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

Vanessa De Santis[1,2]; Catherine Gutmann Roberts[2]; J. Robert Britton[2]

[1]Department of Theoretical and Applied Sciences, University of Insubria, Varese (VA) - Italy

[2]Department of Life and Environmental Sciences, Faculty of Science and Technology, Bournemouth University, Poole, Dorset - UK

Corresponding author: Robert Britton, rbritton@bournemouth.ac.uk

Abstract

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

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

Introduction

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

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

Novel ecological opportunities can enable individual specialisation in resource use to develop within populations (Britton & Andreou 2016), with examples including when terrestrial insects become available for predation by stream fishes (Syrjänen et al. 2011). Individual trophic specialisation results in the population trophic niche becoming diversified, shifting to 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 $\delta^{13}C$) and marine
74 (enriched $\delta^{13}C$) environments (Jardine et al. 2005; Gutmann Roberts et al. 2017). There tends
75 to be considerable differences in the $\delta^{13}C$ 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 $\Delta^{13}C$ with a single formulated food resource was 5.31 ‰. Thus, if the
81 $\Delta^{13}C$ of an individual fish with their putative macroinvertebrate prey exceeds this Δ, it would
82 be assumed that an alternative, highly $\delta^{13}C$ 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 ($\delta^{13}C$, $\delta^{15}N$) 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 $\Delta^{13}C$ 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 ($\delta^{13}C$, $\delta^{15}N$) 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.

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

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

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

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

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

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

between trophic levels and 2 was the trophic position of the baseline organism (Post 2002). The $\delta^{13}C$ data were converted to $\delta^{13}Ccorr$ by $\delta^{13}C_i - \delta^{13}C_{meaninv}/CR_{inv}$, where $\delta^{13}C_{corr}$ was the corrected carbon isotope ratio of the individual fish, $\delta^{13}C_i$ was the uncorrected isotope ratio of that fish, $\delta^{13}C_{meaninv}$ was the mean invertebrate isotope ratio (the 'baseline' invertebrates) and CR_{inv} is the invertebrate carbon range ($\delta^{13}Cmax - \delta^{13}Cmin$; Olsson et al., 2009).

Data analysis and statistical testing

Across the 11 populations, the *B. barbus* samples were collected by electric fishing and/ or angling, comprised of fish between 80 and 850 mm, and were collected in different years. Thus, to understand how river, sampling method, fish length and year of sampling affected the SI data, linear mixed models (LMM) were used. Due to the non-comparable nature of the raw SI data between rivers (due to variable macroinvertebrate SI data; Table 2), the corrected data (Ccorr and TP) had to be used in these models. Correspondingly, they could only be completed using data from the 9 *B. barbus* populations where macroinvertebrate data were available (Table 2). In LMMs, Ccorr or TP was the dependent variable, the independent variable was either sampling method, river or fish length (depending on the test), covariates were sampling, river, year or fish length (depending on the independent variable), and river was used as the random variable (except when the model was testing differences between rivers). Model outputs were the significance of the overall test, the significance of covariates, and the mean values of Ccorr and TP (adjusted for the effects of the covariates) with their pairwise comparisons (with Bonferroni adjustment for multiple comparisons). Where a covariate had consistent non-significant values in all models, it was removed from all final LMMs. The final LMMs were also checked to ensure they met the test assumptions (e.g. the errors have constant variance, are independent, and are normally distributed). Where 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 (Δ^{13}C_macroinvertebrate;
191 Δ^{15}N_macroinvertebrate) by subtracting their δ^{13}C and δ^{15}N values from the mean
192 macroinvertebrate values. The utility of Δ^{13}C_macroinvertebrate and Δ^{15}N_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 Δ^{13}C_macroinvertebrate and Δ^{15}N_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 Δ^{13}C and δ^{13}C (*cf.* Results). The regression
200 coefficients (*a*, *b*) were then used in the equation FM = (Δ^{13}C_macroinvertebrate × *b*) + *a*,
201 where FM = the proportion of marine fishmeal in diet, to predict the proportion of fishmeal in
202 the diet at Δ^{13}C_macroinvertebrate = 5.31 ‰ (Busst & Britton 2016; Gutmann Roberts et al.
203 2017). The Δ^{13}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 Δ^{13}C of *B. barbus* with a known food resource was 5.31 ± 0.09 ‰. Thus, where
206 Δ^{13}C_macroinvertebrate exceeded 5.31 ‰, it was assumed that the main dietary item of that
207 fish could not be macroinvertebrates. The relationship of Δ^{13}C_macroinvertebrate with fish
208 length was then tested across the dataset, enabling the proportion of fish per population
209 whose Δ^{13}C_macroinvertebrate exceeded 5.31 ‰ to be determined. Values of Δ^{13}C_pellet

