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PRIMARY RESEARCH PAPER

Trophic interactions in a lowland river fish community invaded by European barbel *Barbus barbus* (Actinopterygii, Cyprinidae)

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Abstract Following their invasion, non-indigenous fish can potentially exclude native fishes from their original niches through competition, or can partition in their resource use with native species to facilitate co-existence. Here, using stable isotope analysis, the trophic interactions of invasive European barbel *Barbus barbus* and other fishes were tested in an invaded river of relatively low fish species diversity and where no other *Barbus* species were present. Testing was over three distinct life stages: age 0 + (< 38 mm), juveniles (86–231 mm) and adults (> 386 mm). There were strong patterns of isotopic niche partitioning between the juvenile fishes, with some inter-specific niche differences also apparent in 0 + fishes. For adult *B. barbus* and chub *Squalius cephalus*, however, niche convergence was evident. Within the *B. barbus* population, the niches of the adult fish differed significantly from the 0+ and juvenile fish, indicating considerable dietary changes with development. These results suggested that niche partitioning at the most abundant life stages were facilitating the co-existence of invasive *B. barbus* with other fishes in the community, with this most likely driven by inter-specific differences in functional morphology and habitat use.

Keywords Biological invasion · Isotopic niche · Non-indigenous · Stable isotope analysis · Trophic niche

Introduction

Invasions of non-indigenous fishes can result in adverse impacts in native fish communities, including competitive displacement and exclusion (Gozlan et al., 2010). Understanding how an invasive fish can impact native species requires knowledge on their trophic interactions, such as whether they share prey resources, resulting in niche convergence, or exploit different resources, resulting in niche partitioning (Cucherousset et al., 2012; Tran et al., 2015; Copp et al., 2017). Quantifying the feeding relationships of introduced and native fishes is thus important for understanding their ecological risks to the native communities (Cucherousset & Olden, 2011) and facilitates assessment of the ecological impacts that might develop (Gozlan et al., 2010; Tran et al., 2015; Copp et al., 2017).

European barbel *Barbus barbus* (Linneaus, 1758) of the Cyprinidae family is now invasive in many European rivers outside of its native range, especially

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rivers in Italy and Western Britain (Britton & Pegg, 2011). Attaining lengths to approximately 800 mm and weights in excess of 8 kg (Amat-Trigo et al., 2017), they are generally valued for sport angling, with this the primary driver of introductions (Britton & Pegg, 2011). A highly vagile species, they can disperse relatively quickly through river systems (Hunt & Jones, 1974), often leading to rapid colonisation (Carosi et al., 2017). In invaded rivers where they are sympatric with endemic *Barbus* fishes, such as in Italian rivers (e.g. the Tiber basin), long-term data suggest populations of endemic *Barbus tyberinus* Bonaparte, 1839 are being displaced by *B. barbus*, with the mechanism suggested to involve asymmetric competition between the fishes (Carosi et al., 2017). This displacement is in addition to genetic impacts caused by introgression that results in a loss of genetic integrity in the endemic *Barbus* fishes (Meraner et al., 2013; Zaccara et al., 2014). Elsewhere in Europe, invasive *B. barbus* populations are often present in communities where other *Barbus* fishes are absent. Thus, whilst they are sympatric with indigenous cyprinid fishes such as chub *Squalius cephalus* (Linnaeus, 1758), they have lower functionally similarity with these fishes than with congeners. Consequently, the strength of their interactions might be less intense and their invasion might be less likely to incur negative ecological impacts.

Examples of systems invaded by *B. barbus* and where endemic *Barbus* fishes are absent are rivers in Western England. In Britain, *B. barbus* is only indigenous to eastern flowing rivers in England due to their previous connections with mainland Europe at the end of the last glacial period (Wheeler & Jordan, 1990). Research on these indigenous *B. barbus* suggests many of these populations are imperilled due to losses of habitat and river connectivity (Bašić et al., 2017). Consequently, enhancement stocking often supports these populations, with hatchery-reared individuals released at lengths between 120 and 250 mm and age 1+ and 2+ years (Britton et al., 2004; Antognazza et al., 2016). Studies on the trophic interactions of these stocked fish suggest substantial partitioning in their trophic niches with *S. cephalus*, the species that has the most similar functional traits and body sizes as *B. barbus* in these rivers (Bašic & Britton, 2016).

In their invasive range in Western England, populations tend to be more successful than many indigenous populations, with populations being relatively abundant and widespread through basins such as the River Severn (Amat-Trigo et al., 2017). Although knowledge of invasive *B. barbus* trophic interactions with indigenous fishes is limited in these rivers, both Bašić et al. (2015) and Gutmann Roberts et al. (2017) revealed that in rivers in both their native and invasive ranges, angling baits based on marine-derived nutrients can provide a strong trophic subsidy. This results in some individual *B. barbus* and *S. cephalus* (generally > 400 mm) specialising on this allochthonous resource. However, in rivers where angling is less intense and so where this subsidy is lower, and in body sizes that rarely consume these baits (< 400 mm), there remains a distinct knowledge gap on the trophic relationships of invasive *B. barbus* with other species. Moreover, there is also minimal knowledge on how their diet and trophic niche sizes change with increasing body size, and in relation to these changes in the indigenous fishes. This is despite the diet of fish usually being gape limited, where gape size is a function of body length (e.g. Persson et al., 1996), suggesting considerable dietary shifts will occur with increasing body length. Data on the inter- and intraspecific trophic relationships of *B. barbus* are also missing in their invasive range more generally, where competitive interactions between invasive and endemic *Barbus* fishes have, to date, been inferred from relative body condition data (e.g. Carosi et al., 2017).

The aim of this study was to quantify the trophic interactions of a population of invasive *B. barbus* with other fishes in a river where no other *Barbus* fishes were present. The focus was on determining the extent of trophic niche sharing within and between species, and how this altered across a range of life stages (as inferred from body sizes). The River Teme, western England, was the study river, where non-indigenous *B. barbus* have been present since the 1970s (Antognazza et al., 2016). The objective was to determine the trophic niche sizes and overlaps between invasive *B. barbus* and native fishes at three different life stages: age 0+ (young-of-the-year), juveniles and adults. It was hypothesised that (1) due to the consistent patterns of inter-specific trophic partitioning between *B. barbus* and native cyprinid fishes in their indigenous range (Bašic & Britton, 2016), these patterns of interspecific partitioning are present in their non-indigenous, invasive range; and (2) within the fishes, there were significant shifts in the position of the trophic

niches across the three life stages, with populations having a relatively large niche comprising smaller sub-sets.

