Predicting the ecological impacts of an alien invader: experimental approaches reveal the trophic consequences of competition

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Abstract

1. Ecological theory on the trophic impacts of invasive fauna on native competitors is equivocal. While increased inter-specific competition can result in coexisting species having constricted and diverged trophic niches, the competing species might instead increase their niche sizes to maintain energy intakes. Empirical experiments can test invasion theory on competitive interactions and niche sizes across different spatial scales and complexity.

2. The consequences of increased inter-specific competition from a model alien fish *Leuciscus idus* were tested on two taxonomically and trophically similar native fishes, *Squalius cephalus* and *Barbus barbus*. Competitive interactions were tested in tank aquaria using comparative functional responses (CFRs) and cohabitation trials. The consequences of these competitive interactions for the trophic niche sizes and positions of the fishes were tested in pond mesocosms.

3. CFRs revealed that compared to *B. barbus, L. idus* had significantly higher attack and consumption rates; cohabitation trials revealed *B. barbus* growth rates were depressed in sympatry with *L. idus*. For *L. idus* and *S. cephalus,* differences in their functional response parameters and growth rates were not significant.

4. Pond mesocosms used stable isotope metrics to quantify shifts in the trophic niche sizes of the fishes between allopatry and sympatry using a substitutive

experimental design. Isotopic niches were smaller and more divergent in sympatric paired species than predicted by their allopatric treatments, suggesting trophic impacts from inter-specific competition. However, an all-species sympatric treatment revealed similar niche sizes with allopatry. This maintenance of niche sizes in the presence of all species potentially resulted from the buffering of direct competitive effects of the species-pairs by indirect effects.

5. Experimental predictions from tank aquaria assisted the interpretation of the constricted and diverged trophic niches detected in the paired-species sympatric treatments of the pond mesocosms. However, the all-species sympatric treatment of this experiment revealed greater complexity in the outcomes of the competitive interactions within and between the species. These results have important implications for understanding how alien species integrate into food webs and influence the trophic relationships between native species.

Key words: Comparative functional response; inter-specific competition, invasive species, non-native, predator-prey.

Introduction

The ecological impacts of biological invasions are wide ranging and include habitat disruption and genetic introgression with native species (Gozlan et al. 2010). Ecological impacts can also develop through the trophic interactions of the invader with native species, including via predator-prey relationships (Dick et al. 2013; Alexander et al. 2014) and competitive interactions with other consumers (Britton et al. 2018). The intensity of competitive interactions and so the severity of their impacts are predicted to be stronger and more intense when the invader and native species are taxonomically and/ or trophically similar due to their likelihood of exploiting similar prey resources (Dick et al. 2017).

Ecological theory can help predict the trophic consequences of biological invasions (Britton et al. 2018). Hypotheses on trophic niche theory suggest how alien and native species can coexist in food webs (Catford, Jansson & Nilsson 2009). If the alien species utilises resources that are unlimited or unexploited by native species, there will be little change in the competitive pressures of the invaded system, enabling the co-existence of species (Mason et al. 2008; Juncos et al. 2015). Should competitive interactions be more intense due to the alien species exploiting similar and limited prey resources to native species, their niches could constrict in size as the diets of each species becomes more specialized (e.g. Tran et al. 2015; Jackson et al. 2016). These smaller niches might also be divergent if the species exploit alternative resources to minimize their competitive interactions (Busst & Britton 2017; Britton et al. 2018). Competitive exclusion of native species from their original niche could occur if the inter-specific competitive interactions are particularly intense and asymmetric (Bøhn, Amundsen & Sparrow 2008). Conversely, if species diversify their diet in response to increased competition then their niches might increase in size (Britton et al. 2018). The intensity of intra-specific competition can also have considerable influences on trophic niche sizes, with optimal foraging theory predicting that as it intensifies, niche breadths will increase as individuals diversify their diet in response to resource depletion (Svanbäck & Bolnick 2006). Moreover, as competitive interactions are important for structuring the populations of many taxa then understanding how alien species compete with native biota and integrate into native food webs is integral to understanding their ecological impacts (Riccardi et al. 2013; Gallardo et al. 2016).

Across taxa, it remains equivocal as to how these potential shifts in the trophic niches of native species manifest following an invasion (Britton et al. 2018) and so can be investigated further using empirical experiments. Manipulating the abundances of alien and native species enables the outcomes of the altered strength of their competitive interactions to be measured (Britton 2018). For example, cohabitation pond mesocosm experiments can compare the results of inter-specific competition between sympatric alien and native fishes versus allopatric treatments involving only intra-specific competition (Britton 2018). Alterations in niche sizes and trophic positions between allopatry and sympatry can be quantified by stable isotope metrics (Tran et al. 2015; Britton et al. 2018). The competitive relationships between the species can then be informed by aquaria experiments (Britton 2018). Cohabitation aquaria experiments can utilise the same species as pond experiments, but under controlled conditions (Busst & Britton 2016), where higher growth rates within species indicates higher resource acquisition and greater competitive ability (Ward, Webster & Hart 2006). Comparative functional response experiments (CFRs) compare consumption rates as a function of prey density between the alien and native species (Dick et al. 2013, 2014, 2017). A species with a significantly higher consumption rate than a comparator species has the ability to acquire more resources, i.e. their inter-specific interactions will be asymmetric.

The aim here was to use these experimental approaches to empirically predict the trophic impacts of an invasion by a model alien freshwater fish on two trophically and taxonomically similar native fishes. The model area was Great Britain, a temperate region where the model alien fish, ide *Leuciscus idus*, is non-native. The species is, however, present in many lentic environments due to introductions of hatchery reared fish for angling, despite risk assessment suggesting their invasion risk is high in Britain (Britton et al. 2010). It has yet to disperse widely in lotic environments. The species is also taxonomically similar to chub *Squalius cephalus* (synonym: *Leuciscus cephalus*), a native riverine species that tends to coexist with the trophically similar European barbel *Barbus barbus* (Gutmann Roberts & Britton 2018). Consequently, *S. cephalus* and *B. barbus* were the model native fishes. As CFRs tend to predict that high-risk alien species have significantly higher consumption rates than native analogues (Dick et al. 2013), it was predicted that: (i) inter-specific competition between the alien and native fishes would be asymmetric, with *L. idus* the superior competitor; and (ii) this asymmetric competition would result in the native fishes having reduced niche sizes and growth rates when in sympatry compared to allopatry, but with *L. idus* having niche sizes and growth rates similar between allopatry and sympatry.

Materials and Methods

Model fishes

The three model fishes are all species in the Cyprinidae family that are either benthic or bentho-pelagic foragers. Although primarily lotic fishes, they are all also present in a range of lentic habitats (e.g. Jurajda, Ondračková & Reichard 2004; Taylor et al. 2004). Whilst their diets typically comprise of macroinvertebrates, plant material can also be an important food source (Brabrand 1985; Balestrieri et al. 2006; Caffrey et al. 2008). In all experiments, *L. idus*, *S. cephalus* and *B. barbus* were sourced from an aquaculture site in Southern England, with all fish of age 1+ years and 65 to 80 mm starting length (individuals of different lengths were randomly distributed across the experiments). All fish were tagged with 7 mm passive integrated transponder tags (approximate weight: 0.03 g) to enable individual identification. Fish were weighed post-tagging (to 0.1 g). These fish had been pond-reared on a diet of natural and formulated feeds. For aquaria-based experiments, the fish were allowed to acclimate to the aquaria conditions for 28 days at 20 ^{o}C before use. In the aquaria, fish were held in 45 L tanks where water filtration was provided via flow-through systems. When not being used experimentally, the fish were fed a formulated feed based on plant material to standardize prior experience. As different batches of fish were used in each experiment, the fish used in the experimental treatments and replicates were all of similar length and mass to eliminate experimental confounds based on differences in body sizes.

Comparative functional responses (CFRs)

The prey species used in the CFRs were *Gammarus pulex* and Chironomid larvae. In the experiments, individual fish were randomly selected 24 h prior to use and allocated to 10 L experimental tanks at 20 ^{o}C supplied with oxygen to provide constant conditions. They were without food in this period to standardize hunger levels. Individual fish were then presented with a prey species at one of six densities (2, 4, 8, 16, 32 and 64), with a minimum of three replicates generated per density and prey species. Prey exposure was for one hour. The fish were then removed from the tank, the number of prey remaining counted, and the number of prey consumed determined by subtracting this number from the original prey density.

In the CFRs, the comparisons were between the non-native *L. idus* versus the two native fishes. For *B. barbus* and *S. cephalus,* consumption rate data were as per Guo et al. (2017). The *L. idus* consumption rate data were generated at the same time as *B. barbus* and *S. cephalus,* but these data have not been used previously. Analyses of CFRs of all fishes were assessed using the integrated package for functional response analysis in R (‘Frair’) (Pritchard et al. 2017). Logistic regressions of prey density versus the proportion of prey consumed were performed per fish species, with type II functional responses indicated by significant negative first-order terms (Pritchard et al. 2017). Values of the attack rate (a) and handling time (h) were then obtained using maximum likelihood estimation (MLE) in the Random Predator Equation (Rogers 1972), which assumes a Type II response and non-replacement of prey:

$N_e = N_0\,(1 – \exp(a(N_e h\text{-T})))$ (Equation 1)

where N_e is the number of prey eaten, N_0 is the initial density of prey, a is the attack rate, h is the handling time and T is the total time available. Finally, to visualise the uncertainty around the fitted functional responses, bootstrapping (n = 1500) was used to construct empirical 95% confidence intervals of the fitted functional responses (Paterson et al. 2015). These bootstrapped data provided the CFR plots between the species; where there was overlap in their 95 % confidence limits, differences in the functional response curves were considered as not significant (Paterson et al. 2015).

Co-habitation aquaria experiments

The cohabitation experiments in tank aquaria were completed in 45 L tanks arranged on shelving with three tiers (top, middle and bottom shelves) and completed at 18 ^{o}C on 16:8 h light:dark regime. Each species was used in allopatry (N = 10) and then in each two-species sympatric combination (n = 5+5), with three replicates per treatment. Feeding was once per day using a sinking, fishmeal based pellet (1.0 mm diameter; 45 % protein, 20 % oil) at a fixed ration of 2 % mean starting body mass per day. Prior to their release into the tanks, the starting weight of each species per treatment was measured. The experiment ran for 30 days.

At the end of the experimental period, the fish were removed from the tanks and re-weighed. The increase in mass per species and treatment during the experimental period was determined by the ‘specific growth rate’ (SGR):

$([(\ln W_{t+1})\ \ln W_t)/t]/n) \times 100$ (Equation 2)

where W_t = total starting weight of the species in the tank, W_{t+1} = total finishing weight, n = number of fish, and t = number of days between W_t and W_{t+1}. Differences in SGR between treatments and species were tested in a linear mixed effects model. This tested the effect of the interaction of species x treatment on SGR, where tank position (i.e. whether it was on the top, middle or bottom shelf) was used as the random variable and fish starting weight was used initially as a covariate. However, starting weight per species was removed from the final model as its effect was not significant (P > 0.05). Model outputs were the overall significance of the model and the mean SGR values (± 95 % confidence intervals) according to species and treatment.

Co-habitation pond mesocosms

The experimental design was based on substitutive treatments using allopatric and sympatric contexts. There were three allopatric treatments, where each species was used individually (N = 12) and three sympatric treatments using paired species (*L. idus*/ *B. barbus*; *L. idus*/ *S. cephalus*; *B. barbus*/ *S. cephalus*; n = 6+6). A final sympatric treatment then used the three fishes together (n = 4+4+4). All treatments were replicated three times.