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

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

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

Results

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

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

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

Predicting contributions of marine fishmeal to Barbus barbus diet

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

Stable isotope fractionation of Barbus barbus from food resources

The LMM testing the effect of sampling method on Δ^{13}C_macroinvertebrate was not significant (P = 0.89), with the effect of length as a covariate not being significant (P = 0.18). The LMM testing the effect of fish length on Δ^{13}C_macroinvertebrate was significant (P < 0.01), where the effect of sampling method as a covariate was not significant (P = 0.39). This significant influence of fish length on Δ^{13}C_macroinvertebrate was then explored further by a LMM testing the differences in Δ^{13}C_macroinvertebrate between fish of < 300 mm and > 300 mm. The model was significant (P < 0.01), with the effect of sampling method as a covariate also being significant (P = 0.04). The mean Δ^{13}C_macroinvertebrate (adjusted for the effects of covariates) of fish < 300 mm was 2.78 ± 0.84 ‰ versus 5.41 ± 0.34 ‰ for fish > 300 mm.

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

Isotopic niche size

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

Discussion

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

333 The assessments of the influence of marine fishmeal on *B. barbus* diet were completed using
334 calculations of $\Delta^{13}C$. 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 $\Delta^{13}C$ here was
337 possible due to the $\delta^{13}C$ of the marine fishmeal baits being substantially enriched versus
338 freshwater macroinvertebrates (differences approximately 7 to 10 ‰). Thus, despite $\Delta^{13}C$ 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 $\delta^{13}C$ and substantially lower $\Delta^{13}C$_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 $\Delta^{13}C$_macroinvertebrate of 5.31 ‰ (the maximum $\Delta^{13}C$ 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 $\Delta^{13}C$_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 $\delta^{13}C$ depleted and highly distinct from the marine fishmeal
352 resources. Correspondingly, the use here of $\delta^{13}C$ and $\Delta^{13}C$ 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 $\Delta^{13}C$ to the 9 *B. barbus* with macroinvertebrate data available revealed
357 that for fish below 394 mm, $\Delta^{13}C$_macroinvertebrate was always below 5.31 ‰ (the highest

358 $\Delta^{13}C$ of Busst & Britton (2016)). Only at larger body sizes did their values of
359 $\Delta^{13}C$_macroinvertebrate become more $\delta^{13}C$ enriched, with a maximum
360 $\Delta^{13}C$_macroinvertebrate of 10.13 ‰. This $\Delta^{13}C$_macroinvertebrate and $\delta^{13}C$ 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 $\delta^{13}C$ (*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 $\delta^{13}C$ (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 $\Delta^{13}C$_macroinvertebrate values. There were four populations where $\Delta^{13}C$_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 $\Delta^{13}C$_macroinvertebrate
375 values exceeding 5.31 ‰. The Chub and Trout Stream also had no fish with
376 $\Delta^{13}C$_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 $\Delta^{13}C$_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 Δ^{13}C_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

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

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

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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; $\delta^{13}C$ and $\delta^{15}N$ 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 $\delta^{13}C$	$\delta^{13}C$ range	Mean $\delta^{15}N$	$\delta^{15}N$ 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. barbus* fractionation factors sampled from 9 rivers. Note that the mean $\delta^{13}C$ of fishmeal pellets used in the study was -22.12 ± 0.53 ‰ (range -23.19 to -20.17 ‰) and $\delta^{15}N$ was 7.31 ± 1.02 ‰ (range 4.10 to 9.40 ‰).

