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Quantifying trophic interactions and niche sizes of juvenile fishes in an invaded riverine cyprinid fish community

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Abstract

Quantifying feeding interactions between non-indigenous and indigenous fishes in invaded fish communities is important for determining how introduced species integrate into native food webs. Here, the trophic interactions of invasive 0+ European barbel *Barbus barbus* (L.) and the three other principal 0+ fishes in the community, *Squalius cephalus* (L.), *Leuciscus leuciscus* (L.) and *Phoxinus phoxinus* (L.), were investigated in the River Teme, a River Severn tributary in Western England. *Barbus barbus* has been present in the River Teme for approximately 40 years. Analyses of stomach contents from samples collected from three sites between June and September 2015 revealed that, overall, fishes displayed a generalist feeding strategy, with most prey having low frequency of selection. Relationships of diet composition versus body length and gape height were species-specific, with increasing dietary specialisms apparent as the 0+ fishes increased in length and gape height. The trophic niche size of invasive *B. barbus* was always significantly smaller than *S. cephalus* and *L. leuciscus*, and was significantly smaller than *P. phoxinus* at two sites. This was primarily due to differences in the functional morphology of the fishes; 0+ *B. barbus* were generally restricted to foraging on the benthos, whereas the other fishes were able to forage on prey present throughout the water column. Nevertheless, the invasive *B. barbus* were exploiting very similar prey items to populations in their native range, suggesting these invaders were strongly pre-adapted to the River Teme and this arguably facilitated their establishment and invasion.

Introduction

Invasions by non-indigenous fishes can increase inter-specific competition in fish communities, potentially leading to impacted native species having reduced growth and survival rates, and/ or being displaced from their original niche (Gozlan et al. 2010). Quantifying feeding interactions between the invasive and extant fishes in the community is thus important for determining the extent of the invasion-mediated shifts in the trophic structure of the food web (Jackson et al. 2012; Cucherousset et al. 2012; Copp et al. 2016). Ecological theory suggests that these shifts in trophic structure can include the invader occupying an unexploited niche (Shea and Chesson 2002). This will limit their inter-specific competitive interactions and facilitate their integration into the ecological community (Shea and Chesson 2002; Tran et al. 2015). Alternatively, when food resources are more limiting, the niche variation hypothesis suggests that increased inter-specific competition can result in the trophic niches of the competing species to constrict and diverge due to diets becoming more specialised (Van Valen 1965; Olsson et al. 2009; Tran et al. 2015). Conversely, this can result in the trophic niche sizes of competing species to increase, as individuals utilize a wider resource base to maintain their energy requirements (Svanbäck and Bolnick 2007). When invasive and native species coexist for prolonged periods, high overlaps in their trophic niches can suggest a lack of competitive interactions, perhaps due to resources not being limiting, and so facilitating co-existence (Pilger et al. 2010; Guzzo et al. 2013). However, prolonged co-existence can also result in competitive exclusion, where the invader eventually excludes a native species from its original niche and results in its population decline (Bøhn et al. 2008).

The ability of an introduced fish to develop invasive populations depends on their ability to establish sustainable populations, with reproduction and recruitment being key processes. Consequently, the larval and juvenile life-stages of fishes ('0+ fishes') are important in the overall invasion process due to their influence on recruitment (Nunn et al., 2003, 2007a, 2010a). A range of factors influences the growth and survival rates of 0+ fishes, including their ability to capture and ingest the prey items and sizes available (Nunn et al., 2012). If preferred prey items are unavailable, reduced growth rates and/ or starvation can occur, with potentially deleterious consequences for that 0+ cohort (Dickmann et al., 2007; Burrow et al., 2011). Where an introduced fish shares food resources with indigenous fishes and these resources become limiting, this can affect 0+ fish food acquisition and assimilation, and growth and survival rates, and so potentially impedes their ability to recruit and, therefore, establish (Gozlan et al., 2010; Dick et al., 2014, 2017).

The feeding ecology of mature fishes is relatively well understood, including for temperate riverine cyprinid fishes (e.g. Mann, 1974; Nunn et al., 2012). Extant knowledge includes how diet plasticity can assist the establishment of populations of introduced fishes (Basic et al., 2013; Tran et al., 2015). In contrast, the feeding ecology of 0+ fishes is often poorly understood (Nunn et al., 2012), especially within invaded communities (Britton et al., 2009). This is despite developmental shifts in diet often being important for 0+ fish survival (DeVries et al., 1998). In general, most freshwater fishes are planktivorous at the onset of exogenous feeding, with zooplankton being an important larval prey resource (Nunn et al., 2007b, 2010). Thereafter, diets of juvenile riverine cyprinids in temperate regions tend to consist of a mix of cladocerans, copepods and insect larvae, with some species also exploiting adult dipterans and Aufwuchs (the periphyton and associated microfauna that grow on underwater surfaces) (Nunn et al., 2012). However, as individuals increase in body and gape

sizes, there is a general shift towards each species developing specific dietary traits that can result in considerable inter-specific diet and niche differences (Nunn et al., 2007b, 2012). As the ability to assimilate adequate energy has important implications for lengths achieved at the end of the first growth year, this can affect over-winter survival, as larger individuals tend to have higher over-winter survival rates (Nunn et al., 2007a,b, 2010).

The aim of this study was to quantify the trophic interactions of a riverine community of 0+ cyprinid fishes invaded by a non-indigenous fish, European barbel *Barbus barbus* (L.). This fish is indigenous to some European rivers but has been widely introduced outside of their natural range for enhancing angling, in countries including Italy and England (Britton & Pegg, 2011). The study system was the River Teme, a River Severn tributary in western England, where *B. barbus* is non-indigenous and invasive (Wheeler & Jordan, 1990; Antognazza et al., 2016). The introduction of *B. barbus* into the River Severn was in 1956, with the species then dispersing through much of the basin (Wheeler & Jordan, 1990). *Barbus barbus* began to be captured by anglers in the River Teme in the 1970s, indicating they have been present in the study river for approximately 40 years (Antognazza et al. 2016). The fish assemblage of the River Teme is relatively species poor; the only other cyprinids present are minnow *Phoxinus phoxinus* (L.), chub *Squalius cephalus* (L.) and dace *Leuciscus leuciscus* (L.). Some salmonid fishes are also present, including grayling *Thymallus thymallus* (L.).

Through application of stomach contents analyses (SCA) (Hyslop, 1980) to quantify 0+ fish diet on samples collected during 2015, the study objectives were to: (1) quantify diet composition across the community of 0+ fishes, with assessment of inter-specific similarity and spatial patterns; (2) identify shifts in the diet composition of each species and in relation to body length and gape size; and (3) quantify trophic niche sizes per species and according

to gape size, with assessment of the extent of inter-specific niche overlap between invasive *B. barbus* and other fishes. Given that invasive *B. barbus* and the other fishes of the study river have co-existed for approximately 40 years, it was predicted that the trophic niches of the 0+ fishes would be divergent through the fishes having developed strong dietary specialisms, as per the niche variation hypothesis that suggests invasions can result in trophic niche constriction and divergence via the development of dietary specialisms resulting from competitive interactions (Van Valen 1965; Olsson et al. 2009).