The experiment was completed using the treatments within enclosures as per Britton et al. (2018), with the enclosures sitting within a larger, man-made pond (30 x 30 m; 1 m consistent depth) that was located in Southern England. The enclosures comprised of an aluminium frame (length 1.7 m; width: 1.1 m; height: 1.2 m) within a net (mesh: 7 x 7 mm) that prevented fish ingress and egress, but allowed both movements of invertebrates and the growth of macrophytes into the enclosure. The enclosures were placed randomly across the pond, other than in shallow, littoral areas, with approximately 0.5 m between each enclosure. They remained *in-situ* throughout the experimental period. Their placement on top of the substrate enabled macrophytes to grow through them (*Elodea* spp.); all enclosures had similar areal macrophyte coverage during the experiment. Netting (15 x 15 mm mesh) over the enclosures prevented bird predation. The experiment ran for 150 days from April 2017. This provided time for approximately four stable isotope half-lives in the fish dorsal muscle (i.e. at least 94 % isotopic turnover) (Thomas & Crowther 2015). Temperature loggers (TinyTag TGP-4017) in the larger pond revealed the mean water temperature was 17.3 ± 0.8 ^{o}C during the experiment.

On day 150, all the fish were recovered from the enclosures, euthanized (anaesthetic overdose, MS-222) and taken to the laboratory. Samples of putative food resources were taken from the larger pond for stable isotope analysis (SIA) using a sweep net. These focused on the two major macroinvertebrate putative prey species sampled, *Gammarus pulex* and Chironomid larvae (that also ensured consistency with the CFRs). The presence of these macro-invertebrates was checked in each enclosure at the conclusion of the experiment, although their abundances were not accurately quantified. No other macro-invertebrates were detected in sufficient abundances inside or outside of enclosures to warrant their analysis; as their abundances were low outside of enclosures then their low abundance inside enclosures was not considered to be due to fish predation pressure. The other major food resource was plant material ('macrophyte') that was highly abundant in all enclosures, and was also sampled for SIA. All putative food resources were sorted into samples (one sample = 3 to 9 individuals per species for macroinvertebrates), with triplicate samples analysed for each group.

In the laboratory, individuals were identified by their PIT tag and re-weighed, enabling calculation of their SGR (Equation 2). A dorsal muscle sample was taken for SIA. SI sample sizes were a minimum of 12 fish per species per treatment, with a minimum of four fish taken randomly per replicate (Britton et al. 2018). All samples were dried at 60 °C to constant mass before SIA (δ^{13}C, δ^{15}N) at the Cornell University Stable Isotope Laboratory, New York, USA. Prior to analysis, samples were ground to powder and weighed (approximately 1000 µg, but with precise measures taken) in tin capsules. They were then analysed on a Thermo Delta V isotope ratio mass spectrometer (Thermo Scientific, USA) interfaced to a NC2500 elemental analyser (CE Elantach Inc., USA). Analytical precision associated with the δ^{15}N and δ^{13}C sample runs was estimated at 0.42 and 0.15 ‰ respectively. Data outputs were in delta (δ) isotope ratios (‰). The C:N ratios of the analysed samples were between 3.15 and 3.61, indicating relatively low lipid levels (Post et al. 2007). These ratios did not differ significantly between experimental treatments (Supplementary material; Fig. S1). Comparison of original versus lipid-normalised data (Kiljunen et al. 2006) revealed a very strong and significant relationship, indicating that the variability in the original δ^{13}C data was not an artefact of differences in lipid levels (Fig. S2). The shift between the mean original and mean normalised δ^{13}C data per species and treatment was 0.61 to 0.69 ‰ (Table S1), thus had a negligible effect on the relative positions in isotopic space of the species per treatment. In addition, the lipid concentrations of the analysed fish tissues were not a significant predictor of their growth rates, i.e. faster growing fish did not have higher lipid concentrations (Fig. S3). Thus, the original δ^{13}C data were used throughout all analyses, as lipid levels were not a confound in the experiment.

The SI data were used to calculate the trophic niche size of each fish species per treatment using the isotopic niche (Jackson et al. 2011). Whilst closely related to the trophic niche, the isotopic niche is also influenced by factors including growth rate and metabolism, and thus represents a close approximation of the trophic niche (Jackson et al. 2011). The isotopic niche was calculated using standard ellipse areas (SEA) in SIBER (Jackson et al. 2011; Jackson et al. 2012). This is a bivariate measure of the distribution of individuals in isotopic space, with the ellipses enclosing the core 40 % of data, so indicates the typical resource use of the analysed population (Jackson et al. 2011). A Bayesian estimate of SEA (SEA_B) tested differences in niche sizes between treatments per species, calculated using a Markov chain Monte Carlo simulation (10^4 iterations per group) (Jackson et al. 2011; Jackson et al. 2012). Differences in the size of isotopic niches (as SEA_B) were evaluated by calculating the probability that the relative posterior distributions of the niche size of the allopatric treatment were significantly smaller or larger than those of each of their sympatric niches ($\alpha = 0.05$) in SIBER. The SI data were then used to calculate isotopic niche overlap (%) between the species using SEA_C also calculated in SIBER, where subscript '$_C$' indicates a small sample size correction was used (Jackson et al. 2012). Use of SEA_C was mainly to get a representation of the extent of niche overlap between species, as it is more strongly affected by small sample sizes (< 30) than SEA_B (Jackson et al. 2012).

The SI data were then applied to a Bayesian mixing model to predict the relative proportions of the three putative food resources to fish diet per treatment within the package 'Mixing Models for Stable Isotope Analysis in R' (MixSIAR; Stock et al. 2018) Stock & Semmens 2016). The model ran using 'short' run length (chain length: 50,000 iterations with burn-in of 25,000, with posterior thinning (thin: 25) and 3 chains). Model diagnostics were based on Gelman-Rubin and Geweke, with sufficient convergence to accept the results (Stock & Semmens 2013). The isotopic fractionation values between the prey resources and fish were δ^{15}N: 5.10 ± 0.25 ‰; δ^{13}C: 3.8 ± 0.25 ‰, based on the fractionation factors derived for *B. barbus* and *S. cephalus* values on controlled diets based on plant and invertebrate protein sources (Busst & Britton 2016). Mixing model results were reported as means of all feasible solutions, with 5 to 95th percentiles of the distribution ranges.

To assist evaluation of the competition strength within and between species in the treatments, the mean intra- and inter-specific isotopic dissimilarities were calculated (Calizza et al. 2017). For the mean intra-specific isotopic dissimilarity (MND_{ii}), the first step was to calculate intraspecific isotopic dissimilarity (ND_{ii}) for each individual fish per species and treatment, determined as the mean isotopic (Euclidean) distance between each individual and their conspecifics in the treatment. The mean intraspecific isotopic dissimilarity for each species per treatment was then taken as the mean ND_{ii} value of all specimens in that treatment; higher values indicate increased dissimilarity. The same process was followed to determine the mean inter-specific isotopic dissimilarity (MND_{ij}) per species and treatment, except the first step was to calculate the mean isotopic distance of each individual fish from their sympatric species (ND_{ij}) (Calizza et al. 2017).

The SI, predicted diet, isotopic dissimilarity and SGR data were then tested for differences between treatments. Differences in δ^{13}C, δ^{15}N and SGR were tested in linear mixed effects models (LMEM). Enclosure was used as a random effect on the intercept to avoid inflating the degrees of freedom that would occur if individual fish were used as true replicates (Tran et al. 2015). Total starting mass of fish in each enclosure was initially used as a covariate, but was removed from final models as it was not significant (P > 0.05). Outputs from the models were the mean $\delta^{13}C$, $\delta^{15}N$ and SGR per species and treatment. The mean $\delta^{13}C$, $\delta^{15}N$ and SGR data from the models were then used to determine the extent of the change in each species between their allopatric treatment and each sympatric treatment. The extent of the change between allopatry and sympatry was then also determined for isotopic niche size (as SEAc) and the relative assimilation of each food resource from the mixing model outputs. These data were then tested for the significance of their relationships using linear regression. The relationships of MND_{ii} and MND_{ij} with SGR were also tested using linear regression to determine if changes in intra- and/ or inter-specific isotopic dissimilarity were significantly related to growth rates. Initially, multiple regression was used, where the mean isotopic dissimilarity that explained most of the SGR variability was indicated by the highest standardised ß coefficient value; univariate linear regression was then used on both dissimilarity indices. Note that in these tests, only data from sympatric treatments were used, as MND_{ij} could only be determined for treatments involving at least two fish species.

Statistical analyses were performed in R (Version 3.5.2; R Development Core Team 2018). In all results, error around the mean represents 95 % confidence limits. All experiments were completed following ethical review and under the UK Home Office project licence 70/8063.

Results

Comparative functional responses

In the functional response experiments, the first order linear coefficient from logistic regressions revealed the functional responses of all species were Type II and significant (first order linear coefficients from logistic regressions: *G. pulex*: -0.02, -0.04, and -0.06, Chironomid larvae: -0.02, -0.01 and -0.06, for *B. barbus, S. cephalus* and *L. idus* respectively; P < 0.01 in all cases). For *B. barbus* versus *L. idus* using *G. pulex* as prey, *B. barbu*s had a significantly lower attack rate (*a*) and higher handling time (*h*) than *L. idus* (a: 1.18 vs. 3.23, z = -2.64, P < 0.01; h: 0.12 vs. 0.06, z = 2.58, P < 0.01). On Chironomid larvae, *h* was also significantly higher for *B. barbus* (0.03 vs. 0.04, z = 3.93, P < 0.01), but the difference in *a* was not significant (3.38 vs. 4.79, z = -1.42, P = 0.15). In the functional response curves, *L. idus* had higher consumption rates compared with *B. barbus*, with their 95 % confidence limits having minimal overlap (Fig. S4, S5).

For S. *cephalus* versus *L. idus*, differences in *a* were not significant for *G. pulex* (2.09 vs. 3.23, z = -1.65, P = 0.10), but were significantly higher for *L. idus* on Chironomid larvae (1.37 vs. 4.79, z = -4.18, P < 0.01). Handling times were significantly lower in *S. cephalus* on both *G. pulex* (0.03 vs. 0.06, z = -3.84, P < 0.01) and Chironomid larvae (0.01 vs. 0.03, z = -4.16, P < 0.01). For both prey species, the functional response curves revealed high overlap in the 95 % confidence limits of their consumption rates (Fig. S4, S5).

Co-habitation aquaria experiment

Across the three species, there was considerable variation in their specific growth rates, varying between 0.39 ± 0.21 (*B. barbus* in sympatry with *L. idus*) and 1.07 ± 0.21 (*S. cephalus* in sympatry with *B. barbus*). The LMEM testing differences across the treatments was significant ($P < 0.01$). For *S. cephalus* and *L. idus*, differences in SGR between treatments were low, with substantial overlaps in their 95 % confidence limits (Fig. 1A). However, for *B. barbus*, there was a substantial reduction in SGR in sympatry with *L. idus* compared with their SGR in allopatry (Fig 1A).

