

1 **Influences of angler subsidies on the trophic ecology of European barbel *Barbus barbus***

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14

15 **Abstract**

16

17 European barbel *Barbus barbus* is a recreationally important riverine fish that is widely
18 introduced outside of its natural range. Contemporary angling practices for *B. barbus* involve
19 the use of baits based on marine fishmeal (MF). MF is isotopically distinct from freshwater
20 prey via highly enriched $\delta^{13}\text{C}$ and thus its dietary influence on *B. barbus* can be tested via
21 differences in fractionation factors ($\Delta^{13}\text{C}$). Correspondingly, stable isotope data from 11
22 riverine *B. barbus* populations tested how their trophic ecology varied across populations
23 according to MF from angling. $\Delta^{13}\text{C}$ of fish with macroinvertebrate prey resources varied
24 within and between populations (range 0.90 to 10.13 ‰) and indicated that, within
25 populations, up to 71 % of *B. barbus* had relatively high dietary contributions of MF. These
26 contributions were significantly and positively related to fish length, with MF influences
27 increasingly apparent as fish length increased. Population isotopic niche sizes increased as
28 the dietary influence of MF in that population increased. These results indicated that whilst
29 MF from angling can act as a strong trophic subsidy, its influence varies spatially and with
30 fish length, with its use as a food resource by *B. barbus* generally involving dietary
31 specializations of larger-bodied individuals.

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33 Key words: catch-and-release angling; fractionation; marine derived nutrients; stable isotope
34 analysis.

35 **Introduction**

36

37 The European barbel *Barbus barbus* (L.) is a fluvial cyprinid fish typically encountered in the
38 middle reaches of European rivers (Huet 1949). Their populations have high recreational
39 value with catch-and-release anglers (Penczak & Sierakowska 2003; Taylor et al. 2004;
40 Britton & Pegg 2011), with this a driver of introductions into waters outside of their native
41 range (Wheeler & Jordan 1990; Taylor et al. 2004; Antognazza et al. 2016). Areas invaded by
42 *B. barbus* include rivers in Western Britain and Italy (Wheeler & Jordan 1990; Antognazza et
43 al. 2016; Zaccara et al. 2014).

44

45 The natural diet of *B. barbus* tends to comprise of benthic macroinvertebrates (Gutmann
46 Roberts & Britton, 2018). Despite this, contemporary angling practises for *B. barbus* utilise
47 pelletized marine fishmeal ('pellet'; Bašić et al. 2015; Gutmann Robert et al. 2017). These
48 pellets are commonly used in aquaculture, where their feeding in high quantities promotes
49 fast growth rates via their high protein content (Naylor et al. 2000). In angling for *B. barbus*,
50 pellets of up to 21 mm in diameter are used as both an attractant and hook-bait, and so have
51 the potential to supplement fish diet (Grey et al. 2004; Bašić et al. 2015; Gutmann Roberts et
52 al. 2017). The large size of some of these pellets results in their size-selective exploitation of
53 *B. barbus*, with fish below 300 mm rarely captured (Amat Trigo et al. 2017).

54

55 Novel ecological opportunities can enable individual specialisation in resource use to develop
56 within populations (Britton & Andreou 2016), with examples including when terrestrial
57 insects become available for predation by stream fishes (Syrjänen et al. 2011). Individual
58 trophic specialisation results in the population trophic niche becoming diversified, shifting to
59 consist of sub-groups of specialised individuals (Araújo et al. 2011). In four riverine

60 populations in England, the diets of some large bodied *B. barbus* have been shown to
61 comprise of high proportions of pelletized fishmeal, i.e. they are dietary specialists on this
62 allochthonous resource (Bašić et al. 2015). There was, however, high variability in the
63 contribution by fishmeal to the diets of individuals (Gutmann Roberts et al. 2017). As pellets
64 are selective in the sizes of *B. barbus* capture (Amat Trigo et al. 2017), it is also likely that
65 there will be a strong ontogenetic pattern in the extent of their contribution to diet (Gutmann
66 Roberts & Britton 2018), although this has not been tested. Levels of angling exploitation are
67 also not evenly distributed across river fisheries, with disproportionately high levels of
68 angling exploitation focused on relatively small areas where angling quality is perceived to
69 be highest (Parnell et al. 2010; Post & Parkinson 2012). Correspondingly, the extent to which
70 angler baits form an allochthonous trophic subsidy for *B. barbus* might also vary spatially.

71

72 Stable isotope analysis (SIA) enables the energy sources of riverine consumers to be
73 differentiated between resources derived from freshwater (depleted $\delta^{13}\text{C}$) and marine
74 (enriched $\delta^{13}\text{C}$) environments (Jardine et al. 2005; Gutmann Roberts et al. 2017). There tends
75 to be considerable differences in the $\delta^{13}\text{C}$ of marine fishmeal pellets and freshwater prey
76 resources (e.g. between 7 and 10 ‰; Gutmann Roberts et al. (2017)). Correspondingly, if a
77 freshwater fish has consumed large quantities of marine fishmeal, their stable isotope (SI)
78 fractionation factors (Δ) with putative macro-invertebrate prey resources should be highly
79 enriched in ^{13}C . Busst & Britton (2016) revealed that when scale tissue was used for SIA in
80 *B. barbus*, maximum $\Delta^{13}\text{C}$ with a single formulated food resource was 5.31 ‰. Thus, if the
81 $\Delta^{13}\text{C}$ of an individual fish with their putative macroinvertebrate prey exceeds this Δ , it would
82 be assumed that an alternative, highly $\delta^{13}\text{C}$ enriched source has been a strong contributor to
83 its diet, such as marine fishmeal. Whilst mixing models can predict diet composition from SI
84 data of consumers and their putative prey resources (e.g. Jackson et al. 2012), these models

85 require SI data from a range of putative prey. However, for many sampled fish populations,
86 these data are often limited or absent, limiting the application of these models.

87

88 The aim of this study was to thus utilise a SI data-set ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) based on 11 riverine *B.*
89 *barbus* populations to quantify how their trophic ecology varies spatially, and how it varies
90 with fish size (as fish fork length) and in relation to the use of marine fishmeal in angling.
91 Across the populations, the extent of SI data on putative food resources varied considerably,
92 preventing use of mixing models to predict diet composition. Instead, variability in $\Delta^{13}\text{C}$ was
93 used to infer the extent to which *B. barbus* diet was being influenced by freshwater
94 macroinvertebrates versus marine fishmeal (*cf.* Methods, Results). Objectives were to: (1)
95 assess the utility of fractionation factors to discriminate between macroinvertebrate and
96 marine fishmeal in diets of *B. barbus*; (2) test relationships in fractionation factors of *B.*
97 *barbus* with macroinvertebrates and marine fishmeal within and between populations, and
98 according to fish length; and (3) determine trophic (isotopic) niche sizes of populations and
99 test the drivers influencing inter-population differences.

100

101 **Methods**

102

103 *Sample collection and SI analysis*

104 The study was based on the stable isotope data ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) of *B. barbus* sampled from 11
105 rivers in England completed between 2013 and 2017 (Fig. 1; Table 1). Angling for *B. barbus*
106 in these rivers was all catch and release. The dataset included unpublished data as well as
107 some that have been used previously (Table 1), and comprised populations from both the *B.*
108 *barbus* indigenous and non-indigenous range of England (Table 1; Antognazza et al., 2016).
109 The sampled *B. barbus* were collected by electric fishing and/ or catch-and-release angling.

110 During sampling, captured *B. barbuis* were measured (fork length, nearest mm), and between
111 3 and 5 scales removed and transferred to a paper envelope. For 9 of the 11 populations,
112 samples of macro-invertebrates were collected concomitantly by kick-sampling (disturbance
113 of the substrate by kicking, with displaced benthic macroinvertebrates captured downstream
114 in a net) (Table 1).

