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

3

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5

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11

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16

17 **Abstract**

18

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

38 **Introduction**

39

40 Invasions by non-indigenous fishes can increase inter-specific competition in fish
41 communities, potentially leading to impacted native species having reduced growth and
42 survival rates, and/ or being displaced from their original niche (Gozlan et al. 2010).

43 Quantifying feeding interactions between the invasive and extant fishes in the community is
44 thus important for determining the extent of the invasion-mediated shifts in the trophic
45 structure of the food web (Jackson et al. 2012; Cucherousset et al. 2012; Copp et al. 2016).

46 Ecological theory suggests that these shifts in trophic structure can include the invader
47 occupying an unexploited niche (Shea and Chesson 2002). This will limit their inter-specific

48 competitive interactions and facilitate their integration into the ecological community (Shea

49 and Chesson 2002; Tran et al. 2015). Alternatively, when food resources are more limiting,

50 the niche variation hypothesis suggests that increased inter-specific competition can result in

51 the trophic niches of the competing species to constrict and diverge due to diets becoming

52 more specialised (Van Valen 1965; Olsson et al. 2009; Tran et al. 2015). Conversely, this can

53 result in the trophic niche sizes of competing species to increase, as individuals utilize a

54 wider resource base to maintain their energy requirements (Svanbäck and Bolnick 2007).

55 When invasive and native species coexist for prolonged periods, high overlaps in their trophic

56 niches can suggest a lack of competitive interactions, perhaps due to resources not being

57 limiting, and so facilitating co-existence (Pilger et al. 2010; Guzzo et al. 2013). However,

58 prolonged co-existence can also result in competitive exclusion, where the invader eventually

59 excludes a native species from its original niche and results in its population decline (Bøhn et

60 al. 2008).

61

62 The ability of an introduced fish to develop invasive populations depends on their ability to
63 establish sustainable populations, with reproduction and recruitment being key processes.
64 Consequently, the larval and juvenile life-stages of fishes ('0+ fishes') are important in the
65 overall invasion process due to their influence on recruitment (Nunn et al., 2003, 2007a,
66 2010a). A range of factors influences the growth and survival rates of 0+ fishes, including
67 their ability to capture and ingest the prey items and sizes available (Nunn et al., 2012). If
68 preferred prey items are unavailable, reduced growth rates and/ or starvation can occur, with
69 potentially deleterious consequences for that 0+ cohort (Dickmann et al., 2007; Burrow et al.,
70 2011). Where an introduced fish shares food resources with indigenous fishes and these
71 resources become limiting, this can affect 0+ fish food acquisition and assimilation, and
72 growth and survival rates, and so potentially impedes their ability to recruit and, therefore,
73 establish (Gozlan et al., 2010; Dick et al., 2014, 2017).

74

75 The feeding ecology of mature fishes is relatively well understood, including for temperate
76 riverine cyprinid fishes (e.g. Mann, 1974; Nunn et al., 2012). Extant knowledge includes how
77 diet plasticity can assist the establishment of populations of introduced fishes (Basic et al.,
78 2013; Tran et al., 2015). In contrast, the feeding ecology of 0+ fishes is often poorly
79 understood (Nunn et al., 2012), especially within invaded communities (Britton et al., 2009).
80 This is despite developmental shifts in diet often being important for 0+ fish survival
81 (DeVries et al., 1998). In general, most freshwater fishes are planktivorous at the onset of
82 exogenous feeding, with zooplankton being an important larval prey resource (Nunn et al.,
83 2007b, 2010). Thereafter, diets of juvenile riverine cyprinids in temperate regions tend to
84 consist of a mix of cladocerans, copepods and insect larvae, with some species also exploiting
85 adult dipterans and Aufwuchs (the periphyton and associated microfauna that grow on
86 underwater surfaces) (Nunn et al., 2012). However, as individuals increase in body and gape

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

92

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

106

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

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

119

120 **Materials and Methods**

121

122 *Sampling sites and methodology*

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

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

144

145 Due to the restricted 0+ fish habitat of the River Teme and poor riparian access, point-
146 abundance sampling by electric fishing was not an appropriate sampling method (Copp
147 2010). Micro-mesh seine netting was used instead, with acknowledgement that this would
148 limit the proportion of larval fishes <15 mm in samples (Cowx et al. 2001; Copp 2010). On
149 each sampling occasion, the 0+ fish were collected between 07.00 and 11.00, euthanised
150 (MS222) and then preserved in 70 % IMS. Samples were unable to be collected at night for
151 access and safety issues. These samples were then stored at 5 °C prior to their processing in
152 the laboratory. All samples were processed in the laboratory within six months of sampling to
153 minimise issues associated with shrinkage of body lengths related to preservation (Leslie &
154 Moore, 2001).

