline citrate solution. Dissolve 18.5 g sodium hydroxide (NaOH) and 100 g sodium citrate ($C_6H_5O_7Na_3\cdot 2H_2O$) in 1000 ml analytical grade water. Stable indefinitely.

Sodium hypochlorite solution. Commercial bleach (~5% NaOCl). Store at 5 C.

Alkaline hypochlorite solution. Add 10 ml of sodium hypochlorite solution to 90 ml of alkaline citrate solution. Prepare fresh daily.

Ammonia nitrogen 1000 mg/L TAN standard solution. Dissolve 3.8158 g of oven dried (105 ^{o}C) ammonium chloride (NH_4Cl) in 1000 ml analytical grade water with to give 1000 mg/l TAN.

Working TAN standards. The 1000 mg/l TAN stock solution must be diluted to make at least 6 standard solutions with concentrations of 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/l. Firstly, prepare a 100 mg/l TAN solution by pipetting 10 ml of the 1000 mg/l TAN standard in to a 100 ml volumetric flask and making this up to volume (i.e. 100 ml) with analytical grade water. Pipette 2, 4, 6, 8 and 10 mL of the 100 mg/l TAN solution into 5 volumetric flasks (note: that is 2 ml into one flask, 4 ml in the next, 6 ml into the third,

etc.). Make the volumetric flask up to volume and the working standards of 0.2, 0.4, 0.6, 0.8 and 1.0 mg/l have been produced. Use analytical grade water as the 0 mg/l standard.

Procedure

1. Pipet 5.00 ml of the filtered samples, reagent blank and standard solutions into 30 mL polypropylene tubes.

2. Add 0.6 ml of salicylate catalyst solution. Mix well.

3. Add 1.0 ml of alkaline hypochlorite solution. Mix well.

4. Place in a low light area for 1 h.

5. Transfer ~4 mL of solution to a macro-cuvette and measure absorbance at 640 nm after first calibrating the instrument with working standards.

10.2 Appendix B

Table 1 Characteristics of surface sediments from the River Great Ouse. Values derive from 400-count Wolman samples collected at 13 sites. Site reference is as per Figure 22.

Metric / Site	D5 (mm)	D50 (mm)	D95 (mm)	Mean (mm)	Sorting	Skewness	Kurtosis	Fines (%)
1	5.08	19.50	49.70	20.28	0.65	0.91	0.25	1.61
2	2.30	16.24	53.94	15.71	0.68	0.98	0.24	3.50
3	4.45	16.22	35.37	16.92	0.71	0.97	0.24	2.61
4	3.27	14.71	37.38	46.79	0.56	1.43	0.24	3.20
5	7.35	20.08	40.68	20.68	0.68	0.92	0.29	0.74
6	6.47	17.91	37.85	18.51	0.71	0.95	6.47	0.48
7	10.81	33.34	146.78	17.66	0.61	0.94	10.81	0.98
8	6.15	16.23	56.70	18.39	0.64	1.06	6.15	0.49
9	2.78	13.06	35.39	15.97	0.74	1.37	0.18	1.84
10	2.30	18.65	58.90	19.88	0.57	0.84	0.25	3.70
11	4.44	19.85	52.35	20.89	0.67	0.95	0.23	2.23
12	2.66	19.78	56.31	20.74	0.62	0.88	0.24	3.97
13	11.52	25.59	45.88	25.32	0.78	0.92	0.23	0.00

Table 2 Characteristics of subsurface sediments from the River Great Ouse. Values derive from 10 McNeil samples collected at 13 sites. Site reference is as per Figure 22.

Metric / Site	D5 (mm)	D50 (mm)	D95 (mm)	Mean (mm)	Sorting	Skew.	Kurt.	Fines (%)	Sand (%)	Silt (%)	OM (%)
1	0.53	10.57	43.74	12.58	0.41	0.69	0.26	17.41	17.27	0.14	2.64
	0.56	13.24	55.62	16.45	0.36	0.64	0.27	16.62	16.51	0.11	2.58
	0.56	12.22	39.96	13.08	0.52	0.76	0.25	12.91	12.69	0.22	3.89
	0.60	14.91	52.69	16.56	0.41	0.60	0.30	14.20	14.02	0.18	3.57
	0.43	13.71	59.91	15.94	0.37	0.56	0.29	17.85	17.71	0.14	2.57
2	0.22	3.55	31.12	8.07	0.19	0.70	0.29	44.24	43.37	0.88	
	0.27	8.27	105.41	10.93	0.23	0.33	0.24	31.55	30.97	0.58	
	0.18	3.45	25.35	5.55	0.23	0.51	0.27	43.41	42.39	1.02	
	0.22	5.82	33.47	8.47	0.22	0.36	0.28	36.77	35.91	0.86	
	0.26	4.64	45.21	7.82	0.23	0.53	0.23	39.71	39.47	0.24	
	0.34	7.68	34.57	10.04	0.28	0.46	0.31	30.17	29.88	0.29	
	0.21	4.37	34.38	9.36	0.19	0.61	0.31	41.79	41.16	0.62	
	0.28	7.09	31.77	8.10	0.26	0.32	0.28	32.70	32.04	0.66	
	0.23	4.79	24.14	7.20	0.22	0.40	0.32	39.94	39.41	0.53	
	0.22	5.17	26.75	6.55	0.21	0.25	0.30	38.88	38.38	0.50	
	0.22	3.55	31.12	8.07	0.19	0.70	0.29	39.74	39.61	0.13	
3	0.30	4.61	48.05	8.49	0.22	0.60	0.22	27.80	27.66	0.14	1.60
	0.34	8.52	44.52	11.23	0.28	0.47	0.28	19.00	18.89	0.12	2.24
	0.52	14.32	150.79	21.49	0.30	0.68	0.15	32.73	32.55	0.19	2.66
	0.27	7.72	44.62	11.02	0.21	0.32	0.26	35.55	35.41	0.14	1.74
	0.31	5.70	31.03	8.63	0.24	0.48	0.30	27.78	27.57	0.22	1.86
4	0.33	8.20	45.51	10.85	0.29	0.49	0.31	41.03	40.68	0.35	1.85
	0.27	3.73	30.87	7.81	0.23	0.83	0.28	23.67	23.46	0.21	1.92
	0.34	11.56	35.19	12.76	0.32	0.42	0.35	23.92	23.83	0.09	1.91
	0.40	8.35	43.21	10.70	0.34	0.61	0.27	19.41	19.25	0.16	2.96
	0.41	12.88	31.15	14.01	0.42	0.59	0.34	46.87	46.80	0.08	1.72
	0.28	3.04	39.72	9.76	0.15	0.87	0.31	41.46	41.02	0.44	
	0.28	4.60	29.84	7.82	0.23	0.53	0.29	21.79	21.62	0.16	
	0.34	3.84	30.85	9.94	0.41	3.27	0.26	46.64	45.69	0.95	
	0.19	2.69	28.87	6.17	0.20	0.81	0.26	28.14	27.91	0.24	
	0.33	8.16	43.96	10.32	0.29	0.47	0.29	15.17	15.07	0.11	
5	0.46	18.17	47.30	17.64	0.43	0.50	0.30	33.62	33.51	0.11	2.52
	0.31	7.50	29.40	8.44	0.24	0.25	0.30	13.65	13.56	0.09	2.59
	0.63	19.79	45.09	18.57	0.47	0.52	0.30	11.37	11.25	0.12	1.90
	0.51	20.17	40.87	19.25	0.58	0.69	0.27	13.42	13.37	0.05	3.21
	0.78	13.59	42.03	15.14	0.43	0.66	0.32	5.76	5.70	0.06	3.23
	1.61	20.30	40.88	20.16	0.64	0.81	0.26	21.35	21.26	0.09	
	0.40	13.20	68.00	14.32	0.34	0.44	0.30	21.37	21.28	0.09	
	0.35	12.87	37.47	13.04	0.41	0.51	0.30	23.36	23.18	0.18	
	0.35	12.93	42.65	13.18	0.32	0.34	0.32	36.03	35.59	0.43	
	0.28	8.49	31.04	9.51	0.20	0.19	0.32	13.41	13.29	0.12	
6	0.50	12.97	30.65	13.07	0.53	0.69	0.28	45.61	45.19	0.43	1.99
	0.27	3.11	28.91	6.34	0.20	0.63	0.26	26.55	26.47	0.08	3.10
	0.36	9.20	42.28	11.70	0.29	0.45	0.29	14.84	14.76	0.08	1.53
	0.47	9.35	29.95	9.88	0.53	0.76	0.26	12.84	12.75	0.09	2.34
	0.59	9.22	28.63	10.08	0.53	0.82	0.25	15.30	15.17	0.13	2.07
	0.50	11.02	28.28	11.07	0.49	0.63	0.29	12.48	12.39	0.09	
	0.66	10.27	28.94	10.79	0.56	0.80	0.25	15.94	15.86	0.08	
	0.42	9.23	35.84	10.62	0.47	0.78	0.24	9.90	9.82	0.08	
	0.77	12.90	33.37	13.43	0.55	0.77	0.27	14.66	14.59	0.07	
	0.45	11.07	32.16	11.66	0.50	0.70	0.27	16.10	16.05	0.05	
7	0.53	16.67	151.45	26.95	0.33	0.92	0.18	28.06	27.97	0.09	4.23