115

116 The *B. barbuis* SI data were derived from their scale samples, where scales have a longer
117 isotopic turnover rate than their muscle and fin tissue (Busst and Britton 2018). Thus, scale SI
118 data provides information on the long-term diet of the fish (e.g. 6 months, although this will
119 vary with fish size and the different contributions of growth and metabolism to isotopic
120 turnover; Busst & Britton 2018). In the SIA, scale decalcification was not performed prior to
121 their analysis. Whilst comparisons of acidified versus non-acidified scales have revealed
122 significant differences in their isotopic data, the actual changes tend to be minor with, for
123 example, Ventura & Jeppesen (2010) showing that the process produced mean changes in
124 $\delta^{13}\text{C}$ (\pm SD) of 0.18 ± 0.12 and in $\delta^{15}\text{N}$ of -0.21 ± 0.24 , with conclusions that these changes
125 were not biologically relevant. Moreover, these minor changes in SI values by scale
126 acidification compare to the mean differences here between macro-invertebrate and fishmeal
127 pellets (the primary food resources of the *B. barbuis* used here) of 8.16 ± 0.79 ‰ for $\delta^{13}\text{C}$ and
128 5.88 ± 2.23 ‰ for $\delta^{15}\text{N}$ (Table 2). It is, therefore, considered unlikely that the analytical
129 process of the scales had a material influence on the ability of the study to discriminate
130 between fish mainly feeding on macroinvertebrates versus fishmeal pellets.

131

132 Preparation for SI involved the cleaning of scales in distilled water and then, using dissecting
133 scissors, removing the very outer portion of the scale (Bašić et al. 2015). This was to ensure
134 the scale material being analysed was from the most recent growth of each fish (Hutchinson

135 & Trueman 2006). For the macro-invertebrate samples, sorting was to species, with a
136 minimum of three replicate samples analysed per species, and where a sample comprised of
137 between one and three individuals (dependent on body size) (Bašić et al. 2015). Samples
138 from a range of pelletized marine fishmeal ('pellet' hereafter) were also analysed, where a
139 minimum of three samples per product was analysed. All samples were dried to constant
140 mass at 60 °C and then analysed at the Cornell Isotope Laboratory, New York, U.S.A. SI
141 analytical details were as per Busst and Britton (2018), with lipid correction not necessary as
142 C:N ratios indicated very low lipid content (Post et al. 2007).

143

144 Prior to some of the data analyses and testing, the *B. barbus* SI data had to be corrected. This
145 was because of differences between the populations in the values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the
146 macroinvertebrates that meant their data could not be compared without correction (Olsson et
147 al. 2009; Jackson & Britton 2014). For each population, this process involved conversion of
148 $\delta^{15}\text{N}$ to trophic position (TP) and $\delta^{13}\text{C}$ to corrected carbon (Ccorr) (Olsson et al. 2009;
149 Jackson & Britton 2014). Before these calculations could be completed, a common group of
150 macroinvertebrates was identified across all of the samples that were also highly probable to
151 be an important prey item for *B. barbus*. As per Gutmann Roberts and Britton (2018), the
152 chosen macro-invertebrate was the amphipod *Gammarus pulex*. This macroinvertebrate is
153 ubiquitous in British rivers and tends to form an important dietary component for cyprinid
154 fishes (Macneil et al. 1999), including *B. barbus* (Bašić et al., 2015; Gutmann Roberts &
155 Britton, 2018).

156

157 Conversion of $\delta^{15}\text{N}$ to TP was through $\text{TP}_i = [(\delta^{15}\text{N}_i - \delta^{15}\text{N}_{\text{base}})/3.4]+2$, where TP_i was the
158 trophic position of the individual fish, $\delta^{15}\text{N}_i$ was the isotopic ratio of that fish, $\delta^{15}\text{N}_{\text{base}}$ was
159 the isotopic ratio of the primary consumers (macro-invertebrates), 3.4 was the fractionation

160 between trophic levels and 2 was the trophic position of the baseline organism (Post 2002).
161 The $\delta^{13}\text{C}$ data were converted to $\delta^{13}\text{C}_{\text{corr}}$ by $\delta^{13}\text{C}_i - \delta^{13}\text{C}_{\text{meaninv}}/\text{CR}_{\text{inv}}$, where $\delta^{13}\text{C}_{\text{corr}}$ was the
162 corrected carbon isotope ratio of the individual fish, $\delta^{13}\text{C}_i$ was the uncorrected isotope ratio of
163 that fish, $\delta^{13}\text{C}_{\text{meaninv}}$ was the mean invertebrate isotope ratio (the 'baseline' invertebrates) and
164 CR_{inv} is the invertebrate carbon range ($\delta^{13}\text{C}_{\text{max}} - \delta^{13}\text{C}_{\text{min}}$; Olsson et al., 2009).

165

166 *Data analysis and statistical testing*

167 Across the 11 populations, the *B. barbuis* samples were collected by electric fishing and/ or
168 angling, comprised of fish between 80 and 850 mm, and were collected in different years.
169 Thus, to understand how river, sampling method, fish length and year of sampling affected
170 the SI data, linear mixed models (LMM) were used. Due to the non-comparable nature of the
171 raw SI data between rivers (due to variable macroinvertebrate SI data; Table 2), the corrected
172 data (Ccorr and TP) had to be used in these models. Correspondingly, they could only be
173 completed using data from the 9 *B. barbuis* populations where macroinvertebrate data were
174 available (Table 2). In LMMs, Ccorr or TP was the dependent variable, the independent
175 variable was either sampling method, river or fish length (depending on the test), covariates
176 were sampling, river, year or fish length (depending on the independent variable), and river
177 was used as the random variable (except when the model was testing differences between
178 rivers). Model outputs were the significance of the overall test, the significance of covariates,
179 and the mean values of Ccorr and TP (adjusted for the effects of the covariates) with their
180 pairwise comparisons (with Bonferroni adjustment for multiple comparisons). Where a
181 covariate had consistent non-significant values in all models, it was removed from all final
182 LMMs. The final LMMs were also checked to ensure they met the test assumptions (e.g. the
183 errors have constant variance, are independent, and are normally distributed). Where
184 uncorrected data were used in univariate tests at the population level (e.g. differences in the

185 range of *B. barbus* isotope data between sampling methods) then, after checking for
186 normality, either ANOVA (normal distribution) or Mann Whitney U tests (non-normal
187 distribution) were used, with checking that model assumptions were also met.

188

189 The uncorrected SI data for each fish per population were used to calculate their fractionation
190 factor (Δ) with their macro-invertebrate data ($\Delta^{13}\text{C}_{\text{macroinvertebrate}}$;
191 $\Delta^{15}\text{N}_{\text{macroinvertebrate}}$) by subtracting their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from the mean
192 macroinvertebrate values. The utility of $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ and $\Delta^{15}\text{N}_{\text{macroinvertebrate}}$
193 to discriminate between fish feeding primarily on macroinvertebrates and marine fishmeal
194 was tested using data from Gutmann Roberts et al. (2017). In that study, stable isotope
195 Bayesian mixing models had predicted the proportion of marine fishmeal in the diet of *B.*
196 *barbus* sampled from the lower River Teme/ Severn. Here, linear regression tested the
197 relationship between the $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ and $\Delta^{15}\text{N}_{\text{macroinvertebrate}}$ of these fish
198 with their predicted proportion of marine fishmeal in diet. Note that due to the results, all
199 subsequent analyses focused only on use of $\Delta^{13}\text{C}$ and $\delta^{13}\text{C}$ (*cf.* Results). The regression
200 coefficients (a , b) were then used in the equation $\text{FM} = (\Delta^{13}\text{C}_{\text{macroinvertebrate}} \times b) + a$,
201 where FM = the proportion of marine fishmeal in diet, to predict the proportion of fishmeal in
202 the diet at $\Delta^{13}\text{C}_{\text{macroinvertebrate}} = 5.31 \text{ ‰}$ (Busst & Britton 2016; Gutmann Roberts et al.
203 2017). The $\Delta^{13}\text{C}$ of 5.31 ‰ is from Busst & Britton (2016), who determined the fractionation
204 factors of *B. barbus* in relation to a range of formulated feeds and revealed that the maximum
205 $\Delta^{13}\text{C}$ of *B. barbus* with a known food resource was $5.31 \pm 0.09 \text{ ‰}$. Thus, where
206 $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ exceeded 5.31 ‰, it was assumed that the main dietary item of that
207 fish could not be macroinvertebrates. The relationship of $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ with fish
208 length was then tested across the dataset, enabling the proportion of fish per population
209 whose $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ exceeded 5.31 ‰ to be determined. Values of $\Delta^{13}\text{C}_{\text{pellet}}$

210 were then calculated for each fish using a mean $\delta^{13}\text{C}$ value of fishmeal pellets, and with these
211 values then tested for their relationship with $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$.

