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

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Summary

1. The crossing of freshwater ecosystem boundaries by marine derived nutrients (MDN) is usually associated with migratory salmonid fishes returning to natal rivers. An alternative source of MDN in freshwaters is the widespread use of pelletized marine fishmeal ('pellets') by freshwater anglers as they target large bodied cyprinid fishes, such as European barbel *Barbus barbus*.
2. Here, the trophic consequences of MDN from pellets for riverine cyprinid fishes were tested. Approaches used stable isotope analyses in controlled and wild scenarios, using *B. barbus* and chub *Squalius cephalus* as model species. The isotopic niche, measured as standard ellipse area, was used to assess trophic niche size, and mixing models predicted the extent to which MDN contributed to fish diet.
3. In experimental mesocosms, *B. barbus* fed low volumes of pellets (approximately 3 per fish) for 130 days had isotopic niche sizes that were up to four times larger than a control and 'medium' (6 per fish) and 'high' pellet (12 per fish) treatments. Somatic growth rates were significantly higher in the 'medium' and 'high' treatments. In pond enclosure experiments, when juvenile *B. barbus* and *S. cephalus* were fed pellets daily for 100 days, there was a substantial and significant shift in the position of their isotopic niche compared to controls with no pellets fed. However, for each species, there were no significant differences in their somatic growth rates in the presence/ absence of pellets.
4. In a lowland river, high proportions of MDN contributed to the diet of *B. barbus* and *S. cephalus* captured by angling, but with substantial individual variability in those captured by electric fishing. Across all *B. barbus* > 400 mm, MDN dietary contributions ranged between 9 and 71%. This suggested some individual diet

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

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

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

Introduction

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

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

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

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

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

Materials and methods

Model species, experimental designs and field study

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

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

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

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

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

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

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

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

The field study used the invasive *B. barbus* and native *S. cephalus* populations of the River Teme, Worcester (52°10'13" N; 2°14'31" W) to test the influence of MDN from pellets on the diet composition and trophic niche size of wild fishes. The study stretch receives considerable angling pressure for *B. barbus* from both banks throughout the year, but especially between June and October when anglers are present daily, with the majority utilising pellets based on fishmeal. A previous study also indicated *B. barbus* diet elsewhere on the river (approximately 10 km upstream, with separation by a weir of approximately 2.0 m head) consisted of high proportions of pelletized fishmeal (Bašić *et al*., 2015). Here, SIA of the 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. barbus* 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. barbus* 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 $\delta^{13}C$ and $\delta^{15}N$. 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 $\delta^{15}N$ and 0.09 for $\delta^{13}C$, and analytical precision associated with the $\delta^{15}N$ and
230 $\delta^{13}C$ 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': $\delta^{13}C$: -31.86 ± 1.06, $\delta^{15}N$: 5.9 ± 0.66‰; 'non-pellet pond': $\delta^{13}C$: -34.68 ± 1.14,
237 $\delta^{15}N$: 8.49 ± 0.60‰). Therefore, to enable true comparison between the pellet and no pellet
238 treatments, the $\delta^{15}N$ data were transformed to trophic position (TP), using the equation:

239 $$TPi = [(\delta^{15}N_i - \delta^{15}N_{base})/3.4]+2$$

240 where TP_i is the trophic position of the individual fish, $\delta^{15}N_i$ is the isotopic ratio of that fish,
241 $\delta^{15}N_{base}$ 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 $\delta^{13}C$ data were converted to $\delta^{13}Ccorr$ using:

244 $$\delta^{13}Ccorr = \delta^{13}C_i - \delta^{13}C_{meaninv}/CR_{inv}$$

245 where $\delta^{13}C_{corr}$ is the corrected carbon isotope ratio of the individual fish, $\delta^{13}C_i$ is the
246 uncorrected isotope ratio of that fish, $\delta^{13}C_{meaninv}$ is the mean invertebrate isotope ratio (the
247 'baseline' invertebrates) and CR_{inv} is the invertebrate carbon range ($\delta^{13}Cmax$ - $\delta^{13}Cmin$;
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. barbus* 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 10^4 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 ($\delta^{15}N$: 3.4 ± 0.98‰; $\delta^{13}C$: 0.39 ± 1.3‰) (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