Cohabitation pond mesocosms

The largest ranges of $\delta^{13}C$ and $\delta^{15}N$ across the experiment were measured in the allopatric treatments and the sympatric treatment where all the species were together (Table 1; Fig. S6). When two fishes were sympatric, the SI ranges reduced, especially in the *B. barbus*/ *L. idus* treatment (Table 1; Fig. S6). These reduced SI ranges were concomitant with changes in the positions of the isotopic niches between allopatry and sympatry (Fig. 2). The predicted isotopic niche overlap between the species in allopatry was 31 to 39 % (Fig. 2A). When all the fish were in sympatry, these overlaps were reduced to 3 % for *L. idus* versus *B. barbus*, 11 % for *S. cephalus* versus *L. idus*, and 12 % for *S. cephalus* versus *L. idus* (Fig. 2B). This reduction in niche overlap when in sympatry was also apparent in treatments involving two sympatric fishes, where the extent of overlap varied from 5 % for *S. cephalus* versus *B. barbus* (Fig. 2D) to 15 % for *S. cephalus* versus *L. idus* (Fig. 2E). Concomitantly, isotopic niche sizes (as SEAc) reduced, with the posterior distributions of SEA_B revealing these reductions were significant for both native species in sympatry with *L. idus* (Table 2).

The LMEM testing differences in SGR between treatments was significant ($P < 0.01$). Compared to allopatry, *B. barbus* and *L. idus* growth rates were significantly reduced in their sympatric treatments involving paired species. This was, however, not apparent in *S. cephalus* (Fig. 1B), where differences in $\delta^{13}C$ and $\delta^{15}N$ between the species per treatment were also significant ($P < 0.01$). Differences in metrics between allopatry and sympatry per species and treatment revealed that as niche size reduced, $\delta^{13}C$ was significantly enriched ($R^2 = 0.55$, $F_{1,7} = 8.39$, $P = 0.02$; Fig. 3A). This was not apparent for $\delta^{15}N$ ($R^2 = 0.01$, $F_{1,7} = 0.74$, $P = 0.79$). The stable isotope mixing model predicted this shift to enriched $\delta^{13}C$ was through a significant dietary shift away from Chironomid larvae and towards macrophyte and *G. pulex* (Chironomid: $R^2 = 0.92$, $F_{1,7} = 65.54$, $P < 0.01$; *G. pulex*: $R^2 = 0.93$, $F_{1,7} = 79.99$, $P < 0.01$; macrophyte: $R^2 = 0.59$, $F_{1,7} = 8.79$, $P = 0.03$; Fig. 3B). The 5 - 95 % percentiles of the mixing model dietary predictions suggested, however, that these dietary shifts were only significant in sympatric treatments involving *B. barbus* and *L. idus*, but not *S. cephalus* (Table 3).

The multiple regression testing the influence of MND_{ij} and MND_{ii} on SGR was not significant ($R^2 = 0.52$; $F_{2,6} = 3.22$, $P = 0.11$), but with MND_{ii} explaining more of the variability in SGR (standardised ß = 0.69, $P = 0.09$) than MND_{ij} (standardised ß = 0.04, $P = 0.93$). Univariate linear regression revealed the relationship between MND_{ii} and SGR was significant ($R^2 = 0.47$; $F_{1,7} = 6.32$, $P = 0.04$; Fig. 4A), but was not significant for MND_{ij} ($R^2 = 0.28$; $F_{1,7} = 2.65$, $P = 0.14$; Fig. 4B).

Discussion

In general, CFRs predict that ecologically damaging invaders have higher consumption rates than native species (e.g. Dick et al. 2013; Alexander et al. 2014). Here, they predicted that alien *L. idus* had higher attack rates and lower handling times than native *B. barbus*, resulting in significantly higher consumption rates in *L. idus*. In the cohabitation experiments in aquaria, the growth rates of *B. barbus* were significantly depressed in the presence of *L. idus* compared to allopatry. In contrast, the consumption rates of the taxonomically similar *S. cephalus* and *L. idus* were not significantly different and their growth rates did not differ significantly between treatments in the cohabitation experiment. In combination, these results suggest that competitive interactions between *L. idus* and *B. barbus* were asymmetric, as per the prediction. The superior competitor was *L. idus* due to their greater ability to access prey. This asymmetry in inter-specific competition was not, however, apparent between *L. idus* and *S. cephalus*, contrary to the prediction.

A criticism of CFRs for assessing the ecological impacts of alien species is that they do not adequately represent the ecological complexity inherent within more natural systems, where species can utilise multiple prey resources and are competing within a community of species of varying population abundances (e.g. Vonesh et al. 2017). They also cannot easily measure the competitive interactions within and between species directly (Guo et al. 2017). This is despite the potential importance of intra- and inter-specific competition in driving invasion-mediated changes in food web structure (David et al. 2017). Notwithstanding, the CFRs here did provide information on the comparative consumption rates of the fishes on the two major macroinvertebrate prey species used in the SIA of the pond experiment. Correspondingly, their predictions provided a basis for evaluating the competitive interactions of the fish in pond mesocosms.

In the pond mesocosms, there were some significant shifts in the size and position of the isotopic niches of the fishes across the treatments. Comparison of the niche sizes of the species in allopatry versus their paired sympatric treatments revealed some important differences. For *L. idus* and *S. cephalus*, the aquaria experiments predicted their competitive interactions would be symmetric and in the pond experiment, their isotopic niche sizes were both reduced compared to allopatry (significantly so for *S. cephalus*). Whilst both species increased their dietary proportions of *G. pulex* and reduced their proportion of Chironomid larvae, there were sufficient dietary differences to result in their increased niche divergence in sympatry versus allopatry. This result was consistent with other studies that suggest trophic niche constriction and divergence occurs when an invader and competing native species exploit similar food resources (Tran et al. 2015; Jackson et al. 2016). The growth rates of both species in sympatry were, however, similar to allopatry. For *L. idus and B. barbus*, the aquaria experimental predictions of asymmetric competition favouring *L. idus* were not evident in the pond mesocosms. When paired, there were significant reductions in niche sizes in both species, with increased niche divergence, when compared to allopatry. These changes were accompanied by significantly reduced growth rates. These results were, however, also consistent with other studies suggesting increased inter-specific competition is an important determinant of invasion-mediated trophic impacts (e.g. Bøhn et al. 2008; Tran et al. 2015).

The results of the sympatric treatment involving all species in the pond mesocosm experiment revealed that compared with allopatry, there were no significant changes in isotopic niche sizes or growth rates of any species. Also, across the entire experiment, there was a significant relationship between reduced growth rates and reduced mean intra-specific isotopic dissimilarity, but not between growth and mean inter-specific trophic dissimilarity. In combination, these results suggest that inter-specific competition was not the only mechanism responsible for the measured changes in isotopic niche sizes and position across the experiment, with differences in the intensity of intra-specific competition also potentially important. Theory predicts that as intra-specific competition intensifies, individuals should become increasingly opportunistic and thus have greater niche variation (Svanbäck & Bolnick 2006; Rossi et al. 2015). The relatively large niches apparent in all allopatric treatments were consistent with this, where the intensity of intra-specific competitive interactions was assumed to be highest. In the sympatric treatments, however, the smallest isotopic niche sizes occurred when conspecifics were at n = 6, not at n = 4, contrary to theory (Svanbäck & Bolnick 2006). Correspondingly, the interaction of reduced intra- and inter-specific competition in the all-species treatment might have been positively interacting to facilitate the niche expansions (Nelson et al. 2017). Alternatively, in the all-species treatment, the species-pair direct effects that were apparent in the species-pair sympatric treatments might have been buffered by indirect effects (Calizza et al. 2017; David et al. 2017). However, further work is needed to decouple these competition processes to more fully understand why the species-pair direct effects did not scale up and influence niche sizes in the all-species treatment.

The changes in the fish isotopic niche sizes and positions in the pond mesocosms highlight how aquatic invasive species can influence food web structure. In a meta-analysis on the impacts of aquatic invaders, Gallardo et al. (2016) revealed that competition and predation are the key processes driving ecological impacts in aquatic ecosystems, with indirect competitive effects from alien consumers often adversely affecting native species, leading to substantial modifications in food web structure (David et al. 2017). Invasions of alien fishes including *Carassius auratus*, *Cyprinus carpio*, *Pseudorasbora parva* and *Lepomis gibbosus* have all been shown to result in major re-organisations of the isotopic structure of the food web (e.g. Jackson & Britton 2014; Tran et al. 2015; Copp et al. 2017; Britton et al. 2018). Here, the alien *L. idus* also resulted in some food web re-structuring, with the effects involving both direct and indirect competitive effects depending on the number of fishes in the treatments.

Predicting the trophic consequences of invasive species remains an important theoretical and applied research area. Predictions from CFRs are that high-risk alien species tend to have significantly higher consumption rates than native analogues (Dick et al. 2013), with this consistent across fish (Alexander et al. 2014), amphipods (Laverty et al. 2015), snails (Xu et al. 2016) and decapods (Howard et al. 2018). Here, CFRs were used to predict the symmetry of inter-specific competition between species according to comparisons of their consumer-resource dynamics under standardised conditions. The results of the pond mesocosms between allopatry and species-pair treatments then revealed some consistency with the CFR results, especially *S. cephalus* versus *L. idus*. In the all-species treatment, however, there was greater complexity apparent in the results, and this complexity was beyond what the

CFRs could measure and predict. Thus, whilst CFRs have substantially increased understandings of the trophic impacts of invasive species (e.g. Alexander et al. 2014; Howard et al. 2018), their utility for predicting impacts is more limited in complex environments that involve a number of competing consumers. This is important, as competitive processes are important for structuring populations over a wide range of taxa, including snakes (e.g. Luiselli 2006), lizards (e.g. Mitchell 1979) and birds (e.g. Shochat et al. 2004). Moreover, studies across taxa suggest that the outcomes of competitive interactions are also influenced by a range of traits (e.g. body size and foraging behaviours) that then determine the diet of individuals, with food web structure being the sum of these individual diets (Petchey et al. 2008). The experiment here thus makes an important contribution to understanding how alterations in competition strength within and between species can impact the trophic niche sizes and positions of populations, and thus food web structure, whilst controlling for the effects of body size. The results also highlight how alien species integrate into food webs and alter the trophic relationships between native species.

A potential confound within the experiments was the use of hatchery-reared fishes, rather than fish collected from the wild. Hatchery-reared fishes were used due to the difficulty of obtaining sufficient numbers of wild fish to satisfy the experimental designs whilst controlling for size. There were also no wild *L. idus* British populations of sufficient abundance to provide the sample sizes. Literature suggests that there can be differences in the behaviours of hatchery-reared versus wild fish. For example, the movement behaviour and habitat use differed between wild and hatchery reared *S. cephalus* (Bolland et al. 2008), although the hatchery fish could cope with elevated flows and remained close to their stocking locations, as per wild fish (Bolland et al.

2009). Moreover, hatchery-reared fishes that are conditioned with natural stimuli and exposed to natural foods tend to have elevated post-release survival and more natural behaviours (e.g. Brown et al. 2003). The hatchery-reared fishes used in the experiments were all pond-reared, feeding on a mix of natural and supplementary foods. Consequently, as their husbandry used similar conditions to those in the enclosure experiment, and involved pond habitats and natural foods, the fish were considered a strong proxy for testing the interactions of wild fishes.

In summary, three experimental approaches tested the trophic consequences of an alien fish on two native fishes. Aspects of the shifts in isotopic niches and growth rates of fish in relatively complex environments were interpreted using the results of two relatively simple experiments completed in controlled conditions. However, the greater complexity of the pond systems when all the species were present resulted in more complex interactions and less predictable outcomes, and highlighted the direct and indirect interactions that enable alien species to integrate into native food webs.

Authors' contributions

JRB conceived the ideas, designed the methodology and collected data; JRB, FAT, CGR, EN and VDS analysed data; JRB led manuscript writing. All authors contributed critically to drafts and approved submission.

Data accessibility

Data will be provided in Dryad on acceptance.

References

Alexander, M.E., Dick, J.T., Weyl, O.L., Robinson, T.B. & Richardson, D.M. (2014). Existing and emerging high impact invasive species are characterized by higher functional responses than natives. *Biology Letters*, *10*, p.20130946.

