Trophic consequences of competitive interactions in freshwater fish: density dependent effects and impacts of interspecific versus intraspecific competition

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Abstract

1. Determining the comparative impacts of increased intra- versus inter-specific competition is important in freshwater ecosystems for understanding the ecological changes that can result from activities such as fish stocking events (using alien and/ or native fish species), as well as from natural processes that elevate population abundances (e.g increased annual recruitment success). While increased inter-specific competition can result in slower growth rates and/ or reduced population density in the weaker or less abundant competitor, it is important that this is assessed in relation to the impacts of increased intra-specific competition.

2. We tested how the strength of inter-specific competition from a co-existing species varies with abundance, and how this compares with increased intra-specific competition. Fish were the model taxa, as their growth rates strongly correlate with competitive success. Replicated pond mesocosms (150 days) used chub *Squalius cephalus* in an allopatric control (n=5; C5) and allopatric treatment (n=10; C10), and in sympatric treatments (n=5) with European barbel *Barbus barbus* (n=5 (T1), 10 (T2) and 15 (T3)). Treatment effects were tested on fish specific growth rates (SGR), and the size and position of the trophic and isotopic niche (stomach contents and stable isotope analyses (SIA) respectively).

3. Chub SGRs were significantly higher in C5 versus all other treatments, but did not differ among the other allopatric and sympatric treatments. Chub trophic niche sizes in T1 to T3 were significantly smaller than C5, indicating more specialised diets in the presence of barbel. Chub trophic niche size in C10 was, however, larger than C5

and T1, indicating a shift to a more generalised diet as intra-specific competition increased.

4. As SGRs reduced in treatments, so did the predicted extent of fish stable isotope turnover, with SI data in T1 to T3 not at isotopic equilibrium with their diet in the mesocosms at the experiment's end. Following conversion of fish SI data to represent values at 95% isotopic turnover, chub isotopic niches also revealed shifts to a more general diet as intra-specific competition increased, but to more specialised diets as inter-specific competition increased.

5. Increased intra- and inter-specific competition impacts on the trophic and isotopic niches were contrasting; both metrics indicated niche constrictions in sympatry but niche expansions in allopatry. Impacts on fish growth were evident from both. These results emphasise that the trophic consequences of competition in freshwater fish can differ between stocking events involving the release of conspecifics or other species, with this having important considerations for how freshwater fish communities are managed for angling exploitation.

1. Introduction

Anthropogenic activities in freshwater ecosystems frequently manipulate the fish assemblage to either diversify the species present and/ or increase their abundance (Piria et al. 2018; Vitule et al. 2019). This often involves the release of alien species that can ultimately result in an invasion that could have detrimental impacts on native biodiversity (Simerloff et al., 2013; Dominguez Almela et al., 2020). However, it also often involves the release of native species, either translocated from other water or through use of hatchery-reared fish (Cowx and Gerdeaux 2004). Irrespective of whether the released fish are of alien or native origin, they have the potential to impact native species through increased inter-specific competitive interactions (Gozlan et al., 2010; Britton et al., 2018). The intensity of trophic impacts resulting from these interactions can be more severe when the released and native species are taxonomically similar (Ricciardi & Atkinson, 2004; Li et al., 2015) or functionally analogous (Dick et al., 2016, 2017), as it is more probable that the species will share the same prey resources (Buoro et al., 2016). However, increases in the abundance of fish populations can also occur naturally, especially in temperate lowland rivers where the main drivers of recruitment success are abiotic factors such as water temperature and river discharge that fluctuate annually (Nunn et al. 2007).