	D5 (mm)	D50 (mm)	D95 (mm)	Mean (mm)	Sorting	Skew.	Kurt.	Fines (%)	Sand (%)	Silt (%)	OM (%)
	0.43	5.91	30.95	7.20	0.37	0.63	0.24	28.84	28.67	0.17	1.94
	0.32	10.33	69.65	12.59	0.25	0.32	0.28	24.88	24.76	0.11	1.94
	0.42	8.07	53.78	11.19	0.32	0.63	0.21	17.69	17.65	0.04	2.33
	0.48	4.47	72.85	10.78	0.34	2.14	0.18	17.42	17.35	0.07	2.00
	0.43	14.02	36.14	14.46	0.47	0.63	0.31	18.88	18.81	0.08	
	0.46	14.94	44.26	15.69	0.37	0.47	0.30	25.43	25.26	0.17	
	0.42	7.28	58.02	8.65	0.36	0.57	0.21	18.65	18.59	0.07	
	0.52	11.57	131.78	15.66	0.35	0.72	0.15	28.53	28.46	0.07	
	0.38	7.87	107.79	12.98	0.26	0.64	0.26	13.76	13.69	0.07	
8	0.71	13.27	42.59	14.70	0.45	0.69	0.28	17.73	17.60	0.13	4.00
	0.49	9.39	38.95	10.86	0.44	0.73	0.25	20.11	20.05	0.06	2.07
	0.55	8.74	68.18	11.98	0.37	0.81	0.24	22.96	22.82	0.14	2.24
	0.41	8.27	32.46	8.82	0.40	0.54	0.26	20.09	19.95	0.13	2.00
	0.48	10.20	44.47	12.32	0.38	0.64	0.25	23.86	23.77	0.10	2.34
	0.43	7.74	35.48	9.15	0.37	0.60	0.25	20.37	20.27	0.10	2.04
	0.45	9.58	34.58	10.22	0.41	0.57	0.26	17.23	17.10	0.12	2.53
	0.46	11.52	53.21	13.02	0.44	0.69	0.22	24.28	24.20	0.08	2.23
	0.44	8.01	37.39	9.47	0.36	0.56	0.25	16.28	16.21	0.07	
	0.58	9.75	31.18	10.72	0.48	0.73	0.26	10.86	10.85	0.02	
9	0.57	9.53	25.37	9.53	0.86	0.98	0.08	21.09	21.06	0.04	0.87
	0.40	9.46	33.20	10.23	0.40	0.56	0.28	28.08	28.01	0.07	1.21
	0.33	8.05	30.48	8.85	0.31	0.38	1.53	24.86	24.83	0.03	1.37
	0.39	7.82	30.77	9.95	0.34	0.59	0.30	23.35	23.30	0.05	1.37
	0.35	9.87	34.20	10.80	0.35	0.47	0.30	10.71	10.64	0.06	1.12
10	0.88	20.78	44.75	21.00	0.48	0.62	0.33	15.49	15.42	0.07	2.69
	0.59	18.42	48.92	19.59	0.38	0.51	0.35	14.05	14.02	0.03	2.26
	0.70	15.91	70.22	18.69	0.39	0.64	0.31	24.65	24.49	0.16	2.08
	0.38	11.89	56.58	17.85	0.25	0.49	0.33	18.67	18.54	0.14	2.29
	0.42	15.00	74.65	15.13	0.38	0.45	0.22	17.61	17.58	0.03	1.62
11	0.58	11.23	43.48	12.97	0.40	0.64	0.26	10.53	10.42	0.10	1.71
	0.75	17.99	42.73	18.83	0.55	0.78	0.27	13.59	13.35	0.24	3.00
	0.55	17.54	71.44	18.25	0.49	0.68	0.21	17.08	16.94	0.14	3.13
	0.53	14.98	77.64	18.07	0.38	0.64	0.21	14.61	14.49	0.12	2.52
	0.54	12.77	42.12	13.59	0.49	0.70	0.28	20.75	20.64	0.12	2.14
12	0.53	13.68	43.81	14.24	0.35	0.42	0.31	16.79	16.71	0.08	2.10
	0.58	15.27	40.16	15.62	0.43	0.55	0.31	23.25	23.13	0.11	2.38
	0.41	12.40	40.55	13.39	0.32	0.39	0.33	24.03	23.56	0.47	2.87
	0.39	8.97	48.22	10.82	0.33	0.53	0.29	19.09	18.97	0.12	3.55
	0.49	13.95	64.28	15.93	0.35	0.50	0.29	16.77	16.52	0.25	1.87
13	0.34	24.13	51.18	20.42	0.42	0.36	0.33	16.34	16.26	0.08	3.59
	0.61	13.02	38.17	14.46	0.41	0.62	0.34	10.50	10.36	0.14	2.66
	0.77	19.77	51.47	18.92	0.53	0.63	0.27	8.75	8.63	0.11	2.97
	0.91	24.34	44.61	23.33	0.66	0.78	0.24	10.09	10.04	0.05	3.78
	0.90	23.02	87.64	21.40	0.49	0.55	0.23	17.41	17.27	0.14	2.26

Table 3 Hydraulic characteristics from the River Great Ouse collected at 13 sites. Site reference is as per Figure 22.

Metric / Site	Wetted width (m)	Site width (m)	Site length (m)	Bed slope (%)	Water surface slope (%)	Flow depth (m)	Near-bed velocity (m/s)	0.6 depth velocity (m/s)
1	12.30	2.30	8.30	0.31	0.63	0.31	0.35	0.57
	12.20	2.30	9.60			0.29	0.20	0.23
	10.40	2.40				0.24	0.09	0.24
	10.10	2.35				0.18	0.21	0.30
						0.17	0.34	0.42
						0.22	0.23	0.28
						0.31	0.31	0.62
						0.35	0.25	0.41
						0.27	0.61	0.77
						0.23	0.41	0.62
						0.20	0.14	0.45
						0.16	0.16	0.26
						0.12	0.30	0.42
						0.21	0.40	0.51
						0.24	0.41	0.58
						0.26	0.48	0.70
2	11.60	7.6	12.90	0.47	0.08	0.26	0.07	0.14
	11.60	7.6	12.90			0.22	0.41	0.45
	11.80	7.6				0.15	0.28	0.39
	11.00	7.6				0.10	0.03	0.03
						0.20	0.05	0.05
						0.25	0.33	0.54
						0.21	0.23	0.33
						0.30	0.05	0.06
						0.26	0.14	0.20
						0.32	0.16	0.26
						0.20	0.33	0.42
						0.13	0.27	0.28
						0.20	0.24	0.27
						0.10	0.78	0.77
						0.14	0.60	0.45
						0.19	0.14	0.14
3	10.70	6.00	8.90	0.22	0.45	0.10	0.37	0.36
	11.10	6.90	8.55			0.10	0.43	0.51
	10.50	6.90				0.08	0.55	0.65
	10.50	5.90				0.14	0.72	0.87
						0.06	0.25	0.27
						0.11	0.40	0.40
						0.15	0.54	0.58
						0.16	0.55	0.63
						0.07	0.22	0.20
						0.17	0.17	0.28
						0.17	0.41	0.54
						0.17	0.35	0.48
						0.18	0.16	0.27
						0.11	0.53	0.72
						0.13	0.38	0.46
						0.16	0.25	0.29
						0.10	0.37	0.36
						0.10	0.43	0.51
						0.08	0.55	0.65
						0.14	0.72	0.87

