



**Manchester
Metropolitan
University**

Gutmann Roberts, C and Bašić, T and Amat Trigo, F and Britton, JR
(2017) Trophic consequences for riverine cyprinid fishes of angler subsidies
based on marine-derived nutrients. *Freshwater Biology*, 62 (5). pp. 894-905.
ISSN 0046-5070

Downloaded from: <http://e-space.mmu.ac.uk/621420/>

Version: Accepted Version

Publisher: Wiley

DOI: <https://doi.org/10.1111/fwb.12910>

Please cite the published version

<https://e-space.mmu.ac.uk>

1 Accepted for publication in *Freshwater Biology*, 19/01/2017

2

3 **Trophic consequences for riverine cyprinid fishes of angler subsidies based on marine**
4 **derived nutrients**

5

6 Catherine Gutmann Roberts¹, Tea Bašić¹, Fatima Amat Trigo^{1,2}, J Robert Britton^{1*}

7

8 ¹Centre for Conservation Ecology and Environmental Sciences, Faculty of Science and
9 Technology, Bournemouth University, Poole, Dorset, BH12 5BB, UK.

10 ²Departamento de Zoología y Antropología Física, Universidad de Murcia, Spain

11

12

13

14

15

16

17 *Corresponding author: rbritton@bournemouth.ac.uk. (+44)01202965384

18

19 Summary

20

21 1. The crossing of freshwater ecosystem boundaries by marine derived nutrients (MDN)
22 is usually associated with migratory salmonid fishes returning to natal rivers. An
23 alternative source of MDN in freshwaters is the widespread use of pelletized marine
24 fishmeal ('pellets') by freshwater anglers as they target large bodied cyprinid fishes,
25 such as European barbel *Barbus barbus*.

26 2. Here, the trophic consequences of MDN from pellets for riverine cyprinid fishes were
27 tested. Approaches used stable isotope analyses in controlled and wild scenarios,
28 using *B. barbus* and chub *Squalius cephalus* as model species. The isotopic niche,
29 measured as standard ellipse area, was used to assess trophic niche size, and mixing
30 models predicted the extent to which MDN contributed to fish diet.

31 3. In experimental mesocosms, *B. barbus* fed low volumes of pellets (approximately 3
32 per fish) for 130 days had isotopic niche sizes that were up to four times larger than a
33 control and 'medium' (6 per fish) and 'high' pellet (12 per fish) treatments. Somatic
34 growth rates were significantly higher in the 'medium' and 'high' treatments. In pond
35 enclosure experiments, when juvenile *B. barbus* and *S. cephalus* were fed pellets daily
36 for 100 days, there was a substantial and significant shift in the position of their
37 isotopic niche compared to controls with no pellets fed. However, for each species,
38 there were no significant differences in their somatic growth rates in the presence/
39 absence of pellets.

40 4. In a lowland river, high proportions of MDN contributed to the diet of *B. barbus* and
41 *S. cephalus* captured by angling, but with substantial individual variability in those
42 captured by electric fishing. Across all *B. barbus* > 400 mm, MDN dietary
43 contributions ranged between 9 and 71%. This suggested some individual diet

44 specialisations within their population that was associated with feeding on this angler
45 subsidy and that also resulted in a significant increase in the size of their population
46 isotopic niche.

47 5. These results suggested that when pellets containing MDN are used in freshwater
48 angling, they are consumed and assimilated by cyprinid fishes, influencing individual
49 and population trophic positions, and isotopic niche sizes and dietary specialisations.
50 The results also suggested that the extent to which individuals specialise in feeding on
51 pellets potentially influences their vulnerability to capture by anglers.

52

53 **Keywords:** Allochthonous, barbel, fishmeal, MDN, river ecology, stable isotopes

54

55

56 **Introduction**

57

58 Trophic fluxes of energy and nutrient resources can be ecologically significant when they
59 cross the boundaries of ecosystems that differ in their productivity (e.g. Polis & Hurd, 1995;
60 Zhang *et al.*, 2003; Richardson *et al.*, 2016). These cross-system fluxes can maintain the
61 productivity, diversity, and community structure of recipient ecosystems (Schindler *et al.*,
62 2005). Anadromous salmonid fishes are well recognised as playing integral roles in these
63 processes, as they accumulate the majority of their biomass in the ocean and import these into
64 freshwaters during spawning, thus releasing marine derived nutrients (MDN) into the
65 relatively nutrient-poor freshwater systems (Schindler *et al.*, 2003). However, this delivery
66 mechanism is not the only MDN source in freshwaters, as aquaculture and angling activities
67 can also elevate the quantity of MDN to freshwater ecosystems via the release of energy rich
68 foods based on pelletized fishmeal ('pellets') that is derived from marine fishes (Bašić *et al.*,
69 2015).

70

71 The use of marine derived fishmeal pellets in freshwater aquaculture is an integral part of the
72 husbandry process (Naylor *et al.*, 2000). In recreational angling, marine derived fishmeal
73 pellets of up to 21 mm in diameter are used as both an attractant and hook-bait, and thus they
74 can supplement fish diet (Grey, Waldron & Hutchinson, 2004; Jackson *et al.*, 2013; Bašić *et*
75 *al.*, 2015). These inputs of pellets can increase the productivity of freshwater systems due to
76 their nutrient and energy fluxes (Jones *et al.*, 1998; Jefferies, 2000), and thus they can act as a
77 strong allochthonous trophic subsidy (Marcarelli *et al.*, 2011; Sato & Watanabe, 2013). In
78 doing so, they potentially alter food web structure via changes in the trophic interactions of
79 consumers (Jefferies, 2000; Marzacak *et al.*, 2007), and potentially result in resource
80 partitioning between populations (Bašić *et al.*, 2015). The pellets utilised by anglers tend to

81 have high protein levels from fishmeal (typically 40 to 50%) and lipid levels from fish oil
82 (typically 20%) (Naylor *et al.*, 2000; Bašić *et al.*, 2015). These pellets have been used widely
83 for at least 20 years by European freshwater anglers for exploiting the cyprinid fishes
84 common carp *Cyprinus carpio* L. and European barbel *Barbus barbus* (L.) (Jackson *et al.*,
85 2013; Bašić *et al.*, 2015). Substantial quantities can be used, with individual anglers often
86 using in excess of 1 kg per day, with at least 10 anglers often being present daily on some
87 small (< 1 km) stretches of English rivers in summer (Bašić *et al.*, 2015). Arlinghaus and
88 Niesar (2005) estimated that the amount of bait used annually per freshwater angler in
89 Germany was 7.3 kg, indicating that considerable volumes of angler bait might be introduced
90 into freshwaters on an annual basis.

91

92 The provision of novel feeding opportunities, such as the seasonal availability of terrestrial
93 insects for stream fishes (Syrjanen *et al.*, 2011), can result in individual trophic niche
94 specialisation developing within populations (Britton & Andreou, 2016). This is where the
95 population trophic niche consists of sub-groups of trophically specialised individuals that in
96 entirety comprise the population niche (Araújo, Bolnick & Layman, 2011). The attractiveness
97 of pelletized marine-derived fishmeal to many fishes is likely to relate to their provision of an
98 energy rich resource that is relatively easy to assimilate and maximises growth rates (Naylor
99 *et al.*, 2000; Bašić *et al.*, 2015). It was recently established that in four rivers in England, the
100 diet of adult *B. barbus* comprised considerable proportions of pelletized fishmeal (up to 80%;
101 Bašić *et al.*, 2015). However, this study was all based on samples collected from uncontrolled
102 field conditions, with no consideration of how it impacted the population trophic niche of the
103 fish or their somatic growth rates. The aim of this study was thus to quantify how MDN in
104 pelletized fishmeal from angling modifies the population trophic niches, influences individual
105 dietary specialisation, and affects the growth rates of riverine fishes. Following Grey,

106 Waldron & Hutchinson (2004) and Bašić *et al.* (2015), who established that MDN from
107 pellets results in fish isotopic data being distinct within freshwater food webs, objectives
108 were to: (1) assess how MDN modifies the trophic niche size and somatic growth rates of
109 allopatric and sympatric fishes in controlled conditions; and (2) quantify the contribution of
110 MDN to the diet of wild fishes, and assess its role in driving individual trophic niche
111 specialisation and modification of the population trophic niche. It was hypothesised that
112 where available, MDN pellets contribute substantial proportions of the diet of river fishes,
113 resulting in individuals specialising on this trophic subsidy and having faster somatic growth
114 rates.

115

116 **Materials and methods**

117

118 *Model species, experimental designs and field study*

119 The model species were *B. barbuis* and its cyprinid trophic analogue chub *Squalius cephalus*
120 (L.). These fishes are sympatric in many European rivers and achieve relatively similar body
121 sizes (Bašić & Britton, 2016). A mesocosm experiment tested how the variable availability of
122 pellets affected the trophic niche size and somatic growth rates of allopatric *B. barbuis*. A
123 semi-controlled pond experiment determined how pellet availability affected the trophic
124 niche position and size, and somatic growth rates, of *B. barbuis* and *S. cephalus* in allopatry
125 and sympatry. A field study then tested the influence of pellets on the trophic niche and diet
126 composition of wild *B. barbuis* and *S. cephalus*. These studies utilised stable isotope analysis
127 (SIA) to assess trophic niche sizes (as isotopic niches) and the diet composition of the fishes.

128

129 The mesocosm experiment was completed in 12 artificial ponds of 250 L volume, using
130 hatchery-reared juvenile *B. barbuis* across four treatments: control (no supplementary

131 feeding), low (supplementary feeding of approximately three pellets per day per fish),
132 medium (6 pellets per day per fish) and high (12 pellets per day per fish). Each treatment was
133 replicated three times, with five fish used per replicate. The pellets were 2 mm diameter and
134 constituent 45% protein (from marine fishmeal) and 20% fish oil (Dynamite Baits, 2017).
135 Each mesocosm pond was outside, mounted on a concrete base with no overhanging trees
136 nearby, and had a gravel substrate (6 mm diameter), aeration and a filter to maintain water
137 quality. Feeding rates were achieved via automated feeders releasing pellets once per day at
138 20:00, as *B. barbuis* are crepuscular (Britton & Pegg, 2011). The mesocosms were set up in
139 April 2015 and were seeded with macroinvertebrates collected from a local stream
140 (*Gammarus pulex*; 20 per mesocosm). Chironomid larvae naturally colonised all mesocosms.