River	Basin	Mean $\delta^{13}C$	Mean $\delta^{15}N$
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 barbus* 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 barbus* with $\delta^{13}C$ 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.

		All fish		Fish > 300 mm	
River	Basin	% 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).

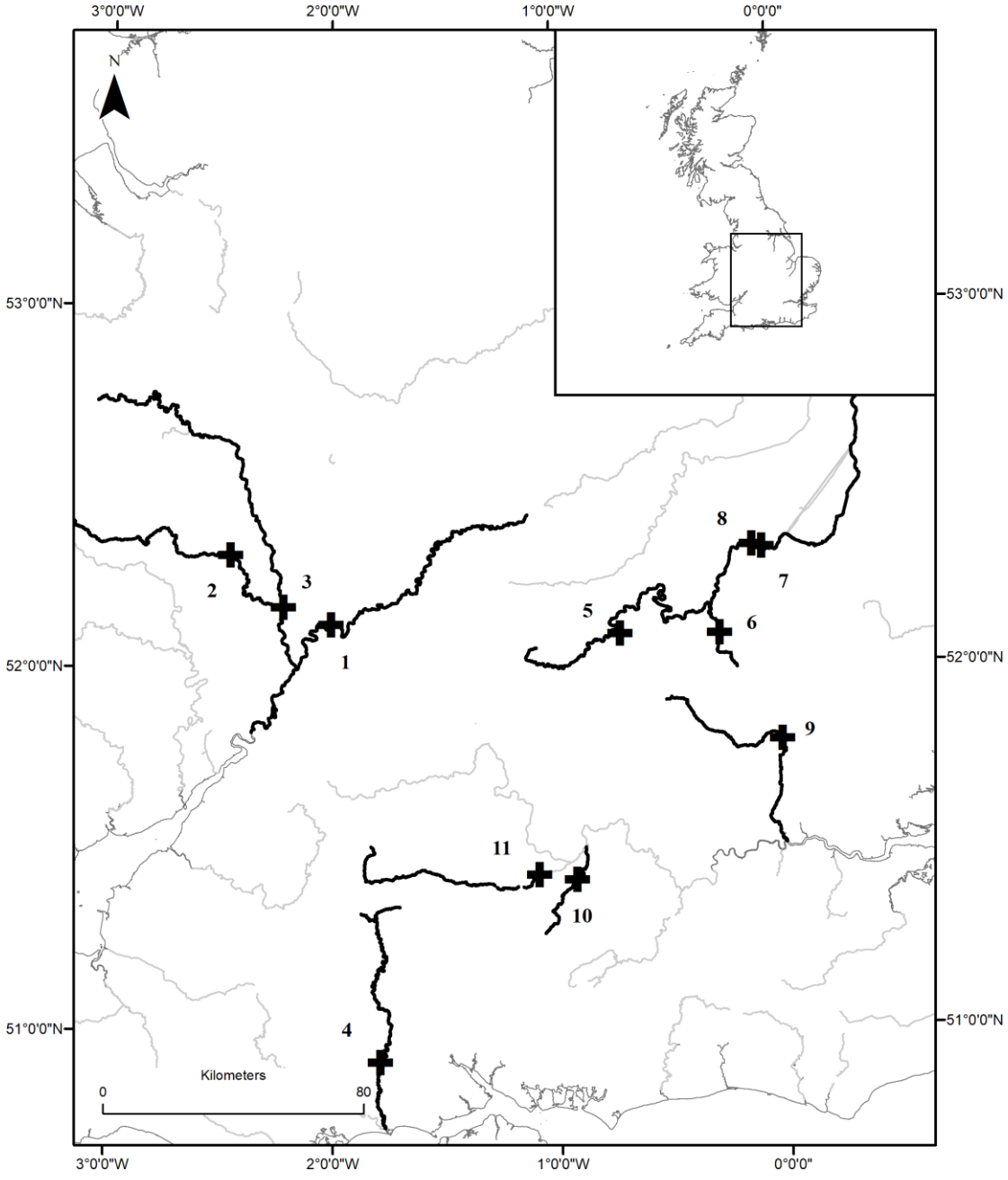
Figure 2. Relationships between length range of *Barbus barbus* per population and the range of their $\delta^{13}C$ and $\delta^{15}N$ data. All ranges represent the difference between the maximum and minimum values in samples. Black circles indicate the sample was only collected by electric fishing,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