Balestrieri, A., Prigioni, C., Remonti, L., Sgrosso, S. & Priore, G. (2006). Feeding ecology of *Leuciscus cephalus* and *Rutilus rubilio* in southern Italy. *Italian Journal of Zoology*, *73*, 129-135.

Bøhn, T., Amundsen, P.A. & Sparrow, A. (2008). Competitive exclusion after invasion? *Biological Invasions, 10,* 359-368.

Bolland, J.D., Cowx, I.G. & Lucas, M.C. (2008). Movements and habitat use of wild and stocked juvenile chub, *Leuciscus cephalus* (L.), in a small lowland river. *Fisheries Management and Ecology*, *15,* 401-407.

Bolland, J.D., Cowx, I.G. & Lucas, M.C. (2009). Dispersal and survival of stocked cyprinids in a small English river: comparison with wild fishes using a multi-method approach. *Journal of Fish Biology*, *74*, 2313-2328.

Brabrand, Å. (1985). Food of roach (*Rutilus rutilus*) and ide (*Leusiscus idus*): significance of diet shift for interspecific competition in omnivorous fishes. *Oecologia*, *66,* 461-467.

Britton, J.R. (2018). Empirical predictions of the trophic consequences of non-native freshwater fishes: a synthesis of approaches and invasion impacts. *Turkish Journal of Fisheries and Aquatic Sciences* http://doi.org/10.4194/1303-2712-v19_6_09

Britton, J.R., Cucherousset, J., Davies, G.D., Godard, M.J. & Copp, G.H. (2010). Non-native fishes and climate change: predicting species responses to warming temperatures in a temperate region. *Freshwater Biology*, *55*, 1130-1141.

Britton, J.R., Ruiz-Navarro, A., Verreycken, H. & Amat-Trigo, F. (2018). Trophic consequences of introduced species: Comparative impacts of increased interspecific versus intraspecific competitive interactions. *Functional Ecology*, *32*, 486-495.

Brown, C., Davidson, T. & Laland, K. (2003). Environmental enrichment and prior experience of live prey improve foraging behaviour in hatchery-reared Atlantic salmon. *Journal of Fish Biology*, *63*, 187-196.

Busst, G. & Britton, J.R. (2017). Comparative trophic impacts of two globally invasive cyprinid fishes reveal species-specific invasion consequences for a threatened native fish. *Freshwater Biology*, *62*, 1587-1595.

Busst, G.M. & Britton, J.R. (2016). High variability in stable isotope diet–tissue discrimination factors of two omnivorous freshwater fishes in controlled ex situ conditions. *Journal of Experimental Biology*, *219*, 1060-1068.

Caffrey, J.M., Acevedo, S., Gallagher, K. & Britton, J.R. (2008). Chub (*Leuciscus cephalus*): a new potentially invasive fish species in Ireland. *Aquatic Invasions*, *3*, 201-209.

Calizza, E., Costantini, M.L., Careddu, G., Rossi, L. (2017). Effect of habitat degradation on competition, carrying capacity, and species assemblage stability. *Ecology and Evolution*, *7*, 5784–5796.

Catford, J.A., Jansson, R. & Nilsson, C. (2009). Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. *Diversity and distributions*, *15*, 22-40.

Copp, G.H., Britton, J.R., Guo, Z., Edmonds-Brown, V.R., Pegg, J., Vilizzi, L. & Davison, P.I. (2017). Trophic consequences of non-native pumpkinseed *Lepomis gibbosus* for native pond fishes. *Biological Invasions*, *19*, 25-41.

David, P., Thebault, E., Anneville, O., Duyck, P.F., Chapuis, E. & Loeuille, N., (2017). Impacts of invasive species on food webs: a review of empirical data. In: *Advances in Ecological Research* (Vol. 56, pp. 1-60). Academic Press.

Dick, J.T., Alexander, M.E., Jeschke, J.M., Ricciardi, A., MacIsaac, H.J., Robinson, T.B.,…Paterson, R.A. (2014). Advancing impact prediction and hypothesis testing in invasion ecology using a comparative functional response approach. *Biological Invasions*, *16*, 735-753.

Dick, J.T., Gallagher, K., Avlijas, S., Clarke, H.C., Lewis, S.E., Leung, S.,… Harrod, C. (2013). Ecological impacts of an invasive predator explained and predicted by comparative functional responses. *Biological Invasions*, *15*, 837-846.

Dick, J.T., Laverty, C., Lennon, J.J., Barrios-O'Neill, D., Mensink, P.J., Britton, J.R,…Caffrey, J.M. (2017). Invader relative impact potential: a new metric to understand and predict the ecological impacts of existing, emerging and future invasive alien species. *Journal of Applied Ecology*, *54*, 1259-1267.

Gallardo, B., Clavero, M., Sánchez, M.I. & Vilà, M. (2016). Global ecological impacts of invasive species in aquatic ecosystems. *Global Change Biology*, *22*, 151-163.

Guo, Z., Sheath, D., Amat Trigo, F. & Britton, J.R. (2017). Comparative functional responses of native and high-impacting invasive fishes: impact predictions for native prey populations. *Ecology of Freshwater Fish*, *26*, 533-540.

Gutmann Roberts, C. & Britton, J.R. (2018) Trophic interactions in a lowland river fish community invaded by European barbel *Barbus barbus* (Actinopterygii, Cyprinidae). *Hydrobiologia*, 819, 259-273.

Howard, B.R., Barrios-O'Neill, D., Alexander, M.E., Dick, J.T., Therriault, T.W., Robinson, T.B. & Côté, I.M. (2018). Functional responses of a cosmopolitan invader demonstrate intraspecific variability in consumer-resource dynamics. *PeerJ*, *6*, p.e5634.

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

Jackson, M.C. & Britton, J.R. (2014). Divergence in the trophic niche of sympatric freshwater invaders. *Biological invasions*, *16*, 1095-1103.

Jackson, M.C., Donohue, I., Jackson, A.L., Britton, J.R., Harper, D.M. & Grey, J. 2012. Population-level metrics of trophic structure based on stable isotopes and their application to invasion ecology. *PloS One, 7*, p.e31757.

Juncos, R., Milano, D., Macchi, P.J. and Vigliano, P.H. (2015). Niche segregation facilitates coexistence between native and introduced fishes in a deep Patagonian lake. *Hydrobiologia*, *747*, 53-67.

Jurajda, P., Ondračková, M. & Reichard, M. (2004). Managed flooding as a tool for supporting natural fish reproduction in man-made lentic water bodies. *Fisheries Management and Ecology*, 11, 237-242.

Kiljunen, M., Grey, J., Sinisalo, T., Harrod, C., Immonen, H. & Jones, R.I. 2006. A revised model for lipid-normalizing δ13C values from aquatic organisms, with implications for isotope mixing models. *Journal of Applied Ecology 43*, 1213-1222.

Laverty, C., Dick, J.T., Alexander, M.E. & Lucy, F.E. (2015). Differential ecological impacts of invader and native predatory freshwater amphipods under environmental change are revealed by comparative functional responses. *Biological Invasions*, *17*, 1761-1770.

Luiselli, L. (2006). Resource partitioning and interspecific competition in snakes: the search for general geographical and guild patterns. *Oikos*, *114*, 193-211.

Mason, N.W., Lanoiselée, C., Mouillot, D., Wilson, J.B. & Argillier, C. (2008). Does niche overlap control relative abundance in French lacustrine fish communities? A new method incorporating functional traits. *Journal of Animal Ecology*, *77*, 661-669.

Mitchell, J.C. (1979). Ecology of southeastern Arizona whiptail lizards (Cnemidophorus: Teiidae): population densities, resource partitioning, and niche overlap. *Canadian Journal of Zoology*, *57*, 1487-1499

Nelson, K.A., Collins, S.F., Sass, G.G. & Wahl, D.H. (2017). A response-surface examination of competition and facilitation between native and invasive juvenile fishes. *Functional Ecology*, *31*, 2157-2166.

Paterson, R.A., Dick, J.T., Pritchard, D.W., Ennis, M., Hatcher, M.J. & Dunn, A.M. (2015). Predicting invasive species impacts: a community module functional response approach reveals context dependencies. *Journal of Animal Ecology*, *84*, 453-463.

Petchey, O.L., Beckerman, A.P., Riede, J.O. & Warren, P.H. (2008). Size, foraging, and food web structure. *Proceedings of the National Academy of Sciences*, *105,* 4191-4196

Pritchard, D.W., Paterson, R.A., Bovy, H.C. & Barrios-O'Neill, D. (2017). Frair: an R package for fitting and comparing consumer functional responses. *Methods in Ecology and Evolution*.

R Development Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ricciardi, A., Hoopes, M.F., Marchetti, M.P. & Lockwood, J.L. (2013). Progress toward understanding the ecological impacts of nonnative species. *Ecological Monographs*, *83*, 263-282.

Rogers, D. (1972). Random search and insect population models. *Journal of Animal Ecology*, *41*, 369-83

Rossi, L., di Lascio, A., Carlino, P., Calizza, E. & Costantini, M.L. (2015). Predator and detritivore niche width helps to explain biocomplexity of experimental detritus-based food webs in four aquatic and terrestrial ecosystems. *Ecological Complexity*, *23,* 14-24.

Shochat, E., Lerman, S.B., Katti, M. & Lewis, D.B. (2004). Linking optimal foraging behavior to bird community structure in an urban-desert landscape: field experiments with artificial food patches. *The American Naturalist*, *164,* 232-243.

Stock, B.C., Jackson, A.L., Ward, E.J., Parnell, A.C., Phillips, D. & Semmens, B.X. (2018). Analyzing mixing systems using a new generation of Bayesian tracer mixing models. *Peer J*, e26884v1.

Svanbäck, R. & Bolnick, D.I. (2006). Intraspecific competition drives increased resource use diversity within a natural population. *Proceedings of the Royal Society B: Biological Sciences*, *274*, 839-844.

Taylor, A.A.L., Britton, J.R. and Cowx, I.G., 2004. Does the stock density of stillwater catch and release fisheries affect the growth performance of introduced cultured barbel? *Journal of Fish Biology*, *65*, 308-313.

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

Tran, T.N.Q., Jackson, M.C., Sheath, D., Verreycken, H. & Britton, J.R. (2015). Patterns of trophic niche divergence between invasive and native fishes in wild communities are predictable from mesocosm studies. *Journal of Animal Ecology*, *84*, 1071-1080.

Vonesh, J., McCoy, M., Altwegg, R., Landi, P. & Measey, J. (2017). Functional responses can't unify invasion ecology. *Biological Invasions*, *19*, 1673-1676.

Ward, A.J., Webster, M.M. & Hart, P.J. (2006). Intraspecific food competition in fishes. *Fish and Fisheries*, *7*, 231-261.

Xu, M., Mu, X., Dick, J.T., Fang, M., Gu, D., Luo, D., Zhang, J., Luo, J. & Hu, Y. (2016). Comparative functional responses predict the invasiveness and ecological impacts of alien herbivorous snails. *PLoS One*, *11*, p.e0147017.

Table 1. Minimum, maximum and ranges of $\delta^{13}C$ and $\delta^{15}N$ per treatment in the pond mesocosm experiment. Note data are combined for all species.