141

142 The fish were measured (fork length, nearest mm) and weighed (to 0.1 g) before their
143 introduction into the mesocosms in June 2015 (Table 1). They were removed in October
144 2015, thus were exposed to their new diets for 130 days. Temperature loggers (TinyTag TGP-
145 4017) in eight mesocosms (2 per treatment) recorded water temperatures twice per day (0.00
146 and 12.00) revealed a mean water temperature (\pm 95% confidence limits) of 19.4 ± 0.7 °C,
147 with no significant differences between mesocosms (ANOVA: $F_{1,6} = 0.56$, $P = 0.48$). For a
148 consumer of starting weight 10 g, estimated half-life at 20 °C is 36 days for $\delta^{13}\text{C}$ and 38 days
149 for $\delta^{15}\text{N}$ (Thomas & Crowther, 2015). These values equate to 92% replacement of both
150 isotopes in the fish after 130 days, with consumers generally considered to have fully
151 equilibrated to their food resources at 94% isotopic replacement (Hobson & Clark, 1992).

152

153 On day 130, the mesocosms were drained and the fish removed, euthanized (over-
154 anaesthesia; MS-222), re-measured, re-weighed and a dorsal muscle sample taken for SIA
155 (Busst, Bašić & Britton, 2015). Samples of putative prey resources were also collected from

156 each mesocosm (*G. pulex* and Chironomid larvae); where possible, these represented
157 triplicate samples per mesocosm (1 sample = 5 individuals). All samples were then oven
158 dried to constant weight at 60°C as preparation for SIA.

159

160 The pond experiment used mesocosms where *B. barbuis* and *S. cephalus* were used in
161 allopatry and sympatry. Thus, three treatments were used in pellet presence and absence: both
162 species in allopatry (n = 10), and a final treatment where they were present in sympatry (n = 5
163 + 5), with three replicates per treatment. All fish were juveniles (starting lengths 60 to 88 mm,
164 starting weights < 10 g) and hatchery reared. Each mesocosm was set up as per Bašić and
165 Britton (2016), thus each comprised of an independent enclosure situated within one of two
166 larger semi-natural, ex-aquaculture ponds (pond size: 30 x 12 m; consistent 1 m depth). Each
167 enclosure comprised of aluminium frames of 1.66 m (length) x 1.05 m (width) x 1.2 m
168 (height) within a net of 7 mm square mesh that prevented fish ingress/ egress but enabled
169 transfer of water and invertebrates. The enclosures provided uniform habitats across the
170 treatments and replicates in which the fish were exposed to the same prey communities. The
171 enclosures in which pellets were fed were located in a separate pond to those with no pellets
172 fed to avoid risk of cross-contamination between treatments. Within their larger ponds, the
173 enclosures were located randomly, with least 0.5 m distance between them for independence.
174 Water temperatures were measured hourly using a temperature logger (TinyTag TGP-4017)
175 placed in the centre of each pond; mean temperature (\pm 95% confidence limits) was $18.2 \pm$
176 0.3 °C in the non-pellet pond and 18.4 ± 0.4 °C in the pellet pond. Anti-predator netting (15
177 mm mesh) was also placed over the top of all enclosures. The enclosures sat on the substrate
178 and macrophytes grew through each of them (primarily *Elodea* spp.)

179

180 The enclosures were placed into the ponds seven days before the fish were introduced, with
181 the experimental period commencing in May 2014 and lasting 100 days. The estimated
182 isotopic turnover was approximately 90% (Thomas & Crowther, 2015). Feeding of pellets
183 used two methods. Firstly, 2 mm pellets were fed via automated feeders (30 per day).
184 Secondly, 3 mm pellets were fed once per week by hand (approximately 60 pellets per
185 replicate). Other than size, the pellets were identical to those used in the first mesocosm
186 experiment, with the same ingredients and constituents (i.e. fishmeal-based, with the same
187 protein and lipid levels; Dynamite Baits, 2017). Following the removal of the enclosures on
188 day 100, the fish were recovered, euthanized (anaesthetic overdose, MS-222) and placed on
189 ice, with samples of macroinvertebrates taken from each enclosure. In the laboratory, fish
190 were re-measured and dorsal muscle samples taken. Macroinvertebrate samples were sorted
191 to species, enabling three samples per species to be dried for SIA (Bašić & Britton, 2016). A
192 random selection of fish dorsal muscle samples (n = 15 to 18 per species and treatment;
193 minimum number of samples per replicate = 5) was then also selected and dried for SIA.

194

195 The field study used the invasive *B. barbuis* and native *S. cephalus* populations of the River
196 Teme, Worcester (52°10'13" N; 2°14'31" W) to test the influence of MDN from pellets on the
197 diet composition and trophic niche size of wild fishes. The study stretch receives considerable
198 angling pressure for *B. barbuis* from both banks throughout the year, but especially between
199 June and October when anglers are present daily, with the majority utilising pellets based on
200 fishmeal. A previous study also indicated *B. barbuis* diet elsewhere on the river
201 (approximately 10 km upstream, with separation by a weir of approximately 2.0 m head)
202 consisted of high proportions of pelletized fishmeal (Bašić *et al.*, 2015). Here, SIA of the
203 fishes utilised scales as only catch and release angling is practised for cyprinid fishes on the

204 river and so the collection of SIA material had to be rapid and non-destructive, but also
205 appropriate for analysis (Hutchinson & Trueman, 2006; Busst & Britton, 2016).

206

207 Samples of *B. barbuis* were captured using a combination of boat mounted electric fishing on
208 the 22nd September 2015 and angling on the 22nd and 23rd September. Samples of *S. cephalus*
209 were captured by angling between 22nd and 30th September 2015. Fish were tagged with
210 passive integrated transponder tags before their release, with no tagged fish recaptured. Each
211 captured fish was measured (fork length (L_f), nearest mm) and three to five scales removed
212 and stored in paper envelopes. Concomitantly, samples of angler bait were taken for SIA.
213 Samples of macroinvertebrates for SIA were collected by kick-sampling. This also provided
214 samples of minnow *Phoxinus phoxinus*, bullhead *Cottus gobio* and stone loach *Barbatula*
215 *barbatula* for SIA (hereafter referred to as ‘small fishes’; all were <40 mm). Triplicate
216 samples were taken of each species, with dorsal muscle samples taken from each ‘small fish’.
217 For SIA, the large body size (> 270 mm) of the sampled *B. barbuis* and *S. cephalus* meant that
218 only material from the very outer portions of scales were used in analyses, i.e. material
219 produced from recent growth (Hutchinson & Trueman, 2006; Bašić *et al.*, 2015).

220

221 *Stable isotope analysis*

222 SIA of all samples was completed at the Cornell Isotope Laboratory, New York, USA, where
223 the dried samples were ground to powder and weighed precisely to ~1000 µg in tin capsules
224 and analysed on a Thermo Delta V isotope ratio mass spectrometer (Thermo Scientific, USA)
225 interfaced to a NC2500 elemental analyser (CE Elantach Inc., USA). Verification for
226 accuracy was against internationally known reference materials and calibrated against the
227 primary reference scales for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Accuracy and precision of the sample runs was
228 tested every 10 samples using a standard animal sample (mink). Overall standard deviation

229 was 0.11‰ for $\delta^{15}\text{N}$ and 0.09 for $\delta^{13}\text{C}$, and analytical precision associated with the $\delta^{15}\text{N}$ and
230 $\delta^{13}\text{C}$ sample runs was estimated at 0.42 and 0.15‰ respectively. Data outputs were in delta
231 (δ) isotope ratios expressed per mille (‰). No lipid correction was applied as C:N ratios
232 indicated very low lipid content (Post *et al.*, 2007).

233

234 In the pond experiment, the 95% confidence limits of the mean SI data for the
235 macroinvertebrates suggested some significant differences between the two larger ponds
236 ('pellet pond': $\delta^{13}\text{C}$: -31.86 ± 1.06 , $\delta^{15}\text{N}$: 5.9 ± 0.66 ‰; 'non-pellet pond': $\delta^{13}\text{C}$: -34.68 ± 1.14 ,
237 $\delta^{15}\text{N}$: 8.49 ± 0.60 ‰). Therefore, to enable true comparison between the pellet and no pellet
238 treatments, the $\delta^{15}\text{N}$ data were transformed to trophic position (TP), using the equation:

$$239 \text{TP}_i = [(\delta^{15}\text{N}_i - \delta^{15}\text{N}_{\text{base}})/3.4]+2$$

240 where TP_i is the trophic position of the individual fish, $\delta^{15}\text{N}_i$ is the isotopic ratio of that fish,
241 $\delta^{15}\text{N}_{\text{base}}$ is the isotopic ratio of the primary consumers (macroinvertebrates), 3.4 is the
242 fractionation between trophic levels and 2 is the trophic position of the baseline organism
243 (Post, 2002). The $\delta^{13}\text{C}$ data were converted to $\delta^{13}\text{C}_{\text{corr}}$ using:

$$244 \delta^{13}\text{C}_{\text{corr}} = \delta^{13}\text{C}_i - \delta^{13}\text{C}_{\text{meaninv}}/\text{CR}_{\text{inv}}$$

245 where $\delta^{13}\text{C}_{\text{corr}}$ is the corrected carbon isotope ratio of the individual fish, $\delta^{13}\text{C}_i$ is the
246 uncorrected isotope ratio of that fish, $\delta^{13}\text{C}_{\text{meaninv}}$ is the mean invertebrate isotope ratio (the
247 'baseline' invertebrates) and CR_{inv} is the invertebrate carbon range ($\delta^{13}\text{C}_{\text{max}} - \delta^{13}\text{C}_{\text{min}}$;
248 Olsson *et al.*, 2009). As stable isotope data from dorsal muscle more closely reflects diet
249 (Grey *et al.*, 2009), then for the fish samples from the field study, their SI scale data were
250 converted to dorsal muscle tissue values before further analysis using conversion values from
251 Busst, Bašić & Britton (2015) that are specific to *B. barbuis* and *S. cephalus*.