The ability of fishes to co-exist within communities is at least partially related to the extent of partitioning of the prey resources between the species, which then relates to how the trophic niche of each species is modified between their allopatric and sympatric contexts (Britton et al., 2018). There are a number of hypotheses regarding how the trophic niches of co-existing species respond to changes in the intensity of their inter-specific competitive interactions (Ricciardi et al., 2013). If the species co-exist in an ecosystem where some prey resources are either unexploited or under-utilised, then the increased exploitation of these resources by at least one of the species should reduce their inter-specific competitive interactions (Okabe & Agetsuma, 2007; Mason et al., 2008; Juncos et al., 2015). Where the resources are either fully exploited or less abundant in the new ecosystem, niche theory suggests that through increased inter-specific competitive interactions, the trophic niche sizes of all species will be reduced compared with their allopatric contexts (Bolnick et al., 2010; Tran et al., 2015; Jackson et al., 2016). Alternatively, this increased inter-specific competition can result in larger niche sizes through the populations exploiting a wider range of prey items (Svanbäck & Bolnick, 2007). If the inter-specific competitive interactions are asymmetric between the coexisting species, the weaker species might be competitively excluded (Tran et al., 2015), leading to trophic niche displacement that potentially results in reduced food intake, slower growth rates and/ or reduced population density (Bøhn, Amundsen & Sparrow, 2008).

Given this apparent importance of inter-specific interactions in driving how competition alters the trophic ecology of populations, it is then important to understand how population density modifies the strength of inter-specific competition (Jackson et al., 2014). In invasion biology, impacts are often assumed to increase in proportion with invader abundance (e.g. Yokomizo et al., 2009, Elgersma & Ehrenfeld, 2011), with studies having a tendency to only compare scenarios of high invader density versus situations where the invader is absent (e.g. Britton et al., 2010). There is increasing evidence that many ecological impacts actually increase non-linearly with fish abundance (Elgersma & Ehrenfeld, 2011), with Jackson et al. (2014) revealing that across a range of population densities of the Asian invasive fish, topmouth gudgeon *Pseudorasbora parva*, impacts were both linear (e.g. on phytoplankton standing stock) and non-linear (e.g. on benthic invertebrate abundance). While testing the extent of alien versus native species can be important, it should also be considered in the context of the strength of increased intra-specific competition, as Buoro et al. (2016) suggested that increased numbers of conspecific fish (e.g. from fish stocking exercises) can have greater ecological consequences than releasing alien fishes, due to the released conspecifics having virtually identical traits to the extant fish that can result in a greater extent of resource sharing.

Therefore, the aim of this study was to test how the trophic ecology (e.g. trophic niche size and position) of a model species is altered by the increased abundance of a co-existing species, and how these impacts relate to those from increased intra-specific competition. The model animals were freshwater fishes, as these are adaptable and tractable animals that provide excellent model systems for experimental competitive studies with, for example, their indeterminate nature of growth enabling correlation with competitive success (Ward et al., 2006; Britton et al., 2019). The model species was chub *Squalius cephalus*, a fish of the Cyprinidae family that is found throughout much of Northwest Europe. Although generally considered a lotic species, it is also encountered in lentic environments. The coexisting species was European barbel *Barbus barbus*, which has been introduced widely outside of its natural range to enhance angling in both lentic and lotic habitats (Taylor et al., 2004; Britton & Pegg, 2011). Alien barbel in rivers in western England usually co-exist with native chub *Squalius cephalus*, where the two fishes tend to be the largest cypriniform fishes present (Gutmann Roberts & Britton, 2018a,b). In rivers in Eastern England, they coexist as native species, as barbel is considered indigenous in these areas due to its post-Pleistocene colonisation of eastern flowing rivers that had connection with the Rhine and Danube (Wheeler & Jordan, 1990; Antognazza et al. 2016). The relatively large body sizes and omnivory of both species suggest they will also share similar prey resources, especially in the absence of recreational angling that can otherwise result in some barbel feeding mainly on angler bait (De Santis et al., 2019). Correspondingly, using a pond mesocosm experiment with chub as the model species and barbel as the co-existing released species, the experiment tested the relative strength of increased inter- and intra-specific competition on chub somatic growth rates, and their trophic and isotopic niche sizes.