Materials and Methods

Sampling sites and methodology

Three sampling sites were used in the non-indigenous range of *B. barbus* in the River Teme (Fig. 1). Due to negligible off-channel habitat throughout the river, each sampling site consisted of areas of reduced flow rates within the river channel. Each site was separated by at least 5 km of river length was thus were considered as independent from each other, with the 0+ fish unable to intentionally move between them. Site 1 was the furthest upstream, located at Tenbury Wells (52°19'N, -2°24'W) (Fig. 1). The sampled areas were located immediately downstream of a road bridge at the downstream end of a large gravel island, near to the right-hand bank. Riparian vegetation included overhanging trees (*Salix* spp.) and, within the river, there was minimal in-stream vegetation, with the river generally running over gravel at depths of < 1m. Sampling areas comprised of large patches of minimal/ negligible flow in marginal areas where depths were generally < 1 m. Site 2 was located at Knightwick (52°12'N, -2°23'W) (Fig. 1), with samples generally collected at the downstream end of an exposed gravel beach where there were shallow patches (< 1 m depth) of low flow over gravel that created nursery habitat for 0+ fishes, but where instream vegetation was

minimal. Site 3 was the most downstream site (52°10'N, -2°14'W) (Fig. 1), with the sampling area located at the downstream end of a gravel riffle used by spawning *B. barbus* and, again, where there were shallow (< 1 m) patches of low and negligible flow over gravel, but with instream vegetation absent. Samples were collected on up to five occasions per site between July and October 2015 (Supplementary material: Table S1), with samples not collected thereafter due to elevated river levels throughout the winter period that prevented safe access to sampling sites.

Due to the restricted 0+ fish habitat of the River Teme and poor riparian access, point-abundance sampling by electric fishing was not an appropriate sampling method (Copp 2010). Micro-mesh seine netting was used instead, with acknowledgement that this would limit the proportion of larval fishes <15 mm in samples (Cowx et al. 2001; Copp 2010). On each sampling occasion, the 0+ fish were collected between 07.00 and 11.00, euthanised (MS222) and then preserved in 70 % IMS. Samples were unable to be collected at night for access and safety issues. These samples were then stored at 5 ^{o}C prior to their processing in the laboratory. All samples were processed in the laboratory within six months of sampling to minimise issues associated with shrinkage of body lengths related to preservation (Leslie & Moore, 2001).

Sample processing and data collection

There were four 0+ fish species, all of the Cyprinidae family, that were captured in sufficient numbers to enable subsequent dietary analyses: *B. barbus*, *S. cephalus*, minnow *Phoxinus phoxinus* (L.) and dace *Leuciscus leuciscus* (L.) (Table S1). In the laboratory, following identification to species level (Pinder, 2001), a maximum of 30 non-indigenous *B. barbus* and 20 individuals of the other fishes per site and per sample date were analysed. These numbers

of analysed fishes were achieved by sub-sampling within the collected samples, with this stratified to ensure the size ranges of fish present in each sample were covered. This involved their measurement using digital callipers (standard length, L_s, to 0.01 mm). The majority of the fishes were already at juvenile stages (a consequence of the sampling method) and thus subsequent dietary analyses focused on these, rather than larval stages (Krupka, 1988; Pinder, 2001). Gape size was measured as the height of the mouth when open at its widest angle, using a stage micro-meter (Lukoschek & McCormick, 2001; Nunn et al., 2007b). The intestine ('gut') was then dissected, with gut fullness (%) estimated and the total gut contents extracted, mounted on a glass slide and fixed using Polyvinyl alcohol-lactic acid-glycerol (PVLG). Prey items were then identified to their lowest practicable taxonomic level using microscopy (to x100 magnification), with their number then counted to provide data on abundance. Periphytic biota (diatoms and similar material that was too small to classify more precisely) were classed as 'Aufwuchs'. The amount of Aufwuchs in each gut was estimated on the basis of their percentage cover on the slide area and converted to a number (0 to 5 scale), similar to other studies (Garner 1996; Mann 1997), so that it was comparable to enumerated prey. As the majority of fishes had low proportions of Aufwuchs in the gut, this scale focused on slide coverage of below 55 % to allow greater discrimination between individual diets and thus greater precision in analyses. Thus, the scale used was: 0 (0 to 1 % coverage), 1 (2 to 3 %), 2 (4 to 7 %), 3 (8 to 20 %), 4 (21 to 55 %) and 5 (56 to 100 %).

A total of 37 distinct prey items were detected across the 0+ fish diets and thus, for some analytical purposes, these were categorised into the following 16 groups according to their taxonomy and functional ecology: Chironomid larvae, Aufwuchs, amphipods, winged insects, chalcid wasp, copepods, Cladocera, nymphs (stonefly and mayfly), Arachindae, Hemipteroids, saucer bugs, caddis larvae, beetles, beetle larvae, springtail (hexapods), seed/

189 spore/ plant material, and fish. The largest prey item in the gut of each individual fish was
190 then measured; for Chironomid larvae this always consisted on measuring the width of the
191 head.
192
193 *Data analysis*
194 Differences in fish standard length between the sites were tested initially using one-way
195 ANOVA with a Tukey post-hoc test. The vacuity index ($\%I_v$) (i.e. the proportion of fish with
196 empty guts) was calculated from: $\%I_v = S_0S_1^{-1}$, where S_0 is the number of fish with empty guts
197 and S_1 is the total number of larval and juvenile fish stomachs examined (Hyslop, 1980).
198 Frequency of occurrence of prey categories (F_i) represented the proportion of all guts that
199 contain that prey category and was determined from: $F_i = N_iN^{-1}$, where N_i is the number of
200 guts in which that prey item i occurred and N is the total number of guts with prey present
201 (Caillet, 1977). Relative abundance of a given prey category ($\%A_i$) represented the
202 proportion of total gut contents from all fish that comprised that prey category and was
203 calculated from: $\%A_i = 100(\Sigma S_iS_t^{-1})$, where S_i is the number of prey items comprising prey i
204 and S_t is the total number of prey in all guts regardless of whether they contained prey item i
205 (Macdonald & Green, 1983). Prey-specific abundance (P_i) represented the proportion of all
206 prey that comprised of a specific prey category and was determined from data from only the
207 guts in which prey items in that category were encountered. It was calculated from: $P_i =$
208 $100(\Sigma S_i\Sigma S_{ti}^{-1})$ here P is the number of prey items comprising prey i and S_{ti} is the total number
209 of prey items in guts that contained prey item i (Amundsen et al., 1996).
210
211 The calculation of frequency of occurrence and prey-specific abundance enabled feeding
212 strategy plots to be produced (Costello, 1990). These plots provided information about the
213 importance of prey categories and feeding strategies of each species via examination of the

distribution of points along the diagonals and the axes of the plot according to: prey importance (represented in the diagonal from the lower left (rare prey) to upper right (dominant prey), feeding strategy (represented in the vertical axis from the bottom (generalization) to top (specialization)), and the relationship between feeding strategy and the between or within-phenotype contributions to the niche width (represented in the diagonal from the lower right (high within-phenotype component, WPC) to upper left (high between-phenotype component, BPC)) (Amundsen et al., 1996; Leunda et al., 2008).

To test whether fish with larger body sizes consumed different prey items to smaller conspecifics, linear regression was used, with standard length as the independent variable and the percentage of specific prey items as the dependent variable. Where assumptions for the test were not met, the percentages of prey data were square-root transformed. Differences in gape height and standard length of the fishes were tested using general linear models, where gape height (μm) or standard length (mm) was the dependent variable and the independent variables were site and species. Differences in the maximum prey size per species were also tested using a general linear model; maximum prey size was the dependent variable, species was the independent variable and standard length was the covariate. This model structure was also used to test differences in maximum prey sizes according to sampling year and site. All general linear models were interpreted with regards to the significance of the independent variable on the dependent variable, the significance of covariates, and the estimated marginal means (i.e. mean values per group, adjusted for effect of covariate) and the significance of their differences according to independently linear pairwise comparisons with Bonferroni adjustment for multiple comparisons. To identify how body length, gape height and their interaction influenced the maximum prey size of each species, multiple regression was used. The outputs were the standardised β coefficients of each independent variable, where higher

values (irrespective of whether they were positive or negative) indicated a stronger correlative effect on the dependent variable, plus their R^2 values and significance.