	$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		
Treatment	Minimum	Maximum	Range	Minimum	Maximum	Range
Allopatric *B. barbus*	-26.29	-23.18	3.11	9.06	9.77	0.71
Allopatric *S. cephalus*	-26.13	-23.40	2.73	8.96	9.65	0.69
Allopatric *L. idus*	-26.12	-23.35	2.77	8.96	9.87	0.91
Sympatric *B. barbus*/ *S. cephalus*	-25.37	-22.84	2.53	9.12	10.23	1.11
Sympatric *S. cephalus/ L. idus*	-25.48	-23.18	2.30	9.22	10.16	0.94
Sympatric *B. barbus*/ *L. idus*	-24.42	-22.84	1.58	9.23	9.80	0.57
All species in sympatry	-26.08	-23.24	2.83	8.88	9.86	0.98

Table 2. Mean stable isotope values, isotopic niche size (as standard ellipse areas, SEAc (c = correction for small sample size) and SEA_B (Bayesian estimate of SEA) of the macroinvertebrate and macrophytes food resources, and for each fish species by treatment in pond mesocosms. For SEA_B, the mean and standard error at a credible interval of 95% (in parentheses) are presented. *Difference in niche size as SEA_B between the treatment and allopatry is significantly different ($P < 0.05$).

Spp.	Treatment	N	Mean $\delta^{13}C$ (‰)	Mean $\delta^{15}N$ (‰)	SEA_c (‰^{2})	SEA_B ‰^{2} (CI 95%)
Gammarus pulex		3	-26.22 ± 0.68	7.38 ± 0.40		
Chironomid larvae		3	-31.37 ± 1.47	5.35 ± 1.47		
Macrophyte		3	-27.82 ± 0.69	1.54 ± 0.56		
L. idus	Allopatry	15	-24.94 ± 0.21	9.41 ± 0.06	0.61	0.51 (0.31-0.93)
	B. barbus	12	-23.51 ± 0.12	9.55 ± 0.04	0.19	0.19 (0.10-0.34)*
	S. cephalus	12	-23.69 ± 0.14	9.49 ± 0.09	0.27	0.32 (0.15-0.51)
	All species	12	-23.93 ± 0.12	9.51 ± 0.07	0.33	0.33 (0.14-0.53)
B. barbus	Allopatry	15	-24.85 ± 0.23	9.34 ± 0.05	0.51	0.64 (0.26-0.81)
	L. idus	12	-23.87 ± 0.11	9.49 ±0.05	0.21	0.22 (0.08-0.27)*
	S. cephalus	12	-23.70 ± 0.15	9.60 ± 0.05	0.24	0.26 (0.12-0.41)*
	All species	12	-24.15 ± 0.19	9.18 ± 0.06	0.49	0.35 (0.22-0.71)
S. cephalus	Allopatry	15	-24.68 ± 0.20	9.46 ± 0.05	0.52	0.50 (0.27-0.80)
	L. idus	13	-24.29 ± 0.14	9.66 ± 0.04	0.26	0.26 (0.13-0.42)*
	B. barbus	12	-24.47 ± 0.20	9.94 ± 0.10	0.70	0.73 (0.33-1.16)
	All species	12	-24.69 ± 0.26	9.46 ± 0.06	0.50	0.65 (0.25-0.85)

Table 3. Predicted dietary proportions of the three putative food resources for the three fishes by treatment in the pond mesocosms.

		Mean predicted dietary proportion (5-95th percentile of distribution range)		
Spp.	Treatment	Chironomidae	*Gammarus pulex*	Macrophyte
B. barbus	Allopatry	0.33 (0.22-0.44)	0.25 (0.18-0.33)	0.42 (0.35-0.48)
	All species	0.18 (0.09-0.27)	0.32 (0.25-0.39)	0.50 (0.44-0.56)
	S. cephalus	0.10 (0.03-0.19)	0.44 (0.38-0.50)	0.46 (0.40-0.51)
	L. idus	0.12 (0.05-0.21)	0.41 (0.35-0.47)	0.47 (0.41-0.52)
S. cephalus	Allopatry	0.31 (0.21-0.42)	0.28 (0.21-0.36)	0.41 (0.34-0.47)
	All species	0.32 (0.21-0.46)	0.28 (0.19-0.36)	0.40 (0.33-0.47)
	L. idus	0.22 (0.13-0.32)	0.37 (0.31-0.44)	0.40 (0.34-0.46)
	B. barbus	0.29 (0.18-0.42)	0.38 (0.29-0.46)	0.33 (0.26-0.39)
L. idus	Allopatry	0.36 (0.24-0.49)	0.24 (0.16-0.33)	0.40 (0.32-0.47)
	All species	0.15 (0.07-0.23)	0.40 (0.34-0.46)	0.46 (0.40-0.51)
	S. cephalus	0.09 (0.03-0.18)	0.43 (0.37-0.48)	0.48 (0.42-0.53)
	B. barbus	0.07 (0.01-0.14)	0.46 (0.40-0.51)	0.47 (0.43-0.53)

Figure captions

Figure 1. Mean specific growth rates of cohabitation experiments completed in (A) tank aquaria, and (B) pond enclosures, where C = control (i.e. each species in allopatry), Ch = sympatry with chub *Squalius cephalus*, Id = sympatry with ide *Leuciscus idus*, Ba = sympatry with barbel *Barbus barbus*, and All = all species in sympatry. Clear circles: barbel, black circles: chub, grey circles: ide. Note differences in axes values between (A) and (B).

Figure 2. Stable isotope bi-plots comparing the standard ellipse area (SEA_c) the fishes in allopatry and sympatry, where A) SEAc of each species in allopatry, B) the species all in sympatry, C) sympatric *L. idus* and *B. barbus,* D) sympatric *B. barbus and S. cephalus*, and E) sympatric *L. idus* and *S. cephalus*. Filled circles/ black dashed line: *L. idus,* filled triangles and black solid lines: *B. barbus*; clear squares, and grey solid lines: *S. cephalus*. The mean SI data for the fish putative food resources were Chironomid larvae: δ^{13}C: -31.37 ± 1.47 ‰, δ^{15}N: 5.35 ± 1.47 ‰; G. pulex: δ^{13}C: -26.22 ± 0.68 ‰, δ^{15}N: 7.38 ± 0.40 ‰; macrophyte: δ^{13}C: -27.82 ± 0.69 ‰, δ^{15}N: 1.54 ± 0.56 ‰.

Figure 3. (A) Relationships of differences in isotopic niche size (as SEAc) between allopatric and sympatric treatments versus their differences in δ^{13}C; and (B) Relationships of differences in mean δ^{13}C between allopatric and sympatric treatments per species versus differences in their predicted dietary proportions per food resource (Chironomid larvae: clear circles, dashed line; *Gammarus pulex*: filled circles, small dashed line; macrophytes: grey circles, solid line). All straight lines

represent the significant linear relationship between the variables (linear regression: $P < 0.03$).

Figure 4. Relationships of the mean intra-specific (A) and intra-specific (B) trophic dissimilarity versus specific growth rate for fishes in sympatric treatments in the pond mesocosm experiment. The solid line represents the significant relationship between the variables according to linear regression.

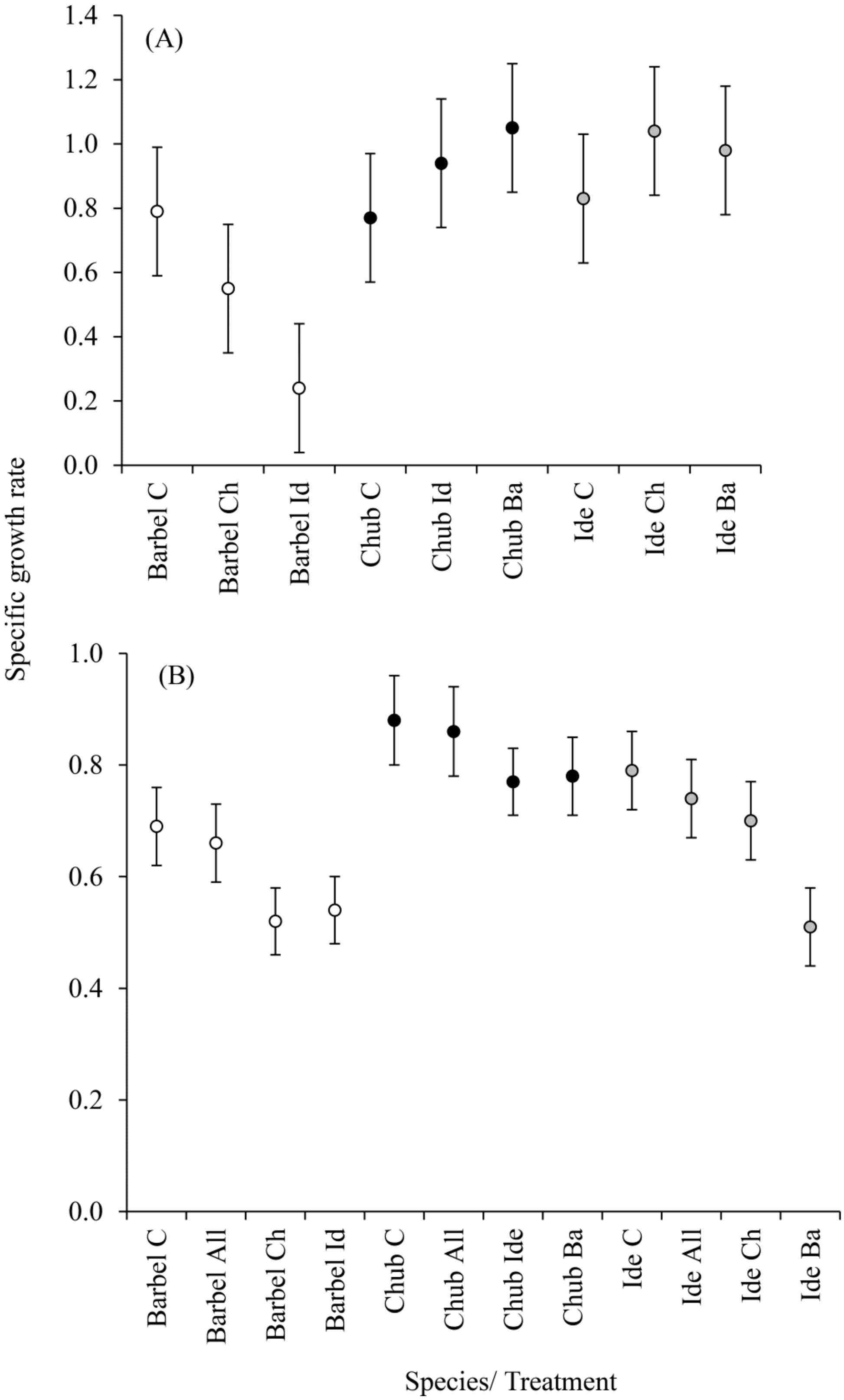
(A)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Barbel C
Barbel Ch
Barbel Id
Chub C
Chub Id
Chub Ba
Ide C
Ide Ch
Ide Ba
Specific growth rate
(B)
1.0
0.8
0.6
0.4
0.2
0.0
Barbel C
Barbel All
Barbel Ch
Barbel Id
Chub C
Chub All
Chub Ide
Chub Ba
Ide C
Ide All
Ide Ch
Ide Ba
Species/ Treatment

Figure 1.