Figure 3. $\Delta^{13}C$_macroinvertebrate (clear circle) and $\Delta^{15}N$_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 $\delta^{13}C$ and $\delta^{15}N$ of macroinvertebrates versus $\delta^{13}C$ of individual *Barbus barbus*, where filled circle = fish of < 300 mm and clear circle = fish ≥ 300 mm. Solid line represents the 1:1 line and the horizontal dashed line represents the maximum $\Delta^{13}C$_macroinvertebrate according to Busst and Britton (2016) (5.31 ‰).

Figure 5. Lengths of individual *Barbus barbus* versus Δ^{13}C_macroinvertebrate. The solid line represents the significant relationship between the variables according to linear regression and the horizontal dashed line represents the maximum Δ^{13}C_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.


3°0'0"W
2°0'0"W
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52°0'0"N
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1
2
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0
80

Figure 1.

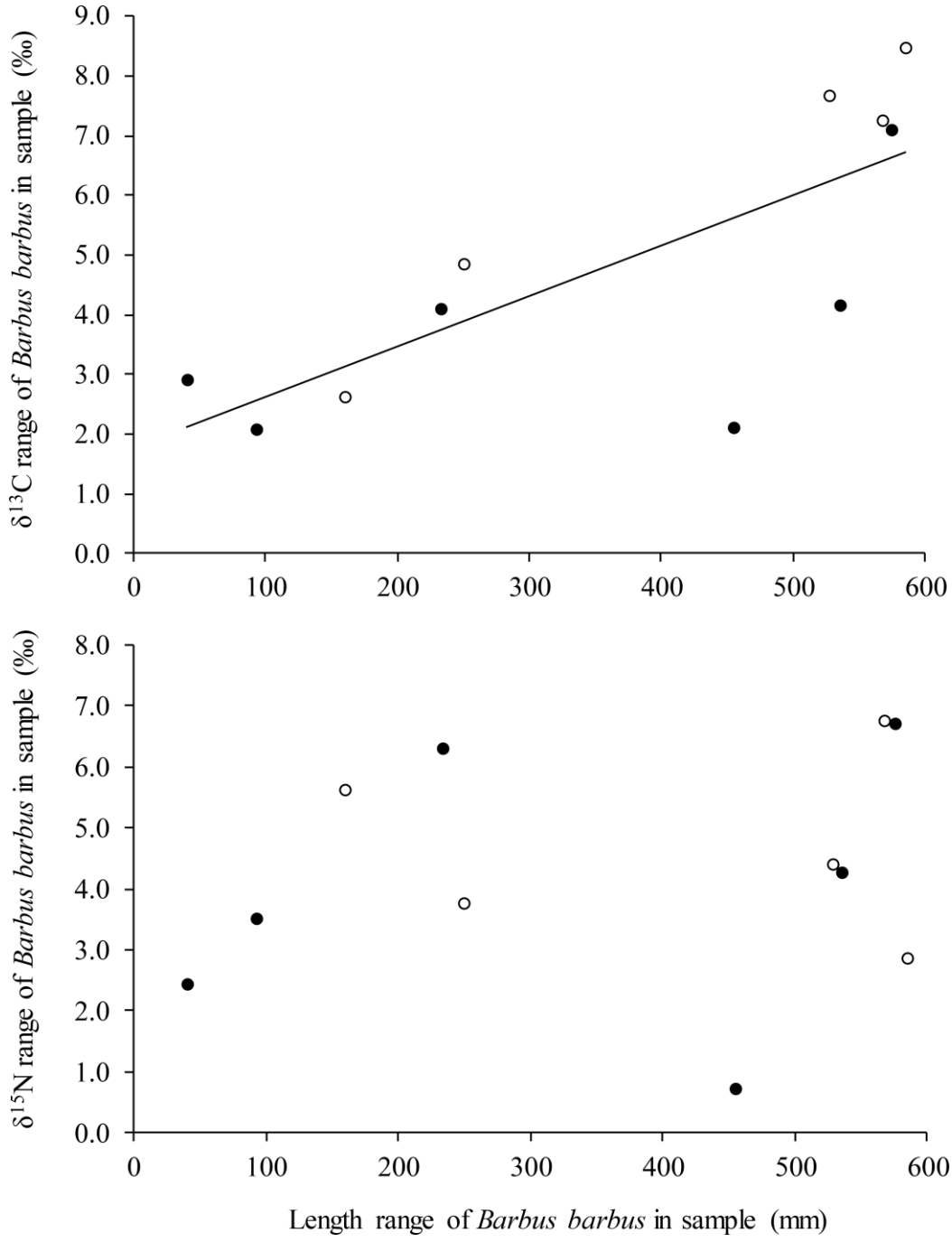
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
δ13C range of Barbus barbus in sample (‰)
0
100
200
300
400
500
600
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
δ15N range of Barbus barbus in sample (‰)
0
100
200
300
400
500
600
Length range of Barbus barbus in sample (mm)