	Wetted width (m)	Site width (m)	Site length (m)	Bed slope (%)	Water surface slope (%)	Flow depth (m)	Near-bed velocity (m/s)	0.6 depth velocity (m/s)
						0.06	0.25	0.27
						0.11	0.40	0.40
						0.15	0.54	0.58
						0.16	0.55	0.63
4	10.00	7.20	9.70	0.47	0.08	0.26	0.07	0.14
	9.10	7.20	9.70			0.22	0.41	0.45
						0.15	0.28	0.39
						0.10	0.03	0.03
						0.20	0.05	0.05
						0.25	0.33	0.54
						0.21	0.23	0.33
						0.30	0.05	0.06
						0.26	0.14	0.20
						0.32	0.16	0.26
						0.20	0.33	0.42
						0.13	0.27	0.28
						0.20	0.24	0.27
						0.10	0.78	0.77
						0.14	0.60	0.45
						0.19	0.14	0.14
5	12.20	7.50	7.30	0.27	0.68	0.34	0.35	0.65
	13.30	7.50	7.30			0.63	0.48	0.55
	12.00	7.50				0.42	0.43	0.52
	14.00	7.50				0.32	0.38	0.46
						0.32	0.41	0.70
						0.36	0.52	0.71
						0.29	0.46	0.73
						0.27	0.58	0.73
						0.15	0.39	0.56
						0.26	0.49	0.76
						0.34	0.35	0.68
						0.32	0.47	0.52
						0.30	0.46	0.89
						0.26	0.39	0.71
						0.19	0.78	0.85
						0.21	0.24	0.67
6	18.60	4.20	6.40	1.09	0.16	0.20	0.66	0.72
	18.70	4.20	6.40			0.22	0.47	0.56
	18.40	4.20				0.16	0.41	0.44
	18.60	4.20				0.19	0.27	0.41
						0.21	0.45	0.44
						0.20	0.24	0.30
						0.13	0.22	0.20
						0.15	0.35	0.40
						0.20	0.46	0.66
						0.09	0.57	0.47
						0.10	0.44	0.49
						0.09	0.53	0.44
						0.09	0.41	0.29
						0.08	0.37	0.44
						0.10	0.44	0.36
						0.11	0.30	0.36
7	14.10	3.50	5.90	2.88	0.34	0.19	0.26	0.15
	14.30	3.50	5.90			0.16	0.33	0.42
	15.30	3.50				0.12	0.46	0.47
	17.30	3.50				0.08	0.00	0.04
						0.23	0.56	0.71
						0.16	0.37	0.81
						0.16	0.57	0.52
						0.12	0.32	0.35

	Wetted width (m)	Site width (m)	Site length (m)	Bed slope (%)	Water surface slope (%)	Flow depth (m)	Near-bed velocity (m/s)	0.6 depth velocity (m/s)
						0.22	0.59	0.75
						0.25	0.47	0.78
						0.16	0.34	0.50
						0.10	0.15	0.15
						0.22	0.21	0.29
						0.27	0.36	0.58
						0.19	0.26	0.15
						0.16	0.33	0.42
8	20.10	6.00	5.70	0.88	0.18	0.29	0.45	0.59
	16.30	5.00	5.70			0.29	0.46	0.58
	16.20	4.60				0.31	0.33	0.47
	16.10	4.20				0.36	0.40	0.52
						0.36	0.34	0.61
						0.28	0.42	0.63
						0.25	0.34	0.67
						0.36	0.35	0.59
						0.36	0.46	0.51
						0.29	0.25	0.65
						0.26	0.29	0.62
						0.27	0.36	0.53
						0.36	0.40	0.63
						0.28	0.31	0.57
						0.24	0.36	0.48
						0.29	0.37	0.61
9	25.00	3.70	5.60	0.98	0.20	0.42	0.36	0.46
	17.40	3.60	5.10			0.44	0.41	0.55
	16.10	3.50				0.48	0.39	0.61
	15.80	2.30				0.47	0.40	0.58
						0.50	0.38	0.67
						0.49	0.40	0.67
						0.45	0.29	0.57
						0.44	0.28	0.51
						0.40	0.43	0.60
						0.46	0.28	0.63
						0.49	0.26	0.73
						0.50	0.47	0.71
						0.52	0.35	0.80
						0.44	0.30	0.81
						0.43	0.43	0.74
						0.40	0.40	0.70
10	6.10	3.90	6.40	1.25	0.31	0.17	0.41	0.64
	6.10	3.70	6.70			0.29	0.35	0.79
	5.60	3.85				0.24	0.38	0.63
	5.95	3.60				0.35	0.39	0.70
						0.20	0.57	0.82
						0.26	0.62	0.89
						0.22	0.64	0.91
						0.23	0.31	0.71
						0.26	0.40	0.78
						0.27	0.43	0.81
						0.21	0.64	0.91
						0.28	0.29	0.50
						0.29	0.25	0.38
						0.27	0.20	0.77
						0.25	0.67	0.87
						0.16	0.34	0.57
11	14.50	5.30	5.15	7.77	0.19	0.41	0.37	0.71
	10.00	5.20	5.15			0.41	0.48	0.56
	9.10	4.70				0.39	0.50	0.66
	9.20	3.60				0.35	0.50	0.72

	Wetted width (m)	Site width (m)	Site length (m)	Bed slope (%)	Water surface slope (%)	Flow depth (m)	Near-bed velocity (m/s)	0.6 depth velocity (m/s)
						0.34	0.58	0.87
						0.32	0.69	0.88
						0.26	0.46	0.89
						0.30	0.57	0.82
						0.28	0.60	0.95
						0.30	0.30	0.86
						0.33	0.27	0.95
						0.26	0.56	0.95
						0.38	0.58	1.18
						0.28	0.51	1.02
						0.28	0.48	0.84
						0.32	0.16	0.65
12	17.00	3.60	7.80	1.03	0.06	0.46	0.26	0.44
	14.90	2.80	7.30			0.48	0.22	0.46
	14.10	3.10				0.44	0.23	0.42
	15.30	2.80				0.48	0.10	0.31
						0.52	0.20	0.33
						0.42	0.19	0.36
						0.36	0.35	0.44
						0.28	0.13	0.41
						0.35	0.06	0.33
						0.37	0.18	0.43
						0.44	0.22	0.40
						0.52	0.15	0.39
						0.50	0.27	0.38
						0.42	0.14	0.35
						0.38	0.22	0.37
						0.35	0.18	0.32
13	18.50	2.10	11.70	0.77	0.17	0.20	0.30	0.40
	17.80	3.00	11.70			0.34	0.51	0.70
	17.40	3.40				0.40	0.37	0.79
	17.10	2.00				0.38	0.51	0.67
						0.36	0.68	0.93
						0.28	0.41	0.84
						0.22	0.75	0.97
						0.12	0.44	0.50
						0.14	0.51	0.45
						0.22	0.39	0.87
						0.28	0.51	0.84
						0.36	0.68	1.06
						0.31	0.44	0.94
						0.22	0.36	0.84
						0.18	0.62	0.76
						0.14	0.59	0.67

Table 4 Characteristics of surface water from the River Great Ouse collected at 4 sites. Site reference is as per Figure 22.

Metric / Site	Temp (° C)	Cond (µS/l)	pH	Dissolved oxygen (mg/l)	Dissolved oxygen (% sat)	TAN (mg/l)	NH_3 (mg/l)
5	16.45	847	8.10	5.70	67.00	0.55	0.02
	16.50	704	8.10	6.30	76.00	0.56	0.02
	16.35	842	8.10	4.80	61.00	1.16	0.04
	16.15	848	8.10	4.50	60.00	0.29	0.01
	16.20	850	8.10	5.10	65.00	1.65	0.06
	16.20	850	8.10	5.10	65.00	0.30	0.01
	16.10	850	8.10	4.50	60.00		
	16.15	850	8.10	4.80	63.00		
	16.20	848	8.10	4.80	63.00		
6	18.30	874	8.01	6.79	71.50	0.10	0.003
	18.00	872	8.01	6.63	70.00	0.08	0.003
	17.90	872	8.02	7.01	73.90	0.03	0.001
	17.90	872	8.02	6.88	72.50	0.06	0.002
	17.90	859	8.03	6.82	71.90	0.08	0.003
	17.80	872	8.03	6.54	69.00		
	17.80	868	8.03	6.58	69.30		
	17.80	854	8.02	6.85	72.00		
	17.80	850	8.03	6.45	67.80		
7	16.40	844	8.03	6.46	65.80	0.06	0.002
	16.70	840	8.01	6.25	64.20	0.07	0.002
	16.70	847	8.02	6.23	63.90	0.15	0.005
	16.80	848	8.02	6.20	63.70	0.12	0.004
	16.60	840	8.01	6.24	63.90		
	16.50	850	8.02	6.37	65.20		
	16.40	840	8.02	6.18	63.10		
	16.20	844	8.02	6.08	61.80		
	16.20	849	8.02	6.23	63.30		
8	18.45	696	8.20	7.40	87.00	0.49	0.02
	17.65	695	8.20	8.30	96.00	0.69	0.03
	17.30	695	8.20	7.60	89.00	0.42	0.02
	17.20	691	8.20	7.60	89.00	0.37	0.02
	17.12	697	8.20	6.40	77.00	1.00	0.05
	16.90	694	8.20	6.50	79.00	0.55	0.03
	16.95	691	8.20	6.70	81.00	0.76	0.04
	16.95	693	8.30	6.50	79.00		
	16.95	695	8.20	6.80	82.00		

Table 5 Characteristics of hyporheic water from the River Great Ouse at 10 cm depth, collected at 6 sites. Site reference is as per Figure 22.