As the *B. barbus* population of the River Teme (and the River Severn basin generally) is an important angling resource (Amat-Trigo et al., 2017), the use of stomach contents analysis via destructive sampling of the juvenile and adult fish was not possible. Consequently, trophic analyses were based on stable isotope analysis (SIA), where the ecological application of carbon (as $\delta^{13}C$) and nitrogen (as $\delta^{15}N$) stable isotopes is based on the predictable relationship between the isotope composition of a consumer and its prey. It thus provides a temporally integrative and powerful tool to analyse trophic interactions between native and non-native fishes (Cucherousset et al., 2012). For comparisons of SI data within and between the fishes, two metrics were used: the significance of differences in $\delta^{15}N$-$\delta^{13}C$ centroids, and core isotopic niche sizes (as a proxy of the trophic niche) and overlaps calculated using standard ellipse areas (Jackson et al., 2011, 2012).

Methods

Sampling details and stable isotope analysis

The study was conducted on the middle reaches of the River Teme, from the town of Tenbury Wells (52°19′N, − 2°24′W) to the village of Bransford (52°10′N, − 2°16′W) (Fig. 1). Across the Teme catchment, altitude varies between 24.3 and 544.5 mAOD, and land-use is primarily grassland (59%), with some horticulture (24%) (CEH, 2018). In the study reach, the river generally comprised sequences of pool and riffles, where maximum depths rarely exceeded 2 m and widths rarely exceeded 15 m. A flow gauging station towards the downstream end of the reach near Bransford had a long-term Q95 of 2.0 $m^3\ s^{-1}$, Q50 of 10.2 $m^3\ s^{-1}$ and Q10 of 42.4 $m^3\ s^{-1}$ (CEH, 2018). In the study reach, the cyprinid fish community had relatively limited diversity, with only invasive *B. barbus*, and *S. cephalus*, dace *Leuciscus leuciscus* (Linnaeus, 1758) and minnow *Phoxinus phoxinus* (Linnaeus, 1758) present. Grayling *Thymallus thymallus* (Linnaeus, 1758) were also present in samples at the upper end of the reach and so were also included in analyses. Other species that were occasionally present in samples but not included in analyses were bullhead *Cottus gobio* Linnaeus, 1758 and stone loach *Barbatula barbatula* (Linnaeus, 1758). Brown trout *Salmo trutta* Linnaeus, 1758 and juvenile Atlantic salmon *Salmo salar* Linnaeus, 1758 are also present in the river but are more prevalent upstream of the town of Ludlow, outside of the study reach (Fig. 1). Compared with the area of river located close to the confluence with the River Severn and that was used by Gutmann Roberts et al. (2017), angling pressure was relatively light in the study reach, and thus inputs of angling baits containing high proportions of pelletized fishmeal were considered as relatively low.

The 0+ fish were sampled from a single area of nursery habitat located close to Bransford (Fig. 1). They were sampled using a micromesh seine net (25 × 2 m) on 12 September 2016. The fish were euthanised via anaesthetic overdose (MS-222) and transported back to the laboratory on ice. In the laboratory, they were identified to species, measured (standard length, nearest mm) and a sample of dorsal muscle tissue removed and dried to constant weight at 50°C. The juvenile and adult fish samples were collected using angling and electric fishing between July and September 2015 and 2016, with SIA based on scales (Busst & Britton, 2016, 2017). Correspondingly, for each captured fish, identification was to species level, followed by measuring (fork length, nearest mm) and the collection of between three and five scales from the area between the base of the dorsal fin and above the lateral line. As scales grow as fish length increases, only the outer portion of scales reflects their most recent growth (Hutchinson & Trueman, 2006; Bašić et al., 2015). Consequently, only the very outer portion of the sampled scales was used in SIA. One scale was prepared per fish, with this involving their thorough washing with distilled water, removal of the scale outer edge using dissection scissors and then drying to constant weight as per the 0+ fish samples. The other scale samples were used to age the fish (Amat-Trigo et al., 2017). Scale decalcification was not performed prior to SIA, since the removal of inorganic carbonates has no significant effect on scale $\delta^{13}C$ and $\delta^{15}N$ values (Sinnatamby et al., 2007, 2010; Woodcock & Walther, 2014).

Concomitantly, qualitative samples for SIA of macroinvertebrates were collected from two areas of the river, 'Area 1′ and 'Area 2′. Samples in Area 1