155

156 *Sample processing and data collection*

157 There were four 0+ fish species, all of the Cyprinidae family, that were captured in sufficient
158 numbers to enable subsequent dietary analyses: *B. barbus*, *S. cephalus*, minnow *Phoxinus*
159 *phoxinus* (L.) and dace *Leuciscus leuciscus* (L.) (Table S1). In the laboratory, following
160 identification to species level (Pinder, 2001), a maximum of 30 non-indigenous *B. barbus* and
161 20 individuals of the other fishes per site and per sample date were analysed. These numbers

162 of analysed fishes were achieved by sub-sampling within the collected samples, with this
163 stratified to ensure the size ranges of fish present in each sample were covered. This involved
164 their measurement using digital callipers (standard length, L_s , to 0.01 mm). The majority of
165 the fishes were already at juvenile stages (a consequence of the sampling method) and thus
166 subsequent dietary analyses focused on these, rather than larval stages (Krupka, 1988; Pinder,
167 2001). Gape size was measured as the height of the mouth when open at its widest angle,
168 using a stage micro-meter (Lukoschek & McCormick, 2001; Nunn et al., 2007b). The
169 intestine ('gut') was then dissected, with gut fullness (%) estimated and the total gut contents
170 extracted, mounted on a glass slide and fixed using Polyvinyl alcohol-lactic acid-glycerol
171 (PVLG). Prey items were then identified to their lowest practicable taxonomic level using
172 microscopy (to x100 magnification), with their number then counted to provide data on
173 abundance. Periphytic biota (diatoms and similar material that was too small to classify more
174 precisely) were classed as 'Aufwuchs'. The amount of Aufwuchs in each gut was estimated
175 on the basis of their percentage cover on the slide area and converted to a number (0 to 5
176 scale), similar to other studies (Garner 1996; Mann 1997), so that it was comparable to
177 enumerated prey. As the majority of fishes had low proportions of Aufwuchs in the gut, this
178 scale focused on slide coverage of below 55 % to allow greater discrimination between
179 individual diets and thus greater precision in analyses. Thus, the scale used was: 0 (0 to 1 %
180 coverage), 1 (2 to 3 %), 2 (4 to 7 %), 3 (8 to 20 %), 4 (21 to 55 %) and 5 (56 to 100 %).

181

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

187 spore/ plant material, and fish. The largest prey item in the gut of each individual fish was
188 then measured; for Chironomid larvae this always consisted on measuring the width of the
189 head.

190

191 *Data analysis*

192 Differences in fish standard length between the sites were tested initially using one-way
193 ANOVA with a Tukey post-hoc test. The vacuity index ($%I_v$) (i.e. the proportion of fish with
194 empty guts) was calculated from: $%I_v = S_0S_1^{-1}$, where S_0 is the number of fish with empty guts
195 and S_1 is the total number of larval and juvenile fish stomachs examined (Hyslop, 1980).
196 Frequency of occurrence of prey categories (F_i) represented the proportion of all guts that
197 contain that prey category and was determined from: $F_i = N_iN^{-1}$, where N_i is the number of
198 guts in which that prey item i occurred and N is the total number of guts with prey present
199 (Caillet, 1977). Relative abundance of a given prey category ($%A_i$) represented the
200 proportion of total gut contents from all fish that comprised that prey category and was
201 calculated from: $%A_i = 100(\sum S_i S_i^{-1})$, where S_i is the number of prey items comprising prey i
202 and S_i is the total number of prey in all guts regardless of whether they contained prey item i
203 (Macdonald & Green, 1983). Prey-specific abundance (P_i) represented the proportion of all
204 prey that comprised of a specific prey category and was determined from data from only the
205 guts in which prey items in that category were encountered. It was calculated from: $P_i =$
206 $100(\sum S_i \sum S_{ii}^{-1})$ here P is the number of prey items comprising prey i and S_{ii} is the total number
207 of prey items in guts that contained prey item i (Amundsen et al., 1996).