Figure 2.

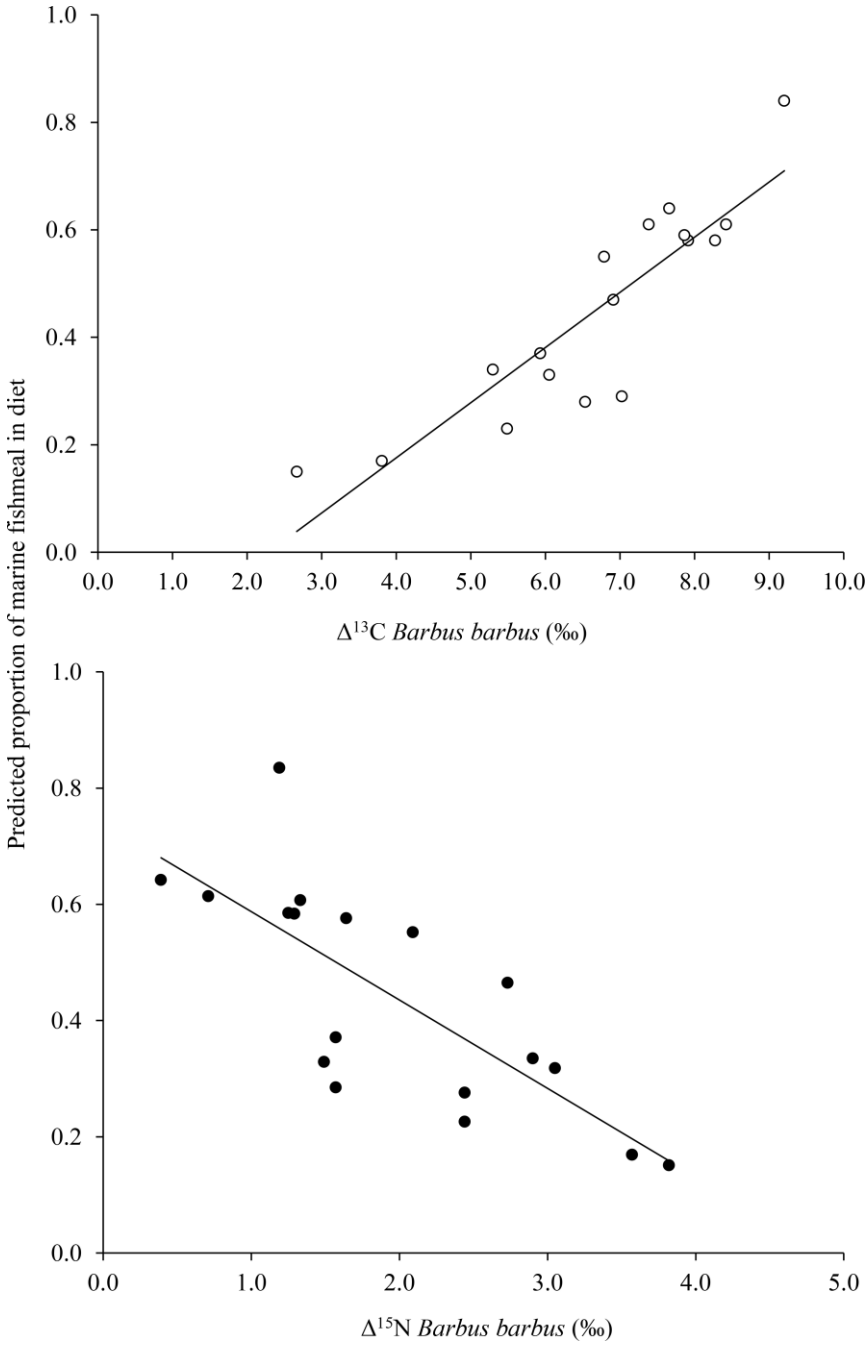
Predicted proportion of marine fishmeal in diet
1.0
0.8
0.6
0.4
0.2
0.0
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
Δ13C Barbus barbus (‰)
1.0
0.8
0.6
0.4
0.2
0.0
0.0
1.0
2.0
3.0
4.0
5.0
Δ15N Barbus barbus (‰)