212

213 The isotopic niches of the *B. barbuis* populations were then estimated using the corrected SI
214 data (Ccorr and TP). These niches were based on ‘standard ellipse areas’ (SEA), calculated
215 using the package ‘Stable Isotope Bayesian Ellipses in R’ (R v 3.4.2; SIBER v 2.1.3; Jackson
216 et al., 2011; Jackson et al., 2012; R Core team, 2014). The SEA metric of each population
217 represents the core 40 % of their isotopic data and so is a bivariate measure of the distribution
218 of individuals in isotopic space that represents a population’s typical resource use (Jackson et
219 al., 2011; Jackson et al., 2012). Two measures of SEA were calculated. The first was SEA_{C} ,
220 whose calculation accounts for small samples sizes that were generally encountered in the
221 datasets (Jackson et al. 2012). The second was SEA_{B} , the Bayesian standard ellipse area, as it
222 enables the 95% credible intervals to be determined around the estimate gained from the
223 posterior distributions. Correspondingly, estimates of SEA_{B} were produced by applying the
224 corrected SI data in a Bayesian framework (*cf.* Parnell et al. 2013). The calculations used
225 vague Inverse-Wishart priors on the covariance matrix and vague normal priors on the means
226 (Parnell et al. 2013). The posteriors were estimated with the software ‘Just Another Gibbs
227 Sampler’ (JAGS v4.3.0., Plummer, 2003), with this run for two chains with 20000 iterations,
228 removing 10000 for burn-in and thinning by a factor of 10. Convergence of the chains was
229 checked with the coda package (Plummer et al., 2006) and the Brooks–Gelman–Rubin
230 diagnostic (Gelman and Rubin, 1992; Brooks and Gelman, 1998). Significant differences in
231 the size of Bayesian isotopic niches between populations were inferred when $\geq 95\%$ of
232 posterior draws for one niche were smaller than the other.

233

234 The influence of variability in Ccorr (as the range (maximum – minimum values) and
235 coefficient of variation of Ccorr per population) on isotopic niche size was then tested using
236 linear regression. Note that throughout the paper, whenever errors around the mean are
237 presented, the values are 95 % confidence limits unless stated otherwise.

238

239 **Results**

240

241 *Influence of fish length, sampling method, year and river on stable isotope data*

242 In the LMMs, the covariate of sampling year always had non-significant effects ($P = 0.83$ to
243 0.97), so was omitted from all final models. The final LMMs testing the effect of sampling
244 method on the corrected stable isotope data were significant (Ccorr: $P < 0.01$; TP: $P < 0.01$),
245 with the effect of fish length as a covariate not significant ($P = 0.38$ and $P = 0.28$
246 respectively). Angled fish had significantly higher values of Ccorr and TP than those sampled
247 by electric fishing (Ccorr: 1.98 ± 0.70 versus 0.59 ± 0.97 , $P < 0.01$; TP: 2.75 ± 0.14 versus
248 2.29 ± 0.22 , $P < 0.01$). The LMMs testing differences in the corrected stable isotope data
249 between rivers were also significant (Ccorr: $P < 0.01$; TP: $P < 0.01$). In the models, the effect
250 of fish length as a covariate was significant for Ccorr ($P < 0.01$) but not TP ($P = 0.41$);
251 sampling method was not a significant covariate in either model (Ccorr: $P = 0.45$; TP: $P =$
252 0.45). Across the rivers, the River Kennet had the highest mean value of Ccorr (adjusted for
253 the effects of covariates) that was significantly higher than all other rivers (Table 3). For TP,
254 fish in the Great Ouse had the highest mean values (4.03 ± 0.32) (Table 3). The LMM testing
255 the effect of fish length on Ccorr was not significant ($P = 0.89$), with the effect of sampling
256 method also not significant ($P = 0.22$). However, the LMM testing the effect of length on TP
257 was significant ($P < 0.02$), where the effect of sampling method was also significant ($P =$
258 0.02).

259

260 The uncorrected stable isotope data over all 11 rivers revealed that as the length range
261 increased in the sampled *B. barbuis*, their $\delta^{13}\text{C}$ range also generally increased ($R^2 = 0.56$; $F_{1,9}$
262 $= 11.57$, $P < 0.01$), but this was not apparent in $\delta^{15}\text{N}$ ($R^2 = 0.03$; $F_{1,9} = 0.30$, $P = 0.60$) (Fig.
263 2). Where the samples contained fish captured by angling, the range of both stable isotopes
264 was not significantly different to samples that only comprised of fish sampled by electric
265 fishing (Mann Whitney U test: $\delta^{13}\text{C}$ $Z = -1.83$, $P = 0.08$; $\delta^{15}\text{N}$: $Z = -0.74$, $P = 0.47$; Fig. 2).

266

267 *Predicting contributions of marine fishmeal to Barbus barbuis diet*

268 The relationship of the predicted proportion of marine fishmeal in the diet of 17 *B. barbuis*
269 from the lower River Teme and Severn (Gutmann Roberts et al., 2017) and the
270 $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ of these fish was significant ($R^2 = 0.78$, $F_{1,15} = 54.44$, $P < 0.01$; Fig.
271 3). Use of the regression coefficients ($a = -0.24$, $b = 0.10$) in the regression equation revealed
272 that the $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ value of 5.31 ‰ was equivalent to a diet comprising 32 %
273 fishmeal; at $\Delta^{13}\text{C}_{\text{macroinvertebrate}} = 10.00$ ‰, this proportion of dietary fishmeal increased
274 to 80 % (Fig. 3). The relationship of the predicted proportion of marine fishmeal in diet and
275 $\Delta^{15}\text{N}_{\text{macroinvertebrate}}$ was also significant ($R^2 = 0.76$, $F_{1,15} = 22.45$, $P < 0.01$; Fig. 3).
276 However, due to the low $\delta^{15}\text{N}$ values of marine fishmeal (mean 4.33 ± 0.26 ‰) versus the
277 macroinvertebrates (12.30 ± 2.51 ‰), then this was a negative relationship. Following Fig. 3,
278 $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ was thus considered a significant predictor of the proportion of
279 marine fishmeal in *B. barbuis* diet. As the ^{13}C stable isotope is also generally used to
280 discriminate between consumer energy sources (especially marine versus freshwater) then the
281 remaining analyses focused on only $\Delta^{13}\text{C}$.

282

283

284

285 *Stable isotope fractionation of Barbus barbuis from food resources*

286 The LMM testing the effect of sampling method on $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ was not
287 significant ($P = 0.89$), with the effect of length as a covariate not being significant ($P = 0.18$).

288 The LMM testing the effect of fish length on $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ was significant ($P <$
289 0.01), where the effect of sampling method as a covariate was not significant ($P = 0.39$). This
290 significant influence of fish length on $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ was then explored further by a
291 LMM testing the differences in $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ between fish of < 300 mm and > 300
292 mm. The model was significant ($P < 0.01$), with the effect of sampling method as a covariate
293 also being significant ($P = 0.04$). The mean $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ (adjusted for the effects
294 of covariates) of fish < 300 mm was 2.78 ± 0.84 ‰ versus 5.41 ± 0.34 ‰ for fish > 300 mm.

295

296 In the 9 populations with macro-invertebrate data available (Table 2), only 53 % of all fish
297 had $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ within 5.31 ‰, the maximum predicted Δ for *B. barbuis* (Fig. 4;
298 Busst and Britton 2016). All *B. barbuis* with $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ exceeding 5.31 ‰ were
299 at least 394 mm in length (Fig. 4). This pattern in $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ was significantly
300 related to fish length ($R^2 = 0.31$, $F_{1,259} = 118.82$, $P < 0.01$); all of the fish with
301 $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ exceeding 5.31 ‰ were at least 394 mm fork length (Fig. 5). The
302 proportions of fish with $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ exceeding 5.31 ‰ also varied between the
303 rivers, ranging from 0 to 71 % (0 to 83 % for fish > 300 mm) (Table 4). For each individual
304 *B. barbuis* with a high $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ value, their $\Delta^{13}\text{C}_{\text{pellet}}$ range ranged from -
305 2.89 to 5.31 ‰ (versus 5.40 to 10.13 ‰ for $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$).