individual mesocosm. However, it also covered *G. pulex*, as some samples were collected from a small proportion of the mesocosms. Their SI data overlapped with Chironomids and so the model could not separate their dietary contributions (mean SI values ± 95% confidence limits (‰): Chironomid: n = 18; $\delta^{13}C$: -24.08 ± 0.36, $\delta^{15}N$: 7.83 ± 0.38; *G. pulex*: n = 6; $\delta^{13}C$: -23.78 ± 0.46, $\delta^{15}N$: 8.29 ± 0.24). In the pond experiments, four putative food resources were used: 2 mm pellet, 3 mm pellet and the macroinvertebrate groups Corixidae and Odonata. In the field study, the putative food resources in the model were pooled according to fish pellet 1, fish pellet 2, small fishes and Arthropoda (*cf.* Bašić *et al*., 2015). In addition to the Bayesian mixing models already outlined, these field study data were then also used to assess individual variability using SOLOSIAR ('siarsolomcmcv4') in the SIAR package in R (Parnell *et al*., 2010; R Core Development Team, 2013). In this model, fractionation values were (mean ± SD): $\delta^{13}C$: 2.57 ± 0.06 for 'small fishes' and both pellets, and 0.80 ± 0.30 for Arthropoda; $\delta^{15}N$: 2.4 ± 0.07 for 'small fishes' and both pellets, and 3.0 ± 0.02 for Arthropoda (Busst, Bašić & Britton, 2015; Busst & Britton, 2016).

Other data analyses

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

303 into models according to their treatments so, for example, *B. barbus* was present in models as
304 (1) allopatric *B. barbus*, (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 $[((\text{total } L_{t+1}) - (\text{total } L_{t+1}))/4]/_t$
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 $100[((\ln W_{t+1}) - (\ln W_t))/4]/_t$
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 $L_{fa} = ([FA\text{-}PA]/SR) \times L_f$.
325 Throughout the results, where error is expressed around the mean, it represents 95%
326 confidence limits unless stated otherwise.

Results

Mesocosm experiments

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

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

352 macroinvertebrates were the principal contributor to *B. barbus* 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. barbus* 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. barbus*) 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 $\delta^{13}C$ data (Ccorr) were
368 primarily between the pellet and no pellet treatments, including between allopatric *B. barbus*
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. barbus* 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

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

Wild fishes

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

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

Discussion

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

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

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

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

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

In summary, across three spatial scales of increasing complexity, it was apparent that the release of fishmeal pellets into freshwaters as an allochthonous trophic subsidy based on MDN had a substantial influence on the isotopic niche (as a proxy of the trophic niche) of riverine fishes. Results from wild *B. barbus*, with some support from the experiments, indicated that individual isotopic niche specialisation resulting from this trophic subsidy was 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
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537
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Table 1. Mean lengths and weights, isotopic niche size (as 95% CL of standard ellipse area, SEA_b) of *Barbus barbus* 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 (±95% CL). Sample sizes were n = 15 per treatment.

							Estimated contribution to diet (%)	
Treatment	Mean length (mm)		Mean weight (g)		SEA_b (‰)	Overlap in isotopic niche with Control (%)	Macroinvertebrate	Pellet
	Start	End	Start	End				
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 (± 95% CL), and mean stable isotope values (± 95% CL).

Treatment	Species	n	Mean starting length (mm)	Mean end length (mm)	Mean $\delta^{13}C$ (‰)	Mean $\delta^{15}N$ (‰)
Allopatry/pellets	*B. barbus*	18	80.1 ± 0.3	117.83 ± 1.99	-24.70 ±0.21	9.39 ± 0.10
Allopatry/pellets	*S. cephalus*	18	81.7 ± 0.4	131.06 ± 1.38	-25.10 ± 0.23	8.44 ± 0.04
Allopatry/no pellets	*B. barbus*	18	77.6 ± 0.2	113.67 ± 1.32	-28.20 ± 0.20	11.18 ± 0.05
Allopatry/no pellets	*S. cephalus*	17	73.9 ± 0.3	124.59 ± 1.69	-30.31 ± 0.19	10.72 ± 0.05
Sympatry/pellets	*B. barbus*	15	82.0 ± 0.4	119.4 ± 1.84	-25.45 ±0.18	9.25 ± 0.09
Sympatry/pellets	*S. cephalus*	15	76.3 ± 0.4	125.27 ± 1.69	-24.94 ± 0.20	8.34 ± 0.04
Sympatry/no pellets	*B. barbus*	15	77.5 ± 0.3	118.94 ± 1.91	-29.05 ± 0.11	10.79 ± 0.05
Sympatry/no pellets	*S. cephalus*	15	76.1 ± 0.4	126.73 ± 1.64	-30.67 ± 0.14	10.81 ± 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 (± 95% CL) from MixSIAR.