252

253 *Testing of stable isotope analysis data*

254 In all cases, the SI data were used to calculate the trophic niche sizes of the fishes, using the
255 isotopic niche. The isotopic niche varies slightly from the trophic niche through factors
256 including growth and metabolic rate of individuals, and thus is used here as an approximation
257 of the trophic niche (Jackson *et al.*, 2011). It was measured using the metric ‘standard ellipse
258 area’ (SEA), a bivariate measure of the distribution of individuals in trophic space (Jackson *et*
259 *al.*, 2012). Each ellipse enclosed ~40% of the data and thus represents the typical resource
260 use within the study population (Jackson *et al.*, 2011; Jackson *et al.*, 2012). Due to relatively
261 small sample sizes, a Bayesian estimate of SEA (SEA_b) was used that utilises a Markov chain
262 Monte Carlo simulation with 10⁴ iterations for each group and provides 95% confidence
263 limits of isotopic niche size (Jackson *et al.*, 2011; R Core Team, 2014). Where appropriate, to
264 indicate how similar fish isotopic niches were in MDN presence/ absence, the extent of niche
265 overlap was also estimated (%).

266

267 Bayesian mixing models then estimated the relative proportions of different food resources
268 contributing to fish diet using the MixSIAR package in R (Parnell *et al.*, 2010; R Core
269 Development Team, 2013; Stock & Semmens, 2013). Correct for isotopic fractionation
270 between resources and consumers used species-specific and tissue-specific fractionation
271 factors between fish and prey ($\delta^{15}\text{N}$: $3.4 \pm 0.98\text{‰}$; $\delta^{13}\text{C}$: $0.39 \pm 1.3\text{‰}$) (Busst, Bašić &
272 Britton, 2015; Busst & Britton, 2016). All models were run using normal run length (chain
273 length: 100,000 iterations with burn-in of 50,000, with posterior thinning (thin: 50) and 3
274 chains). Model diagnostics were based on Gelman-Rubin and Geweke, with sufficient
275 convergence to accept the results (Stock & Semmens, 2013). In mesocosm experiments,
276 models were run with the resources as ‘pellets’ and ‘macroinvertebrates’. The latter was
277 primarily Chironomid larvae, as this was the only putative food resource sampled from each

278 individual mesocosm. However, it also covered *G. pulex*, as some samples were collected
279 from a small proportion of the mesocosms. Their SI data overlapped with Chironomids and
280 so the model could not separate their dietary contributions (mean SI values \pm 95% confidence
281 limits (%): Chironomid: n = 18; $\delta^{13}\text{C}$: -24.08 ± 0.36 , $\delta^{15}\text{N}$: 7.83 ± 0.38 ; *G. pulex*: n = 6; $\delta^{13}\text{C}$:
282 -23.78 ± 0.46 , $\delta^{15}\text{N}$: 8.29 ± 0.24). In the pond experiments, four putative food resources were
283 used: 2 mm pellet, 3 mm pellet and the macroinvertebrate groups Corixidae and Odonata. In
284 the field study, the putative food resources in the model were pooled according to fish pellet
285 1, fish pellet 2, small fishes and Arthropoda (*cf.* Bašić *et al.*, 2015). In addition to the
286 Bayesian mixing models already outlined, these field study data were then also used to assess
287 individual variability using SOLOSIAR ('siarsolomcmc4') in the SIAR package in R
288 (Parnell *et al.*, 2010; R Core Development Team, 2013). In this model, fractionation values
289 were (mean \pm SD): $\delta^{13}\text{C}$: 2.57 ± 0.06 for 'small fishes' and both pellets, and 0.80 ± 0.30 for
290 Arthropoda; $\delta^{15}\text{N}$: 2.4 ± 0.07 for 'small fishes' and both pellets, and 3.0 ± 0.02 for
291 Arthropoda (Busst, Bašić & Britton, 2015; Busst & Britton, 2016).

292

293 *Other data analyses*

294 In the mesocosm and pond experiments, SI data were also tested in linear mixed effect
295 models (LMEM). In the mesocosm experiment, differences were tested in the isotopic data of
296 *B. barbuis* between the four treatments. The dependent variable was $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$, and each
297 model was fitted with mesocosm number as a random effect on the intercept to prevent
298 inflation of the residual degrees of freedom (Tran *et al.*, 2015). The significance of
299 differences in SI data between treatments used estimated marginal means and linearly
300 independent pairwise comparisons with Bonferroni correction for multiple comparisons. In
301 the pond experiment, differences were tested between the species, their allopatric and
302 sympatric treatments, and between the pellet and no pellet treatments. Species were entered

303 into models according to their treatments so, for example, *B. barbuis* was present in models as
304 (1) allopatric *B. barbuis*, (2) in sympatry with *S. cephalus*, and (3) in the presence and absence
305 of pellets. The dependent variable was Ccorr or TP, with each model also fitted with
306 mesocosm number as a random effect. The significance of differences in Ccorr and TP were
307 also determined from the model outputs using linearly independent pairwise comparisons.

308

309 Somatic growth rates were estimated in the mesocosm experiments using incremental length
310 (IL) and specific growth rate (SGR); IL was determined per replicate for each treatment and
311 was expressed as the mean daily growth increment per fish. It was calculated from:

$$312 \quad [((\text{total } L_{t+1}) - (\text{total } L_t)) / 4] / t$$

313 where total L_t and L_{t+1} was the total starting and end lengths of the fish in each replicate, 4
314 represents the number of fish per replicate and t = number of days. Mean specific growth
315 rates (SGR) were determined from:

$$316 \quad 100[(\ln W_{t+1}) - (\ln W_t)] / 4 / t$$

317 where W_t = total starting weight and W_{t+1} = total end weight. In the pond experiments, only
318 incremental length was tested. Using generalised linear models, differences were tested in the
319 growth rate of each species according to their context (allopatric or sympatric) and treatment
320 (pellet or no pellet). In the field study, the scales of the fish were viewed on a projecting
321 microscope and an age estimate derived. Scales measurements of total scale radius (SR) and
322 distance to the penultimate and final annulus (PA and FA respectively) were then taken to
323 enable the last annual length increment (L_{fa}) of the fish to be calculated from:

$$324 \quad L_{fa} = ([FA-PA]/SR) \times L_f.$$

325 Throughout the results, where error is expressed around the mean, it represents 95%
326 confidence limits unless stated otherwise.

327 **Results**

328

329 *Mesocosm experiments*

330 There were no significant differences in starting lengths and weights of the fish across the
331 experimental treatments (generalized linear models: length: Wald $\chi^2 = 0.91$, $P = 0.47$; weight:
332 Wald $\chi^2 = 0.79$, $P = 0.51$). At the conclusion of the experiment, all of the fish were
333 recovered, and their mean length and weight had increased to 120.4 ± 4.1 mm and 18.3 ± 2.0
334 g, with significant differences in final lengths and weights across the treatments (generalized
335 linear model: Wald $\chi^2 = 50.64$, $P < 0.001$). Fish had higher lengths and mass in the Low,
336 Medium and High treatments compared with the Control ($P < 0.001$). The generalized linear
337 model for both SGR and IL was significant (Wald $\chi^2 = 263.9$, $P < 0.001$ and Wald $\chi^2 =$
338 2776.3 , $P < 0.001$ respectively), with growth rates being significantly faster in all treatments
339 compared with the Control ($P < 0.001$; Fig. 1). Both SGR and IL increased as the proportion
340 of pellets fed daily increased (Fig. 1).

341

342 The LMEM revealed significant differences in $\delta^{13}\text{C}$ between *B. barbuis* in the control (mean -
343 $21.4 \pm 0.17\text{‰}$) and the other treatments (Low: $-21.7 \pm 0.2\text{‰}$; Medium: $-22.1 \pm 0.1\text{‰}$; High: -
344 $22.1 \pm 0.1\text{‰}$) ($P < 0.001$; Fig. 2). For $\delta^{15}\text{N}$, the LMEM revealed significant differences
345 between the Control and High treatment (12.4 ± 0.6 vs. $10.6 \pm 1.0\text{‰}$; $P < 0.001$), but not
346 between the Control and the Low and Medium treatments (12.4 ± 0.6 vs. 12.0 ± 1.6 and 11.6
347 $\pm 1.6\text{‰}$ respectively; $P = 1.0$ in all cases; Fig. 2). The 95% confidence limits of the estimates
348 of isotopic niche size (SEA_b) indicated that the niche of the *B. barbuis* in the low treatment
349 was significantly larger than the Control, Medium and High treatments (Table 1; Fig. 2). The
350 isotopic niche of the Control overlapped with that of the Low treatment by 76%, but did not
351 overlap at all with the Medium and High treatments (Table 1; Fig. 2). In the Control,

352 macroinvertebrates were the principal contributor to *B. barbuis* diet, whereas in the Medium
353 and High treatments, pellets contributed up to 48% of diet (Table 1). In the Low treatment,
354 pellets only contributed 23% to estimated diet (Table 1).

355

356 *Pond experiments*

357 Across the treatments, the mean starting lengths of the *B. barbuis* were 77.5 to 82.0 mm and *S.*
358 *cephalus* 73.9 to 81.7 mm (Table 2). At the conclusion of the experiment, 97% of the fish
359 present at the start of the experiment were recovered at the end (174 from 180 fish), with no
360 more than one fish per replicate missing. The length range of the fish had increased to 113.7
361 to 119.4 mm (*B. barbuis*) and 124.6 to 131.1 mm (*S. cephalus*). The generalized linear model
362 testing differences in IL across the species and treatments was significant (Wald $\chi^2 = 105.4$, P
363 = 0.02), with the effect of starting length being a significant covariate ($P = 0.04$). Pairwise
364 comparisons revealed, however, that there were no significant differences in growth rates
365 across the species and their treatments ($P = 0.09$ to 1.0; Fig. 3).