Figure 3.

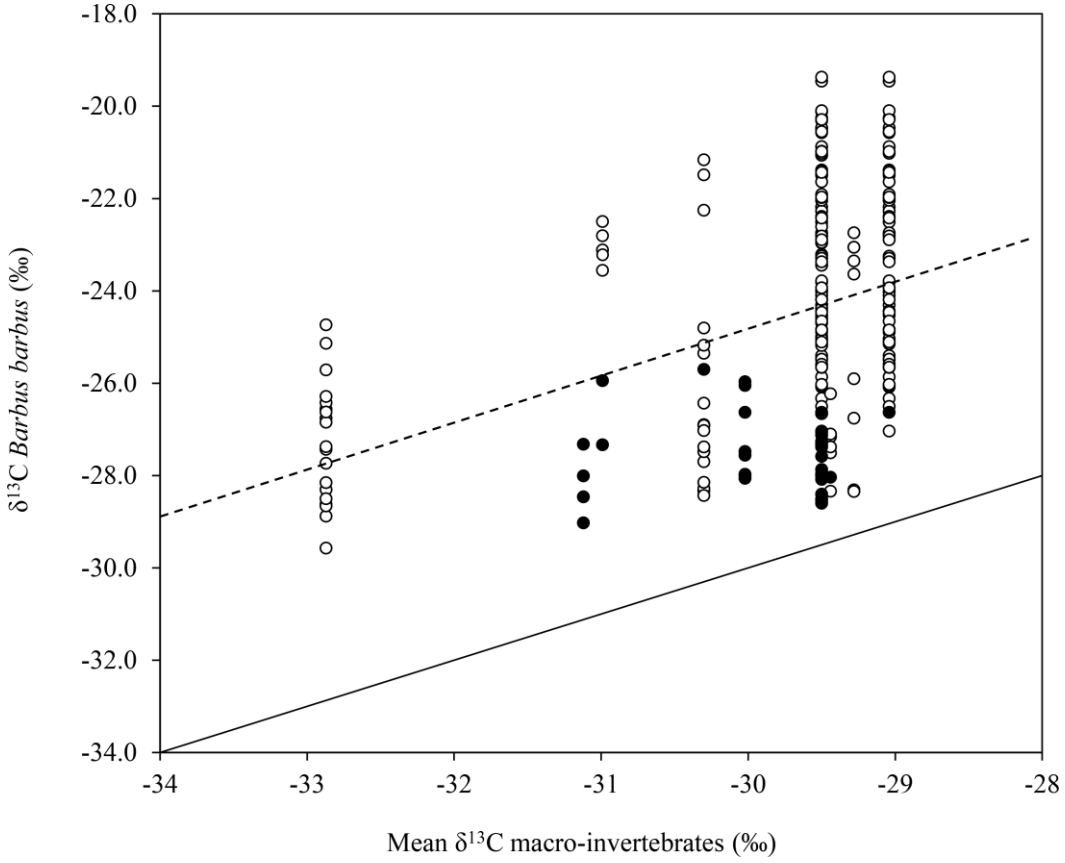
-18.0
-20.0
-22.0
-24.0
-26.0
-28.0
-30.0
-32.0
-34.0
δ13C Barbus barbus (‰)
-34
-33
-32
-31
-30
-29
-28
Mean δ13C macro-invertebrates (‰)