2. Materials and Methods

2.1 Experimental design

The experimental design (hereafter referred to as the 'experiment') used 5 additive and substitutive treatments across a combination of allopatric and sympatric contexts, with each treatment replicated three times (Table 1). Two control treatments used native chub in allopatry ('Allopatry'; N = 5, 10; Table 1). Three substitutive treatments then paired the native chub and non-native barbel in the three different sympatric combinations (Table 1). All the fish used in the treatments were juveniles (starting mass 2.5 to 3.8 g) and sourced from a hatchery in southern England where they had been pond-reared for at least six months prior to the experiment and so were expected to demonstrate natural behaviours. The experiment ran for 150 days between March and July 2018, providing time for the fish to potentially be at isotopic equilibrium with their new diet, given that for fish of starting weight < 10 g, the stable isotope half-life of dorsal muscle is 36 and 38 days for $\delta^{13}C$ and $\delta^{15}N$ respectively (Thomas & Crowther 2015).

The experiment was completed using treatments within enclosures that were located within a larger, man-made pond (30 x 30 m; 1 m consistent depth), located in Southern England. Following Britton et al., (2018), the enclosures that were constructed of an aluminium frame (length 1.7 m; width: 1.1 m; height: 1.2 m) within a net (mesh: 7 mm^2) that prevented fish in-and egress but allowed movements of invertebrates. The enclosures were placed randomly across the pond, with at least 0.5 m between them; they were sufficiently heavy that they remained *in-situ* throughout the experimental period without movement and they sat on the substrate, with macrophytes (primarily *Elodea* spp.) able to grow within each of them (Britton et al., 2018). The enclosures were covered by netting (15 mm mesh) to prevent bird predation. The total mass of fish per species was weighed (nearest 0.1g) prior to release into each replicate per treatment. Temperature loggers (TinyTag TGP-4017) in the larger pond (located in the lower third of the water column) revealed the mean water temperature was 15.6 ± 0.2 °C (range 8.9 to 18.4 °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 on ice. For the purpose of stable isotope analysis (SIA), putative prey samples of the fish were collected from the larger pond, comprising of aquatic macroinvertebrates, terrestrial invertebrates and macrophyte samples. These were sorted into samples (one sample = 3 to 9 invertebrate individuals per species), with triplicate samples taken.

In the laboratory, the fish were measured and weighed, and a dorsal muscle sample taken for SIA. Along with the putative prey resources, all samples were dried at 60°C to constant mass before analysis of $\delta^{13}C$ and $\delta^{15}N$ at the Cornell University Stable Isotope Laboratory, New York, USA, where they were ground to powder and weighed precisely to ~1000 µg in tin capsules and analysed on a Thermo Delta V isotope ratio mass spectrometer (Thermo Scientific, USA) interfaced to a NC2500 elemental analyser (CE Elantach Inc., USA). Data outputs were in the format of delta (δ) isotope ratios expressed per mille (‰). As the C:N ratios indicated very low lipid content (≤ 3.5) (Post et al., 2007), data were analysed without lipid corrections.

2.2 Data analysis

To determine fish growth rates in the experiment, the mean specific growth rate (SGR) in mass per replicate and species was calculated using: $[(\ln W_{t+1}) - (\ln W_t))]/t$, where W_t = mean starting weight of the species in the replicate, W_{t+1} = mean end weight of the species in the replicate, and t = the duration of the experiment (days). A generalized linear model (GLM) tested the differences in SGR between treatments for each species, where SGR was the dependent variable, treatment was the independent variable, and total fish starting mass in each replicate being the covariate. Model outputs were mean SGR per treatment (adjusted for the effect of the covariate) and the significance of differences in SGR between treatments according to pairwise comparisons.