208

209 The calculation of frequency of occurrence and prey-specific abundance enabled feeding
210 strategy plots to be produced (Costello, 1990). These plots provided information about the
211 importance of prey categories and feeding strategies of each species via examination of the

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

219

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

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

239

240 For plots of trophic niche size versus gape height per species, gape heights were classified
241 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
242 groupings were based on the conversion of the stage micro-meter units to the actual gape
243 height of the fishes (in mm). In all analyses, gape heights above 4.8 mm were excluded from
244 analyses as the maximum for *B. barbuis* was 3.1 mm. Trophic niche sizes were expressed as
245 standard deviation ellipses (40%), calculated using detrended correspondence analysis with
246 basic reciprocal averaging that was completed using the 'decorana' function in 'vegan'
247 package v2.4 in R (R Core Team, 2016; Oksanen et al. 2017). This was completed within a
248 Bray-Curtis similarity matrix where all data were square root transformed for normality.
249 Ellipse areas then compared across the gape height classes for each species to determine their
250 influence on the size of the trophic niche.

251

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

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

263

264 **Results**

265

266 *Sample sizes, stages and lengths*

267 Across the four 0+ fishes, SCA was performed on 878 individuals (*B. barbuis*: n = 431; *S.*
268 *cephalus*: n = 174; *L. leuciscus*: n = 81; *P. phoxinus*: n = 192). Across the samples, no fish
269 were present at larval stage 1 and, as there was only one fish at larval stage 2, this individual
270 was removed from subsequent analyses (Table S1). As there were low numbers of fish
271 sampled at larval stages 3 to 5, and relatively high numbers of juvenile fishes (juvenile stages
272 6 to 9), these fish were all grouped together as ‘juveniles’ for analytical purposes (Table S1).
273 The minimum, maximum and mean lengths of these juveniles per species are provided in
274 Table 1. The low number of larvae in samples also meant that testing of ontogenetic diet
275 changes used fish lengths instead of larval stage.

276

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

284

285 *Relative frequency of prey and feeding strategies*

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

297

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

307

308

309

310 *Fish length and gape height influences on diet*

311 The relationship of gape height versus fish length was significant for each species (*B. barbuis*:
312 $R^2 = 0.81$, $F_{1,515} = 2247.0$, $P < 0.01$; *S. cephalus*: $R^2 = 0.86$, $F_{1,185} = 1095.0$, $P < 0.01$; *L.*
313 *leuciscus*: $R^2 = 0.89$, $F_{1,106} = 738.4$, $P < 0.01$; *P. phoxinus*: $R^2 = 0.73$, $F_{1,158} = 435.4$, $P <$
314 0.01). Between the species, there were significant differences in gape height (GLM: Wald χ^2
315 $= 1080.84$, $df = 3$, $P < 0.01$), with standard length a significant covariate ($P < 0.01$). Pairwise
316 comparisons revealed the mean adjusted gape height of *Barbus barbuis* (mean 2.02 ± 0.03
317 mm) was significantly smaller than the other three fishes (*S. cephalus*: 2.81 ± 0.05 mm; *L.*
318 *leuciscus*: 2.38 ± 0.07 mm; *P. phoxinus*: 2.82 ± 0.05 mm; $P < 0.01$ in all cases).

319

320 Maximum prey sizes differed significantly between the fishes (GLM: Wald $\chi^2 = 197.12$, $df =$
321 3 , $P < 0.01$), where the covariate of standard length was significant ($P < 0.01$). The mean
322 maximum prey size of *B. barbuis* (0.51 ± 0.02 mm) was significantly smaller than for *S.*
323 *cephalus* (0.67 ± 0.05 mm; $P < 0.01$), was not significantly different to *L. leuciscus* ($0.53 \pm$
324 0.06 mm; $P = 0.47$), and was significantly larger than *P. phoxinus* (0.35 ± 0.03 mm; $P <$
325 0.01). Multiple regression revealed that for *B. barbuis*, standard length and gape height, and
326 their interaction, were all significant variables, but with length explaining most the variation
327 in the prey size ($P < 0.01$ in all cases) (Table 3). For *S. cephalus*, although gape height and
328 standard length were both non-significant ($P > 0.05$), their interaction was a significant
329 predictor of maximum prey size ($P < 0.01$). In *L. leuciscus*, standard length was the only
330 significant predictor ($P < 0.01$), and none of the variables were significant predictors of
331 maximum prey size in *P. phoxinus* ($P > 0.05$ in all cases), with individuals generally
332 consuming much smaller prey than was possible for their gape height (Table 3).