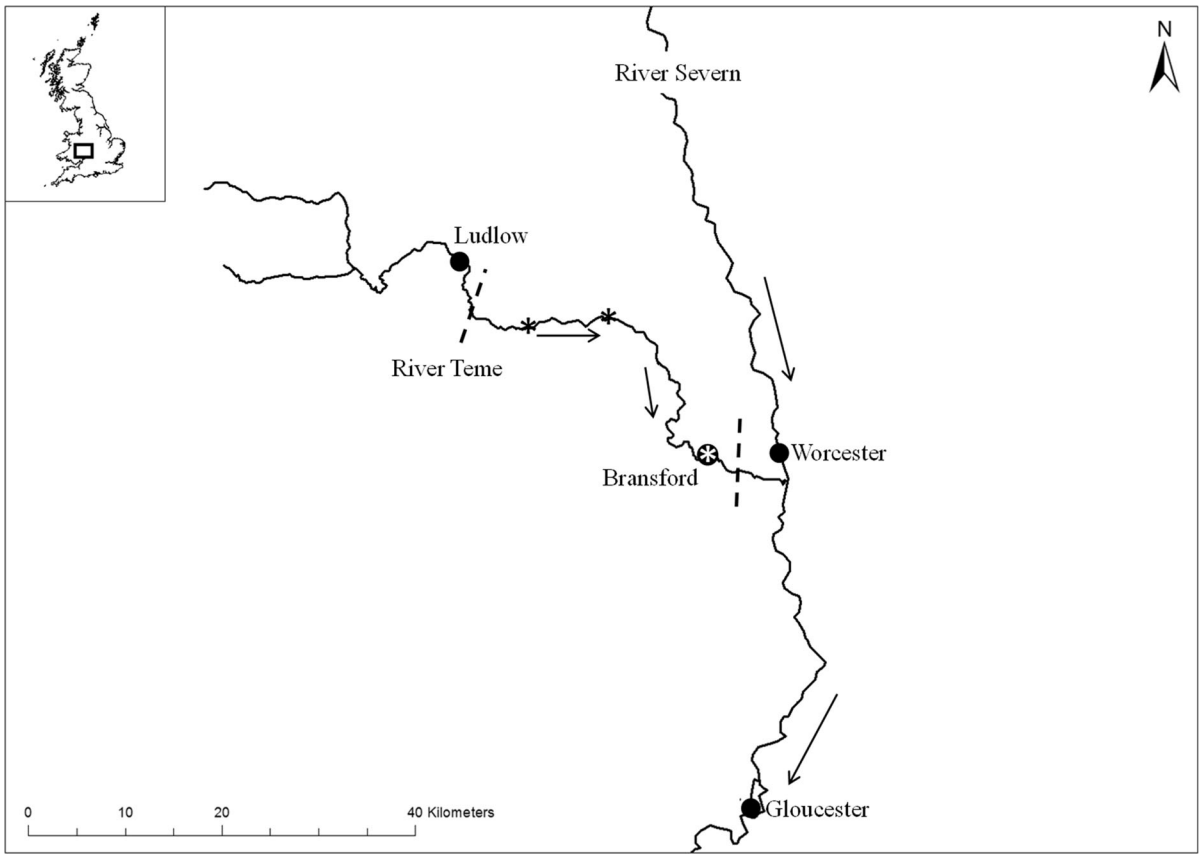

Fig. 1 Inset: location of the River Teme in Great Britain. Main map: The River Teme catchment showing its confluence with the River Severn. Arrows mark the direction of river flow. The study area was the stretch of the river between the two dashed lines. Macroinvertebrates were collected at locations marked with asterisks

were collected from Tenbury Wells (52°19′N, − 2°24′W) and Lindridge (52°32′N, − 2°51′W), and from Area 2 at Bransford (52°10′N, − 2°16′W) in June and September 2015 and 2016. Samples were collected using kick sampling. Macro-invertebrate samples collected in 2015 contained very high proportions of the amphipod *Gammarus pulex* (Linnaeus, 1758) in both sampling areas (> 50%). *Gammarus* spp. are common prey items for riverine fishes generally (e.g. MacNeil et al., 1999), as well as the fishes analysed here more specifically (e.g. Mann, 1974; Bašić et al., 2015). Thus, samples were taken to describe the stable isotope data of fish putative prey in 2015. This sampling was repeated in 2016, with *G. pulex* samples taken for SIA to enable consistent temporal and spatial testing of differences in fish putative prey resources. However, to increase the diversity of these baseline samples, samples of Chironomid larvae (n = 6 per Area) and Trichoptera spp. larvae (n = 3 per Area) were also taken in 2016. All samples were taken back to the laboratory where they were washed in distilled water and dried to constant weight as per the fish samples; note that in each case, one sample comprised between three and six individuals.

The dried muscle, scale and invertebrate samples were then submitted to the Cornell Isotope Laboratory in New York, USA, for SIA. This involved the samples being ground to powder, weighed in tin capsules (nearest 1,000 µg) and analysed on a Thermo Delta V isotope ratio mass spectrometer (Thermo Scientific, USA) interfaced to a NC2500 elemental analyser (CE Elantach Inc. USA). Standards were verified against international reference materials and calibrated against the primary reference scales for $\delta^{13}C$ and $\delta^{15}N$. The accuracy and precision were checked every ten samples using a standard animal sample (mink). The outputs were values of $\delta^{13}C$ and

$\delta^{15}N$ (‰) for each sample. As C:N ratios were below 3.5, indicating low lipid content, there was no need for $\delta^{13}C$ to be lipid corrected (Post et al., 2007; Skinner et al., 2016).

Data analysis

The 0+ fish utilised in the analysis were all between 17 and 38 mm and in their first year of life. The juvenile fish were between 86 and 231 mm and between ages 1+ and 4+ years; note that in this length range, some *L. leuciscus* would have been sexually mature, but with *B. barbus* and *S. cephalus* being immature. The adult fish, comprising only *B. barbus* and *S. cephalus*, were all $\geq$ 386 mm (Table 2). The fish ages were derived by scale ageing using a projecting microscope and accounting annual marks as per Amat-Trigo et al. (2017). For inter-specific data analyses, these length classes were considered separately. This was because the habitat use of these species tended to be quite different, with the 0+ fishes all sampled from marginal areas of the river where flows were minimal, the juvenile fishes were generally captured from relatively shallow and fast-flowing riffle habitats, and the adult fishes have relatively large home ranges in the basin, often exceeding 5 km (Hunt & Jones, 1974). By only completing inter-specific analyses within these groups of lengths, then the data were being tested between fishes of relatively similar body sizes. This meant that these analyses would be more ecologically relevant for testing the hypothesis than comparing data between species of very different length ranges (Bašic & Britton, 2015).

Prior to analysing the stable isotope analysis of the fishes, the stable isotope data of the macro-invertebrate samples were tested for spatial (Area 1 versus Area 2) and temporal (2015 vs. 2016) differences. Testing used generalized linear models (GLM) due to the relatively low sample sizes that were not normally distributed. The GLM revealed some significant differences (*cf.* Results). Thus, to enable the stable isotope data of the juvenile and adult fish to be combined for use across the entire study reach, their isotopic data required 'correction' (Jackson & Britton, 2014). Correspondingly, the $\delta^{15}N$ data were converted to trophic position (TP; Eq. 1) and the $\delta^{13}C$ data were corrected to C_{Corr} (Eq. 2) (Olsson et al., 2009; Jackson & Britton, 2014). To identify the effect of this correction on the stable isotope data, differences were tested in the temporal data in the uncorrected and the corrected data of the juvenile fishes using ANOVA. The juvenile fishes were used in preference to the adult fishes for this, as the diet of the latter was also likely to have had some influence from angling baits containing marine-derived fishmeal (Bašić et al., 2015; Gutmann Roberts et al., 2017). This testing was not completed for the 0+ fishes, as their samples were taken from a single site in 2016. However, their data were also corrected to enable their results to be compared with the juvenile and adult fishes. The stable isotope correction equations were