Fish stomach contents analyses were completed by examining the contents of the entire intestine of each fish under a dissecting microscope (×5 to × 50 magnification). During the analyses, the number of empty intestines was noted and converted to the vacuity index ([number of empty stomachs/ number of stomachs]x100), and the prey items identified to the lowest taxonomic group possible before being grouped into the appropriate categories. The initial analyses were for prey specific abundance (%*Pi*), calculated from $100(\Sigma S_i \times \Sigma S_{ti}^{-1})$, where S_i = the stomach content (number) composed of prey $_i$ and S_{ti} is the total number of prey items in stomachs that contained that item (Leunda et al., 2008). For estimating the trophic niche size, the dietary data were square-root transformed and a Bray Curtis similarity matrix built to enable calculation of the 40% standard deviation ellipses through a non-metric multidimensional scaling (NMDS) approach within the R package '*vegan*' within R 3.4.2 (R Core Team 2017) (Oksanen et al., 2019), where the Bray-Curtis dissimilarity index and 30 maximum numbers of random starts were used to identify a stable solution. Then, to assess whether the experimental treatments were having significant effects on these niche sizes, permutational ANOVA was performed for each species within the treatments using the *adonis* function available in the *vegan* R package. To control for any effect of pond mesocosm position in the model, pond number was used as a covariate. Pairwise comparisons were then used to determine the significance of differences between the treatments.

As the treatments were completed within the same larger pond, all the fish had the same isotopic baseline and thus their SI data and niche data were able to be compared between species and treatments without any baseline corrections. Data per species were combined from replicates for each treatment to provide representative sample sizes sufficient for subsequent analyses. A minimum of four randomly chosen individuals were sampled from each replicate to provide a balanced dataset across the experiment.

The initial analyses using the SI data tested the δ^{13}C and δ^{15}N data per replicate versus their SGR. This relationship was significant, with the fish of lower SGRs having significantly enriched δ^{13}C and δ^{15}N (see Results). This suggested that in some replicates and treatments, the fish had yet to reach isotopic equilibrium with their new diet, which is generally considered to be when the extent of isotopic turnover in tissues is at 95 % (Vander Zanden et al. 2015; Winter et al. 2019). Therefore, the fish SI data were converted to values that represented isotopic equilibrium with their new diet. This required the application of a conversion factor to the SI data that was determined from the relationship between the rate of change in the SI data with the rate of stable isotope turnover as the fish approached dietary equilibrium. This was completed in a three-step process: (i) for each species per replicate, determine the mean SI value by species and predict their mean extent of isotopic turnover during the experiment; (ii) calculate the stable isotope conversion factors each species per replicate; and (iii) apply the replicate-and species-specific conversion factors to each fish in that replicate. These steps were completed as follows:

(i) Following determination of mean $\delta^{13}C$ and $\delta^{15}N$ per species in each replicate ($SI_{\bar{x}}$), the extent of their isotopic turnover in the experiment was then predicted (G_{actual}) using their change in mean mass ($W_{\bar{x}}$) between the start (W_t) and end of the experiment (W_{t+1}). Rates of isotopic turnover can be expressed as a function of change in mass ('G', where $G_{0.5}$ = increase in mass for 50 % isotopic turnover and $G_{0.95}$ = increase in mass for 95 % turnover (Winter et al., 2019). For $\delta^{15}N$ of barbel dorsal muscle, one half-life of isotopic turnover equals 1.39 x body mass ($G_{0.5}$) (Busst & Britton 2018). As equivalent data were unavailable for barbel $\delta^{13}C$, and for $\delta^{13}C$ and $\delta^{15}N$ of chub, then this value of $G_{0.5}$ was applied to both species and isotopes to convert values of $W_{\bar{x}}$ to predicted isotopic turnover rates during the experiment (G_{actual}). This was completed by interpolating to find mass at 95% isotopic turnover (G_{95}) which we considered isotopic equilibrium with the new diet. For example, using $G_{0.5}$ = 1.39 x body mass (Busst & Britton 2018), a fish of starting mass 3.0 g is predicted to be 11.20 g at 93.75% isotopic turnover (4 half-lives), 15.6 g at 96.9% turnover (5 half-lives), and thus 13.0 at G_{95}.