306

307 *Isotopic niche size*

308 The corrected SI data enabled the isotopic niches to be determined for the 9 populations. This
309 revealed variability in the isotopic niche size across the populations (Table 5). The largest
310 niche was for the River Loddon population (Table 5). The Loddon data were omitted from
311 further analyses (it was considered an outlier due to its small sample size in combination with
312 fish present < 100 mm, a contrast to the other populations). Testing using linear regression
313 then revealed that as the range in Ccorr and the coefficient of variation of Ccorr increased, so
314 too did the size of the isotopic niche (Ccorr range: $R^2 = 0.52$; $F_{1,6} = 6.62$, $P = 0.04$; CV: $R^2 =$
315 0.79 ; $F_{1,6} = 23.12$, $P < 0.01$; Fig. 6).

316

317 **Discussion**

318

319 In these *B. barbus* populations, fish that were larger had a greater probability of having
320 enriched values of $\delta^{13}\text{C}$ and whose fractionation factor with macroinvertebrate $\delta^{13}\text{C}$ was
321 elevated. There was, however, high variability within and between rivers over the extent to
322 which the diet of larger fish was based on marine fishmeal, indicating that even where this
323 trophic subsidy was available, only some fish specialised their diet on this subsidy (Gutmann
324 Roberts et al. 2017). Fish captured by angling also had significantly higher
325 $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ values than those electric fished. Between rivers, there were
326 considerable differences in the proportions of fish with elevated $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$
327 values, indicating higher consumption of fishmeal pellets. Whilst this was at least partially
328 related to the sampling method and the lengths of captured from that river, it would also
329 depend on the extent of angling practised on each river, as this determines the amount of
330 pelletized marine fishmeal being released by anglers and so the extent to which it would be
331 available for consumption by *B. barbus* (Gutmann Roberts et al., 2017).

332

333 The assessments of the influence of marine fishmeal on *B. barbus* diet were completed using
334 calculations of $\Delta^{13}\text{C}$. This was used in preference to stable isotope mixing models to predict
335 data composition (Jackson et al. 2012; Phillips et al. 2014), due to differences in the extent of
336 putative prey SI data available across the sampled populations. The use of $\Delta^{13}\text{C}$ here was
337 possible due to the $\delta^{13}\text{C}$ of the marine fishmeal baits being substantially enriched versus
338 freshwater macroinvertebrates (differences approximately 7 to 10 ‰). Thus, despite $\Delta^{13}\text{C}$ of
339 macroinvertebrates and pelletized fishmeal being relatively similar (Busst & Britton 2016), it
340 was initially assumed that fish that fed mainly on macroinvertebrates would have
341 considerably depleted $\delta^{13}\text{C}$ and substantially lower $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ than fish that fed
342 mainly on pelletized fishmeal. This was then tested using data from the River Teme and
343 Severn (Gutmann Roberts et al. 2017), with the results revealing that individual fish with a
344 $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ of 5.31 ‰ (the maximum $\Delta^{13}\text{C}$ recorded in *B. barbus* with a known
345 food resource; Busst & Britton 2016) had a diet predicted to comprise of 32 % pelletized
346 fishmeal that increased to 80 % when $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ was 10.0 ‰. Bašić et al.
347 (2015) did, however, reveal that the diet of adult *B. barbus* can also comprise small fishes
348 and invasive crayfish, yet SI data on these resources were absent for the majority of the
349 populations used here. Although this could have been a concern, in Bašić et al. (2015) the SI
350 data of these prey resources were heavily associated with the freshwater macroinvertebrate
351 energy pathway and were thus $\delta^{13}\text{C}$ depleted and highly distinct from the marine fishmeal
352 resources. Correspondingly, the use here of $\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ to discriminate between influences
353 of freshwater prey versus marine on *B. barbus* diet was still considered highly appropriate,
354 despite the potential for some freshwater prey resources to be missing.

355

356 The application of $\Delta^{13}\text{C}$ to the 9 *B. barbus* with macroinvertebrate data available revealed
357 that for fish below 394 mm, $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ was always below 5.31 ‰ (the highest

358 $\Delta^{13}\text{C}$ of Busst & Britton (2016)). Only at larger body sizes did their values of
359 $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ become more $\delta^{13}\text{C}$ enriched, with a maximum
360 $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ of 10.13 ‰. This $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ and $\delta^{13}\text{C}$ enrichment in
361 the larger fish was thus assumed to be through these fish consuming relatively high quantities
362 of angling-derived marine fishmeal. This assumption was supported by other studies on some
363 of these *B. barbus* populations that had revealed no other putative food resources with such
364 enriched $\delta^{13}\text{C}$ (cf. Bašić et al., 2015; Gutmann Roberts et al., 2017; Gutmann Roberts &
365 Britton, 2018). It was also supported by a number of studies demonstrating that the strong
366 influence of marine fishmeal in the diet and trophic ecology of freshwater fauna can be traced
367 through foodwebs using $\delta^{13}\text{C}$ (Grey et al. 2004; Marcarelli et al. 2011; Jackson et al. 2013;
368 Roussel et al. 2018).

369
370 Across the 9 populations with macroinvertebrate data available, there was high variability in
371 $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ values. There were four populations where $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$
372 values suggested the *B. barbus* prey resources were all primarily of freshwater origin. The
373 samples from the Warwickshire Avon and River Great Ouse both included fish over 394 mm,
374 but only 23 % of fish in the Avon and 0 % from the Great Ouse had $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$
375 values exceeding 5.31 ‰. The Chub and Trout Stream also had no fish with
376 $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ values exceeding 5.31 ‰, but this was most likely related to their
377 samples only comprising fish < 300 mm. In the five other rivers, between 51 and 71 % of all
378 fish had $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ values exceeding 5.31 ‰. These results thus suggest that
379 the dietary utilisation by *B. barbus* of this angling trophic subsidy varied spatially. This was
380 likely to relate to differences in the intensity of *B. barbus* angling effort that affected the
381 quantity of marine fishmeal being released into these rivers. Evidence suggests that
382 recreational anglers allocate fishing effort based on perceived fishing quality and travel time

383 (Post & Parkinson 2012). Whilst the Warwickshire Avon and Great Ouse are both close to
384 urban centres, the Avon has been renowned for the quality of its angling for smaller cyprinid
385 species (Hickley 1986), with angling effort for *B. barbus* being relatively low (personal
386 observations, the authors). Whilst the River Great Ouse has been renown for producing
387 specimen-sized *B. barbus* (e.g. The Times, 2004), genetic analyses have revealed these fish
388 were all stocked (Antognazza et al., 2016). Moreover, these large fish are no longer present
389 due to natural mortality and have not been replaced by either natural recruitment or other
390 stocked fish (Bašić & Britton 2016). This recruitment failure is likely to be due to poor
391 spawning habitat (Bašić et al. 2017; 2018). Consequently, in the last decade, angling effort
392 for *B. barbus*, including the use of marine fishmeal, has declined sharply in the river due to
393 the perception by anglers of decreased angling quality (Post & Parkinson, 2012).

394

395 As well as being variable between populations, values of $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ varied
396 considerably within populations, including in fishes above 394 mm, where values varied
397 between 0.93 and 10.13 ‰. This variability was also apparent in other *B. barbus* studies
398 where mixing models have predicted diet composition from SI data (Bašić et al., 2015;
399 Gutmann Roberts et al., 2017). Thus, where marine fishmeal was present as an angler trophic
400 subsidy, some individual trophic specialisation on this subsidy was apparent (Britton &
401 Andreou, 2016). The consumption of this marine fishmeal by some individuals then increased
402 the sizes of their population niches. This finding aligns to Araújo et al. (2011) who outlined
403 that individual specialisation results in population trophic niches becoming more diversified,
404 shifting to comprise of sub-sets of trophically specialised individuals (Araújo et al., 2011).
405 What was not apparent is why individual fish vary their use of this subsidy and this requires
406 further investigation.