333

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

340

341 *Spatial and inter-specific dietary comparisons*

342 There was a significant difference in niche size between the four species (PERMANOVA: P
343 < 0.01) and across the three sites (PERMANOVA: $P < 0.01$) (Table 4). According to their
344 niche sizes (as 40 % ellipse areas), *S. cephalus* had the largest niche of all species, with this
345 significantly larger than *B. barbuis* in all cases (Fig. 4; Table 5). The size of the *B. barbuis*
346 niche was significantly smaller than *L. leuciscus* at Site 3, and *P. phoxinus* at Site 2 and 3
347 (Table 5).

348

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

355

356

357

358

359 **Discussion**

360

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

371

372 The prediction was that the trophic niches of the 0+ fishes would be divergent, with this
373 divergence developing according to the dietary specialisms of fishes. The results suggested
374 some consistency with this prediction. Although the diets of all the fishes were described as
375 generalist, they became more specialised as their body length and gape height increased. The
376 prediction also included that the inter-specific niche divergence would be driven by
377 competitive interactions, as per the niche variation hypothesis (Van Valen 1965; Olsson et al.
378 2009). Although this was difficult to test, it was considered unlikely, given the increasing and
379 significant ontogenetic differences in the gape size of the fishes, plus their general functional
380 morphological differences (De Silva et al., 1979). For example, the increased dietary
381 specialisations apparent in *B. barbuis* versus *L. leuciscus* were likely to be strongly driven by
382 *B. barbuis* having an inferior mouth that was primarily suited for only feeding on the benthos,
383 with *L. leuciscus* having a terminal mouth and larger gape that enabled their exploitation of a

384 greater diversity of prey (e.g. by also exploiting drifting aerial insects). *Squalius cephalus*
385 also has a terminal mouth that enabled their foraging throughout the water column, and they
386 correspondingly had a very generalist diet and the largest niche of all the fishes at all sites.
387 Given these results, there was no evidence to suggest the prolonged cohabitation of *B. barbuis*
388 with the other fishes in the study river had resulted in the competitive exclusion of a native
389 species from its original niche (Bøhn et al. 2008). This is a contrast to invasive *B. barbuis* in
390 Italy where data suggest they have displaced endemic *Barbus* fishes in invaded river systems
391 via competitive interactions, although dietary data on the fishes are currently absent (Carosi
392 et al., 2017)

393

394 Across the 0+ fishes, trophic niche sizes and composition were most similar between *B.*
395 *barbus* and *P. phoxinus*. The main driver of their trophic similarity was their high dietary
396 proportions of Chironomid larvae. Given that *P. phoxinus* were the most abundant 0+ fish at
397 each site, this suggests some potential for high inter-specific competition for resources with
398 invasive *B. barbuis* (Chase et al., 2016). However, both fishes had other items in their diet,
399 suggesting that had intense competitive interactions resulted in reduced food intake rates,
400 they could have switched to alternative prey (Dill, 1983). Moreover, with *P. phoxinus* the
401 most numerically abundant 0+ fish at all sites and sampling occasions (their analysed sample
402 sizes here of $n = 20$ per site and sampling occasions were derived via sub-sampling), there
403 was no evidence to suggest their high dietary similarity with invasive 0+ *B. barbuis* was
404 having negative consequences at the population level, given their high abundance.