(ii) To calculate the conversion factors for each isotope, species and replicate, the initial step was to determine the mean ratio of $SI_{\bar{x}}$ and G_{actual} per species across all replicates. This was taken as the mean value of all of the ratios ($SIG_{\bar{x}}$) calculated for each replicate ('rep') combination, determined from: $[(SI_{\bar{x}rep}-SI_{\bar{x}rep+1})/ (G_{actualrep}- G_{actualrep+1})]$. The conversion factor (CF) for each isotope, species and replicate was then determined from $[(G_{0.95} - G_{actual}) \times SIG_{\bar{x}}]$.

(iii) The predicted SI data for each fish ($SI_{G0.95}$) was determined from CF x SI_{actual}, where SI_{actual} was the original value of $\delta^{13}C$ or $\delta^{15}N$ of that fish.

The SI data were then used to calculate the trophic niche size of each species per treatment using the isotopic niche (Jackson et al., 2011). Both SI_{actual} and $SI_{G0.95}$ data were used to assess their niche positions and sizes, where they represented these metrics at the end of the experiment (SI_{actual}) and when the fish would have been at isotopic equilibrium had the experiment continued ($SI_{G0.95}$). Whilst closely related to the trophic niche, the isotopic niche is also influenced by factors including growth rate and metabolism (due to their respective effects on stable isotope turnover rates; Busst & Britton 2018), and thus represents a close approximation of the trophic niche (Jackson et al., 2011). It was calculated using standard ellipse areas (SEA) in SIBER (Jackson et al., 2011), a bivariate measure of the distribution of individuals in isotopic space; as each ellipse encloses ≈40% of data, they reveal the population's typical resource use (Jackson et al., 2012). Due to the small samples in the experiment (i.e. <30) a Bayesian estimate of SEA (SEA_B) was used to test differences in niche sizes between species, calculated using a Markov chain Monte Carlo simulation (10^4 iterations per group) (Jackson et al., 2011). Where 95% confidence intervals of SEA_B overlapped between comparator species, the isotopic niches were interpreted as not being significantly different in size. The stable isotope data were then used to calculate isotopic niche overlap (%) between the species in each treatment and across treatments using SEA_c calculated in SIBER, where subscript '$_c$' indicates a small sample size correction was used (Jackson et al., 2012). Use of SEA_c was only 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; Syväranta et al., 2013).

3. Results

3.1 Fish recovery at the end of the experiment

At the conclusion of the experiment, the recovery rate of chub from across the mesocosms was 83.3 %, with the main loss being one replicate of C5 (all fish lost) and one replicate of T1 (1 of 5 fish recovered). Both losses were assumed to be due to netting failure due to a storm the day before the experiment's conclusion, with these replicates removed from subsequent analyses. This resulted in the number of chub being analysed for their stable isotopes in C5 and T1 being constrained to n = 10 and n = 8 respectively (Table 2, 3).

3.2 Specific growth rates and stomach contents data

The GLM testing the effect of the experimental treatments on chub SGR revealed significant differences between the treatments (GLM: Wald $\chi^2 = 81.56$, $P < 0.01$), although the effect of initial fish mass was not significant ($P = 0.65$). SGR was significantly higher in C5 than in all other treatments ($P < 0.01$ in all cases), whereas differences between C10 versus T1 to T3 were not significant ($P = 1.00$) (Fig. 1).

The vacuity indices of the fish stomachs were 0 % for barbel and 1.3 % for chub. The main prey of both species were aquatic insects and macrophytes (Table 2). Prey specific abundances varied between species and treatments that translated into considerable differences in trophic niche sizes between the chub treatments with the smallest niche being in T3 and largest in C10 (Table 2). These differences in chub niche size were significant (PERMANOVA: $F = 8.02$, $P < 0.01$), with pairwise comparisons revealing the niche size in C5 was significantly larger than those in T1, T2 and T3 (Bonferroni adjusted $P = 0.05, 0.01, 0.02$, respectively). The NMDS plot also revealed some inter-specific differences in trophic niche positions, with intra-specific differences also evident in chub, where niche overlap was apparent between C5 with C10 and T1, but with no overlap in C5 versus T2 and T3 (Fig. 2).