366

367 The LMEM revealed that the significant differences in the corrected $\delta^{13}\text{C}$ data (Ccorr) were
368 primarily between the pellet and no pellet treatments, including between allopatric *B. barbuis*
369 (pellet: 1.92 ± 0.09 ; no pellet: 0.68 ± 0.09 ; $P < 0.001$) and allopatric *S. cephalus* (pellet: 1.84
370 ± 0.09 ; no pellet: 0.25 ± 0.09 ; $P < 0.001$) (Fig. 4). The same differences were also apparent
371 for TP, but with additional differences between the two fishes in the presence and absence of
372 pellets ($P < 0.02$ in all cases), where *B. barbuis* were at a higher TP than *S. cephalus* (Fig. 4).
373 Isotopic niche estimates revealed that there was no overlap in the niches of the two fishes in
374 allopatry or sympatry, or in the presence and absence of pellets, but the availability of pellets
375 caused a substantial shift in the position of the isotopic niche of both fishes in both allopatry
376 and sympatry (Fig. 4). This shift was caused by the presence of the pellets in fish diet; where

377 present, their contribution to fish diet was 43 and 58% (Table 3). In terms of isotopic niche
378 size, however, there was considerable overlap in the 95% confidence limits of estimates of
379 SEA_b for the species in the presence/ absence of pellets in their allopatric and sympatric
380 contexts, thus the pellets did not affect isotopic niche size (Table 4).

381

382 *Wild fishes*

383 A total of 31 *B. barbuis* were sampled from the River Teme in September 2015. Of these, 19
384 were captured by electric fishing (mean length 512.1 ± 63.8 mm) and 12 by angling (mean
385 length 616.8 ± 72.7 mm), with the differences in their lengths being significant (ANOVA:
386 $F_{1,29} = 5.56$, $P = 0.03$). Across this dataset, there was also a significant relationship between
387 fish length and SI data ($\delta^{13}C$: $R^2 = 0.42$, $F_{1,29} = 20.61$, $P < 0.001$; $\delta^{15}N$: $R^2 = 0.32$, $F_{1,29}$
388 $= 13.50$, $P < 0.001$). To remove this ontogenetic influence of length on the SI data, the six fish
389 captured by electric fishing of < 400 mm length were removed from the dataset, resulting in
390 the relationships between fish length and SI data now being non-significant ($\delta^{13}C$: $R^2 = 0.10$,
391 $F_{1,23} = 2.30$, $P = 0.13$; $\delta^{15}N$: $R^2 = 0.09$, $F_{1,23} = 2.18$, $P = 0.15$). This also increased the mean
392 length of the electric fished *B. barbuis* to 585.8 ± 55.9 mm ($n = 13$), with this not significantly
393 different to the angler caught fish (ANOVA: $F_{1,23} = 0.96$, $P = 0.34$). In addition, 6 *S. cephalus*
394 were sampled by angling (length range: 400 to 540 mm; mean length 456.7 ± 51.3 mm), with
395 none sampled by electric fishing. Regarding the age of the *B. barbuis* > 400 mm, there was
396 only one individual age at 8+ years, with the remainder all between 11+ and 18+ years. At
397 these ages, their annual length increments were relatively low (mean last annual length
398 increment: 18.7 ± 4.1 mm), with the relationship between length increment and the SI data
399 being non-significant ($\delta^{13}C$: $R^2 = 0.04$, $F_{1,23} = 0.67$, $P = 0.42$; $\delta^{15}N$: $R^2 = 0.08$, $F_{1,23} = 1.56$, P
400 $= 0.23$.

401

402 For the *B. barbuis* > 400 mm sampled by electric fishing, their isotopic niche was
403 significantly larger than the angled fish (95% CL SEA_b: 2.54 to 6.66 vs. 0.66 to 2.30‰; Fig.
404 5). The angled sub-set of *B. barbuis* shared 83% of their isotopic space with those that were
405 electric fished (Fig. 5). The angled *S. cephalus* had an isotopic niche in a similar position to
406 the angled *B. barbuis* and they also had a similar niche size (95% CL SEA_b: 0.63 to 4.28‰;
407 Fig. 5). The estimated dietary contributions from the Bayesian mixing models suggested that
408 the angled *B. barbuis* and *S. cephalus* had total contributions of pellets of 59 and 44%
409 respectively, whereas this was reduced to 39% for the electric fished individuals of > 400 mm
410 (Table 5a). At the individual level, estimated dietary proportions varied by sampling method,
411 but with generally lower proportions of pellets in the diet of electric fished *B. barbuis* (range 9
412 to 62%) than angled (range 40 to 71%) (Table 5b). The coefficient of variation was also
413 higher for all food items for electric fished *B. barbuis*, but this was especially strong for
414 pellets (electric fished: 0.45; angled: 0.17; Table 5b). The overall range of the contribution of
415 pellets to *B. barbuis* diet, irrespective of sampling method, was 9 to 71% (Table 5b).

416

417 **Discussion**

418

419 The two experiments revealed that where fishmeal pellets were present as a food resource for
420 *B. barbuis* and *S. cephalus*, these were generally consumed in sufficient proportions to alter
421 the SI signatures of their tissues, as per the hypothesis, and resulted in major shifts in the
422 position of their population isotopic niche. In wild *B. barbuis*, where fish were sampled by
423 both angling and electric fishing, there was considerable individual variability in the
424 contribution of pellets to diet, ranging between 9 and 71%; where only angled fish were
425 considered then the range was 40 to 71%. High estimates of contributions of pellets to *S.*
426 *cephalus* diet were also apparent, with these all captured by angling. The largest isotopic

427 niches were apparent in the ‘Low’ treatment of the mesocosm experiment and in the wild *B.*
428 *barbus* captured by both angling and electric fishing. This was likely to be the result of the
429 diets of the individual fish comprising of a greater variety of dietary items, in which MDN
430 pellets were important items for only some individuals. Regarding somatic growth rates,
431 whilst these were significantly higher in the ‘medium’ and ‘high’ treatments compared to the
432 control and ‘low’ treatment in the mesocosm experiment, there were no significant
433 differences in the growth rates of the fishes detected in the pond experiment, and there was
434 no relationship between annual length increments and the SI data for the wild fishes. Thus,
435 despite the pellets being consumed and assimilated into the fish tissues across the study
436 approaches, it was only in very controlled conditions where feeding on pellets facilitated
437 faster growth rates, and then only when they were available in relatively high quantities. This
438 finding was generally contrary to the hypothesis.

439

440 Recent studies have suggested that where *B. barbus* populations are enhanced with hatchery
441 reared individuals via stocking then there are strong patterns in isotopic niche partitioning
442 between these fish and other wild fishes, including *S. cephalus* (Bašić & Britton, 2016). This
443 partitioning is also evident between larger individuals, suggesting functional differences
444 between the species result in these trophic differences (Bašić & Britton, 2015, 2016). This
445 isotopic niche partitioning between *B. barbus* and *S. cephalus* was also apparent here, with
446 the species having distinct niches in the presence and absence of pellets. Thus, even where
447 the fishes feed on pellets in relatively high proportions, such as in the ‘pellet pond’ of the
448 pond experiments, their functional differences were still sufficient to result in differences in
449 the position of their isotopic niches. Reasons for these inter-specific isotopic niches
450 differences might relate to differences in the proportions of macroinvertebrates consumed
451 between the species and differences in the stable isotope ecology between *B. barbus* and *S.*

452 *cephalus*, for example through differences in their fractionation factors (Busst, Bašić &
453 Britton, 2015; Busst & Britton, 2016). Irrespective, in this pond experiment, the growth rates
454 and sizes of the isotopic niches of both fishes were not significantly different between their
455 allopatric and sympatric contexts in both pellet presence and absence, suggesting that the
456 fishes were accessing sufficient food resources to maintain their growth rates without having
457 to further alter their diet.

458

459 It was apparent that all of the fish sampled by angling from the River Teme, both here and in
460 Bašić *et al.* (2015), generally had diets comprising relatively high proportions of MDN (up to
461 80% in Bašić *et al.* 2015), yet for *B. barbuis* sampled by electric fishing, there was much
462 greater variability in this MDN contribution, with this independent of body size. This
463 suggests that despite the attractiveness of fishmeal pellets to *B. barbuis* generally, resulting in
464 some individuals developing trophic specialisations, other individuals primarily consumed
465 other items, perhaps through avoiding consuming pellets due to previous angler capture
466 experiences that lead to avoidance (Raaij, 1985; Askey *et al.*, 2006). This also emphasises the
467 potential bias that can result from samples collected by angling alone, as individual
468 variability in the behaviour of individuals can affect capture susceptibility (Klefoth *et al.*,
469 2013).

470

471 It was apparent that the MDN from the pellets was being consumed directly by the fishes,
472 with the stable isotope data of the macroinvertebrates and fish suggesting there was no
473 indirect transfer via prey populations. This is in contrast to the transfer of MDN into
474 freshwaters via migratory salmonid fishes, where the nutrients are more freely available and
475 facilitate the increased production of benthic algae and macroinvertebrates (Schindler *et al.*,
476 2003). This then enhances the food resources available for the larvae and juveniles of the

477 adult migrants, facilitating their feeding, growth and survival in the early life stages (Wipfli *et*
478 *al.*, 2003). The MDN from salmonids can thus be traced through freshwater food webs,
479 enabling assessment of the links between the aquatic and terrestrial food webs. For example,
480 Tonra *et al.* (2015) reported on the removal of Elwha River dam in the USA, which resulted
481 in migratory salmonids returning to the river within 12 months. Following reproduction and
482 death of these fishes, their MDN could be traced through the macroinvertebrate community
483 and then into a bird that preys upon these, the American dipper *Cinclus mexicanus*. Indeed,
484 there are now numerous studies that have traced MDN into terrestrial food webs (e.g.
485 McLoughlin *et al.*, 2016; Richardson *et al.*, 2016), with its influence even affecting the
486 behaviour of terrestrial predator and scavenger species (Schindler *et al.*, 2013).