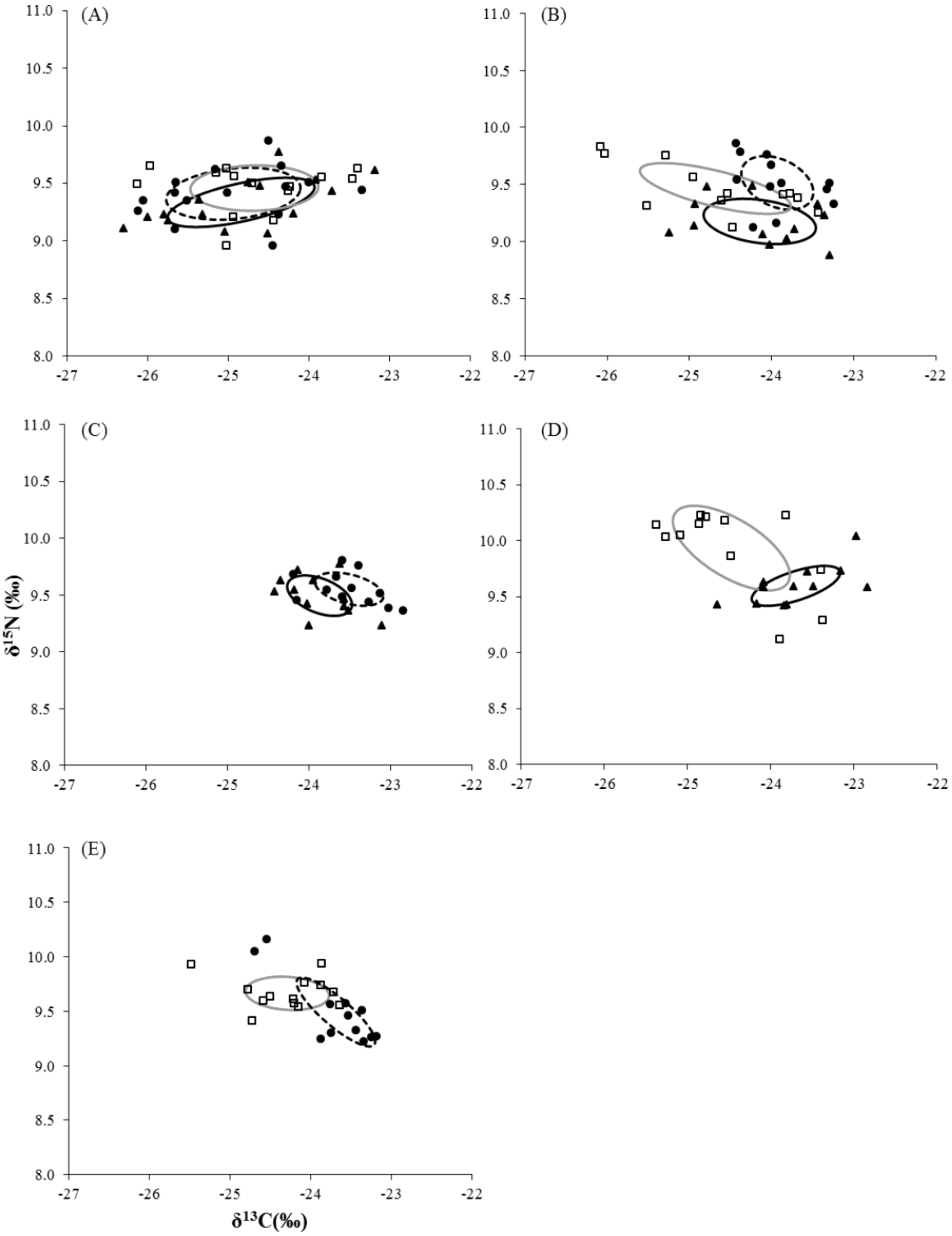

Figure 2.

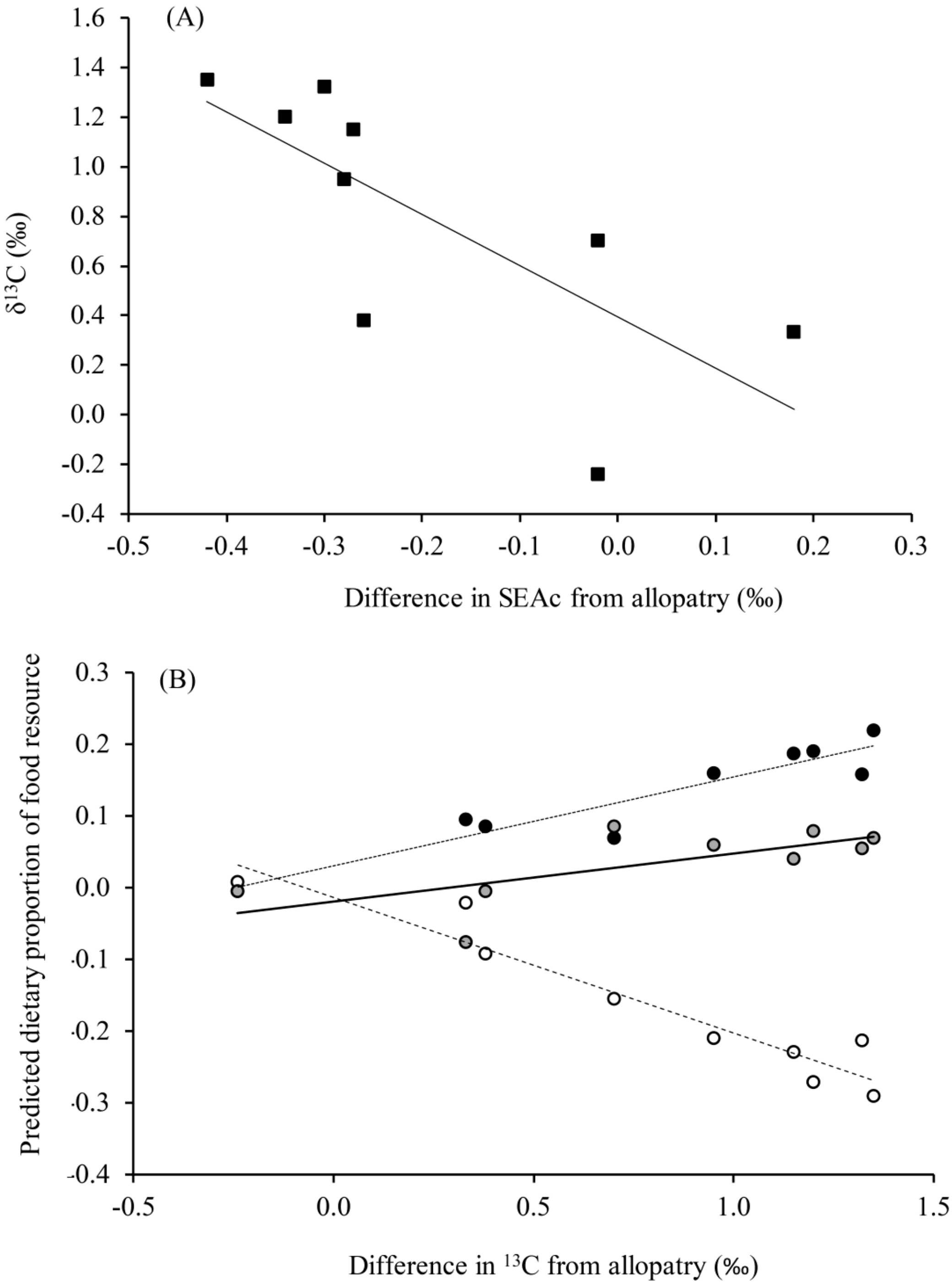
(A)
δ^{13}C (‰)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.5
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
Difference in SEAc from allopatry (‰)
(B)
Predicted dietary proportion of food resource
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
0.0
0.5
1.0
1.5
Difference in ^{13}C from allopatry (‰)

Figure 3.

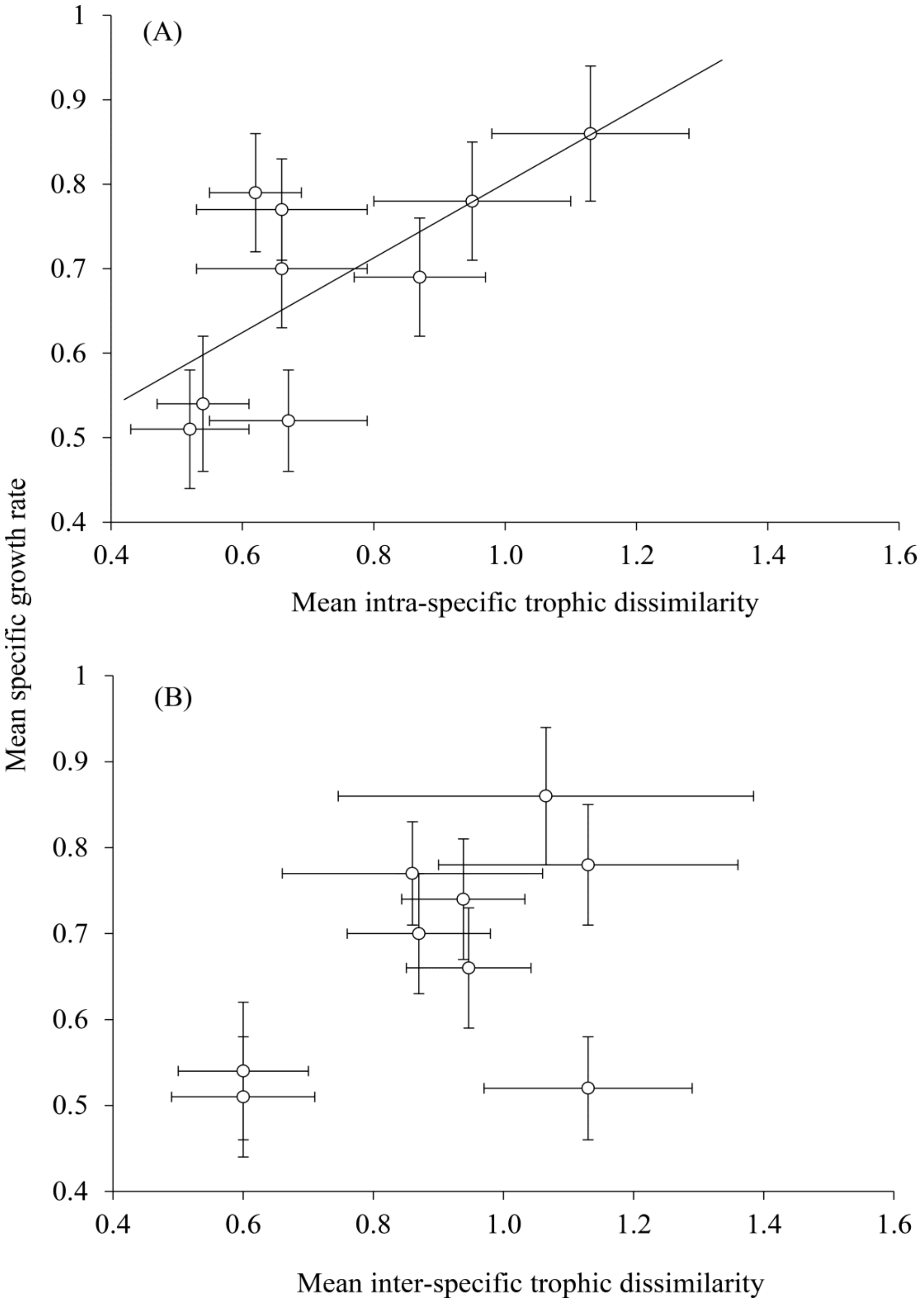
Mean specific growth rate
(A)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.4
0.6
0.8
1.0
1.2
1.4
1.6
Mean intra-specific trophic dissimilarity
(B)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.4
0.6
0.8
1.0
1.2
1.4
1.6
Mean inter-specific trophic dissimilarity

Figure 4.