Metric / Site	Temp (° C)	Cond (µS/l)	pH	Dissolved oxygen (mg/l)	Dissolved oxygen (% sat)	TAN (mg/l)	NH_3 (mg/l)
2	17.55	675	6.40	5.50	75.00	0.08	0.0001
	17.30	679	6.80	5.40	68.00	0.15	0.0002
	16.95	674	6.30	5.40	67.00		
4	17.65	672	6.40	6.90	82.00	0.43	0.002
	17.50	678	7.10	5.60	70.00	2.13	0.009
	17.45	690	6.70	4.70	61.00	0.14	0.001
	17.50	677	7.30	5.90	74.00	0.69	0.003
	17.25	681	7.40	4.50	59.00	0.82	0.004
	17.15	675	7.60	6.30	79.00	0.05	0.0002
	17.50	672	6.90	6.70	80.00		
	17.30	675	7.40	7.30	87.00		
	17.75	681	7.30	5.80	71.00		
5	16.25	851	8.10	4.80	63.00	0.69	0.03
	16.15	850	8.10	4.70	62.00	0.61	0.02
	16.25	851	8.20	4.90	64.00	0.50	0.02
	16.15	850	8.10	4.70	62.00	0.53	0.02
	16.20	848	8.10	4.60	62.00	0.98	0.04
	16.15	853	8.20	5.10	64.00	0.95	0.03
	16.35	859	8.00	2.90	45.00	0.56	0.02
	16.25	865	8.10	4.00	56.00		
	16.35	851	8.10	4.70	62.00		
6	17.70	844	7.94	6.24	65.40	0.03	0.001
	17.60	850	7.99	7.10	74.20	0.04	0.001
	17.60	843	7.90	6.66	69.70	0.04	0.001
	17.60	841	8.00	6.50	67.90	0.06	0.002
	17.50	846	7.97	6.38	66.50	0.03	0.001
	17.30	839	8.00	6.38	66.30	0.01	0.0003
	17.40	844	7.96	5.72	59.50		
	17.40	844	7.99	6.50	67.50		
	17.20	840	7.99	6.46	67.00		
7	16.70	839	8.04	6.21	63.10	0.70	0.02
	16.70	844	8.01	6.18	63.40	0.05	0.002
	16.50	833	8.01	6.23	63.70	0.21	0.006
	16.70	850	8.03	6.19	63.50	1.41	0.04
	16.90	835	7.92	5.70	58.60	0.01	0.0003
	16.80	835	7.83	4.72	48.60	0.01	0.0003
	16.60	845	8.09	6.56	67.30		
	16.80	845	8.06	6.25	64.30		
	17.00	847	8.09	6.48	66.80		
8	16.95	692	8.20	6.50	79.00	0.62	0.03
	16.95	693	8.20	6.40	78.00	0.70	0.03
	16.80	693	8.20	6.10	75.00	0.51	0.02
	16.90	693	8.20	5.80	72.00	0.87	0.04
	17.00	700	8.10	5.50	70.00	0.74	0.04
	16.80	695	8.20	5.40	69.00	0.49	0.02
	16.70	695	8.20	5.60	71.00		
	17.00	702	8.10	4.80	63.00		
	17.15	700	8.20	5.70	71.00		

Table 6 Characteristics of hyporheic water from the River Great Ouse at 20 cm depth, collected at 6 sites. Site reference is as per Figure 22.

Metric / Site	Temp (° C)	Cond (µS/l)	pH	Dissolved oxygen (mg/l)	Dissolved oxygen (% sat)	TAN (mg/l)	NH_3 (mg/l)
2	17.80	675	6.80	5.40	67.00	0.80	0.001
	16.95	677	6.80	5.40	67.00	0.82	0.001
	16.90	675	6.30	5.50	68.00	0.07	0.0001
4	17.35	685	7.70	6.10	74.00	1.09	0.020
	17.35	690	7.80	6.10	75.00	0.22	0.004
	17.30	702	7.60	5.00	63.00	0.08	0.002
	16.55	674	7.50	6.20	76.00	1.08	0.020
	16.15	679	7.90	6.40	78.00	1.35	0.025
	16.15	674	7.90	5.30	68.00	0.14	0.003
	16.40	678	7.80	4.70	62.00	0.29	0.005
	16.30	674	8.10	5.20	67.00		
	16.40	677	7.80	5.10	65.00		
5	16.20	871	8.00	3.00	46.00	1.42	0.05
	16.30	848	8.10	4.30	58.00	0.46	0.02
	16.25	863	8.10	4.40	59.00	0.65	0.02
	16.30	850	8.10	4.50	60.00	0.73	0.03
	16.35	853	8.10	4.70	62.00	0.41	0.02
	16.35	851	8.10	0.40	22.00	0.5	0.02
	16.15	860	8.10	3.50	51.00		
	16.25	876	8.00	3.70	53.00		
	16.15	852	8.20	4.70	62.00		
6	17.60	843	7.93	6.00	62.70	0.06	0.002
	17.60	839	7.89	5.92	61.90	0.07	0.002
	17.60	844	7.99	6.75	70.70	0.08	0.002
	17.50	844	7.93	6.46	67.50	0.04	0.001
	17.40	835	7.84	5.75	60.00	0.06	0.002
	17.40	845	7.99	6.20	64.60	0.03	0.001
	17.40	849	8.00	6.53	68.00		
	17.30	847	7.85	5.20	54.00		
	17.30	837	7.78	4.09	42.60		
7	16.60	844	8.04	6.13	62.60	0.21	0.006
	16.70	834	7.93	5.46	56.00	0.12	0.004
	16.50	840	8.03	6.43	65.70	0.01	0.0003
	16.80	835	7.95	5.94	61.10	0.07	0.002
	16.80	843	7.97	5.79	59.50	0.05	0.002
	16.70	844	7.96	6.10	62.40	0.09	0.002
	16.90	846	8.00	6.13	63.20		
	16.90	840	7.89	5.62	58.00		
	16.90	835	8.01	6.17	63.60		
8	16.90	693	8.20	6.10	75.00	0.62	0.02
	16.85	693	8.20	6.10	75.00	0.60	0.02
	16.85	693	8.20	5.70	72.00	0.39	0.01
	16.90	699	8.10	5.10	65.00	0.70	0.03
	17.00	694	8.20	5.50	69.00	0.32	0.01
	16.70	721	8.10	3.70	52.00	0.56	0.02
	16.65	704	8.00	3.30	49.00		
	17.15	705	8.10	4.80	63.00		
	17.05	716	8.10	4.20	57.00		

Table 7 Characteristics of hyporheic water from the River Great Ouse at 30 cm depth, collected at 6 sites. Site reference is as per Figure 22.

Metric / Site	Temp (° C)	Cond (µS/l)	pH	Dissolved oxygen (mg/l)	Dissolved oxygen (% sat)	TAN (mg/l)	NH_3 (mg/l)
2	17.50	675	6.00	5.40	67.00	0.04	0.00003
	16.90	675	6.80	5.30	66.00	0.04	0.00003
	16.70	675	6.40	5.40	68.00		
4	17.25	758	7.30	5.70	70.00	0.24	0.001
	16.85	712	7.20	5.70	68.00	1.63	0.010
	17.45	674	7.70	6.30	77.00	0.74	0.010
	16.15	675	8.10	5.80	72.00	0.98	0.010
	15.90	699	8.00	4.90	65.00	0.62	0.004
	16.15	674	6.90	5.00	65.00	0.93	0.010
	15.45	673	6.90	4.80	62.00		
	15.80	689	7.00	4.30	58.00		
	16.00	837	6.70	3.00	46.00		
5	16.20	853	8.10	4.80	63.00	0.58	0.02
	16.20	858	8.10	4.10	56.00	0.83	0.03
	16.25	886	7.90	3.30	49.00	1.30	0.05
	16.20	858	8.00	2.80	44.00	0.24	0.01
	16.25	871	8.10	4.30	58.00	0.07	0.003
	16.35	851	8.20	4.50	60.00	0.43	0.02
	16.25	853	8.10	4.50	60.00		
	16.25	896	8.00	2.80	44.00		
	16.20	855	8.10	4.70	62.00		
6	17.40	818	7.71	3.15	32.90	0.06	0.002
	17.30	836	7.74	5.02	52.50	0.04	0.001
	17.50	836	7.98	7.01	73.20	0.05	0.001
	17.50	836	8.01	6.86	71.50	0.07	0.002
	17.50	846	7.99	6.83	71.30	0.03	0.001
	17.20	832	7.74	3.80	39.30	0.09	0.002
	17.40	840	7.91	6.28	65.30		
	17.20	850	7.91	5.93	61.40		
	17.10	833	7.94	6.23	64.60		
7	16.70	844	8.01	6.34	66.10	0.07	0.002
	16.60	844	7.98	6.25	64.00	0.04	0.001
	16.70	837	8.02	6.35	65.00	0.14	0.004
	16.80	849	8.02	6.47	66.60	0.10	0.003
	16.80	836	7.95	5.83	59.90	0.01	0.0003
	16.60	846	8.02	6.00	61.50	0.03	0.001
	16.90	843	8.03	6.43	66.30		
	17.00	837	7.77	4.63	47.60		
	16.90	849	7.99	5.94	61.30		
8	16.85	701	8.10	5.00	65.00	0.68	0.03
	16.85	693	8.20	5.70	71.00	0.80	0.03
	16.90	693	8.30	5.70	71.00	0.63	0.02
	16.80	701	8.00	4.50	60.00	0.38	0.01
	16.95	700	8.20	5.20	66.00	0.59	0.02
	16.75	696	8.20	5.40	69.00	0.53	0.02
	16.95	700	8.10	4.40	59.00		
	17.10	696	8.20	5.10	65.00		
	17.00	734	7.90	2.20	38.00		