For plots of trophic niche size versus gape height per species, gape heights were classified into five size groups: 0.8 to 1.4, 1.5 to 2.2, 2.3 to 3.1, 3.2 to 3.9 and 4.0 to 4.8 mm. These groupings were based on the conversion of the stage micro-meter units to the actual gape height of the fishes (in mm). In all analyses, gape heights above 4.8 mm were excluded from analyses as the maximum for *B. barbus* was 3.1 mm. Trophic niche sizes were expressed as standard deviation ellipses (40%), calculated using detrended correspondence analysis with basic reciprocal averaging that was completed using the 'decorana' function in 'vegan' package v2.4 in R (R Core Team, 2016; Oksanen et al. 2017). This was completed within a Bray-Curtis similarity matrix where all data were square root transformed for normality. Ellipse areas then compared across the gape height classes for each species to determine their influence on the size of the trophic niche.

Finally, to determine the differences in trophic niche sizes between species and sites, an ANOVA was carried out using a permutational approach. This analysis was carried out in R (R Core Team, 2017) using the vegan package (Oksanen et al. 2017), with the adonis function used to complete a PERMANOVA analysis. All vacuous guts and guts containing only diatoms were removed from the dataset prior to these analyses, plus three dietary items that only occurred once. As the dietary composition data were expressed as percentages, they were square-root transformed, followed by construction of a resemblance matrix with Bray-Curtis similarity that enabled the PERMANOVA analysis to be calculated between species and sites. To identify inter-specific differences, pairwise comparisons were carried out to

identify the significance of differences in niche sizes (Martinez Arbizu 2017). Drivers of inter-specific difference by site were determined using a SIMPER analysis (PRIMER 7).

Results

Sample sizes, stages and lengths

Across the four 0+ fishes, SCA was performed on 878 individuals (*B. barbus*: n = 431; *S. cephalus*: n = 174; *L. leuciscus*: n = 81; *P. phoxinus*: n = 192). Across the samples, no fish were present at larval stage 1 and, as there was only one fish at larval stage 2, this individual was removed from subsequent analyses (Table S1). As there were low numbers of fish sampled at larval stages 3 to 5, and relatively high numbers of juvenile fishes (juvenile stages 6 to 9), these fish were all grouped together as 'juveniles' for analytical purposes (Table S1). The minimum, maximum and mean lengths of these juveniles per species are provided in Table 1. The low number of larvae in samples also meant that testing of ontogenetic diet changes used fish lengths instead of larval stage.

Across the dataset, the standard length of *B. barbus* differed significantly between sites (ANOVA: $F_{2,428} = 3.97$, $P = 0.02$), with fish at Site 1 being significantly larger than those at Site 2 (Table 2). Similarly, *S. cephalus* at Site 2 were significantly smaller than the other sites (ANOVA; $F_{2,156} = 8.87$, $P < 0.01$; Table 2). *Phoxinus phoxinus* were significantly smaller at Site 3 than the other sites (ANOVA; $F_{2,174} = 17.9$, $P < 0.01$). As *L. leuciscus* was only sampled at Site 3, no spatial comparisons were possible. Vacuity indices were generally low, with the highest values in *S. cephalus* (up to 6 %) and lowest in *B. barbus* (0 to 0.6 %) (Table 2).

Relative frequency of prey and feeding strategies

Chironomid larvae were the most important prey item across the species, with values ranging between 44 % (*S. cephalus*) and 83 % (*B. barbus*) of diet, with Aufwuchs also a prominent item for all fishes (Table 2). There was variability in the contributions of prey categories between the fishes with, for example, Hemipteroids comprising of 7 % and 24 % of the diet of *S. cephalus* and *L. leuciscus* respectively, but less than 1 % for both *B. barbus* and *P. phoxinus*. Spatially, there was low variability in the relative frequencies of prey items in *B. barbus* diet, with Chironomid larvae being the dominant prey at all sites. In contrast, there was greater spatial variability in *S. cephalus* diet, for example in the proportion of hemipteroids (1 % at Site 3, > 10 % at other sites). For *P. phoxinus*, the major spatial differences were in the proportions of Chironomid larvae and Aufwuchs, although when combined, these prey categories still comprised between 85 and 94 % of their diet (Table 2).

Feeding strategy plots for each species suggested they were all generalists, with the majority of prey items having prey specific abundances of < 50 % with relatively low frequency of occurrences (Fig. 2). The relative high proportion of Chironomid larvae across the diet of each species was, however, strongly reflected in the feeding strategy plots, where their prey specific abundances ranged between 52 and 83 %. The most varied diet was in *L. leuciscus*, although the majority of prey categories had low frequency of occurrences and low prey specific abundances (Fig. 2). Spatially, there was little variability in the feeding strategy plots for *B. barbus* (Fig. S1), but with greater variability apparent for *P. phoxinus* and *S. cephalus* (Fig. S2, S3).

312 *Fish length and gape height influences on diet*

313 The relationship of gape height versus fish length was significant for each species (*B. barbus*:
314 $R^2 = 0.81$, $F_{1,515} = 2247.0$, $P < 0.01$; *S. cephalus*: $R^2 = 0.86$, $F_{1,185} = 1095.0$, $P < 0.01$; *L.*
315 *leuciscus*: $R^2 = 0.89$, $F_{1,106} = 738.4$, $P < 0.01$; *P. phoxinus*: $R^2 = 0.73$, $F_{1,158} = 435.4$, $P <$
316 0.01). Between the species, there were significant differences in gape height (GLM: Wald χ^2
317 = 1080.84, df = 3, $P < 0.01$), with standard length a significant covariate ($P < 0.01$). Pairwise
318 comparisons revealed the mean adjusted gape height of *Barbus barbus* (mean 2.02 ± 0.03
319 mm) was significantly smaller than the other three fishes (*S. cephalus*: 2.81± 0.05 mm; *L.*
320 *leuciscus*: 2.38 ± 0.07 mm; *P. phoxinus*: 2.82 ± 0.05 mm; $P < 0.01$ in all cases).
321
322 Maximum prey sizes differed significantly between the fishes (GLM: Wald $\chi^2 = 197.12$, df =
323 3, $P < 0.01$), where the covariate of standard length was significant ($P < 0.01$). The mean
324 maximum prey size of *B. barbus* (0.51 ± 0.02 mm) was significantly smaller than for *S.*
325 *cephalus* (0.67 ± 0.05 mm; $P < 0.01$), was not significantly different to *L. leuciscus* (0.53 ±
326 0.06 mm; $P = 0.47$), and was significantly larger than *P. phoxinus* (0.35 ± 0.03 mm; $P <$
327 0.01). Multiple regression revealed that for *B. barbus*, standard length and gape height, and
328 their interaction, were all significant variables, but with length explaining most the variation
329 in the prey size ($P < 0.01$ in all cases) (Table 3). For *S. cephalus*, although gape height and
330 standard length were both non-significant ($P > 0.05$), their interaction was a significant
331 predictor of maximum prey size ($P < 0.01$). In *L. leuciscus*, standard length was the only
332 significant predictor ($P < 0.01$), and none of the variables were significant predictors of
333 maximum prey size in *P. phoxinus* ($P > 0.05$ in all cases), with individuals generally
334 consuming much smaller prey than was possible for their gape height (Table 3).
335

Increases in gape height did not necessarily result in the development of a larger trophic niche across the 0+ fishes (Fig. 3). In *B. barbus* and *S. cephalus*, whilst the size of their trophic niches altered with gape height, it was largest *S. cephalus* at gape height of 2.5 to 3.1 mm and for *B. barbus* at 1.6 to 2.2 mm, with reductions thereafter (Fig. 3). For *P. phoxinus*, their largest trophic niches occurred in the two smallest gape height classes, suggesting their diet became more specialised as their gape height increased (Fig. 3).