	Corixidae	Odonata	2mm pellet	3mm pellet	Total pellet*
Allopatric *B. barbus* (n=18)	0.34 ± 0.11	0.21 ± 0.13	0.27 ± 0.06	0.18 ± 0.06	0.45
Allopatric *S. cephalus* (n=15)	0.26 ± 0.04	0.16 ± 0.05	0.33 ± 0.04	0.25 ± 0.04	0.58
Sympatric *B. barbus* (n=18)	0.32 ± 0.11	0.22 ± 0.12	0.25 ± 0.06	0.22 ± 0.07	0.47
Sympatric *S. cephalus* (n=15)	0.25 ± 0.09	0.15 ± 0.10	0.33 ± 0.09	0.27 ± 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 barbus* 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 *B. barbus*	18	0.02 – 0.05	0.03 – 0.09
Sympatric *B. barbus*	18	0.01 – 0.03	0.02 – 0.04
Allopatric *S. cephalus*	15	0.02 – 0.05	0.02 – 0.05
Sympatric *S. cephalus*	15	0.01 – 0.02	0.01 – 0.04

Table 5. (a) Mean contributions to fish diet of putative food resources (0 – 1 scale; ± 95% CL) of *Barbus barbus* and *Squalius cephalus* in the River Teme by sampling method, estimated by MixSIAR; (b) minimum, maximum, mean (± 95% CL) and coefficient of variation (CV) of estimates of contributions to individual *B. barbus* 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. barbus* of > 400 mm length were used in analyses.

(a)

Species	n	Arthropoda	'Small fishes'	Pellet 1	Pellet 2	Total pellet*
Electric fished *B. barbus*	13	0.39 ± 0.10	0.26 ± 0.09	0.10 ± 0.04	0.26 ± 0.04	0.36
Angled *B. barbus*	12	0.22 ± 0.07	0.20 ± 0.06	0.11 ± 0.03	0.48 ± 0.04	0.59
Angled *S. cephalus*	6	0.23 ± 0.11	0.24 ± 0.10	0.15 ± 0.06	0.39 ± 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)

	Minimum		Maximum		Mean		CV	
Dietary item	EF	A	EF	A	EF	A	EF	A
Arthropod	0.07	0.13	0.45	0.30	0.19 ± 0.09	0.18 ± 0.05	0.82	0.68
Small fish	0.18	0.16	0.50	0.43	0.23 ± 0.10	0.24 ± 0.05	0.81	0.69
Pellet	0.09	0.40	0.62	0.71	0.38 ± 0.09	0.59 ± 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 barbus* 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 barbus* 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 barbus* (filled circles) and *Squalius cephalus* (clear circles) per treatment in the pond enclosure experiment. BAP: allopatric *B. barbus* with pellets; BAN: allopatric *B. barbus*, no pellets; BSP: sympatric *B. barbus* with pellets; BSN: sympatric *B. barbus*, 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, Ccorr) 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 barbus* 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. barbus* 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 barbus* (electric fishing; length range 401 to 770 mm; n = 13): data points: black circles, solid black line: isotopic niche; *Barbus barbus* (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.