Figure 4.

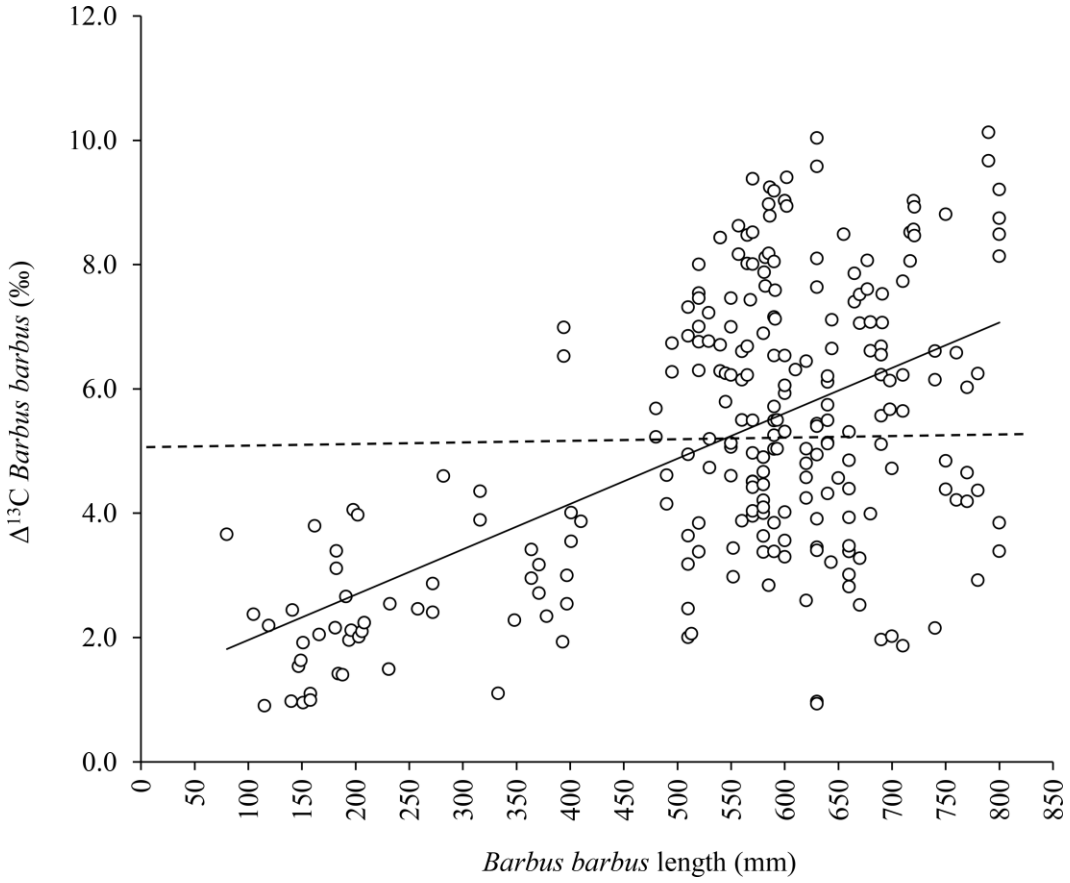
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Δ13C Barbus barbus (‰)
0
50
100
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850
Barbus barbus length (mm)

Figure 5.