Spatial and inter-specific dietary comparisons

There was a significant difference in niche size between the four species (PERMANOVA: $P < 0.01$) and across the three sites (PERMANOVA: $P < 0.01$) (Table 4). According to their niche sizes (as 40 % ellipse areas), *S. cephalus* had the largest niche of all species, with this significantly larger than *B. barbus* in all cases (Fig. 4; Table 5). The size of the *B. barbus* niche was significantly smaller than *L. leuciscus* at Site 3, and *P. phoxinus* at Site 2 and 3 (Table 5).

At Site 1, the niches of the three fishes present were generally discrete with low overlap (Fig. 4). At Site 2, the large niche of *S. cephalus* did not overlap with *B. barbus*, but the *B. barbus* niche sat within the larger niche of *P. phoxinus* (Fig. 4). At Site 3, the only site with all four fishes present, the niche of *B. barbus* had some overlap with all the other species, but with the niches of the other fishes having some differences, especially between *S. cephalus* and *L. leuciscus* (Fig. 4).

Discussion

This study successfully described the diet composition of 0+ fishes in a cyprinid fish community of low species richness that has been invaded by non-indigenous *B. barbus*. Overall, the 0+ fishes displayed a generalist feeding strategy, with most (but not all) prey categories having low selectivity according to feeding strategy plots. For some prey items in the diet, there were strong relationships with fish length, indicating the importance of increasing body size as a driver of dietary changes. There were, however, some differences in how the effects of body length and gape height manifested on diet composition, with dietary shifts in *B. barbus* and *S. cephalus* influenced strongly by their interaction, whereas in *L. leuciscus*, increased length was the only significant explanatory variable in their dietary changes.

The prediction was that the trophic niches of the 0+ fishes would be divergent, with this divergence developing according to the dietary specialisms of fishes. The results suggested some consistency with this prediction. Although the diets of all the fishes were described as generalist, they became more specialised as their body length and gape height increased. The prediction also included that the inter-specific niche divergence would be driven by competitive interactions, as per the niche variation hypothesis (Van Valen 1965; Olsson et al. 2009). Although this was difficult to test, it was considered unlikely, given the increasing and significant ontogenetic differences in the gape size of the fishes, plus their general functional morphological differences (De Silva et al., 1979). For example, the increased dietary specialisations apparent in *B. barbus* versus *L. leuciscus* were likely to be strongly driven by *B. barbus* having an inferior mouth that was primarily suited for only feeding on the benthos, with *L. leuciscus* having a terminal mouth and larger gape that enabled their exploitation of a

386 greater diversity of prey (e.g. by also exploiting drifting aerial insects). *Squalius cephalus*
387 also has a terminal mouth that enabled their foraging throughout the water column, and they
388 correspondingly had a very generalist diet and the largest niche of all the fishes at all sites.
389 Given these results, there was no evidence to suggest the prolonged cohabitation of *B. barbus*
390 with the other fishes in the study river had resulted in the competitive exclusion of a native
391 species from its original niche (Bøhn et al. 2008). This is a contrast to invasive *B. barbus* in
392 Italy where data suggest they have displaced endemic *Barbus* fishes in invaded river systems
393 via competitive interactions, although dietary data on the fishes are currently absent (Carosi
394 et al., 2017)
395
396 Across the 0+ fishes, trophic niche sizes and composition were most similar between *B.*
397 *barbus* and *P. phoxinus*. The main driver of their trophic similarity was their high dietary
398 proportions of Chironomid larvae. Given that *P. phoxinus* were the most abundant 0+ fish at
399 each site, this suggests some potential for high inter-specific competition for resources with
400 invasive *B. barbus* (Chase et al., 2016). However, both fishes had other items in their diet,
401 suggesting that had intense competitive interactions resulted in reduced food intake rates,
402 they could have switched to alternative prey (Dill, 1983). Moreover, with *P. phoxinus* the
403 most numerically abundant 0+ fish at all sites and sampling occasions (their analysed sample
404 sizes here of n = 20 per site and sampling occasions were derived via sub-sampling), there
405 was no evidence to suggest their high dietary similarity with invasive 0+ *B. barbus* was
406 having negative consequences at the population level, given their high abundance.
407
408 The diet composition of these invasive 0+ *B. barbus* in the River Teme was relatively similar
409 to their diets in rivers in their indigenous range. For example, in the River Seig, Germany,
410 larvae of Chironomids, caddisfly and mayfly were also all present in 0+ *B. barbus* diet

411 (Bischoff & Freyhof, 1998). Similarly, in the River Trent, Eastern England, the diet of *B.*
412 *barbus* in their late larval stages was also strongly dependent on Chironomid larvae (Nunn et
413 al., 2007b). In the River Lee, England, Copp et al. (2005) also reported 0+ *B. barbus*
414 predating upon similar items, including larvae of caddis fly and Chironomid larvae. Thus,
415 there appears to be high similarity in *B. barbus* diet between their indigenous and non-
416 indigenous ranges. When coupled with their diet similarities with the indigenous and highly
417 abundant *P. phoxinus*, these results suggest some consistency with the pre-adaptation
418 hypothesis of invasion biology. This hypothesis suggests that the probability of invasion by
419 an introduced species is elevated when they share similar ecological traits and behaviours
420 with indigenous species (Duncan & Williams, 2002). These similar traits and behaviours can
421 include similar abilities to acquire resources (Duncan & Williams, 2002; Ricciardi & Mottiar,
422 2006). Invasion probability is also increased when the introduced species expresses their
423 traits and behaviours in a similar manner to populations in their natural range (Duncan &
424 Williams, 2002; Ricciardi & Mottiar, 2006; Buoro et al., 2016). The results here suggest that
425 0+ *B. barbus* underwent minimal shifts in their foraging behaviours to adapt to the River
426 Teme, given their diet similarities to both their natural range and the other species in their
427 new range. It is suggested that these factors assisted their establishment in, and invasion of,
428 the River Teme.
429
430 There was a very low proportion of small-bodied (< 15 mm) and early larval stages in the 0+
431 fish samples. This was likely to have related to sampling bias resulting from the micromesh
432 seine net, with it being inefficient to capture fishes of these lengths and life-stages (Cowx et
433 al., 2001). If future studies require increased numbers of larval fishes in their analyses then an
434 alternative sampling method would be required, such as point abundance sampling using
435 electric fishing. This method can potentially sample larvae as small as 5 mm length (Copp,

2010). Notwithstanding, at the free embryo stage and when they emerge from within spawning gravels, *B. barbus* larvae can be between 8 and 13 mm (Vilizzi & Copp, 2013). Thus, to capture early larval stages might require sampling methods capable of catching fish within the spawning gravels. Although the use of preservation of fish samples enabled enhanced dietary analyses in the laboratory, this can potentially result in shrinkage of body lengths (Fox, 1996). However, Leslie & Moore (2001) suggested shrinkage effects are relatively low when using similar preservation methods, providing samples are processed within a year of collection, as was completed here. Consequently, the relationships between diet and fish lengths in our study were considered valid. Finally, in our study, spatial comparisons were made in diet of each species, with differences between sites likely to have related to differences in food availability. However, the food availability of each site was not quantified accurately (given the presence of 37 items across the diets), preventing further analysis. Although these data on resource availability might also have assisted more precise testing of whether diets were generalist or specialist, assumptions on this were made from the feeding strategy plots (Amundsen et al. 1996). From these plots, all the fishes were described as generalists. However, across the four species, there was variation in the extent of this dietary generalism. *Barbus barbus* generally had the narrowest diet and smallest niche, and so they have also been described as being the species with the most specialist diet of the analysed fishes.