487

488 In contrast, the apparent direct transfer of MDN from fishmeal pellet to *B. barbuis* and *S.*
489 *cephalus* in this study suggested that this nutrient subsidy might have only minor impacts on
490 the non-fish communities. In the wild, the fish consuming these pellets tend to be large-
491 bodied and thus are only likely to be predated upon by large piscivores, including otter *Lutra*
492 *lutra*, although otters tend to prefer to consume high abundances of smaller bodied fishes
493 (Britton *et al.*, 2006). Unlike salmonid fishes, *B. barbuis* and *S. cephalus* are relatively long-
494 lived (> 15 years; Britton, 2007; Britton *et al.* 2013), reproducing annually following sexual
495 maturity (Britton & Pegg, 2011), and thus there is no large post-spawning die-off.
496 Consequently, they might be acting as MDN sinks, with low rates of nutrient transfer to
497 higher trophic levels. However, determining the extent of MDN transfer to higher trophic
498 levels requires further work. There might also be some alternative ecological benefits of this
499 MDN subsidy. For example, in many European rivers, including the River Teme, *B. barbuis* is
500 a large-bodied invasive fish that potentially impacts prey populations and competes with
501 functional analogues (Antognazza *et al.*, 2016). Whilst recent studies suggest some trophic

502 (isotopic) partitioning between *B. barbus* and other fishes in riverine communities (Bašić &
503 Britton, 2015, 2016), the high proportion of fishmeal pellets detected in the diet of wild
504 fishes, both here and in Bašić *et al.* (2015), suggests this trophic subsidy could potentially
505 lead to further partitioning between fish populations across the fish communities. This is also
506 likely to reduce invasive *B. barbus* predation pressure on macroinvertebrate communities, as
507 their dietary requirements are primarily met by the consumption of this angler subsidy.

508

509 These results add to an increasing literature base on the role of subsidies from fishery
510 activities in the trophic ecology of freshwater communities. For example, Grey, Waldron &
511 Hutchinson (2004) demonstrated that approximately 65% of *Daphnia* spp. and over 80% of
512 roach *Rutilus rutilus* body carbon was ultimately derived from pellet material originating
513 from an *in situ* fish farm in Esthwaite Water, England. These data suggest that the MDN were
514 more freely available within the lake via the breakdown of the pellets, with a number of other
515 studies also revealing their integration into the food web more generally (Fernandez-Jover *et*
516 *al.*, 2011a,b; Demétrio *et al.*, 2012; Jackson *et al.*, 2013). Thus, further work is suggested in
517 riverine systems where fishmeal pellets are used by anglers to identify whether there is
518 greater transfer of MDN in the food web than suggested here.

519

520 In summary, across three spatial scales of increasing complexity, it was apparent that the
521 release of fishmeal pellets into freshwaters as an allochthonous trophic subsidy based on
522 MDN had a substantial influence on the isotopic niche (as a proxy of the trophic niche) of
523 riverine fishes. Results from wild *B. barbus*, with some support from the experiments,
524 indicated that individual isotopic niche specialisation resulting from this trophic subsidy was
525 strongly apparent, with its development potentially associated with behavioural differences

526 between individual fish that leads to variability in their avoidance/ consumption of pellets and
527 thus their likelihood of angler capture.

528

529 **Acknowledgments**

530

531 CGR was supported by a studentship funded by the Severn Rivers Trust and TB was
532 supported by a studentship funded by the Environment Agency and Barbel Society. F.A-T
533 holds a doctoral fellowship from the Spanish Ministry of Education (FPU13/00235). We
534 specifically thank Brecht Morris, Alan Henshaw and Pete Reading for their assistance in
535 obtaining samples from the River Teme. All work was completed under project licence and
536 personal licences from the UK Home Office.

537

538 **References**

539

540 Antognazza C.M., Andreou D., Zaccara S. & Britton J.R. (2016) Loss of genetic integrity and
541 biological invasions result from stocking and introductions of *Barbus barbus*: insights
542 from rivers in England. *Ecology and Evolution*, **6**, 1280-1292.

543 Araújo M.S., Bolnick D.I. & Layman C.A. (2011) The ecological causes of individual
544 specialisation. *Ecology Letters*, **14**, 948-958.

545 Arlinghaus R. & Niesar N. (2005) Nutrient digestibility of angling baits for carp, *Cyprinus*
546 *carpio*, with implications for groundbait formulation and eutrophication control. *Fisheries*
547 *Management and Ecology*, **12**, 91-97

548 Askey P.J., Richards S.A., Post J.R. & Parkinson E.A. (2006) Linking angling catch rates and
549 fish learning under catch-and-release regulations. *North American Journal of Fisheries*
550 *Management*, **26**, 1020-1029.

551 Bašić T. & Britton J.R. (2015) Utility of fish scales from stock assessment surveys in stable
552 isotope analysis for initial assessments of trophic relationships in riverine fish
553 communities. *Journal of Applied Ichthyology*, **31**, 296-300.

554 Bašić T., Britton J.R., Jackson M.C., Reading P. & Grey J. (2015) Angling baits and invasive
555 crayfish as important trophic subsidies for a large cyprinid fish. *Aquatic Sciences*, **77**, 153-
556 160.

557 Bašić T. & Britton J.R. (2016) Characterising the trophic niches of stocked and resident
558 cyprinid fishes: consistency in partitioning over time, space and body sizes. *Ecology and
559 Evolution* **6**, 5093-5104.

560 Britton J.R., Pegg J., Shepherd J.S. & Toms S. (2006) Revealing the prey items of the otter
561 *Lutra lutra* in South West England using stomach contents analysis. *Folia Zoologica*, **55**,
562 167-174

563 Britton J.R. (2007) Reference data for evaluating the growth of common riverine fishes in the
564 UK. *Journal of Applied Ichthyology*, **23**, 555-560.

565 Britton J.R. & Pegg J. (2011) Ecology of European Barbel *Barbus barbus*: Implications for
566 river, fishery, and conservation management. *Reviews in Fisheries Science*, **19**, 321-330.

567 Britton J.R., Davies G.D. and Pegg J. (2013) Spatial variation in the somatic growth rates of
568 European barbel *Barbus barbus*: a UK perspective. *Ecology of Freshwater Fish*, **22**, 21-
569 29.

570 Britton J.R. & Andreou D. (2016) Parasitism as a Driver of Trophic Niche Specialisation.
571 *Trends in Parasitology*, **32**, 437-445.

572 Busst G., Bašić T. & Britton J.R. (2015) Stable isotope signatures and trophic-step
573 fractionation factors of fish tissues collected as non-lethal surrogates of dorsal muscle.
574 *Rapid Communications in Mass Spectrometry*, **29**, 1535-1544.

575 Busst G.M. & Britton J.R. (2016) High variability in stable isotope diet–tissue discrimination

576 factors of two omnivorous freshwater fishes in controlled ex situ conditions. *Journal of*
577 *Experimental Biology*, **219**, 1060-1068.

578 Demétrio J.A., Gomes L.C., Latini J.D. & Agostinho A.A. (2012) Influence of net cage
579 farming on the diet of associated wild fish in a Neotropical reservoir. *Aquaculture*, **330-**
580 **333**, 172-178.

581 Dynamite Baits (2017) Marine Halibut Pellets.
582 <http://www.dynamitebaits.com/products/p/marine-halibut-pellets>. Last accessed
583 17/01/2017.

584 Fernandez-Jover D., Arechavala-Lopez P., Martinez-Rubio L., Tocher D.R., Bayle-Sempere
585 J.T., Lopez-Jimenez J.A., Martinez-Lopez F.J. & Sanchez-Jerez P. (2011a) Monitoring the
586 influence of marine aquaculture on wild fish communities: benefits and limitations of fatty
587 acid profiles. *Aquaculture Environment Interactions*, **2**, 39-47.

588 Fernandez-Jover D., Martinez-Rubio L., Sanchez-Jerez P., Bayle-Sempere J.T., Lopez
589 Jimenez J.A., Martínez Lopez F.J., Bjørn P.A., Uglem I. & Dempster T. (2011b) Waste
590 feed from coastal fish farms: a trophic subsidy with compositional side-effects for wild
591 gadoids. *Estuarine and Coastal Shelf Science*, **91**, 559-568.

592 Grey J., Waldron S. & Hutchinson R. (2004) The utility of carbon and nitrogen isotope
593 analyses to trace contributions from fish farms to the receiving communities of freshwater
594 lakes: a pilot study in Esthwaite Water, UK. *Hydrobiologia*, **524**, 253-262.

595 Grey J., Graham C.T., Britton J.R. & Harrod C. (2009) Stable isotope analysis of archived
596 roach (*Rutilus rutilus*) scales for retrospective study of shallow lake responses to nutrient
597 reduction. *Freshwater Biology*, **54**, 1663-1670.

598 Hobson K.A. & Clark R.G. (1992) Assessing avian diets using stable isotopes I: turnover of
599 ¹³C in tissues. *Condor*, **94**, 181-188.

600 Hutchinson J.J. & Trueman C.N. (2006) Stable isotope analyses of collagen in fish scales:
601 limitations set by scale architecture. *Journal of Fish Biology*, **69**, 1874-1880.

602 Jackson A.L., Inger R., Parnell A.C. & Bearhop S. (2011) Comparing isotopic niche widths
603 among and within communities: SIBER–Stable Isotope Bayesian Ellipses in R. *Journal of*
604 *Animal Ecology*, **80**, 595-602.

605 Jackson M.C., Donohue I., Jackson A.L., Britton J.R., Harper D.M. & Grey J. (2012)
606 Population-level metrics of trophic structure based on stable isotopes and their application
607 to invasion ecology. *PLoS ONE* **7**:e31757.

608 Jackson M.C., Allen R., Pegg J. & Britton J.R. (2013) Do trophic subsidies affect the
609 outcome of introductions of a non-native freshwater fish? *Freshwater Biology*, **58**, 2144-
610 2153.

611 Jefferies R.L. (2000) Allochthonous inputs: integrating population changes and food-web
612 dynamics. *Trends in Ecology and Evolution*, **15**, 19-22.

613 Jones R.I., Grey J., Sleep D. & Quarmby C. (1998) An assessment, using stable isotopes, of
614 the importance of allochthonous organic carbon sources to the pelagic food web in Loch
615 Ness. *Proceedings of the Royal Society of London: Biological Sciences*, **265**, 105-110.