$$TP_i = \frac{\delta^{15}N_i - \delta^{15}N_{base}}{3.4} + 2 \quad (1)$$

$$\delta^{13}C_{corr} = \frac{\delta^{13}C_i - \delta^{13}C_{meaninv}}{CR_{inv}} \quad (2)$$

where TP_i is the trophic position of the fish, $\delta^{15}N_i$ is the isotopic ratio of the fish, $\delta^{15}N_{base}$ is the isotopic ratio of primary consumers, 3.4 is the fractionation between trophic levels and 2 is the trophic position of the baseline organism (Post, 2002); and $\delta^{13}C_{corr}$ is the corrected carbon isotope ratio of the fish, $\delta^{13}C_i$ is the uncorrected isotope ratio of the fish, $\delta^{13}C_{meaninv}$ is the mean invertebrate isotope ratio and CR_{inv} is the invertebrate carbon range ($\delta^{13}C_{max} - \delta^{13}C_{min}$) (Olsson et al., 2009).

The initial analyses using the corrected SI data tested differences in TP and C_{Corr} between species and between different life stages of the same species using ANOVA or Welch's test, with the latter used where the data were normally distributed but violated the assumption of homogeneity of variance. For each life stage, the corrected SI data were then used to test the significance of differences in their $\delta^{15}N$-$\delta^{13}C$ centroids, and differences in the positions and overlaps of their core trophic niches. For testing differences in the $\delta^{15}N$-$\delta^{13}C$ centroids per life stage and species, the SIA data were normalised by square root transformation and a resemblance matrix computed using Euclidean distances (Dethier et al., 2013). A PERMANOVA model was then fitted to this distance matrix using the *adonis* function in the vegan package in R. This calculated the significance of the differences in $\delta^{15}N$-$\delta^{13}C$ centroids per group (Oksanen et al., 2007; R Core Team, 2017). As the *adonis* function is similar to traditional ANOVA, it provided a pseudo *F*-statistic and *P* value

based on 999 permutations of the data (Dixon, 2003). Using the same method, it was then determined whether different life stages within *B. barbus* and *S. cephalus* had significant differences in their δ^{15}N-δ^{13}C centroids. With more than two life stages of fish being used per test, pairwise comparisons tested the significance of differences between the groups, with Bonferroni adjustment for multiple comparisons.

To compare 'core' trophic niche size and overlaps within and between species, the isotopic niche was used, where the isotopic niche is an approximation of the trophic niche. It is acknowledged that the isotopic niche varies slightly from the trophic niche due to it being influenced by factors other than diet (Jackson et al., 2011), such as growth and metabolic rate of individuals (Busst & Britton, 2017). It was calculated using the metric 'standard ellipse area' (SEA), a bivariate measure of the distribution of individuals in isotopic space (Jackson et al., 2012). To examine the size and overlap of the 'core' isotopic niches of each size group by species, ellipses were plotted that enclosed 40% of the predicted data and thus the typical resource use of that life stage of fish. The ellipses were calculated within the R package SIBER v2.1.3 (Jackson et al., 2011, 2012) and, due to some relatively small sample sizes, a corrected Bayesian estimate of Standard Ellipse Area (SEA_c) was calculated. This was followed by a calculation utilising a Markov chain Monte Carlo simulation with 10^4 iterations for each analysed group that provided 95% confidence limits (SEA_b) of the isotopic niche size (Jackson et al., 2011; R Core Team, 2017). Using SEA_c, the extent of niche overlap (%) between species and life stages was then also estimated. This was determined using the maximum likelihood fitted standard ellipses, with the extent of the overlap between two groups thus represented by the overlap of their core niches. This was calculated using Bayesian modelling in the SIBER package, with the denominator being the sum of non-overlapping area of the two ellipses (Jackson et al., 2011).

Results

Stable isotope correction for macro-invertebrate temporal and spatial differences

Comparison of spatial differences in SI data of the Chironomid larvae and *Trichoptera spp*. in 2016 revealed minimal differences in mean values, with overlaps in their 95% confidence limits (Chironomid: δ^{13}C: Area 1: -31.36 ± 0.39, Area 2: 31.76 ± 0.42‰; δ^{15}N: Area 1: 9.88 ± 0.35, Area 2: 9.74 ± 0.12‰; Trichoptera: δ^{13}C: Area 1: 32.36 ± 0.45, Area 2: 32.25 ± 0.38‰; δ^{15}N: Area 1: 9.20 ± 0.31, Area 2: 8.86 ± 0.42‰). In *G. pulex*, however, some spatial and temporal differences in their SI data were apparent that, when tested in GLMs, revealed significant differences (δ^{13}C: Wald $\chi^2 = 12.05$, $P < 0.01$; δ^{15}N: Wald $\chi^2 = 23.5$, $P < 0.01$; Table 1A). Pairwise comparisons of the mean SI values from both models revealed these significant differences were both spatial and temporal for both stable isotopes (Table 1B, C). Consequently, the use of Eqs. 1 and 2 to correct the fish SI data used the *G. pulex* SI data only (Table 1A). Prior to data correction, there were significant differences in the juvenile fish stable isotope data between years (ANOVA: δ^{13}C $F_{1,45} = 5.05$, $P = 0.03$; δ^{15}N $F_{1,45} = 11.56$, $P < 0.01$; Fig. 2A). However, these significant differences were no longer apparent following isotopic correction (ANOVA: δ^{13}C $F_{1,45} = 0.10$, $P = 0.75$; δ^{15}N $F_{1,45} = 1.11$, $P = 0.30$; Fig. 2B). Note that in the tests, SI data from *L. leuciscus* were not included as they were only present in samples in 2016.

Intra- and inter-specific stable isotope relationships

The lengths of each species were very similar across the 0+ fishes. There was greater natural variation between the lengths of fishes as juveniles (*T. thymallus* were smaller than other fishes) and adults (*S. cephalus* were generally smaller than *B. barbus*) (Table 2). The only species with all life stages represented in analyses were *B. barbus* and *S. cephalus*. For *B. barbus*, C_{corr} was significantly higher in adults than the 0+ fish and juveniles ($P < 0.01$), whilst TP was significantly lower for adults versus the 0+ fish ($P < 0.01$) (Table 3). For *S. cephalus*, the 0+ fish had significantly lower C_{corr} than juveniles and adults ($P < 0.01$) and significantly higher TP ($P < 0.01$) (Table 3).