Supplementary material

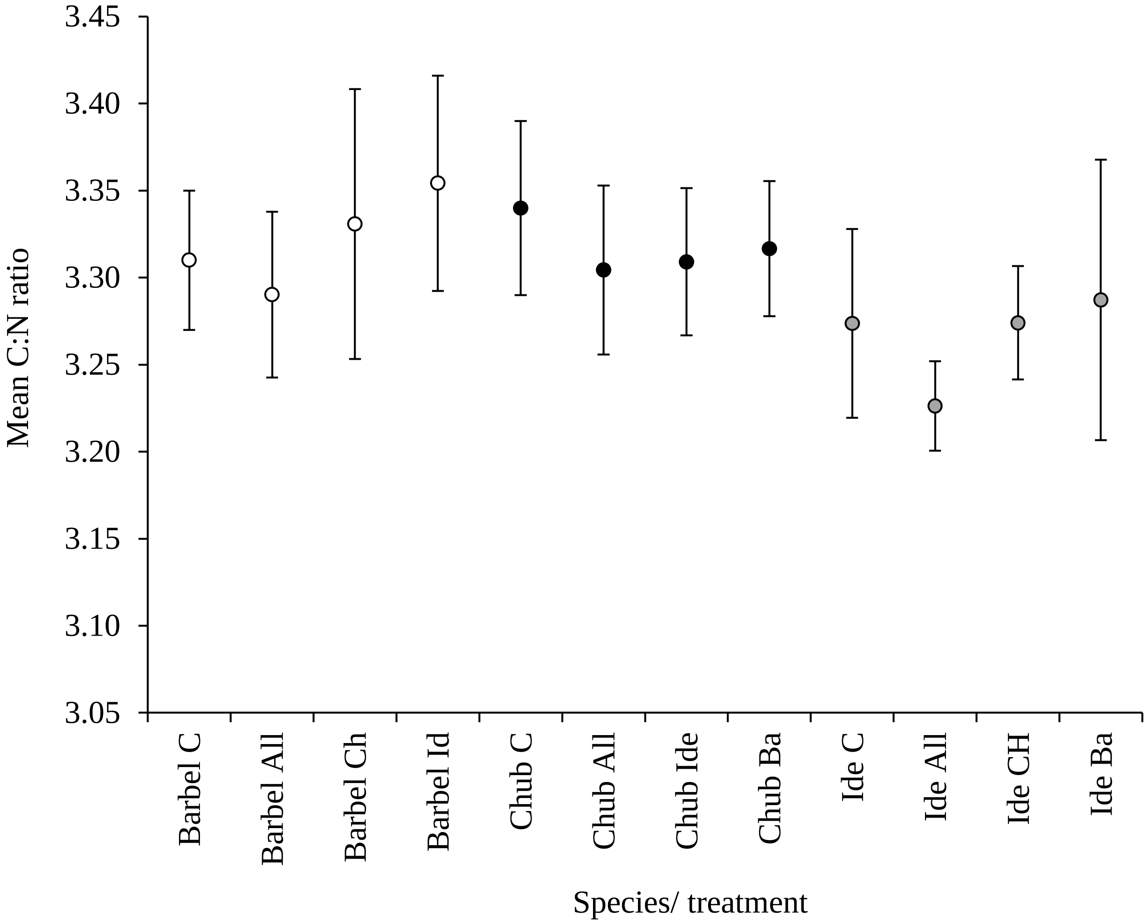

Figure S1. Mean C:N per species and treatment in the pond enclosures, where C = control, Ch = sympatry with chub *Squalius cephalus*, Id = sympatry with ide *Leuciscus idus*, Ba = sympatry with barbel *Barbus barbus*, and All = all species in sympatry. Clear circles: barbel, black circles: chub, grey circles: ide. Note differences in axes values between (A) and (B). Differences in C:N ratios between the species per treatment were not significant ($F_{1,152}$ = 1.74, P = 0.10).

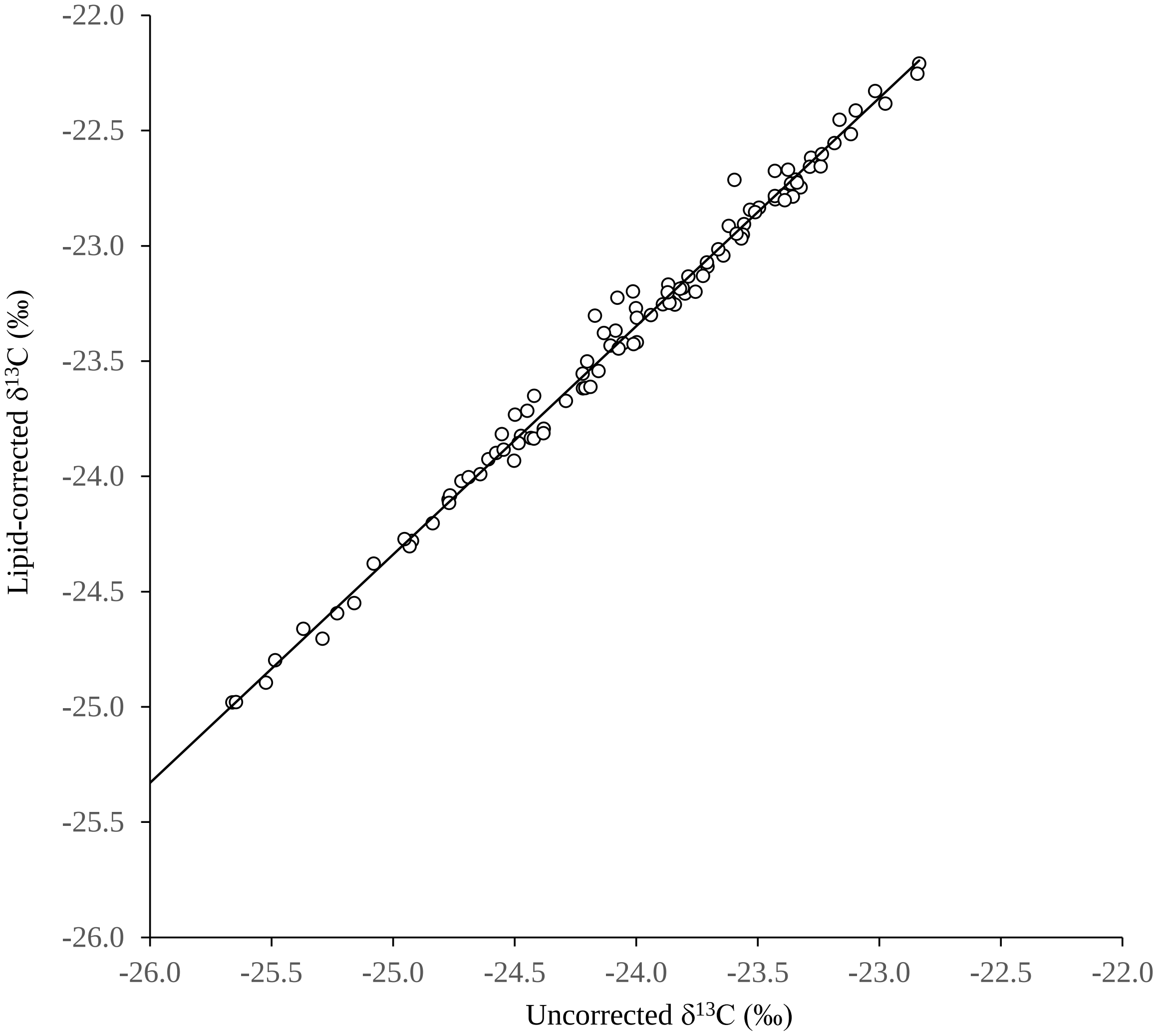

Figure S2. Relationship of uncorrected versus lipid corrected δ^{13}C for all fish samples (Kiljunen et al. 2006), where the solid line is the significant relationship according to linear regression ($R^2 > 0.99$, $F_{1,152} = 15066.9$, $P < 0.001$).

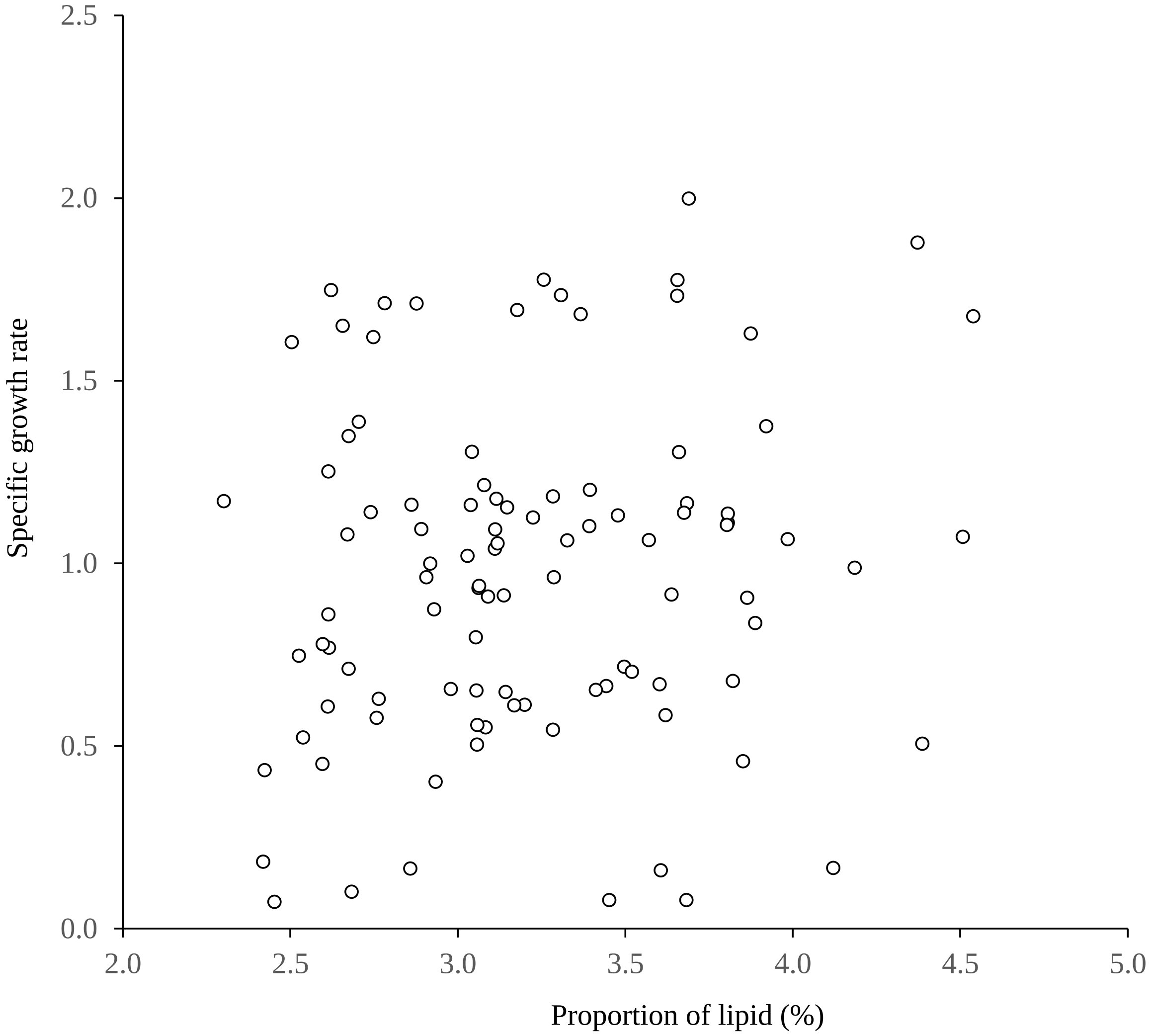

Figure S3. Relationship of proportion of lipid in the analysed dorsal muscle samples of each individual fish, as calculated $\delta^{13}C$ and C:N ratios (Post et al. 2007), versus their specific growth rates. The relationship was not significant according to linear regression (R^2 = 0.02, $F_{1,152}$ = 2.18, P = 0.14).