407

408 Contemporary angling practises for other cyprinid fishes (such as carp *Cyprinus carpio*) now
409 also include the use of energy rich, formulated feeds (Mehner et al. 2018). Substantial
410 quantities of these feeds are now released into many European freshwaters. For example,
411 individual freshwater anglers in Germany have been estimated as using 7.3 kg bait year⁻¹
412 (Arlinghaus 2004). For anglers specifically targeting large *C. carpio* in Germany, the average
413 amount of bait released was 215 kg per angler per year (Niesar et al. 2004). Per hour of
414 fishing, freshwaters anglers introduce approximately 150 g of bait (Niesar et al., 2004;
415 Arlinghaus, 2004). Consequently, the release of energy-rich angler baits into freshwaters
416 provides a strong trophic subsidy that can supplement fish diet (Specziár et al. 1997;
417 Arlinghaus & Niesar 2005; Bašić et al. 2015). Whether this is considered beneficial for the
418 fish and fishery might then depend on the fishery management objectives. If the management
419 objective is to provide faster growing fishes to enhance catch-and-release angling via
420 increasing the opportunity for anglers to capture larger individuals then this trophic subsidy
421 can be viewed positively, with encouragement for anglers to introduce more of this bait. This
422 is because these subsidies can directly increase fish production (Schreckenbach & Brämick
423 2003; Niesar et al. 2004), potentially also altering population demographics via increasing the
424 body mass of individual fishes (Arlinghaus & Niesar, 2005). Indeed, in *B. barbuis*, individuals
425 increased in condition and had higher food conversion ratios when fed a formulated feed
426 rather than Chironomid larvae (Kamiński et al. 2010). However, if the management
427 objectives are to provide more natural angling experiences, such as for anglers whose main
428 motivations for angling are non-catch related (Arlinghaus 2006), then the use of these baits as
429 a trophic subsidy might be viewed as being less beneficial as it results in fish diet becoming
430 associated with anthropogenic enhancement.

431

432 In summary, the application of $\Delta^{13}\text{C}$ to a number of *B. barbus* populations enabled the
433 influence of marine trophic subsidies on their isotopic ecology to be assessed. The results
434 suggested that where present as a trophic subsidy, marine fishmeal had some substantial
435 influences on *B. barbus* diet and, correspondingly, their isotopic niche size. However, this
436 influence varied spatially and with body size, indicating its exploitation as a dietary resource
437 by *B. barbus* was not universal and involved large bodied individuals specializing on this
438 subsidy.

439

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Table 1. Overview of the 11 *Barbus barbus* populations used in the study. (In ‘River’, W. Avon = Warwickshire Avon, H. Avon = Hampshire Avon; ‘Basin’, S = River Severn, GO = Great Ouse, HA = Hampshire Avon, TH = Thames; ‘Range’, NI = non-indigenous, I = non-indigenous; Method, A = angling, EF = electric fishing. Note L = fork length, mm; $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are all in ‰, ‘MI’ = macroinvertebrate; and ‘Source’ indicates whether the SI data have been used previously; U = unpublished, 1 Gutmann Roberts et al., (2017); 2 Gutmann Roberts & Britton (2018); 3 Bašić & Britton (2016); 4 Bašić et al., (2015).

River	Basin	Range	n	Method	Mean L	L range	Mean $\delta^{13}\text{C}$	$\delta^{13}\text{C}$ range	Mean $\delta^{15}\text{N}$	$\delta^{15}\text{N}$ range	MI sample	Source
W. Avon	S	NI	18	A	637 ± 62	282 - 850	-26.06 ± 1.07	-28.43 - -21.17	16.19 ± 0.92	11.94 - 18.68	Y	U
Teme	S	NI	122	A/ EF	400 ± 79	105 - 690	-25.37 ± 0.87	-28.60 - -20.12	12.27 ± 0.23	10.66 - 13.51	Y	1
Severn	S	NI	69	A	591 ± 27	272 - 800	-23.40 ± 0.47	-27.04 - -19.37	12.57 ± 0.25	10.48 - 14.88	Y	1,2
H. Avon	HA	NI	25	A	660 ± 30	550 - 800	-26.92 ± 0.54	-29.57 - -24.73	11.44 ± 0.47	9.97 - 13.71	Y	4
Great Ouse	GO	I	7	EF	399 ± 107	188 - 643	-27.39 ± 0.51	-28.34 - -26.23	20.52 ± 0.20	20.09 - 20.83	Y	3
Ivel	GO	I	11	EF	513 ± 118	250 - 785	-26.22 ± 0.86	-28.28 - -24.10	21.41 ± 0.67	19.50 - 23.77	N	3
Chub Stream	GO	I	8	EF	204 ± 20	166 - 258	-27.22 ± 0.61	-28.06 - -25.97	16.50 ± 0.77	15.42 - 18.93	Y	3
Trout Stream	GO	I	6	EF	159 ± 17	142 - 197	-22.77 ± 0.66	-24.11 - -22.03	13.42 ± 0.78	12.23 - 14.94	Y	3
Lee	TH	I	20	EF	319 ± 44	202 - 435	-25.65 ± 0.67	-27.88 - -23.76	17.85 ± 0.85	14.35 - 20.64	N	U
Loddon	TH	I	7	A	403 ± 182	80 - 655	-23.64 ± 1.74	-27.33 - -20.22	13.1 ± 1.85	10.31 - 17.02	Y	U
Kennet	TH	I	9	A	631 ± 37	550 - 710	-25.02 ± 1.52	-28.35 - -22.74	11.34 ± 0.60	10.23 - 12.86	Y	4

Table 2. Mean stable isotope data of macro-invertebrates per river (‰) used to calculate *B. barbuis* fractionation factors sampled from 9 rivers. Note that the mean $\delta^{13}\text{C}$ of fishmeal pellets used in the study was -22.12 ± 0.53 ‰ (range -23.19 to -20.17 ‰) and $\delta^{15}\text{N}$ was 7.31 ± 1.02 ‰ (range 4.10 to 9.40 ‰).

River	Basin	Mean $\delta^{13}\text{C}$	Mean $\delta^{15}\text{N}$
W. Avon	S	-30.30 ± 1.36	14.83 ± 0.42
Teme	S	-29.50 ± 0.81	10.31 ± 0.51
Severn	S	-29.04 ± 0.43	12.30 ± 2.51
H. Avon	HA	-32.87 ± 1.53	9.52 ± 0.81
Great Ouse	GO	-29.44 ± 0.86	14.15 ± 0.71
Chub Stream	GO	-30.02 ± 1.31	17.12 ± 1.12
Trout Stream	GO	-31.12 ± 0.87	16.24 ± 0.57
Loddon	TH	-30.99 ± 0.50	16.55 ± 0.15
Kennet	TH	-29.28 ± 0.24	7.65 ± 0.18

Table 3. Mean values (adjusted for the effects of covariates in LMMs) of corrected carbon (Ccorr) and trophic position (TP) for *Barbus barbuis* sampled from 9 rivers.

River	Mean Ccorr	TP
W. Avon	1.28 ± 0.72	2.42 ± 0.20
Teme	3.42 ± 0.49	2.58 ± 0.26
Severn	2.26 ± 0.38	2.65 ± 0.11
H. Avon	0.52 ± 0.72	2.59 ± 0.20
Great Ouse	6.71 ± 1.15	4.03 ± 0.32
Chub Stream	2.40 ± 0.90	1.25 ± 0.25
Trout Stream	2.97 ± 1.05	3.56 ± 0.29
Loddon	4.86 ± 1.17	1.12 ± 0.32
Kennet	9.39 ± 0.97	3.10 ± 0.28

Table 4. Proportion of *Barbus barbuis* with $\delta^{13}\text{C}$ fractionation factors with macro-invertebrates within the range of the species (Busst & Britton 2016) (NP) and those exceeding the maximum fractionation factor with macroinvertebrates (P) for all fish and then only those exceeding 300 mm in length.

River	Basin	All fish		Fish > 300 mm	
		% NP	% P	% NP	% P
W. Avon	S	77.8	22.2	76.5	23.5
Teme	S	49.2	50.8	39.2	60.8
Severn	S	49.3	50.7	48.5	51.5
H. Avon	HA	42.1	57.9	42.1	57.9
Great Ouse	GO	100.0	0.0	100.0	0.0
Chub Stream	GO	100.0	0.0	-	-
Trout Stream	GO	100.0	0.0	-	-
Loddon	TH	28.6	71.4	16.7	83.3
Kennet	TH	44.4	55.6	44.4	55.6

Table 5. Isotopic niche sizes (as standard ellipse areas, SEA) of 9 populations of *Barbus barbus*. Details on basin and range as per Table 1.