In summary, these results indicated how invasive 0+ *B. barbus* had successfully integrated into a 0+ cyprinid fish community via their diet and feeding ecology. The results highlighted that the 0+ *B. barbus* were consuming similar items to conspecifics in their indigenous range, suggesting some consistency with the pre-adaptation hypothesis of invasion biology. As the 0+ fishes all increased in their lengths and gape sizes, their diets became increasingly

461 dissimilar, especially between *B. barbus* and other fishes. This was primarily due to
462 differences in their functional morphology and resulted in the *B. barbus* niche sizes generally
463 being significantly smaller than the other fishes. This invaded fish community thus represents
464 a strong case study of how the invasion of a river system by a non-indigenous fish was
465 facilitated by the utilisation of their pre-adapted foraging behaviours.
466
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472
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Table. 1. Sample size (n), standard length (LS) range (Min LS/ Max LS) and mean standard length (mm) (± 95% confidence intervals) for *Barbus barbus*, *Squalius cephalus*, *Leuciscus leuciscus* and *Phoxinus phoxinus*.

Species	n	Min LS (mm)	Max LS (mm)	Mean LS (mm)
B. barbus	427	12.3	36.8	21.7 ±0.49
S. cephalus	147	11.2	33.9	19.7 ± 0.75
L. leuciscus	77	23.7	48.9	37.2 ± 1.46
P. phoxinus	142	12.7	33.8	21.4 ± 0.71

Table 2. Relative frequency (%) of prey items, vacuity index (%I_v) and mean standard length (mm) ± CI for 0+ fishes in the samples collected from Sites 1,2, and 3: barbel *Barbus barbus* chub *Squalius cephalus*, , minnow *Phoxinus phoxinus* and dace *Leuciscus leuciscus.*

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

Table 3. Output from multiple regression to determine significant explanatory variables of maximum prey size for each species (GH = gape height; L_S = standard length; GH: L_S interaction between gape height and standard length)

	df	Standardised β	F value	P
Barbus barbus				
GH	1	-0.11	44.94	< 0.01
L_S	1	0.46	12.99	< 0.01
GH: L_S	1	-0.17	18.66	< 0.01
Residuals	513			
Squalius cephalus				
GH	1	0.02	2.05	0.15
L_S	1	-0.07	2.71	0.10
GH: L_S	1	-0.23	14.19	< 0.01
Residuals	183	0.21		
Leuciscus leuciscus				
GH	1	-0.57	0.92	0.34
L_S	1	0.72	7.33	< 0.01
GH: L_S	1	0.02	0.04	0.84
Residuals	104	-0.02		
Phoxinus phoxinus				
GH	1	-0.08	0.02	0.89
L_S	1	0.06	0.15	0.70
GH: L_S	1	0.03	0.23	0.63
Residuals	156	-0.03		

Table 4. Comparison of diet between the 0+ fishes, site and the interaction of site and species (PERMANOVA)

Factor	Df	F	R^2	*P*
Species	3	80.75	0.24	< 0.01
Site	2	4.06	0.01	< 0.01
Species: site	4	6.56	0.03	< 0.01
Residuals	736		0.73	
Total	745		1.00	

Table 5. Sample sizes, mean standard length, 40% standard error ellipse area and pairwise comparisons and significance (PERMANOVA) testing in niche size differences between *Barbus barbus* and the other fishes, as calculated in 'vegan' package v2.4 in R (R Core Team, 2016).

Site (S)/ species	n	Average LS (mm) ± 95% CL	Within group similarity	40% Ellipse area	R^2	P_{adj}
S1						
B. barbus	140	22.6 ± 0.9	75%	0.28		
S. cephalus	43	20.7 ± 1.6	47%	1.29	0.21	0.04
P. phoxinus	47	22.6 ± 1.3	83%	0.18	0.08	0.04
S2						
B. barbus	151	21.0 ± 0.7	76%	0.13		
S. cephalus	44	17.9 ± 1.2	34%	8.72	029	0.04
P. phoxinus	51	22.9 ± 0.6	51%	1.29	0.08	0.04
S3						
B. barbus	136	21.5 ± 0.9	79%	0.18		
S. cephalus	54	21.0 ± 0.9	40%	1.76	0.24	0.04
P. phoxinus	42	22.0 ± 0.7	74%	0.41	0.10	0.04
L. leuciscus	33	30.9 ± 1.4	48%	1.35	0.25	0.04

Figure captions

Figure 1. Inset: Location of the Rivers Severn and Teme in England and Wales; main: location of the samplings sites on the River Teme, where Site 1 was downstream of Tenbury Wells, Site 2 was at Knightwick and Site 3 was downstream of Powick.

Figure 2. Feeding strategy plots for four 0+ fishes from the River Teme, where (a) *Squalius cephalus*, (b) *Barbus barbus*, (c) *Phoxinus phoxinus* and (d) *Leuciscus leuciscus*. Points represent prey categories: Aufwuchs (□); chironomid larvae (◊); amphipod (🔲); winged insects (×); chalcid wasp (■); copepod (●); Cladocera (+); nymphs (▬); water arachnids (-); hemipteroid assemblage (✶); saucer bug (♦); caddisfly larvae (●); beetle (▲); beetle larvae (○); springtail (♦); seed/ spore (🔲); and fish (🔲).

Figure 3. Gape height (GH) versus trophic niche size, plotted as MDS plots with 40% confidence interval ellipses for describing niche size, for (a) *Barbus barbus*, (b) *Squalius cephalus*, (c) *Leuciscus leuciscus*, and (d) *Phoxinus phoxinus*. On each plot, the ellipses represent groupings of gape heights according to: 0.8 – 1.4 (solid line), 1.5 – 2.2 (short dashes), 235 – 3.1 (dotted), 3.2 – 3.9 (dash dot) and 4.0 – 4.8 (long dashes).

Figure 4. Non-metric MDS plots (Square root transformation, Bray Curtis similarity) 40% ellipses from Site (1), (2) and (3); *Barbus barbus* (solid line), *Squalius cephalus* (long dashed line), *Phoxinus phoxinus* (dotted line) and *Leuciscus leuciscus* (short dashed) between 12.3 and 37.6 mm.