3.3 Stable isotope analyses

The relationships of SGR versus both $\delta^{13}C$ and $\delta^{15}N$ (as SI_{actual}) were both negative and significant, with enriched values of both isotopes as SGR decreased ($\delta^{13}C$: $R^2 = 0.88$, $F_{1,12} = 84.08$, $P < 0.01$; $\delta^{15}N$: $R^2 = 0.82$, $F_{1,12} = 54.66$, $P < 0.01$; Fig. 3A). Conversion of SGR to the predicted isotopic turnover rate (G_{actual}) revealed the number of half-lives (according to the change in fish mass over the experiment; $G_{0.5}$) varied between 3.4 and 4.7, with this also significantly related to both $\delta^{13}C$ and $\delta^{15}N$, where more enriched isotope values were associated with lower $G_{0.5}$ values ($\delta^{13}C$: $R^2 = 0.86$, $F_{1,12} = 78.28$, $P < 0.01$; $\delta^{15}N$: $R^2 = 0.68$, $F_{1,12} = 25.85$, $P < 0.01$; Fig. 3B). The relationship between SGR and $G_{0.5}$ was also significant, best described by polynomial regression ($R^2 = 0.97$, $F_{2,11} = 88.21$, $P < 0.01$; Fig. 3C). Due to these significant relationships of SGR, $G_{0.5}$ and the SI_{actual} data (Fig. 3), values of SI_{actual} were converted to their predicted values at $G_{0.95}$ ($SI_{G0.95}$). The conversion had the effect of depleting the $\delta^{13}C$ and $\delta^{15}N$ values of the fish in T1 and T3 (Fig. 4A,B) and brought the fractionation factors of the experimental fish with their putative prey resources across the experiment to values generally within the range of those expected in both species (Busst & Britton 2016) (Table 3; Fig. 4A,B).

The standard ellipse areas (as SEA_B) of both SI_{actual} and $SI_{G0.95}$ data revealed that differences in the isotopic niches of C5 versus all other treatments were not significantly different, with overlap evident in the 95 % intervals around their means (Table 4). In all treatments, there were considerable inter-specific differences in the positions of these niches in isotopic space, with no overlap between chub and barbel in T1, T2 and T3 for both SI_{actual} and $SI_{G0.95}$ data

(Fig. 5A,B). In addition, there were shifts in chub isotopic niche position between C5 and T1 to T3; C5 overlapped with C10 by 99 %, by 19 % with T1, but not overlap at all with T4 and T5 (Fig. 5B). For C10, their 95 % intervals around mean SEA_B of $SI_{G0.95}$ was significantly larger than T1 and T2, but not C5 and T3, and had some overlap with all of them (17 to 40 %; Fig. 5B).

4. Discussion

The presence of coexisting barbel in the sympatric treatments had marked impacts on the growth, isotopic turnover rates and trophic niche sizes of the chub when compared to the allopatric controls. Specific growth rates were significantly reduced in all treatments compared to the C5 control, with these lower growth rates being significantly related to decreased isotopic turnover in the treatments, resulting in the diet of the sympatric chub not being at isotopic equilibrium with their diet in the mesocosms. When the fish stable isotope values were corrected to represent 95 % isotopic turnover since the start of the experiment (i.e. at diet equilibrium), the chub in the sympatric treatments had smaller isotopic niches than C5, with this also evident in their trophic niches (from stomach contents data). Conversely, the isotopic and trophic niches of chub in the allopatric control of C10, where there was twice the number of fish per replicate versus C5, were both larger, despite the reduced growth rates of the fish. These results suggest a fundamental difference in how the ecological consequences of intra- versus inter-specific interactions can manifest (Buoro et al., 2016; Britton et al., 2018).