10.3 Appendix C

Table 8 Surface metrics 24 hours post gravel jetting at riffle scale collected at 5 sites. Site reference is as per Figure 30.

Metric / Site	D5 (mm)	D50 (mm)	D95 (mm)	Mean (mm)	Sorting	Skewness	Kurtosis
1	5.94	21.78	65.8	23.20	0.66	0.96	0.22
2	2.49	22.48	44.85	22.41	0.68	0.85	0.24
3	12.09	27.91	44.52	28.55	0.78	0.98	0.26
4	11.13	25.03	43.63	24.97	0.77	0.93	0.24
5	10.31	24.19	57.11	25.10	0.72	0.97	0.25

Table 9 Surface metrics 1 year post gravel jetting at riffle scale collected at 4 sites. Site reference is as per Figure 30.

Metric / Site	D5 (mm)	D50 (mm)	D95 (mm)	Mean (mm)	Sorting	Skewness	Kurtosis
2	4.90	17.9	40.10	18.46	0.68	0.92	0.27
3	7.05	20.19	40.20	20.61	0.69	0.91	0.29
4	5.45	17.39	42.48	19.15	0.67	1.03	0.25
5	5.40	16.50	47.19	17.69	0.64	0.95	0.24

Table 10 D5 (mm), D50 (mm) and D95 (mm) percentiles per site (± SE) at control and jetted patches, 1 hour, 3 months and 9 months post-jetting. Site reference is as per Figure 30.

		Site reference		
1 hour		**3**	**5**	**6**
Control	D5	6.24 ± 0.23	7.74 ± 0.59	5.49 ± 1.90
	D50	21.47 ± 2.92	18.70 ± 0.64	24.42 ± 1.68
	D95	40.57 ± 0.86	40.81 ± 1.22	42.41 ± 0.75
Treatment	D5	6.80 ± 1.65	10.10 ± 0.84	13.77 ± 2.02
	D50	22.50 ± 0.91	24.70 ± 2.65	30.07 ± 0.49
	D95	41.87 ± 0.54	55.88 ± 6.99	53.22 ± 4.28
3 months		**3**	**5**	**6**
Control	D5	3.08 ± 0.51	2.63 ± 0.45	1.61 ± 0.42
	D50	13.75 ± 0.75	9.80 ± 0.55	18.82 ± 1.24
	D95	28.32 ± 0.39	29.71 ± 0.58	34.14 ± 3.49
Treatment	D5	4.61 ± 1.21	4.56 ± 0.49	7.30 ± 1.44
	D50	13.22 ± 0.70	14.08 ± 1.75	17.41 ± 1.19
	D95	28.98 ± 0.22	33.40 ± 2.02	31. 19 ± 0.60
9 months		**3**	**5**	**6**
Control	D5	1.72 ± 0.77	0.89 ± 0.17	1.54 ± 0.15
	D50	13.74 ± 0.71	9.36 ± 1.23	18.61 ± 0.87
	D95	30.31 ± 6.34	35.45 ± 1.36	40.47 ± 1.07
Treatment	D5	3.11 ± 2.24	0.69 ± 0.06	2.45 ± 0.49
	D50	17.74 ± 1.60	8.75 ± 1.51	15.23 ± 1.57
	D95	37.72 ± 1.43	28.66 ± 5.16	36.50 ± 2.94

Table 11 Mean (mm), sorting, skewness and kurtosis parameters per site (± SE) at control and jetted patches, 1 hour, 3 months and 9 months post-jetting. Site reference is as per Figure 30.

		Site reference		
1 hour		**3**	**5**	**6**
Control	Mean	20.47 ± 1.14	19.67 ± 0.95	23.64 ± 2.08
	Sorting	0.68 ± 0.03	0.71 ± 0.01	0.72 ± 0.04
	Skewness	0.82 ± 0.10	0.98 ± 0.03	0.84 ± 0.07
	Kurtosis	0.28 ± 0.02	0.26 ± 0.00	0.25 ± 0.01
Treatment	Mean	22.32 ± 0.76	25.69 ± 2.72	31.23 ± 0.63
	Sorting	0.72 ± 0.02	0.70 ± 0.02	0.79 ± 0.01
	Skewness	0.89 ± 0.04	0.96 ± 0.01	1.02 ± 0.01
	Kurtosis	0.26 ± 0.01	0.25 ± 0.01	0.26 ± 0.01
3 months		**3**	**5**	**6**
Control	Mean	14.26 ± 0.29	11.21 ± 0.75	18.32 ± 1.90
	Sorting	0.68 ± 0.02	0.62 ± 0.03	0.72 ± 0.02
	Skewness	0.93 ± 0.05	1.05 ± 0.03	0.85 ± 0.08
	Kurtosis	0.29 ± 0.02	0.25 ± 0.03	0.23 ± 0.02
Treatment	Mean	14.22 ± 0.34	15.07 ± 1.45	17.40 ± 1.40
	Sorting	0.68 ± 0.02	0.66 ± 0.01	0.74 ± 0.04
	Skewness	1.01 ± 0.11	0.99 ± 0.08	0.90 ± 0.05
	Kurtosis	0.28 ± 0.01	0.27 ± 0.02	0.26 ± 0.003
9 months		**3**	**5**	**6**
Control	Mean	15.14 ± 0.69	10.12 ± 1.59	18.75 ± 0.69
	Sorting	0.59 ± 0.05	0.56 ± 0.04	0.59 ± 0.04
	Skewness	0.94 ± 0.12	0.83 ± 0.08	0.78 ± 0.05
	Kurtosis	0.27 ± 0.03	0.25 ± 0.02	0.28 ± 0.01
Treatment	Mean	18.88 ± 1.44	9.91 ± 1.66	15.70 ± 1.39
	Sorting	0.68 ± 0.04	0.52 ± 0.01	0.64 ± 0.02
	Skewness	0.97 ± 0.03	0.88 ± 0.06	0.88 ± 0.01
	Kurtosis	0.26 ± 0.02	0.27 ± 0.01	0.25 ± 0.01

Table 12 Subsurface metrics 24 hours post gravel jetting at riffle scale collected at 5 sites. Site reference is as per Figure 30.