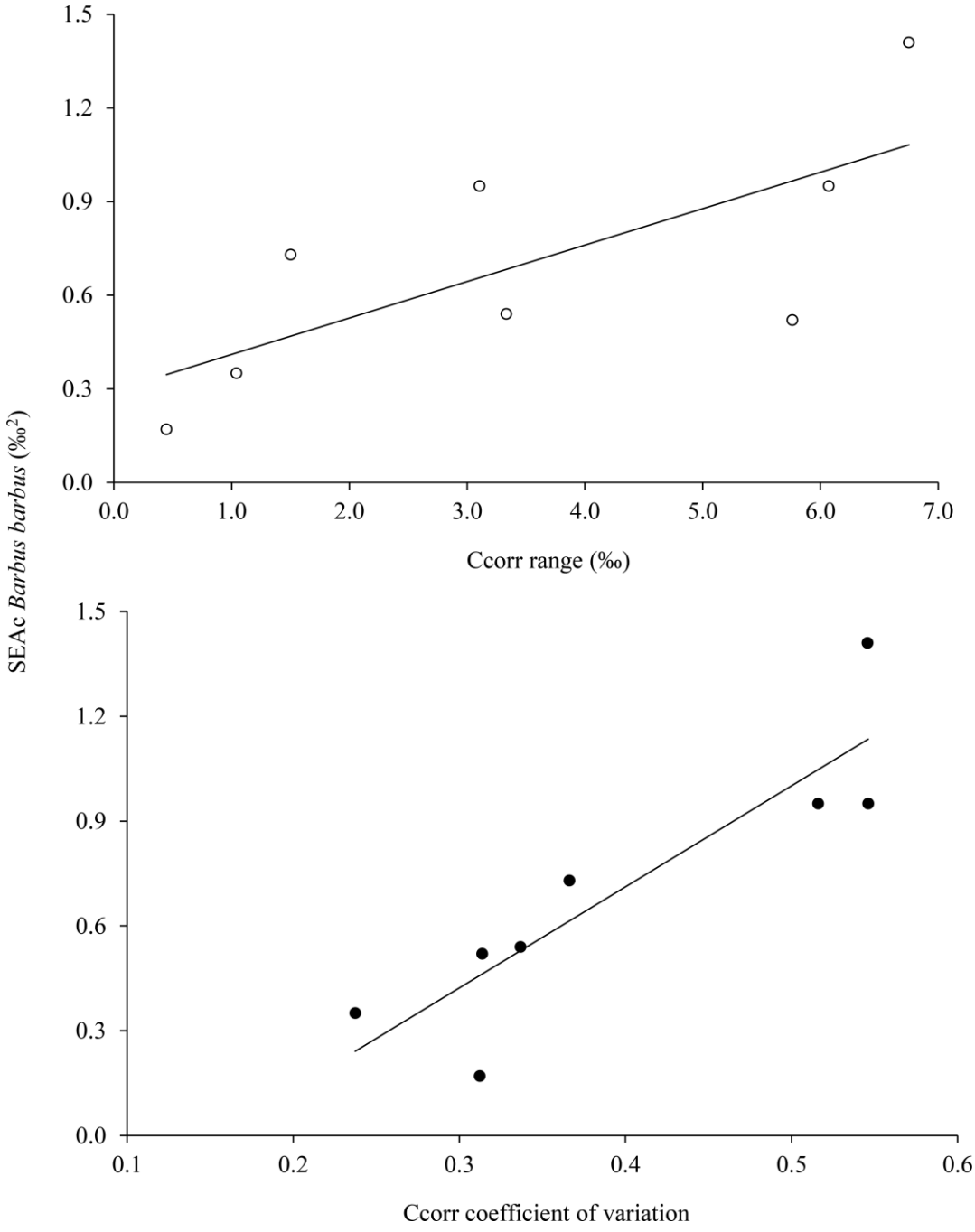
SEAc Barbus barbus (‰²)
1.5
1.2
0.9
0.6
0.3
0.0
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
Ccorr range (‰)
1.5
1.2
0.9
0.6
0.3
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Ccorr coefficient of variation

Figure 6.