Between the species, differences in C_{corr} between 0+ *B. barbus* and 0+ *S. cephalus* were not significant, but was between both of these 0+ fishes and 0+ *P. phoxinus* ($P < 0.01$; Table 3). The TP of 0+ *B. barbus* was significantly higher than both *S. cephalus*

Table 1 (A) Mean stable isotope data of *Gammarus pulex* from the two sampled areas of the River Teme in 2015 and 2016, where values are estimates (± 95% CL) from the generalized linear model (GLM), and their significance of differences (as *P* values) according to pairwise comparisons with Bonferroni adjustment in the GLM for (B) $\delta^{13}C$ and (C) $\delta^{15}N$

(A)

Area	Year	*n*	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
1	2015	6	− 30.68 ± 0.58	10.78 ± 0.57
	2016	4	− 29.44 ± 0.82	8.73 ± 0.71
2	2015	3	− 29.10 ± 0.82	10.22 ± 0.82
	2016	6	− 29.86 ± 0.82	9.16 ± 0.82

(B)

$\delta^{13}C$	Area 1, 2015	Area 1, 2016	Area 2, 2015	Area 2, 2016
Area 1, 2015	–			
Area 1, 2016	**0.05**	–		
Area 2, 2015	**0.01**	1.00	–	
Area 2, 2016	0.67	1.00	1.00	–

(C)

$\delta^{13}N$	Area 1, 2015	Area 1, 2016	Area 2, 2015	Area 2, 2016
Area 1, 2015	–			
Area 1, 2016	**< 0.01**	–		
Area 2, 2015	1.00	**0.04**	–	
Area 2, 2016	**< 0.01**	1.00	0.43	–

Sample number (*n*) represents the number of samples analysed, where one sample = 3–6 individual gammarids

Values in bold are significant at $P \leq 0.05$

and *P. phoxinus* ($P < 0.01$), although it was not significantly different between *S. cephalus* and *P. phoxinus* ($P > 0.05$; Table 3). In juveniles, C_{corr} of *B. barbus* was significantly lower than all other fishes ($P < 0.01$; Table 3). The TP of juvenile *B. barbus* was significantly higher than *T. thymallus*, significantly lower than *L. leuciscus*, but not significantly different to *S. cephalus* (Table 3). There were no significant differences in C_{corr} and TP between adult *B. barbus* and *S. cephalus* ($P > 0.05$; Table 3).

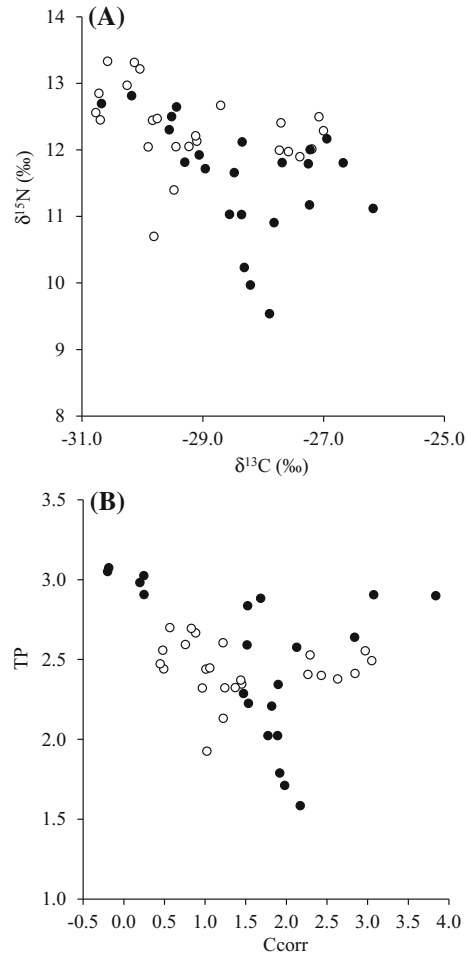

Fig. 2 Uncorrected (**A**) and corrected (**B**) stable isotope data of 'juvenile' fish (except *Leuciscus leuciscus*) sampled in 2015 (clear circle) and 2016 (filled circle)

Differences in $\delta^{15}N$-$\delta^{13}C$ centroids

The overall test for differences in the positions of the $\delta^{15}N$-$\delta^{13}C$ centroids of the 0+ fishes was significant ($F_{2,58} = 15.72$, $P < 0.01$); pairwise comparisons indicated the significant differences were between *P. phoxinus* and the other fishes ($P < 0.01$ in both cases), but with differences between *B. barbus* and *S. cephalus* being not significant ($P > 0.05$; Table 4). For the juvenile fishes, the overall model was significant ($F_{3,76} = 18.41$, $P < 0.01$), with significant differences between all species ($P < 0.01$; Table 4). This

Table 2 The number, fish length ranges, mean lengths (95% CL) and measurement type (*SL* standard length, *FL* fork length) of each life stage of fish analysed for their stable isotopes across the two sampling areas

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

Table 3 Outputs of ANOVA/Welch's test of corrected carbon (C_{corr}) and trophic position (TP) for comparisons between life stages and species for *Barbus barbus* and S*qualius cephalus*

Species	Test	Testing	df	*F*	*P*
C_{corr}					
Barbus barbus	ANOVA	Life stage	2,64	32.76	<0.01
Squalius cephalus	Welch's	Life stage	2,31	17.66	<0.01
0+	ANOVA	Species	3,60	10.01	<0.01
Juvenile	Welch's	Species	3,36	16.61	<0.01
Adult	ANOVA	Species	1,40	2.09	0.16
Species/ life stage	Test	Testing	df	*F*	*P*
TP					
Barbus barbus	ANOVA	Life stage	2,64	47.17	<0.01
Squalius cephalus	ANOVA	Life stage	2,49	6.52	<0.01
0+	ANOVA	Species	3,60	12.36	<0.01
Juvenile	Welch's	Species	3,30	60.53	<0.01
Adult	ANOVA	Species	1,40	0.02	0.90

Note data for all sites and years are combined

was in contrast to the adult *B. barbus* and *S. cephalus*, which were not significantly different ($F_{1,41} = 1.77$, $P = 0.18$).