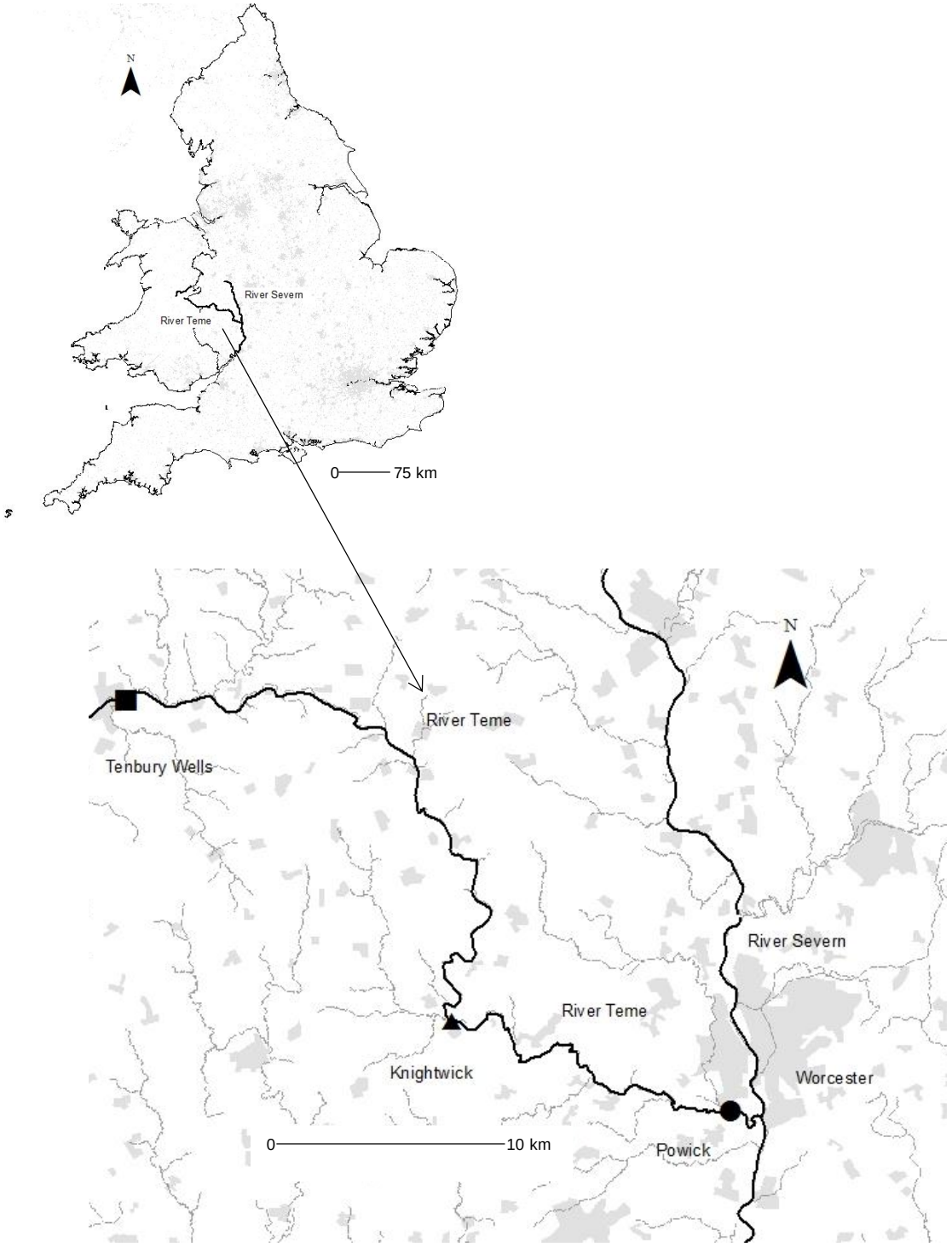
N
River Severn
River Teme
0
75 km
N
River Teme
Tenbury Wells
River Severn
River Teme
Knightwick
Worcester
0
10 km
Powick

Figure 1.

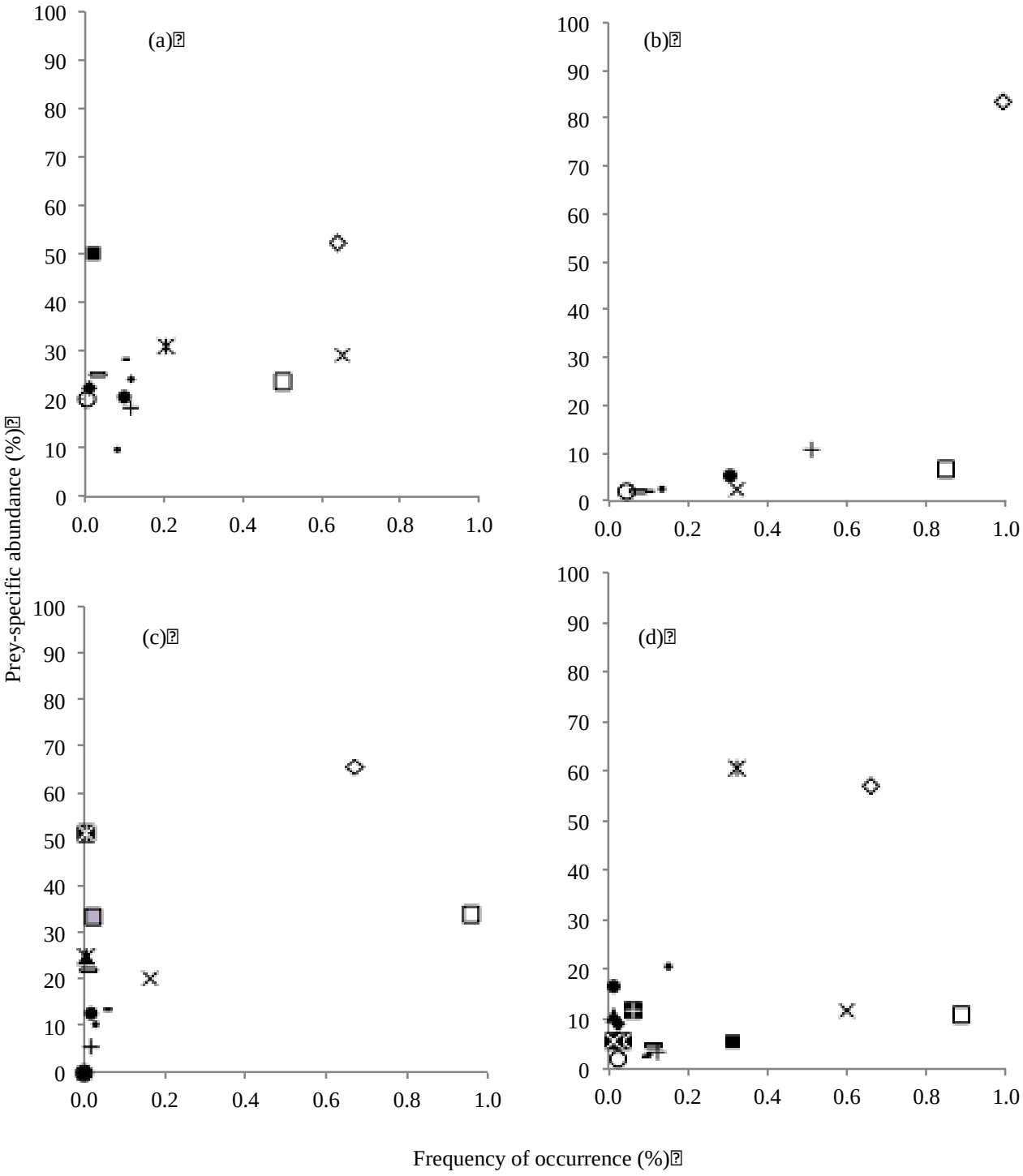
(a)
(b)
(c)
(d)
Prey-specific abundance (%)
Frequency of occurrence (%)
0
10
20
30
40
50
60
70
80
90
100
0.0
0.2
0.4
0.6
0.8
1.0

Figure 2.