Metric / Site	D5 (mm)	D50 (mm)	D95 (mm)	Mean (mm)	Sorting	Skewness	Kurtosis	Fines (%)	Sand (%)	Silt (%)
1	0.34	10.34	44.53	12.65	0.30	0.46	0.29	24.46	24.25	0.21
	0.77	16.93	56.08	17.67	0.51	0.71	0.26	10.81	10.71	0.10
	0.58	10.94	41.66	12.30	0.48	0.77	0.26	14.96	14.85	0.11
	0.92	16.25	72.00	18.80	0.48	0.81	0.21	9.21	9.09	0.12
	0.65	11.72	30.94	12.67	0.53	0.80	0.27	11.14	10.97	0.17
	0.50	12.84	39.96	13.54	0.49	0.69	0.28	13.15	12.96	0.19
	0.37	7.85	31.06	9.04	0.34	0.50	0.29	25.84	25.62	0.22
	0.65	11.00	29.70	11.97	0.50	0.75	0.29	12.81	12.45	0.36
	0.33	9.60	36.55	10.59	0.32	0.41	0.29	25.05	24.85	0.20
	0.64	13.48	59.67	14.31	0.52	0.75	0.23	12.15	12.08	0.07
2	0.42	6.79	31.05	10.15	0.31	0.70	0.30	27.56	27.45	0.11
	0.51	12.59	39.36	14.05	0.39	0.56	0.34	18.96	18.85	0.11
	0.56	11.1	43.98	12.60	0.43	0.67	0.29	16.89	16.80	0.09
	0.44	10.02	35.95	11.20	0.39	0.58	0.29	20.62	20.52	0.10
	0.55	14.21	40.89	15.07	0.45	0.63	0.30	15.60	15.55	0.05
	0.46	11.11	39.95	12.08	0.43	0.63	0.27	18.30	18.14	0.16
	0.75	13.46	39.94	14.75	0.43	0.64	0.30	13.94	13.89	0.05
	0.42	11.98	38.58	13.45	0.37	0.53	0.34	20.03	19.94	0.09
	0.35	5.88	40.22	8.59	0.24	0.45	0.26	35.62	35.39	0.23
	0.21	10.08	51.65	10.92	0.34	0.44	0.29	23.96	23.91	0.05
3	0.60	16.42	44.86	16.75	0.46	0.61	0.29	14.13	14.07	0.06
	0.47	1.90	30.02	6.41	0.34	4.17	0.23	54.07	54.01	0.06
	0.47	12.18	36.22	12.83	0.37	0.47	0.33	20.19	20.11	0.08
	0.48	19.12	43.39	17.36	0.46	0.48	0.30	15.17	15.12	0.05
	0.53	14.17	65.94	15.09	0.40	0.54	0.30	17.22	17.13	0.09

	D5 (mm)	D50 (mm)	D95 (mm)	Mean (mm)	Sorting	Skewness	Kurtosis	Fines (%)	Sand (%)	Silt (%)
	0.52	12.41	38.67	13.22	0.41	0.56	0.30	17.63	17.55	0.08
	0.69	18.68	42.25	18.74	0.57	0.74	0.27	10.40	10.31	0.09
	0.99	15.99	38.75	17.31	0.59	0.90	0.28	8.35	8.30	0.05
	0.43	14.31	40.63	14.84	0.38	0.48	0.33	18.89	18.80	0.09
	0.54	8.49	32.57	9.45	0.44	0.68	0.27	18.83	18.79	0.04
4	0.78	13.6	33.19	13.93	0.58	0.80	0.26	8.81	8.73	0.08
	0.45	10.77	34.1	11.98	0.43	0.64	0.29	18.53	18.49	0.04
	0.55	10.02	29.75	11.13	0.49	0.78	0.27	14.80	14.74	0.06
	0.54	8.49	32.57	9.45	0.44	0.68	0.27	18.83	18.79	0.04
	0.39	7.26	25.77	8.04	0.41	0.60	0.29	23.36	23.24	0.12
	1.45	13.31	39.75	14.13	0.59	0.86	0.25	6.82	6.77	0.05
	0.47	8.69	29.23	9.31	0.52	0.77	0.25	16.29	16.20	0.09
	0.46	8.95	40.27	10.57	0.41	0.70	0.25	19.64	19.61	0.03
	0.47	9.16	28.22	9.89	0.50	0.75	0.26	15.77	15.72	0.05
	1.24	12.14	30.70	13.31	0.56	0.87	0.28	7.93	7.89	0.04
5	0.47	7.91	63.87	10.56	0.36	0.73	0.22	22.46	22.36	0.10
	0.67	8.78	38.29	10.76	0.43	0.80	0.26	16.25	16.18	0.07
	0.76	10.43	35.44	12.11	0.48	0.81	0.27	13.34	13.26	0.08
	0.55	9.69	33.20	11.17	0.38	0.60	0.30	20.40	20.30	0.10
	0.63	9.90	33.88	10.76	0.44	0.65	0.27	17.52	17.43	0.09
	1.07	14.85	122.5	17.59	0.50	0.89	0.14	9.08	9.02	0.06
	0.95	11.96	70.69	14.00	0.47	0.80	0.24	12.01	11.96	0.05
	0.49	8.68	52.36	9.98	0.40	0.63	0.21	20.99	20.90	0.09
	0.40	6.72	55.05	8.73	0.32	0.58	0.24	28.13	28.02	0.11
	0.85	12.02	41.93	14.52	0.50	0.93	0.27	11.07	11.01	0.06

Table 13 Subsurface metrics 1 year post gravel jetting at riffle scale collected at 4 sites. Site reference is as per Figure 30.

Metric / Site	D5 (mm)	D50 (mm)	D95 (mm)	Mean (mm)	Sorting	Skewness	Kurtosis	Fines (%)	Sand (%)	Silt (%)
1	0.42	12.98	38.60	13.91	0.38	0.50	0.34	19.16	19.08	0.08
	0.41	9.89	35.65	11.30	0.40	0.62	0.29	20.21	20.07	0.14
	0.26	7.19	31.98	8.87	0.24	0.30	0.30	32.3	31.74	0.56
	0.30	6.02	29.17	8.01	0.31	0.56	0.28	30.82	30.28	0.54
	0.65	13.62	41.68	14.08	0.50	0.68	0.28	12.94	12.86	0.08
3	0.74	13.50	41.25	15.12	0.45	0.70	0.30	12.72	12.68	0.04
	0.41	11.34	31.56	12.18	0.37	0.48	0.33	21.08	20.95	0.13
	0.28	6.59	36.88	8.78	0.22	0.30	0.27	36.94	36.6	0.34
	0.69	14.10	40.31	15.52	0.41	0.60	0.32	14.66	14.62	0.04
	0.41	9.34	35.57	11.04	0.32	0.48	0.31	24.53	24.44	0.09
4	0.93	14.21	35.39	14.92	0.56	0.80	0.28	8.48	8.41	0.07
	0.87	11.49	31.17	12.27	0.57	0.85	0.26	9.04	8.97	0.07
	0.39	7.31	33.49	9.02	0.38	0.67	0.28	23.52	23.45	0.07
	0.59	13.11	43.54	14.61	0.43	0.65	0.30	15.37	15.32	0.05
	0.57	10.56	36.30	11.66	0.46	0.69	0.27	16.03	16.01	0.02
5	0.52	11.11	43.17	13.04	0.46	0.78	0.27	15.33	15.16	0.17
	0.44	10.35	48.30	13.65	0.35	0.67	0.28	19.71	19.61	0.10
	0.74	10.06	40.50	12.25	0.45	0.82	0.25	14.77	14.73	0.04
	0.66	10.25	34.67	11.17	0.48	0.72	0.26	15.76	15.70	0.06
	0.34	8.17	44.21	11.17	0.27	0.48	0.26	28.68	28.50	0.18
	0.55	7.23	32.26	8.39	0.39	0.62	0.27	23.32	23.24	0.08
	1.09	11.12	57.79	13.71	0.49	0.95	0.22	10.18	10.14	0.04
	0.48	7.57	35.00	10.11	0.35	0.70	0.28	23.44	23.36	0.08

Table 14 Subsurface metrics per site (± SE) at control and jetted patches, 1 hour post-jetting. Site reference is as per Figure 30.

		Site reference		
		3	**5**	**6**
Control	D5	0.48 ± 0.14	0.57 ± 0.09	0.57 ± 0.132
	D50	10.48 ± 2.04	10.51 ± 0.31	18.97 ± 3.23
	D95	36.56 ± 2.81	43.99 ± 2.29	46.94 ± 4.39
	Mean	12.03 ± 1.84	12.98 ± 0.41	17.93 ± 1.79
	Sorting	0.34 ± 0.07	0.42 ± 0.03	0.45 ± 0.04
	Skewness	0.50 ± 0.12	0.76 ± 0.05	0.54 ± 0.09
	Kurtosis	0.30 ± 0.02	0.27 ± 0.01	0.31 ± 0.02
	Sand	23.41 ± 7.02	16.50 ± 1.56	14.38 ± 2.01
	Silt	0.17 ± 0.09	0.10 ± 0.04	0.16 ± 0.05
	Organic matter	2.34 ± 0.22	2.77 ± 0.62	3.07 ± 0.27
Treatment	D5	0.88 ± 0.33	0.62 ± 00.13	1.86 ± 1.22
	D50	15.51 ± 1.99	10.08 ± 0.96	21.15 ± 4.09
	D95	39.40 ± 1.93	41.32 ± 2.01	77.45 ± 32.16
	Mean	15.92 ± 1.84	12.32 ± 0.52	21.81 ± 4.15
	Sorting	0.53 ± 0.02	0.42 ± 0.05	0.54 ± 0.09
	Skewness	0.72 ± 0.04	0.76 ± 0.01	0.70 ± 0.10
	Kurtosis	0.28 ± 0.01	0.26 ± 0.002	0.24 ± 0.04
	Sand	11.49 ± 4.70	17.04 ± 4.65	9.60 ± 7.39
	Silt	0.05 ± 0.04	0.14 ± 0.11	0.05 ± 0.05
	Organic matter	2.24 ± 0.08	2.67 ± 0.39	2.04 ± 0.19

Table 15 Surface water quality parameters collected post jetting at 5 sites. Site reference is as per Figure 30.