The model testing differences in $\delta^{15}N$-$\delta^{13}C$ centroids between the different life stages of *B. barbus* was significant ($F_{2,64} = 28.89$, $P < 0.01$), with pairwise comparisons indicating the significant differences were between adults and the other life stages ($P < 0.01$; Table 4). Whilst the overall model was also significant in *S. cephalus* ($F_{2,49} = 17.31$, $P < 0.01$), pairwise comparisons indicated the significant differences were only between the 0+ fishes and the other life stages ($P < 0.01$; Table 4).

Core isotopic niches (standard ellipse areas)

The 95% confidence intervals of the core isotopic niches (as standard ellipse areas) at each life stage suggested they were similar in size between the species (Table 5). In general, the core isotopic niches of the 0+ fishes had low overlap (maximum 7% between *B. barbus* and *S. cephalus*), the juvenile fishes had no niche overlap, but in adult *B. barbus* and *S. cephalus*, their niches overlapped by 55% (Fig. 3). Within *B. barbus*, there was no overlap in their core niches between the 0+, juvenile and adult fish (Fig. 3). In *S. cephalus*, there was no niche overlap between 0+ and juveniles, with this increasing to 2% between

Table 4 The significance of differences in $\delta^{15}N$-$\delta^{13}C$ centroids between the different life stages of the fishes, as represented by *P* values (with Bonferroni adjustment for multiple comparisons) derived in PERMANOVA

	0+ *B. barbus*	0+ *S. cephalus*	0+ *P. phoxinus*	J *B. barbus*	J *S. cephalus*	J *L. leuciscus*	J *T. thymallus*	A *B. barbus*	A *S. cephalus*
0+ *B. barbus*	-								
0+ *S. cephalus*	0.74	–							
0+ *P. phoxinus*	< 0.01*	< 0.01*	–						
J *B. barbus*	0.22	–	–	–					
J *S. cephalus*	–	< 0.01*	–	0.01*	–				
J *L. leuciscus*	–	–	–	0.02*	0.01*	–			
J *T. thymallus*	–	–	–	0.01*	0.01*	0.02*	–		
A *B. barbus*	< 0.01*	–	–	< 0.01*	–	–	–	–	
A *S. cephalus*	–	< 0.01*	–	–	0.62	–	–	0.18	–

Table 5 Mean stable isotope data (± 95% CL) and standard ellipse areas (as SEAc; ± 95% CI SEAb) for the sampled fishes in the study river and across the three life stages (0+, juvenile and adult)

Lifestage and species	C_{corr}	TP	SEAc (± 95% CL)
0+ *B. barbus*	0.06 ± 0.38	3.30 ± 0.08	0.77 ± 0.28
0+ *S. cephalus*	0.49 ± 0.65	2.92 ± 0.13	0.96 ± 0.51
0+ *P. phoxinus*	− 1.49 ± 0.49	3.02 ± 0.11	0.73 ± 0.36
Juvenile *B. barbus*	0.39 ± 0.58	2.70 ± 0.13	0.54 ± 0.28
Juvenile *S. cephalus*	2.57 ± 0.75	2.63 ± 0.14	0.59 ± 0.30
Juvenile *L. leuciscus*	1.42 ± 0.39	3.03 ± 0.05	0.44 ± 0.16
Juvenile *T. thymallus*	1.57 ± 0.20	2.13 ± 0.14	0.28 ± 0.14
Adult *B. barbus*	2.52 ± 0.40	2.62 ± 0.15	1.34 ± 0.58
Adult *S. cephalus*	3.22 ± 0.45	2.61 ± 0.15	1.89 ± 0.83

0 + and adults, and then the juvenile niche sitting entirely within the adult niche (Fig. 4).

Discussion

Hypothesis 1 tested whether there were consistent inter-specific patterns of trophic partitioning between *B. barbus* and the other fishes. It was formulated due to these patterns of niche partitioning being evident between the fishes in the *B. barbus* indigenous range (Bašic & Britton, 2016). An alternative to this hypothesis would be the fishes having high niche overlap, as has been suggested between invasive and endemic *Barbus* fishes in Italian rivers, where it appears to have resulted in the competitive displacement of the endemics (Carosi et al., 2017). Hypothesis 1 was tested using two analyses, $\delta^{15}N$-$\delta^{13}C$ centroids and core isotopic niches. The centroids were calculated using all SI data per life stage and species, whereas cores niches are based on a predicted 40% of the SI data to indicate typical resource use (Jackson et al., 2011, 2012). There were some consistent results from these analyses that aligned with Hypothesis 1, especially in the juvenile fishes where there were significant differences in $\delta^{15}N$-$\delta^{13}C$ centroids between all species and no overlaps in their core niches. In the 0+ fishes, there was less consistency in the results of both analyses, with isotopic niches showing low inter-specific overlap, but with $\delta^{15}N$-$\delta^{13}C$ centroids showing significant differences only between *P. phoxinus* and the other fishes. Whilst there was poor alignment of the results in the adult fishes with Hypothesis 1, both analyses provided consistent results; the $\delta^{15}N$-$\delta^{13}C$ centroids of the adult *B. barbus* and *S. cephalus* were not significantly different and their core niches had