River	Basin	Range	Length range (mm)	SEA _c	SEA _B (95% CI)
W. Avon	S	NI	282 - 850	0.75	0.95 (0.52-1.43)
Teme	S	NI	105 - 690	0.94	0.95 (0.65-1.26)
Severn	S	NI	272 - 800	0.53	0.54 (0.42-0.67)
H. Avon	HA	NI	550 - 800	0.35	0.35 (0.19-0.52)
Great Ouse	GO	I	188 - 643	0.52	0.52 (0.17-0.96)
Chub Stream	GO	I	166 - 258	0.15	0.17 (0.07-0.30)
Trout Stream	GO	I	142 - 197	0.49	0.73 (0.32-1.24)
Loddon	TH	I	80 - 655	2.62	2.75 (0.94-5.16)
Kennet	TH	I	550 - 710	0.77	1.41 (0.59-2.40)

Figure captions

Figure 1. Inset: Study area in Great Britain. Main image: approximate locations in England of the 11 *B. barbus* populations used in the study (black crosses) and where: 1: Warwickshire Avon, 2: River Teme, 3: River Severn, 4: Hampshire Avon, 5: River Great Ouse, 6: River Ivel, 7: Chub Stream, 8: Trout Stream, 9: River Lee, 10: River Loddon and 11: River Kennet (*cf.* Table 1).

Figure 2. Relationships between length range of *Barbus barbus* per population and the range of their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data. All ranges represent the difference between the maximum and minimum values in samples. Black circles indicate the sample was only collected by electric fishing, clear circles indicate the sample included fish captured by angling.

Figure 3. $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ (clear circle) and $\Delta^{15}\text{N}_{\text{macroinvertebrate}}$ (filled circle) versus predicted proportion of marine fishmeal in the diet of 17 *B. barbus* from the lower River Teme/ Severn, where the solid line represents the significant relationship between the variables according to linear regression.

Figure 4. Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of macroinvertebrates versus $\delta^{13}\text{C}$ of individual *Barbus barbus*, where filled circle = fish of < 300 mm and clear circle = fish \geq 300 mm. Solid line represents the 1:1 line and the horizontal dashed line represents the maximum $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ according to Busst and Britton (2016) (5.31 ‰).

Figure 5. Lengths of individual *Barbus barbuis* versus $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$. The solid line represents the significant relationship between the variables according to linear regression and the horizontal dashed line represents the maximum $\Delta^{13}\text{C}_{\text{macroinvertebrate}}$ according to Busst and Britton (2016) (5.31 ‰).

Figure 6. Range of the corrected carbon stable isotope (Ccorr; clear circle) and coefficient of variation of Ccorr versus the isotopic niche size (as SEAc). The solid line represents the significant relationship between the variables according to linear regression.

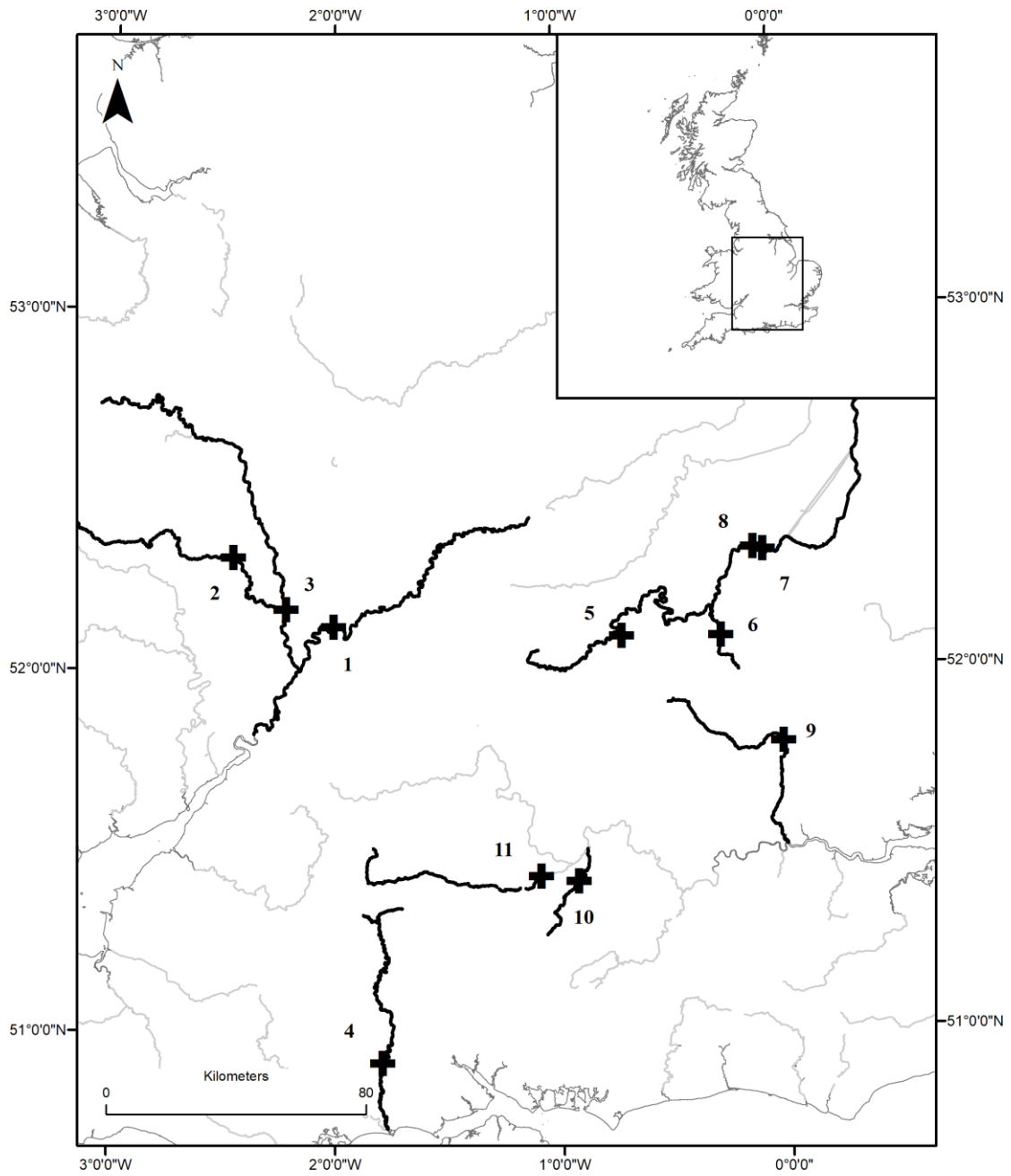


Figure 1.