405

406 The diet composition of these invasive 0+ *B. barbuis* in the River Teme was relatively similar
407 to their diets in rivers in their indigenous range. For example, in the River Seig, Germany,
408 larvae of Chironomids, caddisfly and mayfly were also all present in 0+ *B. barbuis* diet

409 (Bischoff & Freyhof, 1998). Similarly, in the River Trent, Eastern England, the diet of *B.*
410 *barbus* in their late larval stages was also strongly dependent on Chironomid larvae (Nunn et
411 al., 2007b). In the River Lee, England, Copp et al. (2005) also reported 0+ *B. barbus*
412 predated upon similar items, including larvae of caddis fly and Chironomid larvae. Thus,
413 there appears to be high similarity in *B. barbus* diet between their indigenous and non-
414 indigenous ranges. When coupled with their diet similarities with the indigenous and highly
415 abundant *P. phoxinus*, these results suggest some consistency with the pre-adaptation
416 hypothesis of invasion biology. This hypothesis suggests that the probability of invasion by
417 an introduced species is elevated when they share similar ecological traits and behaviours
418 with indigenous species (Duncan & Williams, 2002). These similar traits and behaviours can
419 include similar abilities to acquire resources (Duncan & Williams, 2002; Ricciardi & Mottiar,
420 2006). Invasion probability is also increased when the introduced species expresses their
421 traits and behaviours in a similar manner to populations in their natural range (Duncan &
422 Williams, 2002; Ricciardi & Mottiar, 2006; Buoro et al., 2016). The results here suggest that
423 0+ *B. barbus* underwent minimal shifts in their foraging behaviours to adapt to the River
424 Teme, given their diet similarities to both their natural range and the other species in their
425 new range. It is suggested that these factors assisted their establishment in, and invasion of,
426 the River Teme.

427

428 There was a very low proportion of small-bodied (< 15 mm) and early larval stages in the 0+
429 fish samples. This was likely to have related to sampling bias resulting from the micromesh
430 seine net, with it being inefficient to capture fishes of these lengths and life-stages (Cowx et
431 al., 2001). If future studies require increased numbers of larval fishes in their analyses then an
432 alternative sampling method would be required, such as point abundance sampling using
433 electric fishing. This method can potentially sample larvae as small as 5 mm length (Copp,

434 2010). Notwithstanding, at the free embryo stage and when they emerge from within
435 spawning gravels, *B. barbuis* larvae can be between 8 and 13 mm (Vilizzi & Copp, 2013).
436 Thus, to capture early larval stages might require sampling methods capable of catching fish
437 within the spawning gravels. Although the use of preservation of fish samples enabled
438 enhanced dietary analyses in the laboratory, this can potentially result in shrinkage of body
439 lengths (Fox, 1996). However, Leslie & Moore (2001) suggested shrinkage effects are
440 relatively low when using similar preservation methods, providing samples are processed
441 within a year of collection, as was completed here. Consequently, the relationships between
442 diet and fish lengths in our study were considered valid. Finally, in our study, spatial
443 comparisons were made in diet of each species, with differences between sites likely to have
444 related to differences in food availability. However, the food availability of each site was not
445 quantified accurately (given the presence of 37 items across the diets), preventing further
446 analysis. Although these data on resource availability might also have assisted more precise
447 testing of whether diets were generalist or specialist, assumptions on this were made from the
448 feeding strategy plots (Amundsen et al. 1996). From these plots, all the fishes were described
449 as generalists. However, across the four species, there was variation in the extent of this
450 dietary generalism. *Barbus barbuis* generally had the narrowest diet and smallest niche, and
451 so they have also been described as being the species with the most specialist diet of the
452 analysed fishes.

453

454 In summary, these results indicated how invasive 0+ *B. barbuis* had successfully integrated
455 into a 0+ cyprinid fish community via their diet and feeding ecology. The results highlighted
456 that the 0+ *B. barbuis* were consuming similar items to conspecifics in their indigenous range,
457 suggesting some consistency with the pre-adaptation hypothesis of invasion biology. As the
458 0+ fishes all increased in their lengths and gape sizes, their diets became increasingly

459 dissimilar, especially between *B. barbuis* and other fishes. This was primarily due to
460 differences in their functional morphology and resulted in the *B. barbuis* niche sizes generally
461 being significantly smaller than the other fishes. This invaded fish community thus represents
462 a strong case study of how the invasion of a river system by a non-indigenous fish was
463 facilitated by the utilisation of their pre-adapted foraging behaviours.

464

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470

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