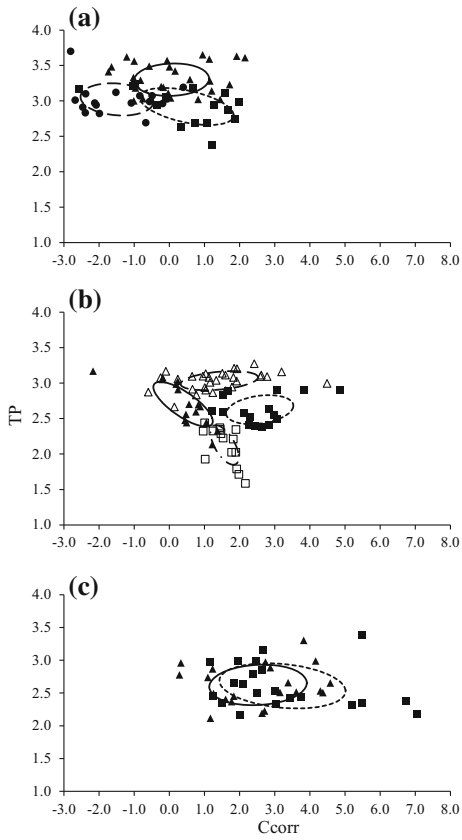

Fig. 3 Corrected Carbon (C_{corr}) versus trophic position (TP) for *Barbus barbus* (filled triangle), *Squalius cephalus* (filled square), *Leuciscus leuciscus* (delta) and *Thymallus thymallus* (open square), and the positions of their core isotopic niches (as SEA_c), where solid line: *B. barbus*, small dashed line: *S. cephalus*, long dashed line: *L. leuciscus*, and dash/dot line: *T. thymallus*. (**a**) 0+ fishes; (**b**) juvenile fishes; and (c) adult fishes

relatively high overlap (55%). Across all life stages and analyses, there was no evidence to support the alternative hypothesis that invasion by *B. barbus* had resulted in competitive displacement of native fishes, as suggested by Carosi et al. (2017) for endemic *Barbus*. It is, however, acknowledged that this was not tested implicitly here, given the absence of data from the pre-invaded period or from sites with *B. barbus* absent.

The pattern of isotopic niche partitioning between *B. barbus* and other fishes was thus consistent with a number of isotopic studies completed on populations in their indigenous range (Bašić et al., 2015; Bašić and Britton, 2015, 2016). These studies all suggested that *B. barbus* and *S. cephalus* have distinct core isotopic niches, with minimal inter-specific resource sharing. This pattern was evident in rivers that had been stocked with hatchery-reared *B. barbus* at sizes below 250 mm and remained evident in adult fishes (Bašić and Britton, 2016). In contrast, Gutmann Roberts et al. (2017) revealed that in the lower reaches of the study river, there was high overlap in the core isotopic niches of adult *B. barbus* and *S. cephalus*, primarily the result of individual fishes specialising in the consumption of pelletized marine fishmeal utilised by anglers. This niche overlap was also evident in the adult fishes here, where inter-specific niche differences were only significant in the 0+ and juvenile fishes. In *B. barbus*, there were strong and significant patterns in niche partitioning between their different life stages, suggesting considerable ontogenetic shifts in their diet that resulted in their population having a relatively large core isotopic niche that was composed of at least three distinct sub-sets. This was consistent with Hypothesis 2, although the $\delta^{15}N$-$\delta^{13}C$ centroids suggested differences were not significant between the 0+ and juvenile fish. In contrast, the isotopic niches of *S. cephalus* were more similar over their three studied life stages, with only the niche of the 0+ fish being distinct from the other life stages, with the juvenile and adult niches overlapping completely, contrary to Hypothesis 2.

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

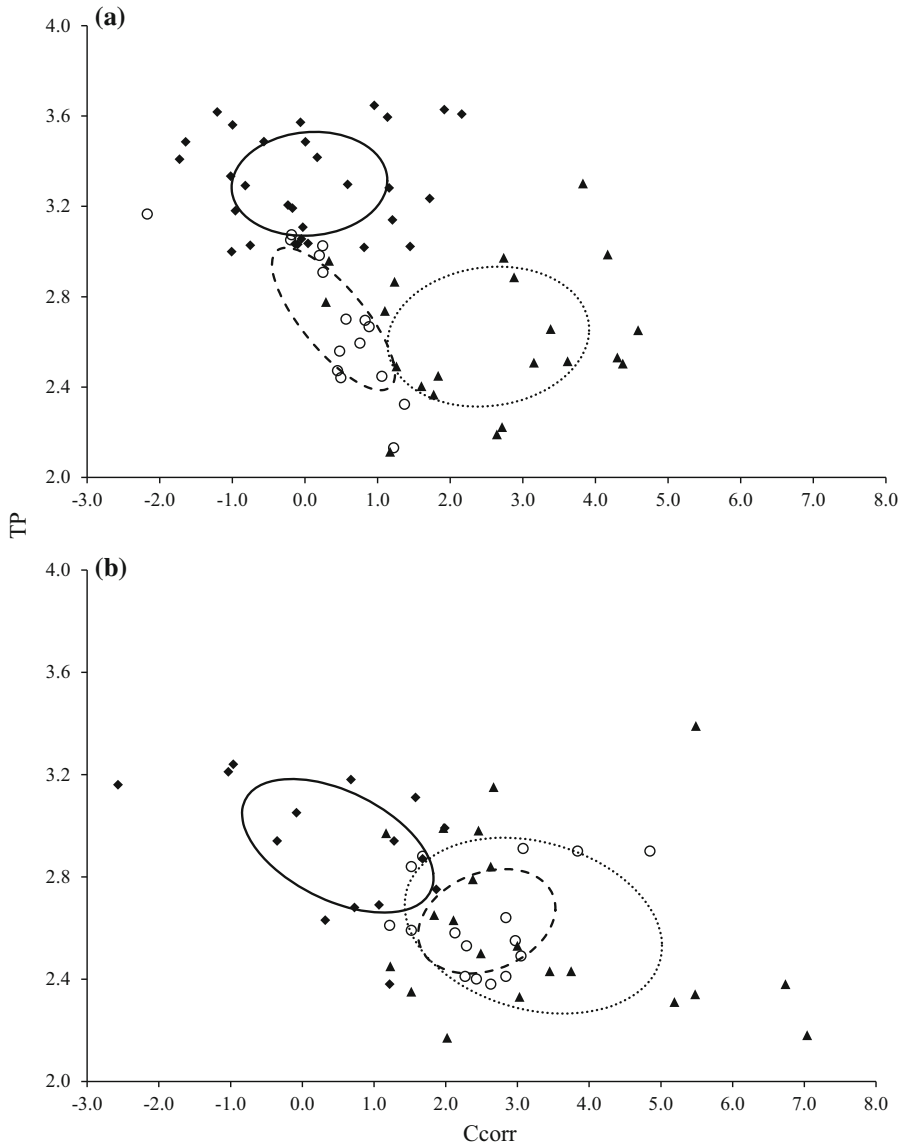

Fig. 4 Intra-specific comparisons of Corrected Carbon (C_{corr}) versus trophic position (TP) and positions of core isotopic niches (as SEA_c) for (**a**) *Barbus barbus* and (**b**) *Squalius cephalus*, and where filled diamond, solid line: 0+ fish; open circles, dashed line: juvenile fish; filled triangle, dotted line: adult fish