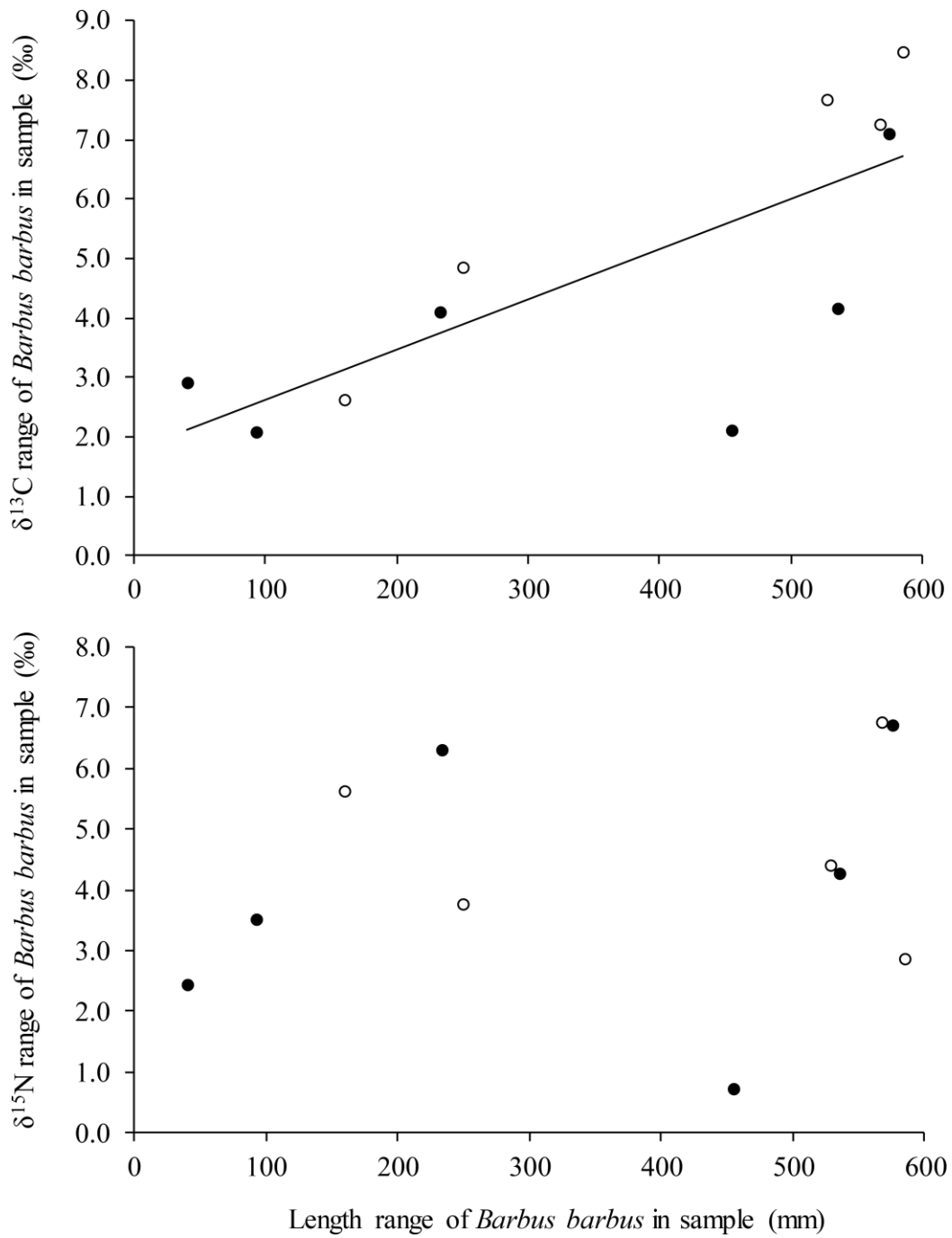


Figure 2.

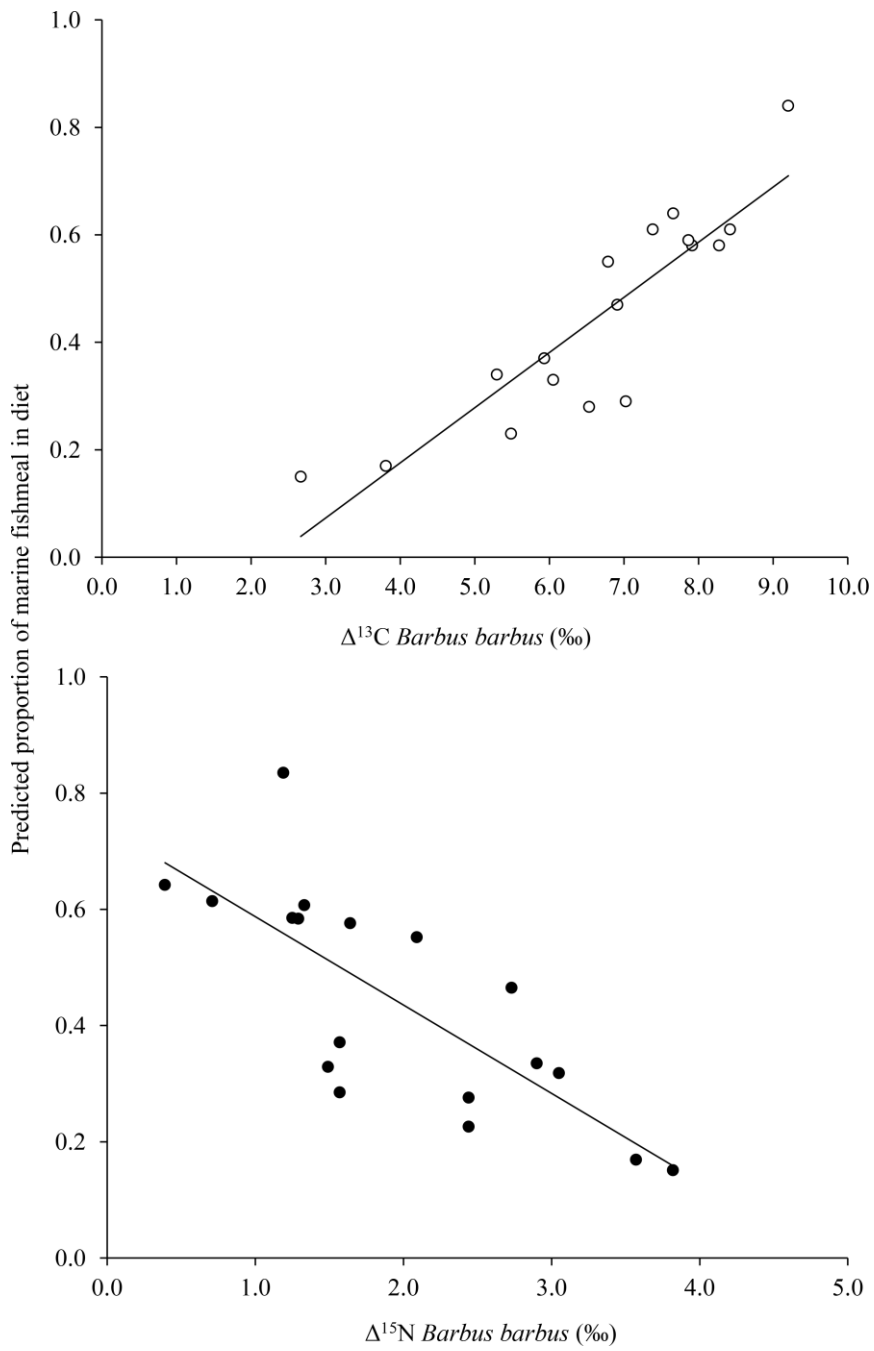


Figure 3.