The results of this experiment that revealed substantial differences in chub growth rates between C5 and the other treatments were considered to primarily be an impact of the increased competition for prey that resulted from the higher fish densities, but with the effect of this being the same for intra- and inter-specific competitive interactions. These results are consistent with Britton et al. (2018), where similar effects were seen in the growth rates of native tench *Tinca tinca* in allopatry versus sympatry with carp *Cyprinus carpio* and goldfish *Carassius auratus*. For *B. barbus*, previous tank-based experiments revealed their growth rates were strongly impacted by density, but with the density-dependent impacts being independent of species (Pegg & Britton, 2011). Across these studies, there is consistency in reduced fish growth rates as the extent of their competitive interactions increase, i.e. the growth is density-dependent (Ward et al., 2006). However, in contrast to here, the differences in density dependent growth did not differ between intra- and inter-specific competitive interactions suggesting some context dependency and/ or species-specific responses in these outcomes.

In contrast to the specific growth rates, there were some marked patterns in the trophic responses of the fish in the treatments. Compared with the allopatric chub treatment C5, the stomach contents data revealed significantly smaller dietary niches in the species when in sympatry with the alien barbel. Whilst this also had some support from the stable isotope data, there were some overlaps in the extent of the 95 % confidence intervals of $\mathrm{SEA_B}$. These reduced niche sizes suggested chub shifted to be a more specialised diet when sympatric with barbel, a result consistent with the niche variation hypothesis that predicts populations become less generalized in their diet under conditions of increased inter-specific competition (Van Valen, 1965; Thomson, 2004; Olsson et al., 2009). Similar outcomes were evident in native fish communities invaded by *P. parva*, where strong patterns of niche divergence and constriction were detected across a range of spatial scales (Jackson & Britton, 2014; Tran et al., 2015), which were at least partially explained by some of the low threshold, non-linear impacts of *P. parva* on their prey communities (Jackson et al., 2014). However, this niche constriction was only detected in the presence of inter-specific competition; comparison of the trophic niche results of the chub allopatric controls of C5 versus C10 revealed increased niche sizes as intra-specific competition increased. This is also consistent with trophic niche theory that suggests that as resource competition increases, species will exploit a wider dietary base to maintain their energetic requirements (Svanbäck & Bolnick, 2007). Thus, a major finding of this experiment was this fundamental difference between the impact of increased competition between allopatric (niche expansion) and sympatric (niche constriction) contexts.

These differences in the trophic and isotopic niche sizes of chub between their allopatric and sympatric treatments were despite the isotopic niches of the two species being strongly partitioned (irrespective of whether the uncorrected or corrected SI data were used). These results suggest that the changes detected in chub niche sizes were less likely to relate to their ability to continue to consume their core dietary items, but more likely to be due to the reduced availability of less important items that contributed to their diet on a more occasional basis. However, the experimental design precluded this from being tested. Irrespective, this trophic and isotopic niche partitioning is also evident in other studies that have analysed these species in both experimental and wild settings (e.g. Bašić & Britton, 2016; Gutmann Roberts et al., 2017). For example, in the River Teme, Western England, where alien barbel have been sympatric with chub since the 1970s, the trophic and isotopic niches of the two species tend to be partitioned, with the niche divergence being apparent in their juvenile life-stages (Gutmann Roberts & Britton, 2018a) and then remaining throughout life (Gutmann Roberts & Britton, 2018b). Despite this partitioning, the species do overlap in some aspects of their resource use, such as when they are juveniles when they both consume chironomid larvae

(Gutmann Roberts & Britton, 2018a), supporting the suggestion that the species were competing directly for at least some of the prey resources available in the pond mesocosms here.