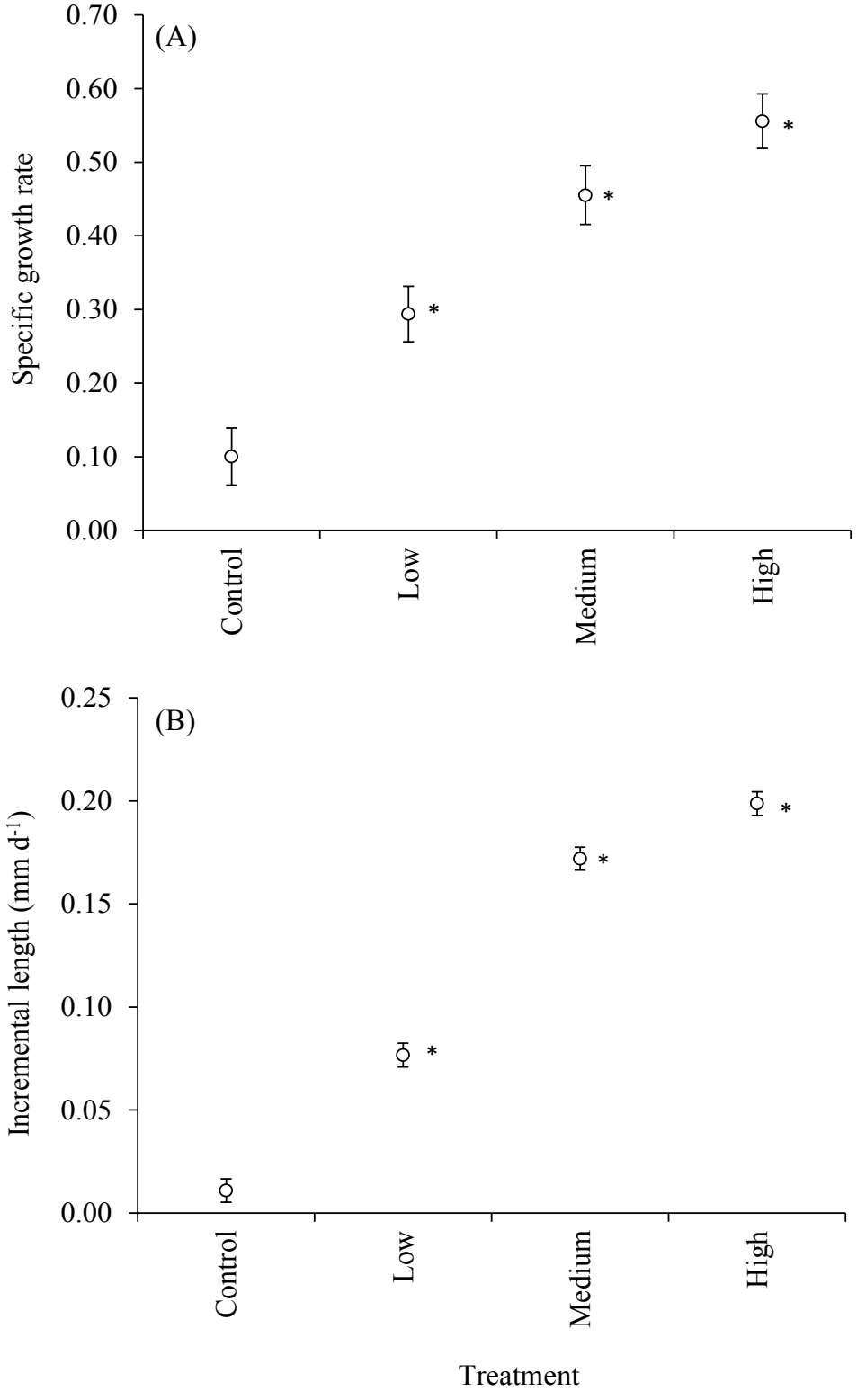
(A)
Specific growth rate
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Control
Low
Medium
High
(B)
Incremental length (mm d-1)
0.25
0.20
0.15
0.10
0.05
0.00
Control
Low
Medium
High
Treatment

Figure 1.