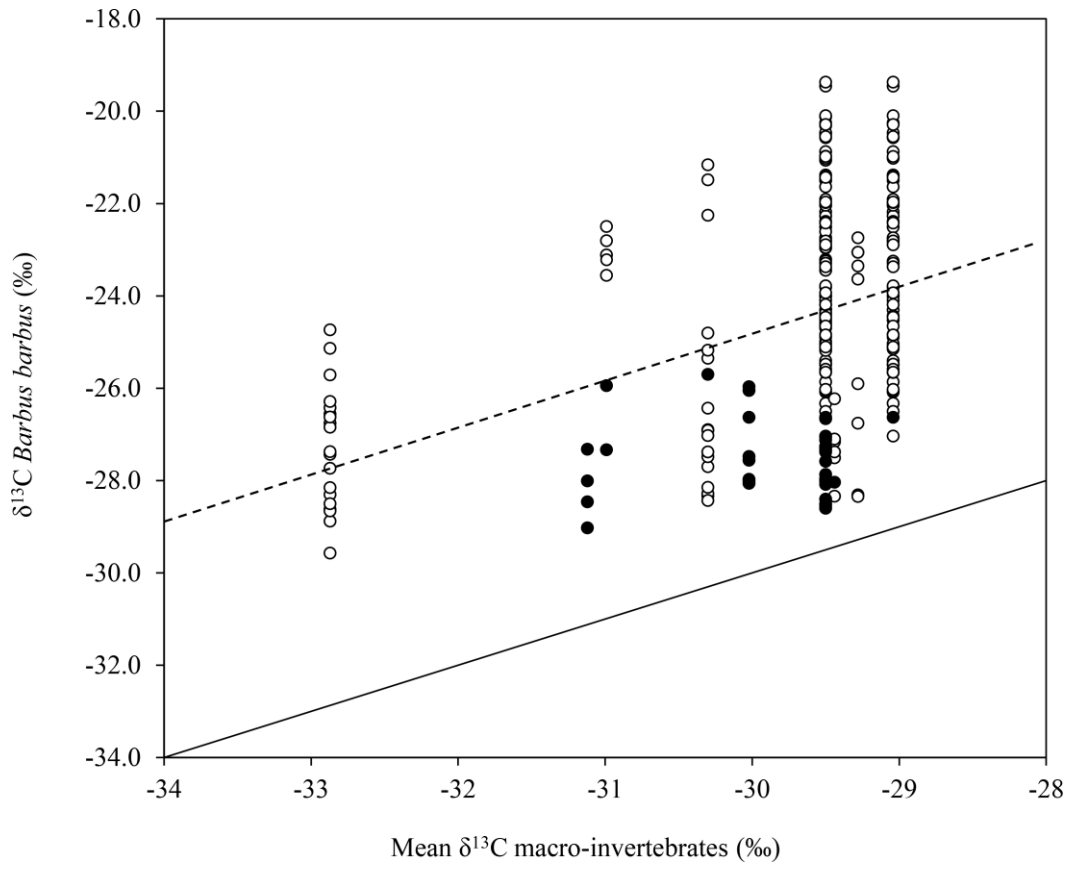


Figure 4.

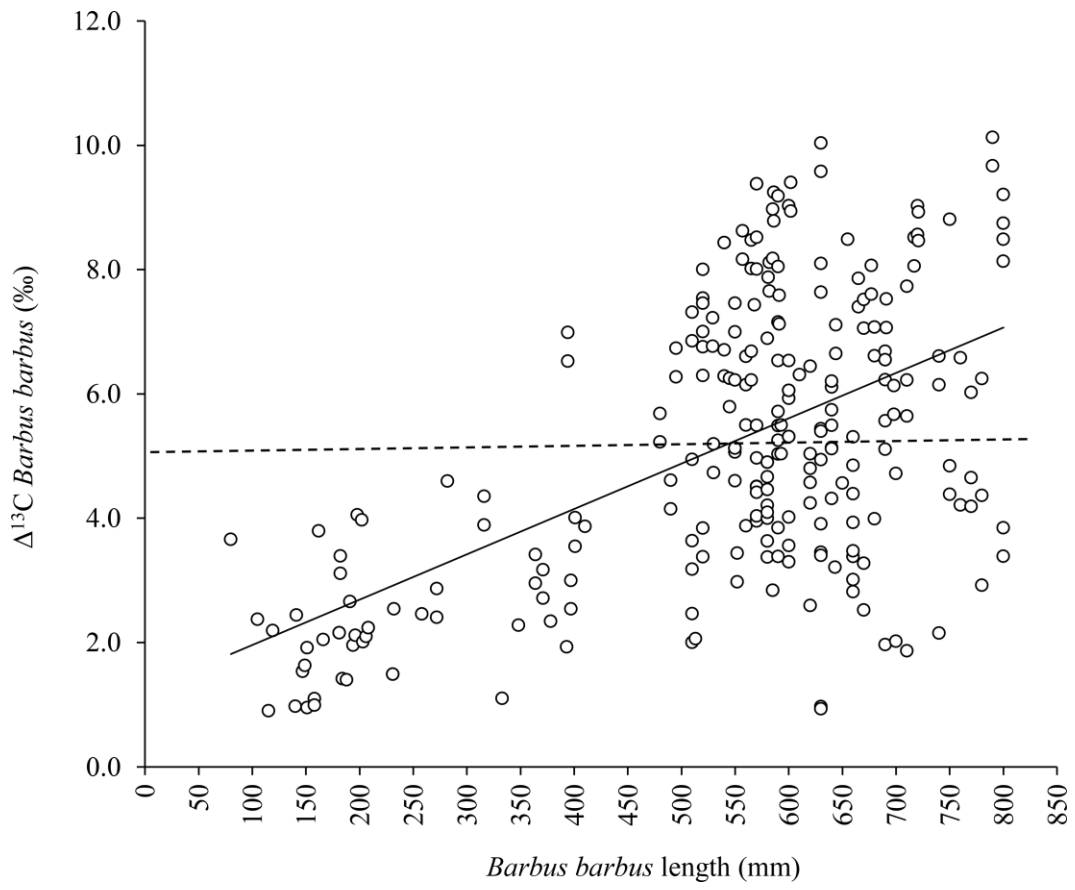


Figure 5.

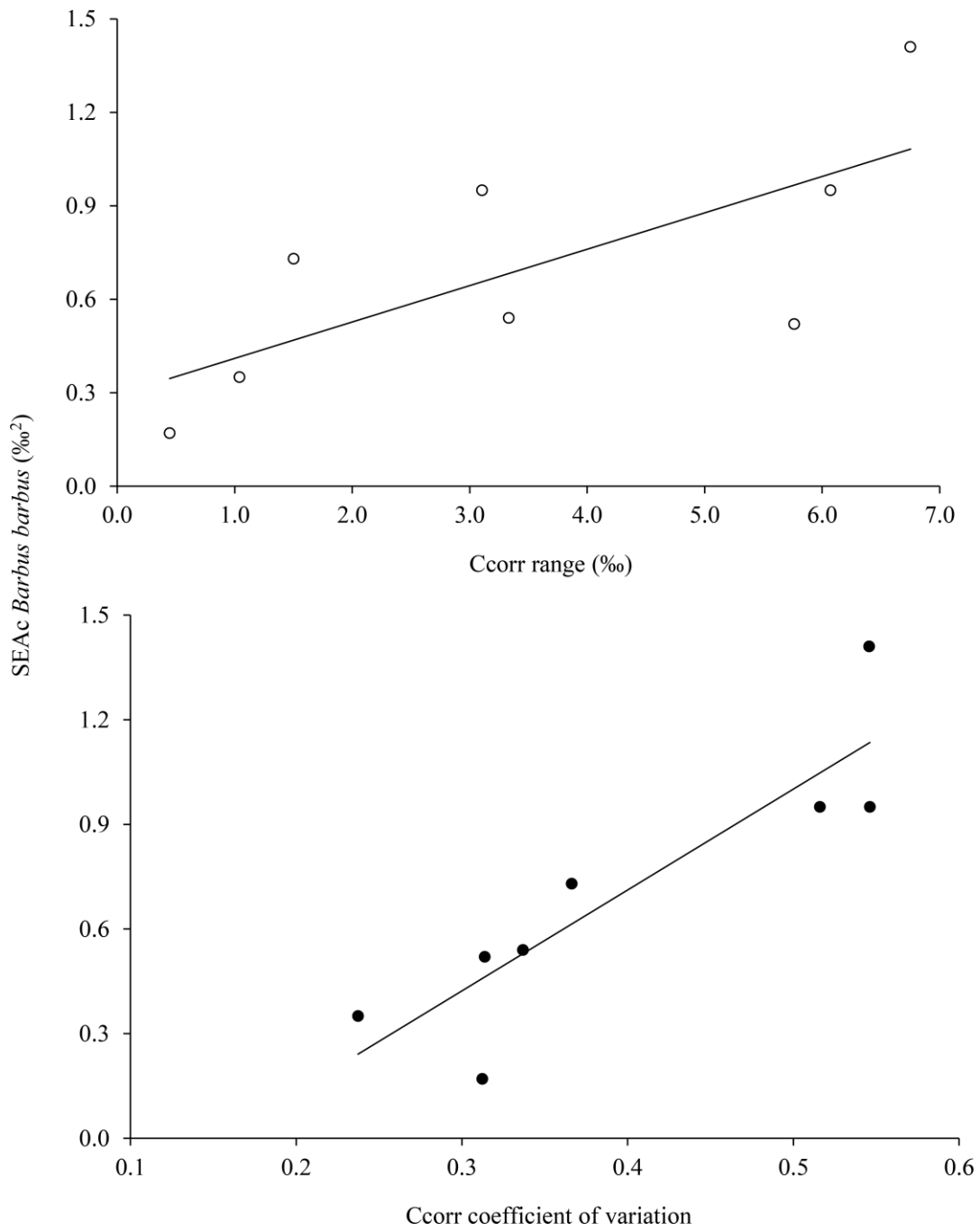


Figure 6.