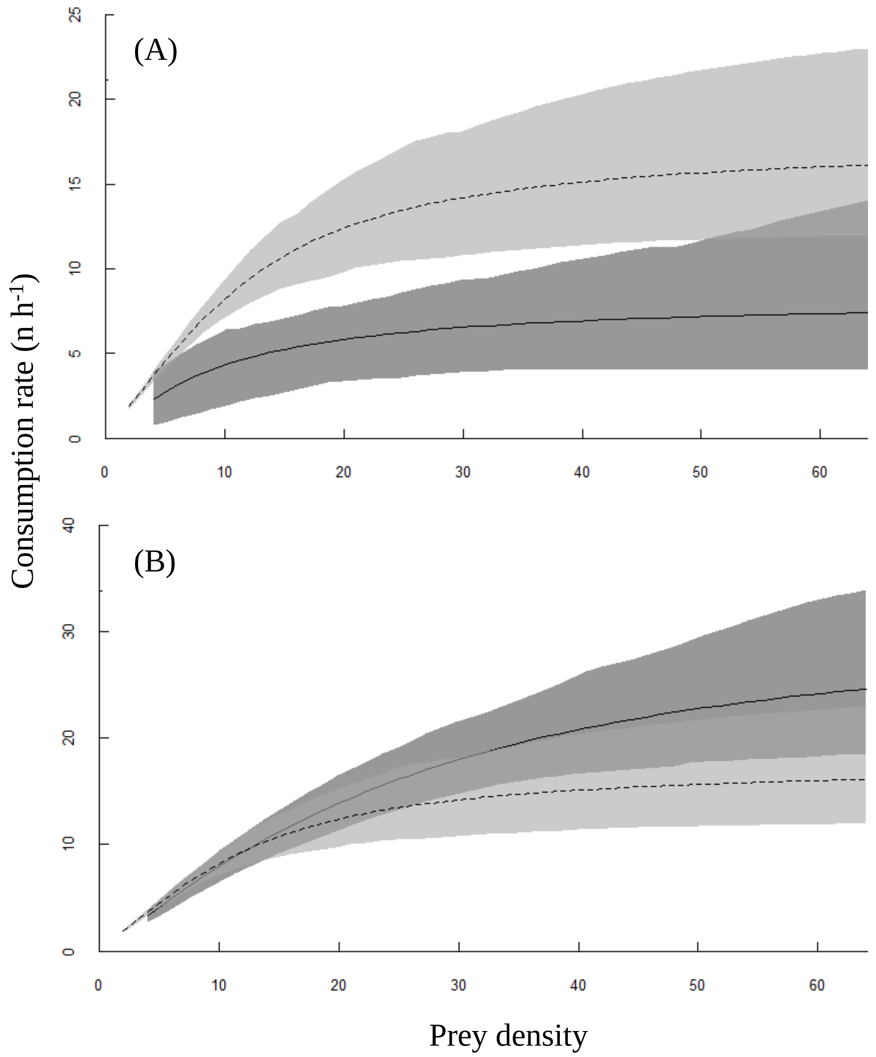

Figure S4. Comparative functional response curves for *Gammarus pulex* as prey, comparing *Leuciscus idus* (dashed line) versus (A) *Barbus barbus* (solid line) and (B) *Squalius cephalus* (solid line). Shaded areas around the curves represent 95 % confidence intervals generated by boot-strapping. Note differences in values on the Y axis.

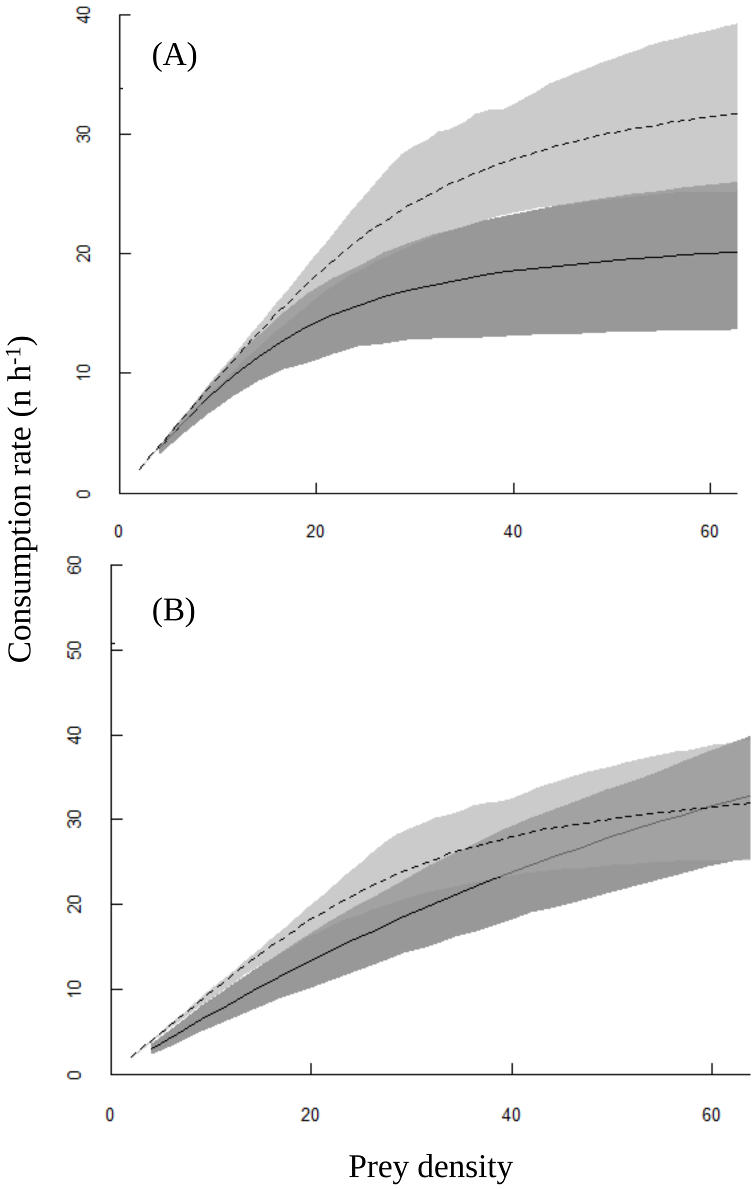

Figure S5. Comparative functional response curves for Chironomid larvae as prey, comparing *Leuciscus idus* (dashed line) versus *Barbus barbus* (solid line) (A) and (B) *Squalius cephalus* (solid line). Shaded areas around the curves represent 95 % confidence intervals generated by boot-strapping. Note differences in values on the Y axis.

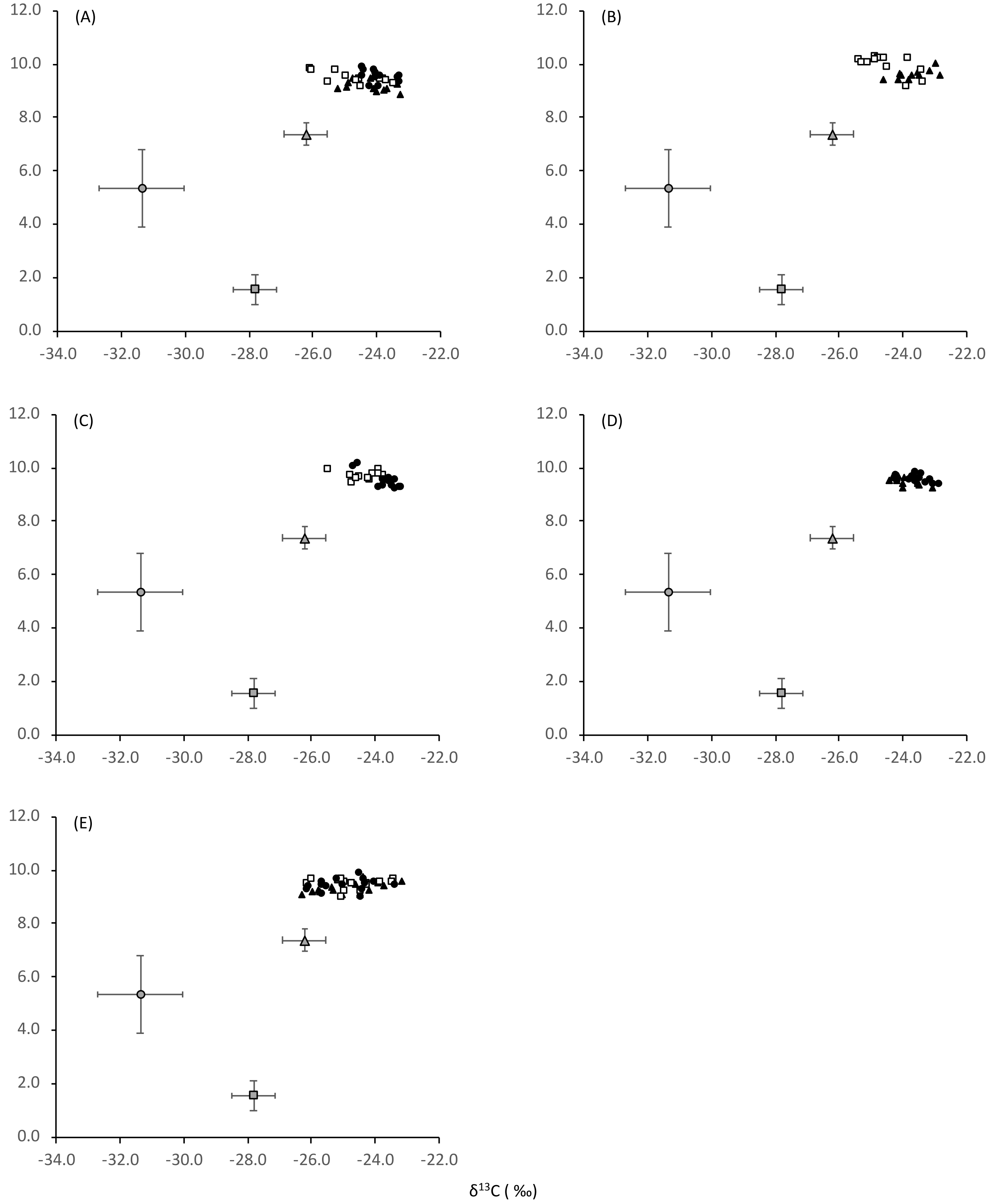

Figure S6. Stable isotope biplots for (A) All species sympatric treatment; (B) *Barbus barbus*/ *Squalius cephalus* species pair treatment; (C) *S. cephalus*/ *Leuciscus idus* species pair treatment; (D) *B. barbus/ L. idus* species pair treatment; and (E) All species in allopatry. For fish, filled circles: *L. idus*; filled triangles: *B. barbus*; clear squares: *S. cephalus*. For putative prey used in the stable isotope mixing models to predict fish diet, grey circle = Chironomid larvae; grey triangle = *Gammarus pulex*; grey square = macrophyte. Error bars represent 95 % confidence limits.

References

Kiljunen, M., Grey, J., Sinisalo, T., Harrod, C., Immonen, H. & Jones, R.I. 2006. A revised model for lipid-normalizing δ13C values from aquatic organisms, with implications for isotope mixing models. *Journal of Applied Ecology 43*, 1213-1222.

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