616 Klefoth T., Pieterek T. and Arlinghaus R. (2013) Impacts of domestication on angling
617 vulnerability of common carp, *Cyprinus carpio*: the role of learning, foraging behaviour
618 and food preferences. *Fisheries Management and Ecology*, **20**, 174-186.

619 Marcarelli A.M., Baxter C.V., Mineau M.M. & Hall R.O. (2011) Quantity and quality:
620 unifying food web and ecosystem perspectives on the role of resource subsidies in
621 freshwaters. *Ecology*, **92**, 1215-1225.

622 Marczak L.B., Thompson R.M. & Richardson, J.S. (2007) Meta-analysis: trophic level,
623 habitat, and productivity shape the food web effects of resource subsidies. *Ecology*, **88**,
624 140-148.

625 McLoughlin P.D., Lysak K., Debeffe L., Perry T. & Hobson K.A. (2016) Density-dependent
626 resource selection by a terrestrial herbivore in response to sea-to-land nutrient transfer by
627 seals. *Ecology*, **97**, 1929-1937.

628 Naylor R.L., Goldburg R.J., Primavera J.H., Kautsky N., Beveridge M.C.M., Clay J., Folke
629 C., Lubchenco J., Mooney H. & Troell M. (2000) Effect of aquaculture on world fish
630 supplies. *Nature*, **405**, 1017-1024.

631 Olsson K., Stenroth P., Nyström P. & Graneli W. (2009) Invasions and niche width: Does
632 niche width of an introduced crayfish differ from a native crayfish? *Freshwater Biology*,
633 **54**, 1731–1740.

634 Parnell A.C., Inger R., Bearhop S. & Jackson A.L. (2010) Source partitioning using stable
635 isotopes: Coping with too much variation. *PLoS ONE*, **5**, e9672.

636 Polis G.A. & Hurd S.D. (1995) Extraordinarily high spider densities on islands: flow of
637 energy from the marine to terrestrial food webs and the absence of predation. *Proceedings*
638 *of the National Academy of Sciences USA*, **92**, 4382-4386.

639 Post D.M. (2002) Using stable isotopes to estimate trophic position: Models, methods, and
640 assumptions. *Ecology*, **83**, 703-718.

641 Post D.M., Layman C.A., Arrington D.A., Takimoto G., Quattrochi J. & Montana C.G.
642 (2007) Getting to the fat of the matter: models, methods and assumptions for dealing with
643 lipids in stable isotope analyses. *Oecologia*, **152**, 179-189.

644 R Development Core Team (2013) R: A language and environment for statistical computing.

645 Raat A.J.P. (1985) Analysis of angling vulnerability of common carp, *Cyprinus carpio* L., in
646 catch-and-release angling in ponds. *Aquaculture Research*, **16**, 171-187.

647 Richardson D.P., Kohler A.E., Hailemichael M. & Finney B.P. (2016) The fate of marine-
648 derived nutrients: tracing $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ through oligotrophic freshwater and linked

649 riparian ecosystems following salmon carcass analog additions. *Canadian Journal of*
650 *Fisheries and Aquatic Sciences*, **73**, 1-15.

651 Sato T. & Watanabe K. (2013) Do stage-specific functional responses of consumers dampen
652 the effects of subsidies on trophic cascades in streams? *Journal of Animal Ecology*, **83**,
653 907-915.

654 Schindler D.E., Scheuerell M.D., Moore J.W., Gende S.M., Francis T.B. & Palen W.J. (2003)
655 Pacific salmon and the ecology of coastal ecosystems. *Frontiers in Ecology and the*
656 *Environment*, **1**, 31-37.

657 Schindler D.E., Leavitt P.R., Brock C.S., Johnson S.P. & Quay P.D. (2005) Marine-derived
658 nutrients, commercial fisheries and production of salmon and lake algae in Alaska.
659 *Ecology*, **86**, 3225-3231.

660 Schindler D.E., Armstrong J.B., Bentley K.T., Jankowski K., Lisi P.J. & Payne L.X. (2013)
661 Riding the crimson tide: mobile terrestrial consumers track phenological variation in
662 spawning of an anadromous fish. *Biology Letters*, **9**, p.20130048.

663 Stock B.C. & Semmens B.X. (2013). MixSIAR GUI User Manual. Version 3.1.
664 <https://github.com/brianstock/MixSIAR/>. doi:10.5281/zenodo.47719. Last accessed
665 17/09/2016.

666 Syrjanen J., Korsu K., Louhi P., Paavola R. & Muotka T. (2011) Stream salmonids as
667 opportunistic foragers: the importance of terrestrial invertebrates along a stream-size
668 gradient. *Canadian Journal of Fisheries and Aquatic Sciences*, **68**, 2146-2156.

669 Thomas S.M. & Crowther T.W. (2015) Predicting rates of isotopic turnover across the animal
670 kingdom: a synthesis of existing data. *Journal of Animal Ecology*, **84**, 861-870.

671 Tonra C.M., Sager-Fradkin K., Morley S.A., Duda J.J. & Marra P.P. (2015) The rapid return
672 of marine-derived nutrients to a freshwater food web following dam removal. *Biological*
673 *Conservation*, **192**, 130-134.

- 674 Tran T.N.Q., Jackson M.C., Sheath D., Verreycken H. & Britton J.R. (2015) Patterns of
675 trophic niche divergence between invasive and native fishes in wild communities are
676 predictable from mesocosm studies. *Journal of Animal Ecology*, **84**, 1071–1080.
- 677 Wipfli M.S., Hudson J.P., Caouette J.P. & Chaloner D.T. (2003) Marine subsidies in
678 freshwater ecosystems: salmon carcasses increase the growth rates of stream-resident
679 salmonids. *Transactions of the American Fisheries Society*, **132**, 371-381.
- 680 Zhang Y., Negishi J.N., Richardson J.S. & Kolodziejczyk R. (2003) Impacts of marine-
681 derived nutrients on stream ecosystem functioning. *Proceedings of the Royal Society of
682 London B: Biological Sciences*, **270**, 2117-2123.

Table 1. Mean lengths and weights, isotopic niche size (as 95% CL of standard ellipse area, SEA_b) of *Barbus barbuis* per treatment and the extent of their overlap between treatments, and the estimated contributions of putative foods to their diet (0 – 1 scale), as predicted in MixSIAR ($\pm 95\%$ CL). Sample sizes were n = 15 per treatment.

Treatment	Mean length (mm)		Mean weight (g)		SEA _b (‰)	Overlap in isotopic niche with Control (%)	Estimated contribution to diet (%)	
	Start	End	Start	End			Macroinvertebrate	Pellet
Control	106.5 ± 8.5	108.2 ± 8.3	9.9 ± 1.8	11.2 ± 2.2	0.06 – 0.21	n /a	0.97 ± 0.02	0.03 ± 0.02
Low	103.8 ± 5.9	113.3 ± 6.6	10.2 ± 1.2	14.7 ± 2.5	0.39 – 1.31	76	0.77 ± 0.02	0.23 ± 0.02
Medium	105 ± 3.9	127.3 ± 3.9	12.3 ± 1.0	22.9 ± 2.5	0.10 – 0.33	0	0.52 ± 0.02	0.48 ± 0.02
High	106.6 ± 4.1	132.7 ± 6.6	11.6 ± 0.9	24.3 ± 3.4	0.08 – 0.28	0	0.54 ± 0.02	0.47 ± 0.02

Table 2. Number of fish per species and treatment analysed for stable isotope analysis from the pond enclosure experiment, their start and end mean lengths (\pm 95% CL), and mean stable isotope values (\pm 95% CL).

Treatment	Species	n	Mean starting length (mm)	Mean end length (mm)	Mean $\delta^{13}\text{C}$ (‰)	Mean $\delta^{15}\text{N}$ (‰)
Allopatry/pellets	<i>B. barbuis</i>	18	80.1 \pm 0.3	117.83 \pm 1.99	-24.70 \pm 0.21	9.39 \pm 0.10
Allopatry/pellets	<i>S. cephalus</i>	18	81.7 \pm 0.4	131.06 \pm 1.38	-25.10 \pm 0.23	8.44 \pm 0.04
Allopatry/no pellets	<i>B. barbuis</i>	18	77.6 \pm 0.2	113.67 \pm 1.32	-28.20 \pm 0.20	11.18 \pm 0.05
Allopatry/no pellets	<i>S. cephalus</i>	17	73.9 \pm 0.3	124.59 \pm 1.69	-30.31 \pm 0.19	10.72 \pm 0.05
Sympatry/pellets	<i>B. barbuis</i>	15	82.0 \pm 0.4	119.4 \pm 1.84	-25.45 \pm 0.18	9.25 \pm 0.09
Sympatry/pellets	<i>S. cephalus</i>	15	76.3 \pm 0.4	125.27 \pm 1.69	-24.94 \pm 0.20	8.34 \pm 0.04
Sympatry/no pellets	<i>B. barbuis</i>	15	77.5 \pm 0.3	118.94 \pm 1.91	-29.05 \pm 0.11	10.79 \pm 0.05
Sympatry/no pellets	<i>S. cephalus</i>	15	76.1 \pm 0.4	126.73 \pm 1.64	-30.67 \pm 0.14	10.81 \pm 0.03

Table 3. Estimated contributions (0 – 1) of each putative food item to fish diet in the ‘pellet’ treatments of the pond enclosure experiment. Values represent mean estimated dietary proportions (\pm 95% CL) from MixSIAR.

	Corixidae	Odonata	2mm pellet	3mm pellet	Total pellet*
Allopatric <i>B. barbuis</i> (n=18)	0.34 \pm 0.11	0.21 \pm 0.13	0.27 \pm 0.06	0.18 \pm 0.06	0.45
Allopatric <i>S. cephalus</i> (n=15)	0.26 \pm 0.04	0.16 \pm 0.05	0.33 \pm 0.04	0.25 \pm 0.04	0.58
Sympatric <i>B. barbuis</i> (n=18)	0.32 \pm 0.11	0.22 \pm 0.12	0.25 \pm 0.06	0.22 \pm 0.07	0.47
Sympatric <i>S. cephalus</i> (n=15)	0.25 \pm 0.09	0.15 \pm 0.10	0.33 \pm 0.09	0.27 \pm 0.11	0.60

* derived from additional of the modal estimations of the 2mm and 3mm pellet and so no estimate of error around the values are provided.