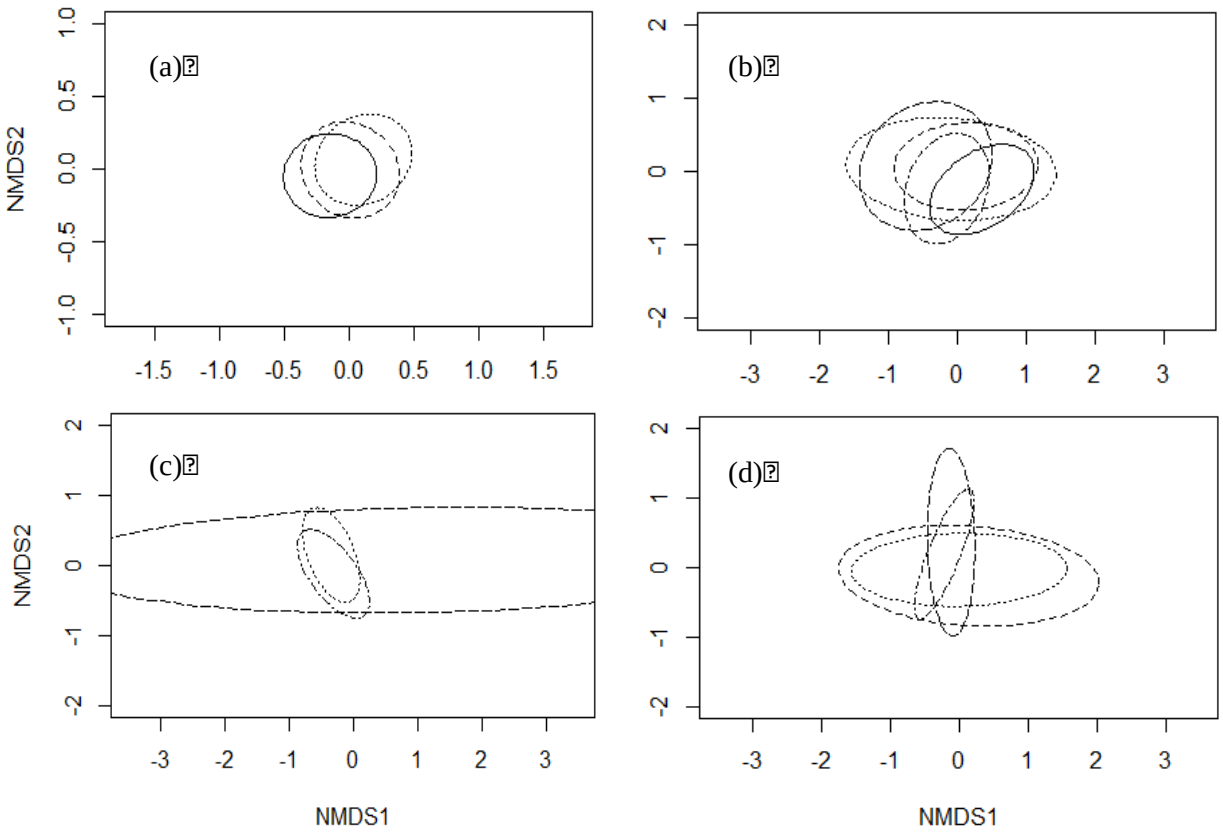

Figure 3.

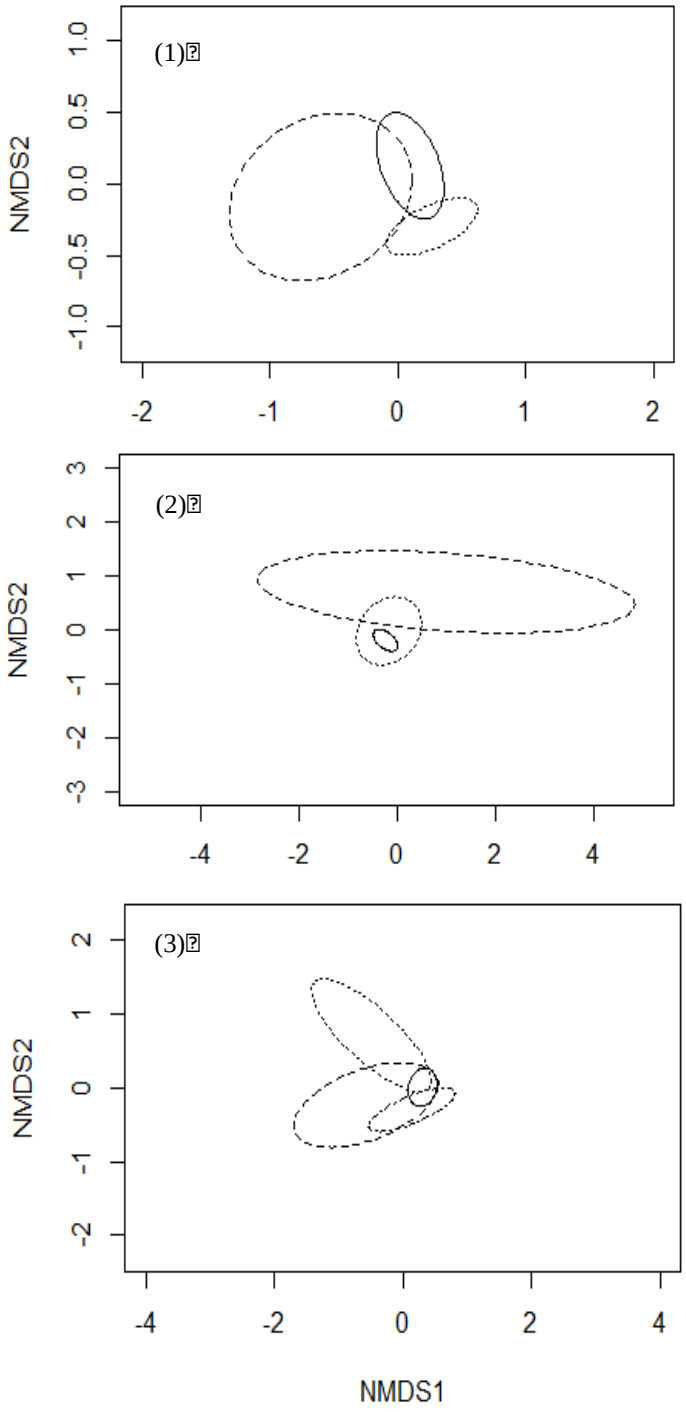

Figure 4.

Supplementary material

Table S1. Number (N) of larval and juvenile fish utilised for dietary analysis for 0+ fish (*Barbus barbus*, *Squalius cephalus*, *Phoxinus phoxinus* and *Leuciscus leuciscus*) at Site 1, 2 and 3, River Teme. Fish classed as larval stages L3, L4, L5 or juvenile (J).

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

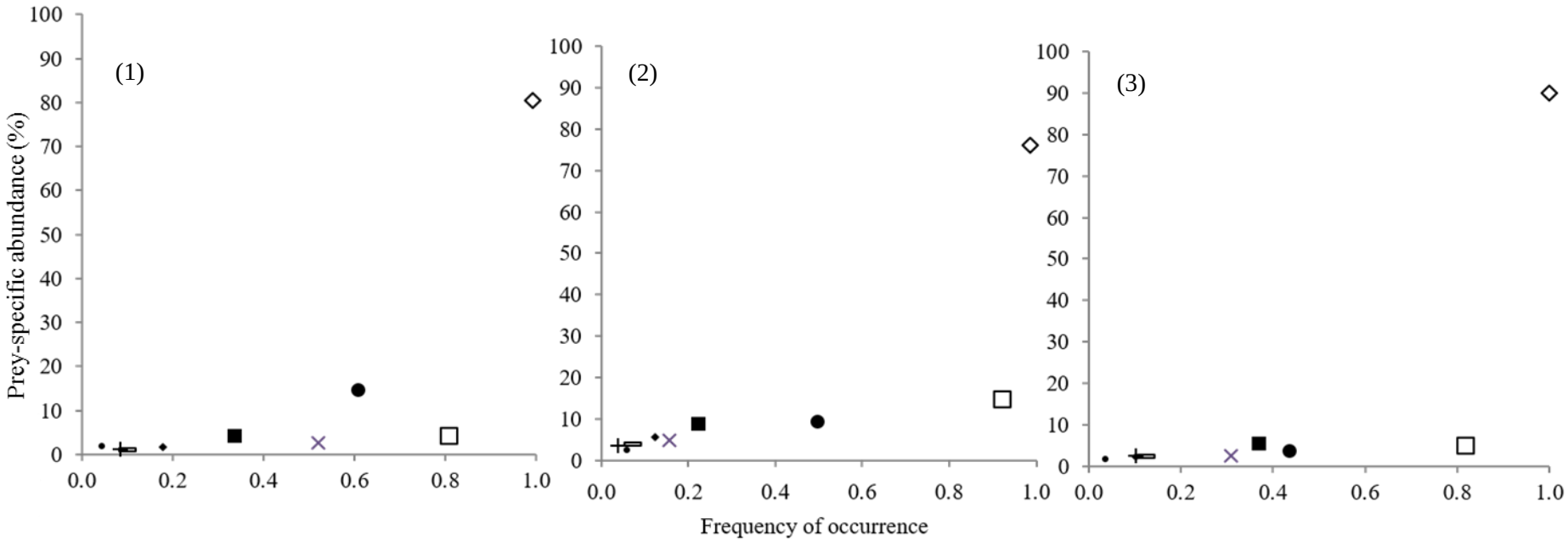

Figure S1. Feeding strategy plots for 0+ *Barbus barbus* by site (1), (2) and (3) on the River Teme. Points represent prey categories: Aufwuchs (□); chironomid larvae (◊); winged insects (×); copepod (■); Cladocera (●); nymphs (+); water arachnids (▬); caddisfly larvae (♦) and beetle larvae (•)