Metric / Site	Temp (° C)	Cond (µS/l)	pH	Dissolved oxygen (mg/l)	Dissolved oxygen (% sat)	TAN (mg/l)	NH_3 (mg/l)
1	14.70	764	8.30	6.80	80.00	0.22	0.01
	14.70	764	8.30	6.50	78.00	0.88	0.04
	14.65	764	8.30	5.80	71.00	0.19	0.01
	14.65	764	8.30	5.70	71.00	0.79	0.04
	14.60	764	8.30	5.80	72.00	0.06	0.003
	14.55	764	8.30	6.20	76.00	0.06	0.003
	15.10	763	8.30	5.90	72.00		
	14.95	763	8.30	6.40	72.00		
	14.80	765	8.30	6.10	78.00		
2	15.20	766	8.30	6.40	78.00	0.28	0.01
	14.90	782	8.30	5.90	71.00	0.37	0.02
	15.80	733	8.30	6.90	82.00	0.32	0.02
	14.75	777	8.30	6.90	82.00	0.33	0.02
	15.25	777	8.30	6.20	78.00	0.11	0.01
	15.55	779	8.30	6.40	77.00	0.06	0.003
	15.10	777	8.30	6.70	81.00		
	15.00	768	8.30	6.30	76.00		
	14.85	775	8.30	6.60	78.00		
3	18.45	696	8.20	7.40	87.00	0.06	0.003
	17.65	695	8.20	8.30	96.00	0.09	0.005
	17.30	695	8.20	7.60	89.00	0.05	0.003
	17.20	691	8.20	7.60	89.00	0.14	0.007
	17.15	697	8.20	6.40	77.00	0.05	0.003
	16.90	694	8.20	6.50	79.00		
	16.95	691	8.20	6.70	81.00		
	16.95	693	8.30	6.50	79.00		
	16.95	695	8.20	6.80	82.00		
4	16.00	881	8.04	6.46	65.40	0.02	0.001
	16.30	849	8.04	6.26	63.70	0.26	0.009
	16.50	861	8.04	6.25	63.80	0.03	0.001
	16.50	862	8.04	6.27	64.10	0.02	0.001
	16.60	856	8.04	6.12	62.70	0.15	0.005
	16.60	859	8.05	6.21	63.60	0.26	0.009
	16.60	857	8.05	6.35	65.00		
	16.60	857	8.05	6.18	63.30		
	16.60	861	8.06	6.29	64.30		
5	17.95	805	8.20	5.10	65.00	0.10	0.005
	17.70	812	8.20	5.20	66.00	0.06	0.003
	17.65	810	8.20	5.20	66.00	0.06	0.003
	17.60	808	8.20	5.60	70.00	0.06	0.003
	17.50	811	8.30	5.30	67.00	0.06	0.003
	17.55	809	8.20	5.30	67.00	0.04	0.002
	17.70	807	8.20	5.90	73.00		
	17.65	809	8.20	5.40	68.00		
	17.70	809	8.20	5.40	88.00		

Table 16 Hyporheic water quality parameters collected post jetting at 6 sites at 10 cm depth. Site reference is as per Figure 30.

Metric / Site	Temp (° C)	Cond (µS/l)	pH	Dissolved oxygen (mg/l)	Dissolved oxygen (% sat)	TAN (mg/l)	NH_3 (mg/l)
1	15.65	770	8.30	6.10	75.00	0.33	0.02
	15.90	772	8.30	6.50	78.00	0.84	0.04
	14.85	767	8.30	6.20	76.00	0.26	0.01
	14.70	765	8.30	5.20	67.00	0.21	0.01
	14.60	764	8.40	5.60	69.00	0.83	0.04
	14.75	767	8.30	5.60	71.00	0.16	0.01
	14.90	772	8.30	5.60	70.00		
	15.30	763	8.10	5.50	69.00		
	14.65	764	8.30	5.50	69.00		
2	15.50	768	8.30	5.50	70.00	0.73	0.04
	15.30	764	8.30	6.50	79.00	0.24	0.01
	15.35	777	8.30	6.80	81.00	0.16	0.01
	15.60	773	8.30	5.50	69.00	0.68	0.04
	15.35	770	8.30	6.00	72.00	0.99	0.05
	15.35	779	8.30	6.20	76.00	1.05	0.06
	15.30	769	8.30	6.40	78.00		
	14.85	771	8.30	5.70	71.00		
	14.70	777	8.40	5.90	73.00		
3	17.65	812	8.10	4.80	62.00	0.04	0.002
	17.55	811	8.00	4.70	61.00	0.08	0.003
	17.65	812	8.10	4.70	61.00	0.02	0.001
	18.00	817	8.10	4.90	63.00	0.03	0.001
	17.80	817	8.10	4.60	60.00		
	17.60	805	8.00	4.20	56.00		
	17.95	818	8.10	4.40	58.00		
	18.05	816	8.10	4.60	61.00		
	17.65	812	8.10	4.80	62.00		
4	16.70	867	8.07	6.24	64.60	0.28	0.010
	16.80	858	8.04	6.22	63.90	0.03	0.001
	16.80	855	8.07	6.75	69.40	0.03	0.001
	16.90	863	8.07	6.39	65.80	0.04	0.001
	17.00	859	8.03	6.43	66.30	0.01	0.0003
	17.00	859	8.05	6.39	66.00	0.002	0.0001
	16.90	865	8.08	6.46	66.60		
	16.90	858	8.06	6.38	65.80		
	16.90	854	8.08	6.51	67.10		
5	17.50	812	8.30	5.90	73.00	0.03	0.002
	17.55	809	8.30	4.60	60.00	0.05	0.003
	17.75	806	8.30	5.20	66.00	0.02	0.001
	17.70	805	8.30	5.20	66.00	0.04	0.002
	17.80	812	8.30	5.60	70.00	0.03	0.002
	17.90	809	8.30	5.70	71.00	0.04	0.002
	17.95	810	8.30	5.40	68.00		
	17.85	811	8.20	6.00	74.00		
	17.90	808	8.20	5.50	69.00		

Table 17 Hyporheic water quality parameters collected post jetting at 6 sites at 20 cm depth. Site reference is as per Figure 30.

Metric / Site	Temp (° C)	Cond (µS/l)	pH	Dissolved oxygen (mg/l)	Dissolved oxygen (% sat)	TAN (mg/l)	NH_3 (mg/l)
1	15.60	772	8.20	5.50	70.00	0.56	0.03
	15.50	769	8.30	6.10	75.00	0.07	0.004
	14.85	768	8.30	5.90	72.00	1.27	0.06
	14.80	764	8.30	5.00	64.00	0.52	0.03
	14.80	769	8.30	5.30	68.00	1.33	0.07
	14.80	767	8.30	5.30	68.00	0.12	0.01
	14.95	772	8.30	5.30	68.00		
	14.75	768	8.30	5.80	72.00		
	14.80	775	8.30	5.30	68.00		
2	15.50	775	8.30	5.80	72.00	0.43	0.02
	15.40	779	8.20	6.30	77.00	0.38	0.02
	15.20	772	8.30	6.20	75.00	0.38	0.02
	15.35	776	8.30	6.50	79.00	0.50	0.03
	15.20	767	8.30	5.90	73.00	1.07	0.05
	15.35	776	8.30	6.10	75.00	0.09	0.01
	14.90	770	8.30	5.70	71.00		
	14.65	771	8.40	5.70	72.00		
	14.70	759	8.10	3.30	48.00		
3	17.50	807	8.00	4.20	56.00	0.02	0.001
	17.70	810	8.10	4.60	60.00	0.02	0.001
	17.55	811	7.90	4.30	57.00	0.01	0.0003
	17.70	810	8.00	4.70	61.00	0.02	0.001
	17.75	813	8.00	4.60	60.00	0.06	0.002
	17.85	815	8.00	4.50	60.00	0.08	0.003
	17.75	813	8.10	5.10	65.00		
	18.10	811	8.00	4.20	56.00		
	17.70	795	7.90	2.20	37.00		
4	16.80	832	7.83	4.01	41.30	0.02	0.001
	16.80	860	8.06	6.54	67.20	0.10	0.003
	16.80	858	8.00	6.26	64.40	0.04	0.001
	16.90	856	7.94	5.81	59.90	0.04	0.001
	17.00	857	7.95	6.16	63.60	0.08	0.002
	17.00	856	7.95	6.19	63.80	0.14	0.004
	16.80	857	8.09	6.31	65.00		
	16.90	858	8.07	6.31	65.00		
	16.90		7.93	5.55	57.10		
5	17.45	802	8.20	5.70	71.00	0.02	0.001
	17.55	807	8.30	5.80	72.00	0.01	0.001
	17.35	805	8.30	4.70	61.00	0.03	0.002
	17.60	805	8.30	5.00	64.00	0.02	0.001
	17.95	808	8.30	5.40	68.00	0.02	0.001
	17.70	801	8.20	5.10	65.00	0.04	0.002
	17.85	810	8.30	5.90	73.00		
	17.60	805	8.10	4.70	61.00		
	17.70	790	8.10	0.80	23.00		

Table 18 Hyporheic water quality parameters collected post jetting at 6 sites at 30 cm depth. Site reference is as per Figure 30.