Table 4. Isotopic niche size, as 95% CL of SEA_b (‰) for *Barbus barbuis* and *Squalius cephalus* in the different treatments of the pond enclosure experiment, and as calculated from corrected stable isotope data. Sample sizes were as per Table 3.

	n	No fishmeal pellet	Fishmeal pellet
Allopatric <i>B. barbuis</i>	18	0.02 – 0.05	0.03 – 0.09
Sympatric <i>B. barbuis</i>	18	0.01 – 0.03	0.02 – 0.04
Allopatric <i>S. cephalus</i>	15	0.02 – 0.05	0.02 – 0.05
Sympatric <i>S. cephalus</i>	15	0.01 – 0.02	0.01 – 0.04

Table 5. (a) Mean contributions to fish diet of putative food resources (0 – 1 scale; \pm 95% CL) of *Barbus barbuis* and *Squalius cephalus* in the River Teme by sampling method, estimated by MixSIAR; (b) minimum, maximum, mean (\pm 95% CL) and coefficient of variation (CV) of estimates of contributions to individual *B. barbuis* diet (0 – 1) of the putative foods per sampling method (EF: electric fishing; A: angling), estimated by SOLOSIAR, where mean pellet data represents the sum of mean Pellet 1 and mean Pellet 2 per individual fish. Only *B. barbuis* of > 400 mm length were used in analyses.

(a)

Species	n	Arthropoda	'Small fishes'	Pellet 1	Pellet 2	Total pellet*
Electric fished <i>B. barbuis</i>	13	0.39 \pm 0.10	0.26 \pm 0.09	0.10 \pm 0.04	0.26 \pm 0.04	0.36
Angled <i>B. barbuis</i>	12	0.22 \pm 0.07	0.20 \pm 0.06	0.11 \pm 0.03	0.48 \pm 0.04	0.59
Angled <i>S. cephalus</i>	6	0.23 \pm 0.11	0.24 \pm 0.10	0.15 \pm 0.06	0.39 \pm 0.08	0.54

* derived from additional of the modal estimations of the 2mm and 3mm pellet and so no estimate of error around the values are provided.

(b)

Dietary item	Minimum		Maximum		Mean		CV	
	EF	A	EF	A	EF	A	EF	A
Arthropod	0.07	0.13	0.45	0.30	0.19 \pm 0.09	0.18 \pm 0.05	0.82	0.68
Small fish	0.18	0.16	0.50	0.43	0.23 \pm 0.10	0.24 \pm 0.05	0.81	0.69
Pellet	0.09	0.40	0.62	0.71	0.38 \pm 0.09	0.59 \pm 0.06	0.45	0.17

Figure captions

Figure 1. Somatic growth rates, as specific growth rate (A) and incremental length (B) per treatment for *Barbus barbuis* in the mesocosm experiment. Values represent estimated marginal means from the generalized linear models and * indicates the difference in growth rate is significant at $P < 0.001$) between the treatment and the control according to linearly independent pairwise comparisons. Error bars represent 95% confidence limits.

Figure 2. Stable isotope bi-plot of *Barbus barbuis* in the 250 L mesocosms and their isotopic niche (as standard ellipse area, SEA_c), where clear triangles are the control fish and solid black line is their isotopic niche, filled triangles are the low treatment fish and the dashed black line is their isotopic niche, clear circles are the medium treatment fish and the solid light grey line is their isotopic niche, and grey circles are the high treatment fish and the dark grey line is their isotopic. × represent Chironomid larvae and + represent the fishmeal pellets fed daily.

Figure 3. Somatic growth rates, as incremental length, of *Barbus barbuis* (filled circles) and *Squalius cephalus* (clear circles) per treatment in the pond enclosure experiment. BAP: allopatric *B. barbuis* with pellets; BAN: allopatric *B. barbuis*, no pellets; BSP: sympatric *B. barbuis* with pellets; BSN: sympatric *B. barbuis*, no pellets; CAP: allopatric *S. cephalus* with pellets; CAN: allopatric *S. cephalus*, no pellets; CSP: sympatric *S. cephalus* with pellets; CSN: sympatric *S. cephalus*, no pellets. Error bars represent 95% confidence limits.

Figure 4. Stable isotope biplots (of corrected stable isotope data to trophic position and corrected carbon, C_{corr}) showing individual data points (as symbols) and the isotopic niche (as standard ellipse area, SEA_c) for (A) allopatric *Squalius cephalus* in the no pellet (clear

circle, solid black line) and pellet treatment (filled circle, dashed black line); (B) allopatric *Barbus barbuis* in the no pellet (clear square, solid grey line) and pellet treatment (filled square, dashed grey line); and (C) sympatric *S. cephalus* in the no pellet (clear circle, solid black line) and pellet treatment (filled circle, dashed black line), and sympatric *B. barbuis* in the no pellet (clear square, solid grey line) and pellet treatment (filled square, dashed grey line).

Figure 5. Stable isotope bi-plot of the lower River Teme, showing individual data points and isotopic niches (as standard ellipse areas). *Barbus barbuis* (electric fishing; length range 401 to 770 mm; n = 13): data points: black circles, solid black line: isotopic niche; *Barbus barbuis* (angling, length range 520 to 721 mm; n = 12): data points: clear circles, dashed black line: isotopic niche; *Squalius cephalus* (angling, length range 400 to 540 mm; n = 6): data points: clear squares, solid grey line: isotopic niche, Grey circles are combined data for 'small fishes' (*Cottus gobio*, *Barbatula barbatula*, *Phoxinus phoxinus*); + fishmeal pellet 1; × fishmeal pellet 2; black triangle: Arthropoda.

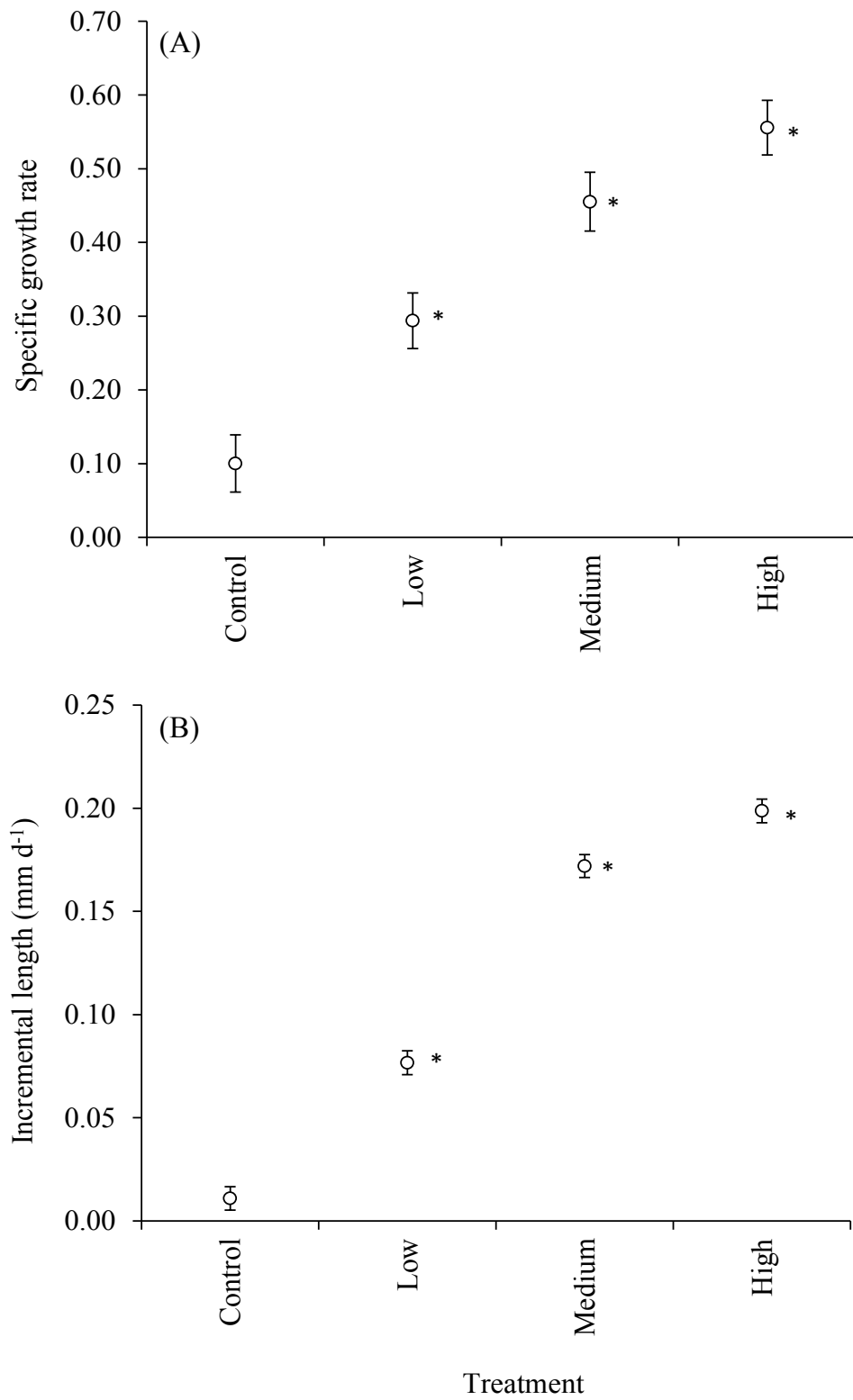


Figure 1.