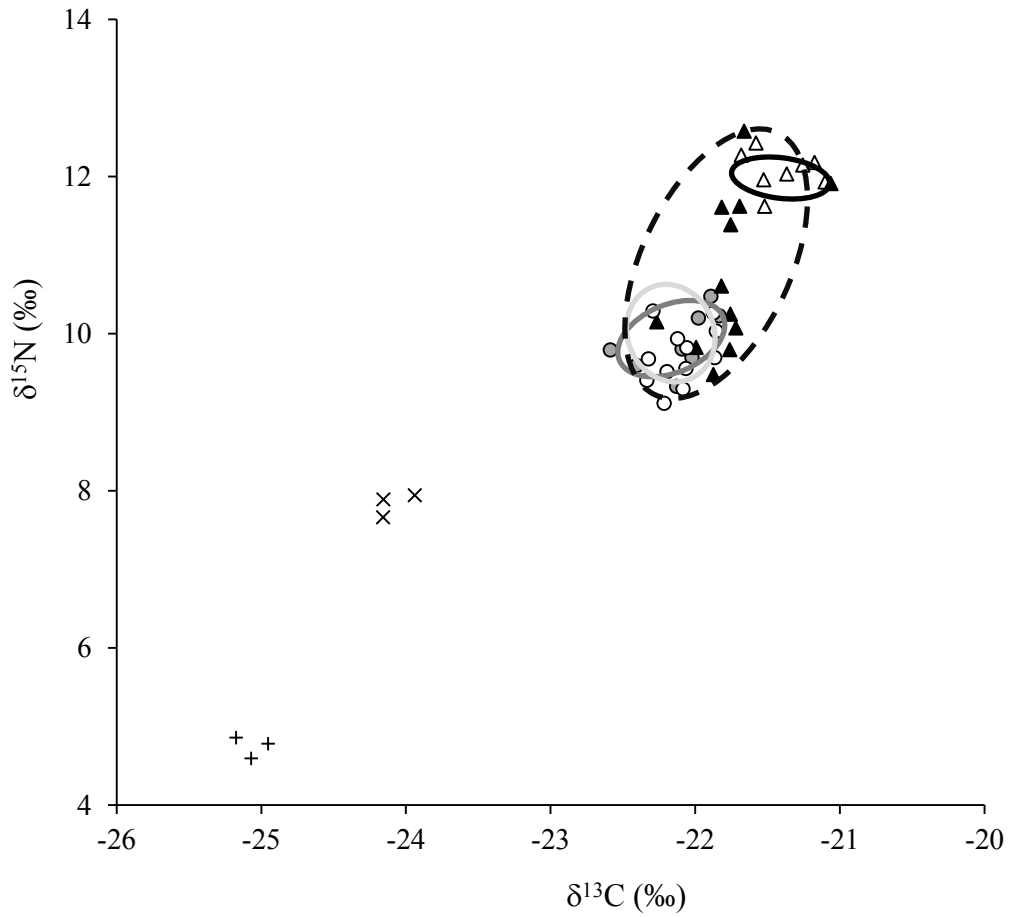

Figure 2.

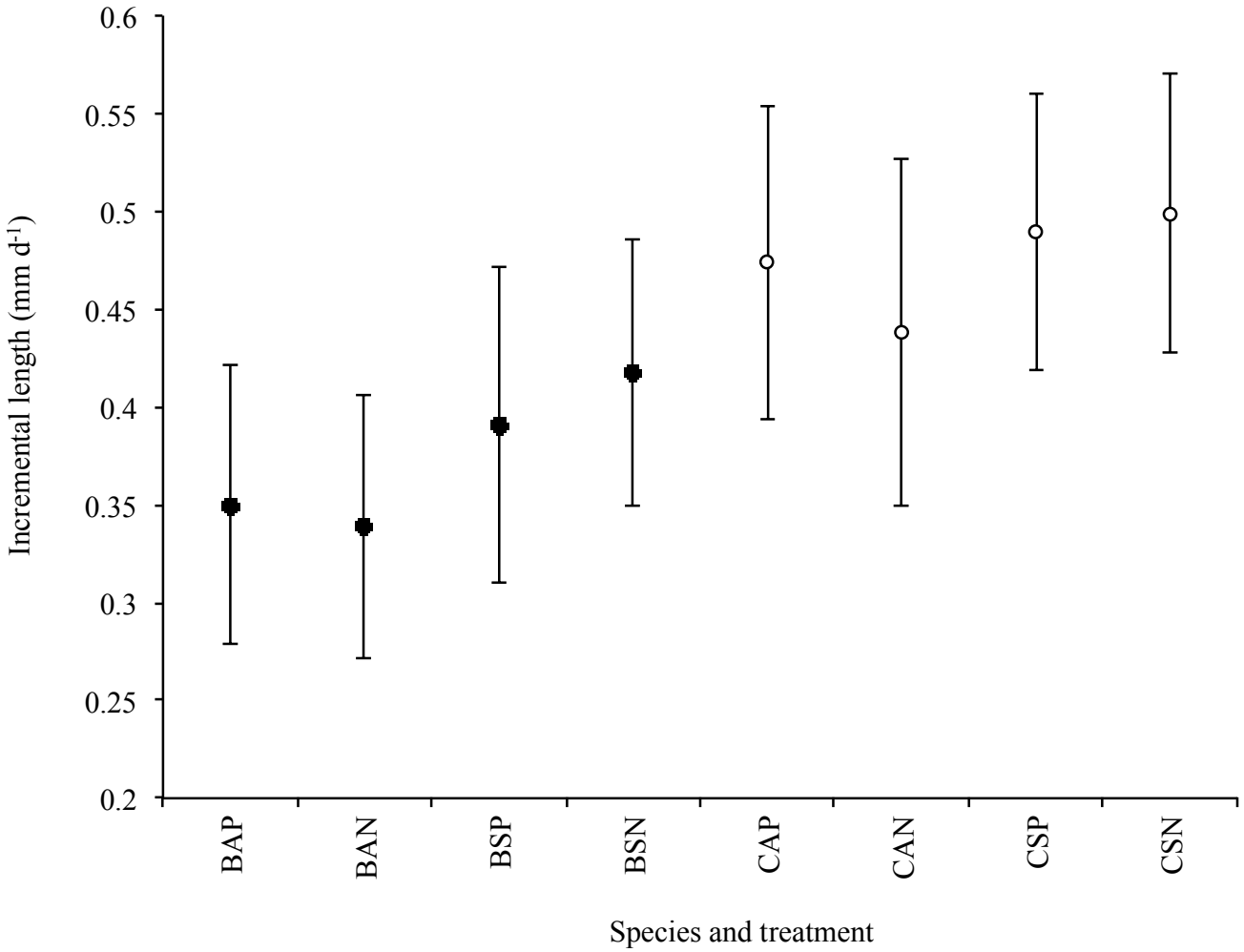
0.6
0.55
0.5
0.45
0.4
0.35
0.3
0.25
0.2
Incremental length (mm d-1)
BAP
BAN
BSP
BSN
CAP
CAN
CSP
CSN
Species and treatment