Metric / Site	Temp (° C)	Cond (µS/l)	pH	Dissolved oxygen (mg/l)	Dissolved oxygen (% sat)	TAN (mg/l)	NH_3 (mg/l)
1	15.25	766	8.20	5.40	69.00	0.34	0.02
	14.95	773	8.30	5.60	70.00	0.40	0.02
	14.85	772	8.30	5.60	70.00	0.45	0.02
	14.80	772	8.30	5.60	70.00	1.65	0.08
	14.85	770	8.30	5.90	72.00	0.98	0.05
	14.75	769	8.30	5.40	68.00	0.05	0.002
	14.85	780	8.00	4.70	62.00		
	14.65	774	8.30	5.80	72.00		
	14.70	765	8.30	5.60	71.00		
2	15.50	827	7.80	4.90	69.00	0.46	0.01
	15.25	772	8.30	6.10	74.00	0.32	0.01
	15.30	775	8.20	6.00	74.00	0.55	0.01
	15.25	770	8.20	5.40	79.00	0.38	0.01
	15.60	769	8.30	6.20	76.00	0.85	0.02
	15.15	797	8.10	5.50	70.00	0.83	0.02
	14.85	761	8.20	5.10	67.00		
	14.75	783	8.00	3.80	54.00		
	14.45	770	8.30	5.30	68.00		
3	17.60	810	8.00	4.40	58.00	0.52	0.020
	17.40	794	7.90	2.20	37.00	0.02	0.001
	17.50	809	7.80	3.40	48.00	0.02	0.001
	17.70	811	8.00	4.40	58.00	0.08	0.002
	17.80	804	8.00	4.10	55.00	0.08	0.002
	17.70	810	8.00	3.90	53.00	0.04	0.001
	17.85	808	8.00	4.30	58.00		
	17.80	806	8.00	4.30	57.00		
	17.65	810	8.00	1.00	26.00		
4	16.90	820	7.78	3.35	31.50	0.04	0.001
	16.80	856	8.06	6.46	66.50	0.04	0.001
	16.90	858	7.88	5.25	54.10	0.02	0.001
	17.00	854	7.81	4.81	49.70	0.21	0.006
	17.10	848	7.82	3.95	40.80	0.01	0.0003
	17.00	859	8.03	6.35	65.60	0.01	0.0003
	16.80	856	8.06	6.42	66.20		
	17.00	856	7.97	6.20	64.70		
	16.90		8.04	6.55	67.50		
5	17.35	809	8.30	5.50	69.00	0.13	0.006
	17.65	805	8.20	5.30	67.00	0.06	0.003
	17.65	807	8.20	6.00	74.00	0.03	0.001
	17.65	805	8.30	5.30	67.00	0.03	0.001
	17.95	808	8.30	5.70	71.00	0.02	0.001
	17.75	808	8.30	5.10	65.00	0.04	0.002
	17.60	803	8.00	3.50	73.00		
	17.70	803	8.10	4.30	61.00		
	17.60	779	8.00	1.90	34.00		

10.4 Appendix D

Table 19 Water quality parameters measured daily during the experiment (Mean ± SE).

Date	Temp. (° C)	O_2 (mg/l)	O_2 (%)	pH	Conductivity (Us/cm)	Unionized NH_3 (mg/l)
24/05/2015	16.27 ± 0.03	8.45 ± 0.05	85.84 ± 0.56	7.96 ± 0.002	719.30 ± 3.14	0.03 ± 0.02
25/05/2015	16.45 ± 0.02	8.49 ± 0.06	86.62 ± 0.53	8.00 ± 0.001	723.70 ± 4.15	0.02 ± 0.00
26/05/2015	16.68 ± 0.03	8.62 ± 0.06	87.93 ± 0.62	8.02 ± 0.002	724.27 ± 0.78	0.03 ± 0.001
27/05/2015	16.79 ± 0.04	8.78 ± 0.04	89.97 ± 0.32	8.00 ± 0.003	727.80 ± 1.24	0.03 ± 0.001
28/05/2015	16.58 ± 0.02	8.77 ± 0.03	90.21 ± 0.33	8.03 ± 0.00	727.13 ± 1.23	0.03 ± 0.00
29/05/2015	17.33 ± 0.07	8.22 ± 0.04	86.62 ± 0.38	8.06 ± 0.003	743.00 ± 2.98	0.03 ± 0.001
30/05/2015	17.42 ± 0.01	8.29 ± 0.05	86.89 ± 0.53	8.04 ± 0.002	738.87 ± 0.82	0.03 ± 0.00
31/05/2015	17.51 ± 0.03	8.31 ± 0.05	87.98 ± 0.59	8.09 ± 0.002	739.40 ± 1.99	0.03 ± 0.002
01/06/2015	17.58 ± 0.02	8.03 ± 0.03	84.57 ± 0.33	8.08 ± 0.005	745.87 ± 1.14	0.04 ± 0.001
02/06/2015	17.60 ± 0.02	8.05 ± 0.03	85.86 ± 0.37	8.07 ± 0.002	743.07 ± 1.06	0.03 ± 0.001
03/06/2015	17.76 ± 0.02	8.36 ± 0.05	87.43 ± 0.57	8.04 ± 0.002	746.47 ± 1.12	0.03 ±0.001
04/06/2015	18.11 ± 0.08	7.97 ± 0.11	83.74 ± 1.33	8.06 ± 0.01	750.07 ± 1.22	0.03 ± 0.002
05/06/2015	18.13 ± 0.12	8.14 ± 0.04	86.45 ± 0.41	8.00 ± 0.004	750.67 ± 2.67	0.03 ± 0.001
06/06/2015	17.90 ± 0.07	8.13 ± 0.09	85.24 ± 0.83	8.08 ± 0.01	743.20 ± 4.31	0.04 ± 0.002
07/06/2015	17.80 ± 0.07	8.38 ± 0.06	86.75 ± 0.53	8.07 ± 0.001	746.70 ± 2.53	0.04 ± 0.001
08/06/2015	17.70 ± 0.00	8.31 ± 0.09	85.68 ± 0.90	8.08 ± 0.002	742.40 ± 2.25	0.04 ± 0.002
09/06/2015	17.60 ± 0.00	7.92 ± 0.05	81.26 ± 0.51	8.07 ± 0.002	737.00 ± 0.32	0.04 ± 0.002
10/06/2015	17.61 ± 0.01	8.39 ± 0.05	86.72 ± 0.57	8.07 ± 0.002	728.80 ± 9.44	0.03 ± 0.001
11/06/2015	18.05 ± 0.12	8.10 ± 0.09	85.13 ± 0.73	8.07 ± 0.002	675.10 ± 14.48	0.04 ± 0.001
12/06/2015	17.74 ± 0.05	8.09 ± 0.02	85.43 ± 0.17	8.05 ± 0.002	734.30 ± 5.19	0.04 ± 0.002
13/06/2015	17.60 ± 0.03	7.95 ± 0.05	83.89 ± 0.55	8.06 ± 0.02	744.60 ± 1.68	0.04 ± 0.001
14/06/2015	17.75 ± 0.02	7.99 ± 0.04	84.16 ± 0.38	8.01 ± 0.01	750.60 ± 1.96	0.04 ± 0.001
15/06/2015	18.15 ± 0.12	8.25 ± 0.11	86.72 ± 0.96	8.02 ± 0.002	757.10 ± 2.85	0.04 ± 0.002
16/06/2015	17.86 ± 0.02	8.03 ± 0.22	81.68 ± 0.67	7.98 ± 0.02	749.40 ± 1.94	0.04 ± 0.002
17/06/2015	18.20 ± 0.00	8.43 ± 0.08	89.04 ± 0.82	8.01 ± 0.002	760.00 ± 1.84	0.03 ± 0.00
18/06/2015	17.94 ± 0.02	8.12 ± 0.09	85.32 ± 0.96	8.05 ± 0.01	749.00 ± 1.45	0.04 ± 0.00