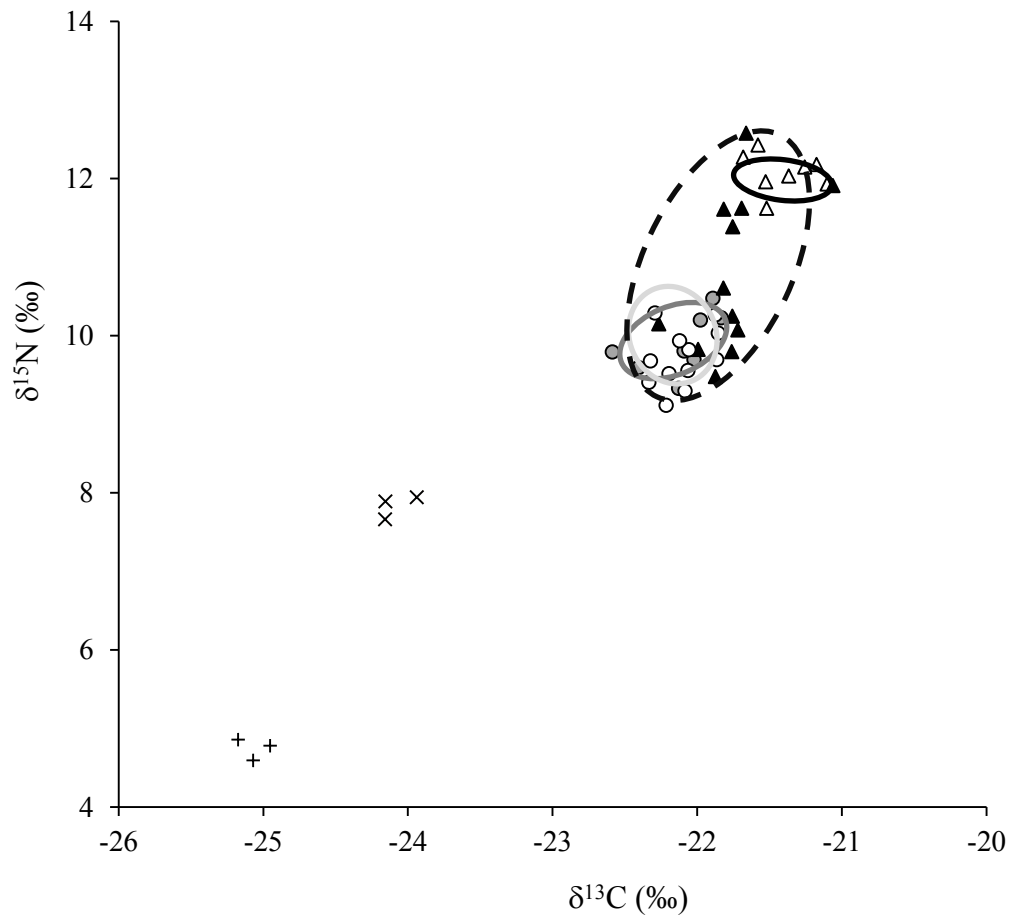


Figure 2.

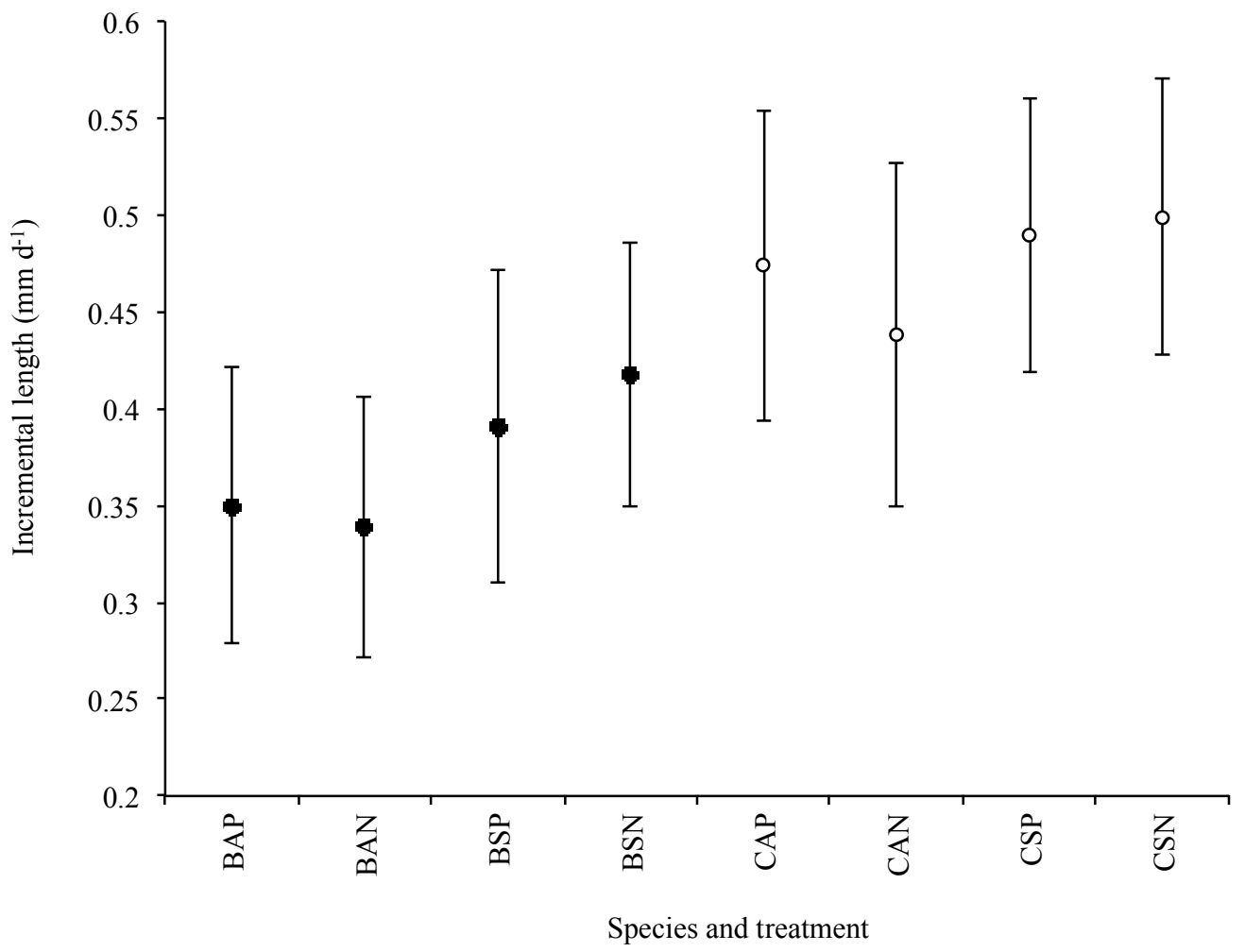


Figure 3.

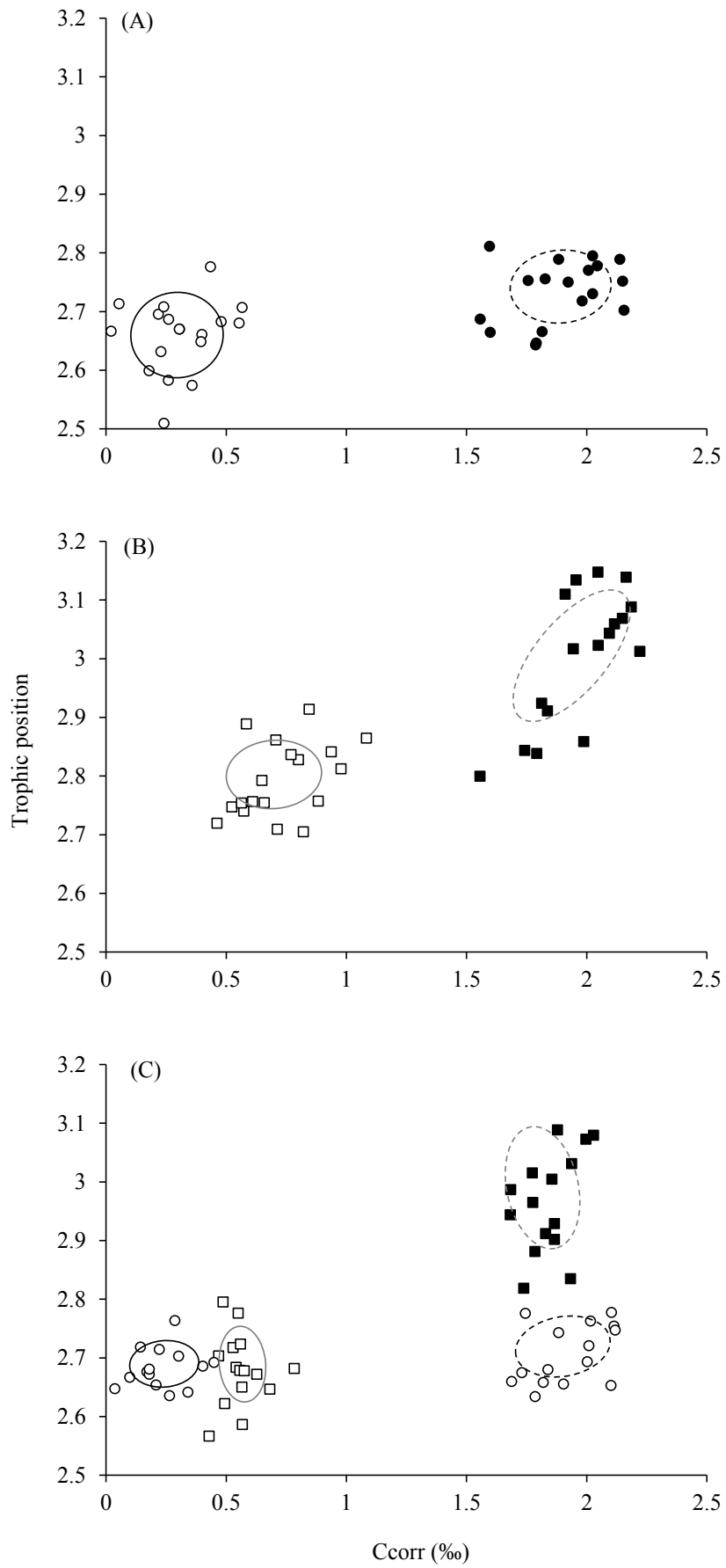


Figure 4.