Figure 3.

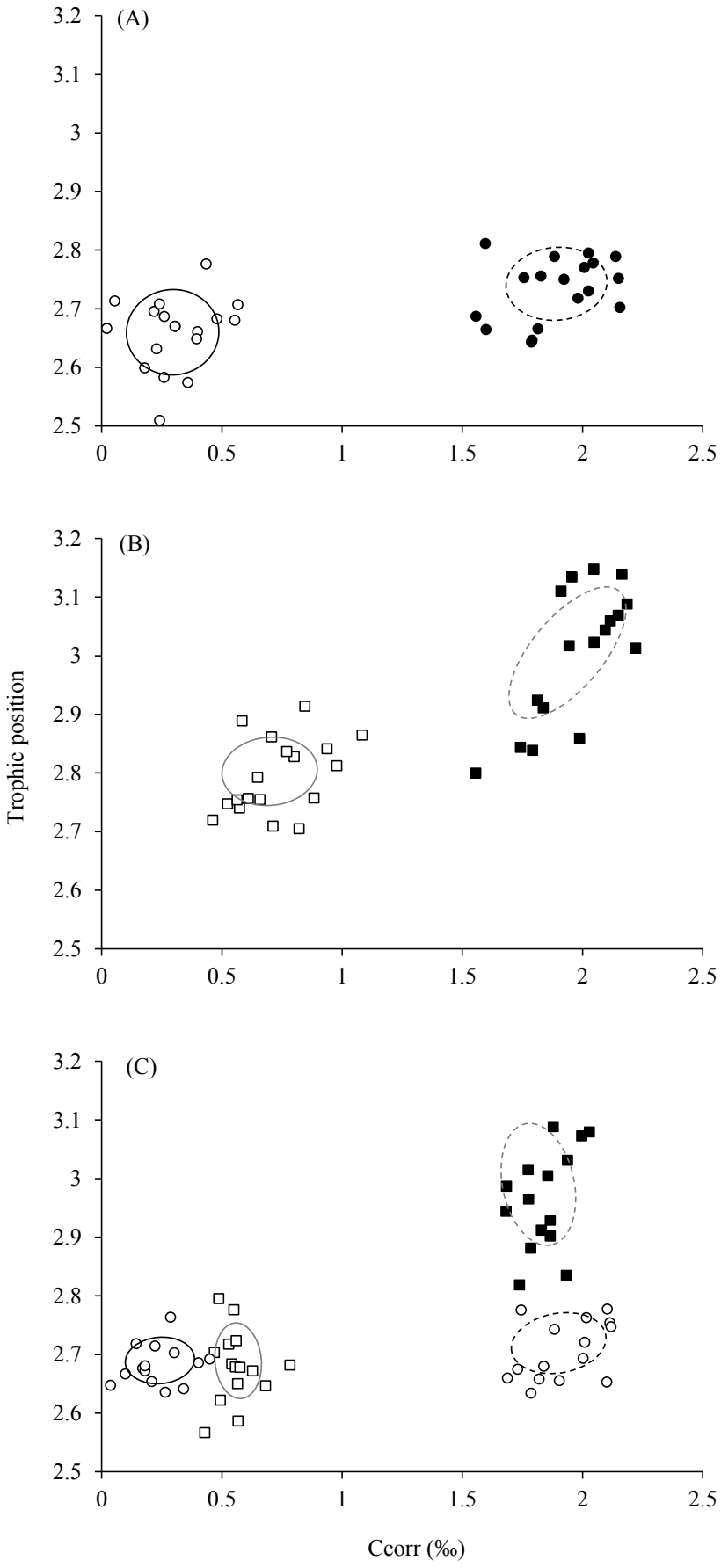

Figure 4.

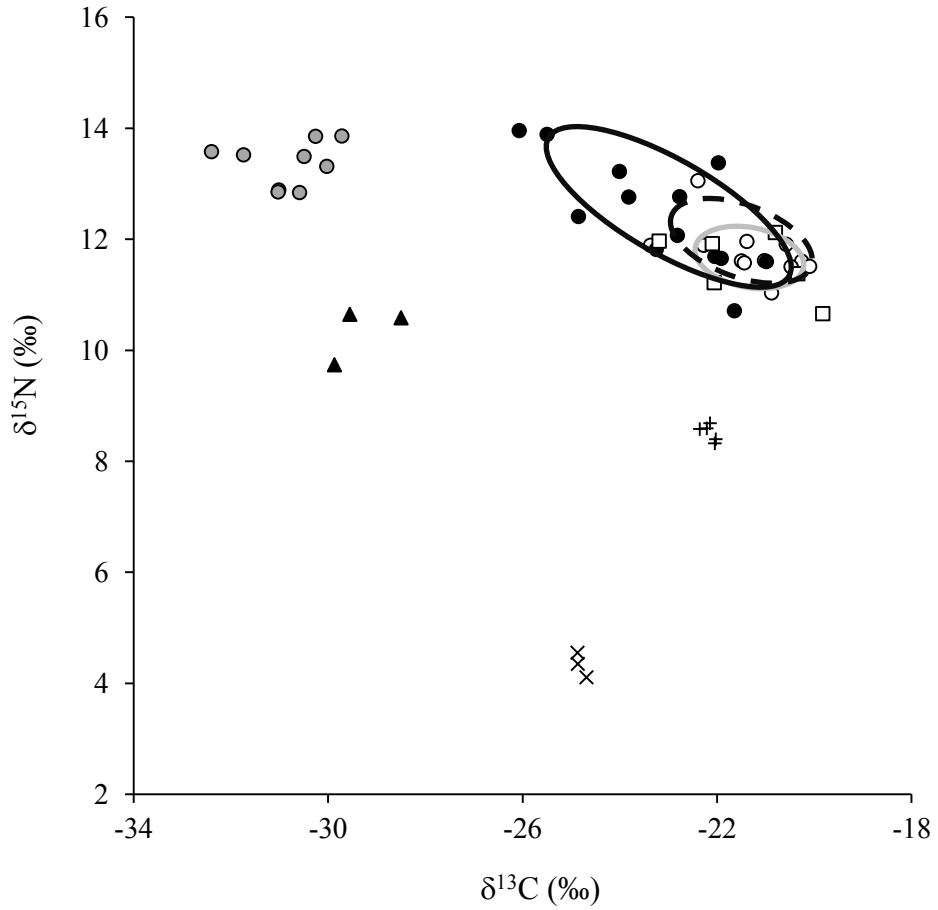

Figure 5.