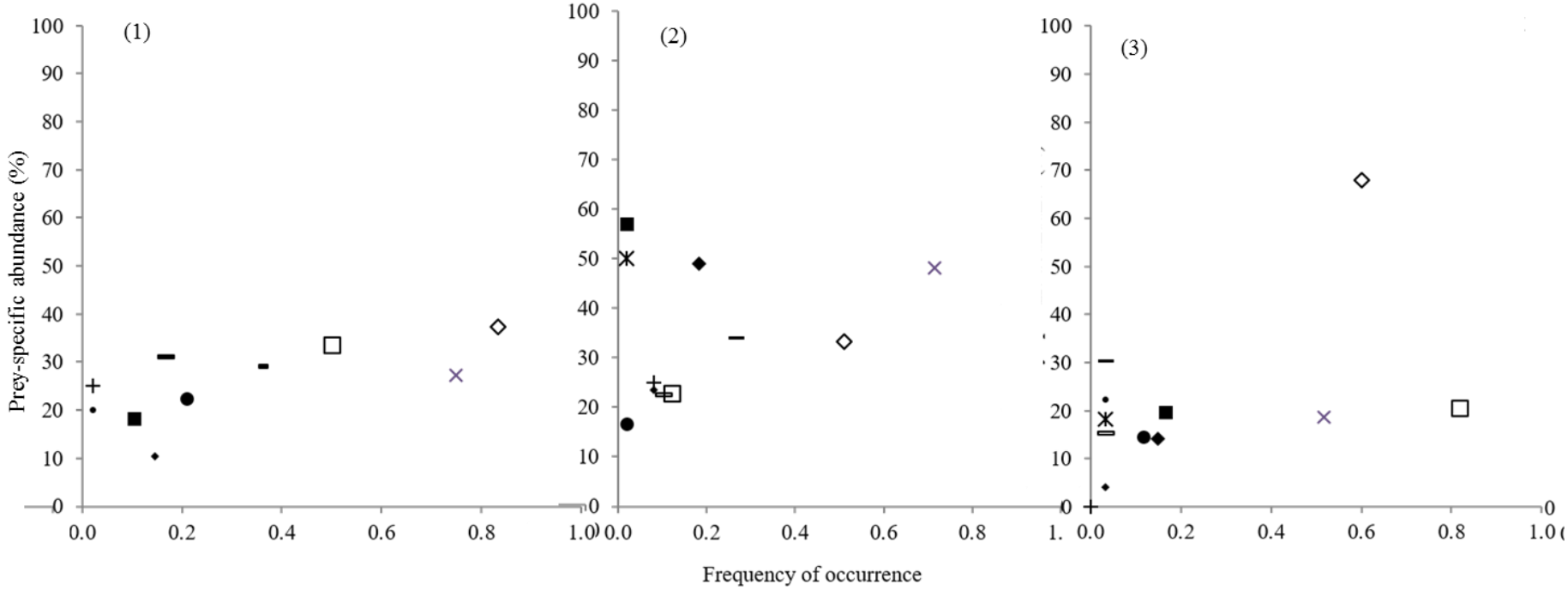

Figure S2. Feeding strategy plots for 0+ *Squalius cephalus* by site (1), (2) and (3) on the River Teme. Points represent prey categories: Aufwuchs (□); chironomid larvae (◊); winged insects (×); copepod (■); Cladocera (●); nymphs (+); water arachnids (▬); caddisfly larvae (♦); beetle larvae (●); hemipteroid assemblage (-); chalcid wasp (✳) and saucer bug (♦)

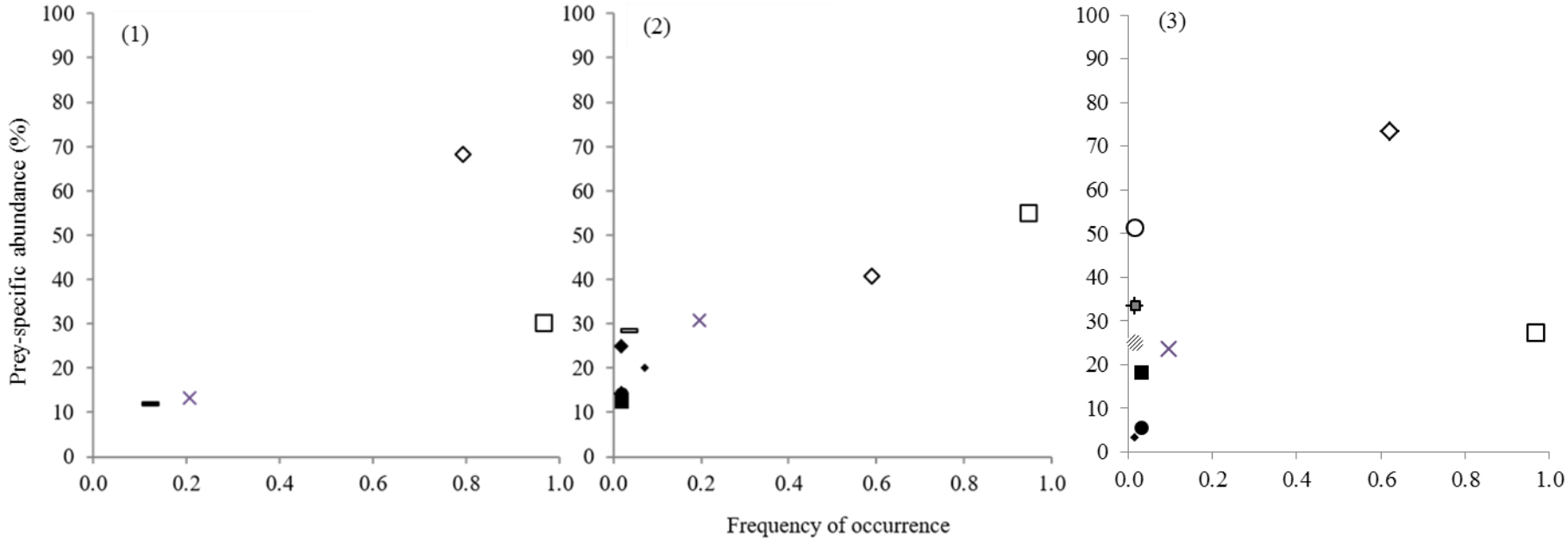

Figure S3. Feeding strategy plots for 0+ *Phoxinus phoxinus* by site (1), (2) and (3) on the River Teme. Points represent prey categories: Aufwuchs (□); chironomid larvae (◊); amphipod (▣); winged insects (×); copepod (■); Cladocera (●); nymphs (+); water arachnids (▬); caddisfly larvae (⬧); beetle (⧫); hemipteroid assemblage (⫽); seed/spore (○)