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

In this study, the fish SI data were 'corrected' to enable data to be combined across two sampling years and relatively long stretch of river. The use of standard equations to 'correct' SI data in this manner is well established (e.g. Olsson et al., 2009; Jackson & Britton, 2014). However, this correction relies upon an adequate description of the SI data of the fish prey resources. Here, correction focused on use of *G. pulex* SI data. The rationale for this was they were present in all samples from all areas sampled (in contrast to other macro-invertebrate taxa), there were significant differences in their isotopic data between years and sites (whereas data from other macro-invertebrate taxa revealed similar values, at least spatially), and literature suggests *Gammarus* spp. are an important prey item for many fishes (e.g. MacNeil et al., 1999). Correction of the juvenile fish SI data using the *G. pulex* data was also demonstrated as removing significant temporal differences. To enable the SI data of the 0+ fishes to be comparable to the other fishes, they were also corrected using the *G. pulex* data, although

stomach contents analyses (SCA) for the 0+ fishes had suggested that these amphipods were a minor prey item (Gutmann Roberts & Britton, 2018). However, individuals of > 20 mm standard length in all the 0+ fish species analysed had infections of the intestinal parasite *Pomphorhynchus tereticollis* (C. Gutmann Roberts, unpublished data). This parasite has gammarids as its intermediate host (Kaldonski et al., 2008), suggesting *G. pulex* might have been consumed in greater proportions in the 0+ fishes than suggested by SCA. Moreover, in the River Severn basin, *Pomphorhynchus* spp. has been reported as prevalent in all the fishes studied here (Brown 1984), suggesting gammarids are a common and important prey item of fish in the river. Therefore, although it is acknowledged that the macro-invertebrate baseline SI data used to correct the data here could have utilised a wider range of taxa, especially in 2015, it is strongly argued that the use of *G. pulex* SI data to correct the fish SI data was justified and appropriate.

Following introductions of non-native fishes, adverse ecological impacts often develop through increased inter-specific competition for food resources between invasive and sympatric native fishes (Gozlan et al., 2010; Cucherousset et al., 2012). Given the relatively similar size ranges of the invasive *B. barbus* with other cyprinid fishes across the different life stages (albeit with some inter-specific length differences within life stages), this suggests there was considerable potential for inter-specific competitive interactions, especially given the fishes were from relatively similar functional guilds (Bašic & Britton, 2016). However, the lack of overlap in the isotopic niches of the 0+ and juvenile fishes—the life stages when their abundances tend to be highest—suggested low dietary overlap, with only the isotopic niches of the adult fishes indicating some dietary overlap. Schulze et al. (2012) suggested that species within the same ecological guild can only coexist when they respond differently to limited resource availability with, for example, specialised species only persisting if their competitors are generalists. Evidence in literature supports this, with reduced trophic niche sizes in many co-existing fishes when compared to allopatry (Bolnick et al., 2010; Tran et al., 2015). In the study river, however, even where the isotopic niches of the fishes were partitioned, the niches were similarly sized. Although this suggests there had not been any niche constriction in the native fishes in *B. barbus* presence, it is acknowledged that this is speculative given that isotopic niche sizes of the native fishes were unable to be measured in *B. barbus* absence. Notwithstanding, the inter-specific isotopic niche partitioning evident in the study suggests that despite their similar ecological guilds and sharing similar habitats (especially the 0+ fishes), there were sufficient differences between the fishes in their functional traits and/or habitat utilisation to enable substantial differentiation in their core isotopic niches to occur (Robinson et al., 1993; Borcherding et al., 2013; Negus & Hoffman, 2013).

On one hand, the results here could suggest that the ecological impacts of invasive *B. barbus* are relatively minor in the river, as there was little evidence to suggest there was high diet similarity in the fishes at their most abundant life stages (0+ and juveniles). This inference is supported by other recent studies on native *B. barbus* that have revealed strong patterns of inter-specific core isotopic niche partitioning (e.g. Bašic & Britton, 2015, 2016). However, these studies were all limited to assessing trophic interactions via stable isotope analysis, with studies suggesting that when compared with other dietary analysis methods, such as stomach contents analyses, different results can occur, resulting from differences between items ingested (stomach contents) and assimilated (SIA) (e.g. Locke et al., 2013). Consequently, some caution is necessary if isotopic niche overlaps are to be used to infer the strength of competitive interactions. Moreover, invasive *B. barbus* can potentially result in other ecological concerns, such as causing habitat alterations, given recent work has demonstrated that in their native range, *B. barbus* act as 'zoogeomorphic agents' (Pledger et al., 2014, 2016). This is where their benthic foraging activities can reduce bed material stability, increase bedload transport, and impact microtopographic roughness and sediment structure (Pledger et al., 2014, 2016). This benthic foraging could then also impact upon aspects of the macro-invertebrate communities, such as decreased abundance via predation or reduced species richness via disturbance. However, these aspects were beyond the scope of this study and so further research is required to provide increased understandings of how *B. barbus* invasions affect macro-invertebrate communities and sediment structure. Finally, it should also be noted that the study river was low in fish species richness, with a cyprinid fish community comprising only four species

(including *B. barbus*), with other fish taxa being very limited in diversity and abundance in the study reach, and with no other *Barbus* species present. Consequently, if *B. barbus* are introduced into a river with considerably higher native fish species richness, irrespective of the presence of any other *Barbus* species, there is the possibility that there will be a greater probability of higher niche overlaps within species in the fish community and thus higher potential for ecological impacts to result. It is therefore recommended that such introductions proceed only with caution and full risk assessment (Roy et al., 2018).

In summary, across three life stages of invasive *B. barbus*, there were some strong patterns of isotopic niche partitioning with native fishes, with this partitioning initially evident between some fishes during their first year of life that became strongly apparent at juvenile life stages. These invasive *B. barbus* thus integrated into this riverine food web via exploiting different food resources to the native fishes that facilitated their co-existence.

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