The utilisation of two complementary methods of trophic analyses in the study was helpful given that there was an inherent issue with the use of stable isotope data in some of the treatments that related to the extent of their isotopic turnover during the experiment. The turnover rate of stable isotopes within animal tissues varies between tissue types, with faster turnover rates evident in blood and blood plasma compared with white muscle (Vander Zanden et al., 2015; Mohan et al., 2016). In fish, the isotopic turnover rates of $\delta^{13}C$ and $\delta^{15}N$ tend to be slowest in scales, with the rates increasing for fin tissue and then dorsal muscle, but with these rates often varying considerably by species and context (Busst & Britton, 2018). Epidermal mucus tends to show the fastest turnover rates (Church et al., 2009; Winter et al., 2019). Dorsal muscle was used here as the tissue of choice for the SIA, with muscle tending to be the usual tissue used in fish-based studies (Grey, 2006). Also, it was justified by the *a priori* prediction that the fish (due to their size and the duration of the experiment) would complete approximately 4 stable isotope half-lives during the experiment, i.e. approximately 94 % isotopic turnover, where 95 % is considered to be at equilibrium with the new diet (Thomas & Crowther, 2015), with the predicted chub isotopic turnover rates being 94.8 to 95.9 in C5. In T1 to T3, however, these reduced to 89.7 to 93.0 %, resulting in substantially enriched $\delta^{13}C$ and $\delta^{15}N$ versus the putative prey resources, presumably due to the remaining influence of their previous diet. Although these data were then corrected, the calculations were based on the *B. barbus* $\delta^{15}N$ turnover rate of Busst & Britton (2018), and thus assumed that: (i) chub has a similar stable isotope turnover rate to barbel; and (ii) the turnover rate of $\delta^{13}C$ in both species is similar to $\delta^{15}N$. Whilst these assumptions were made due to the absence of any other data available on the stable isotope turnover rates for these species, it is acknowledged that this is potentially an issue within these analyses. Nevertheless, the difference in the isotopic niche results were relatively similar for the corrected and uncorrected data, and were consistent with the trophic niche results from the stomach contents data, and so this issue was not considered to be a confound in the experiment.

In summary, this experiment revealed that the impacts of the increasing abundances of coexisting species include increased inter-specific competition that results in dietary specialisation and suppressed somatic growth rates in native species. This result has applicability to manipulations of fish assemblages for angling, whether the species released to enhance fishery performance is of native or non-native origin, and in situations where there are temporal increases in fish abundance through increased annual recruitment success (Nunn et al. 2007). Although depressed growth rates also result from increased intra-specific competition, increased intra-specific competition resulted in trophic niche expansion and so a shift to a more generalized diet, whereas increased inter-specific competition resulted in niche constriction, so a shift to a more specialised diet. These results thus indicate some important ecological differences in how competitive interactions can manifest within and between species in freshwater fish communities.

Data sharing statement: The data that support the findings of this study will be provided in Bournemouth University's data repository on acceptance.

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Table 1. Overview of the experimental design, including the name used for each treatment in analyses, where Chub n = number of chub per replicate, Barbel n = number of barbel per replicate, and N = total fish number of fish per replicate. Each treatment was replicated three times.

	Code	Chub n	Barbel n	N
Allopatric control (5)	C5	5	0	5
Allopatric control (10)	C10	10	0	10
Sympatric treatment 1	T1	5	5	10
Sympatric treatment 2	T2	5	10	15
Sympatric treatment 3	T3	5	15	20

Table 2. Prey-specific abundance (%P_i) of principal prey items in fish diet and the associated trophic niche size ('Niche'; as 40% standard deviation ellipses) in barbel and chub between the treatments (C5, C10, T1 to T3), where n = number of fish analysed per treatment and 'Insects' are unidentified aquatic insects.

				%P_i								
		n	Niche	Insect	Macrophyte	Corixid	Diptera	Cladocera	Chironomid	Hydracarina	Chaoboridae	Gastropoda
Barbel	T1	12	0.640	48	32	5	5	8	17	18	5	15
	T2	29	0.498	36	29	8	8	21	29	18	0	2
	T3	41	0.623	48	45	9	5	10	23	10	20	5
Chub	C5	10	0.539	37	23	8	3	5	40	8	22	13
	C10	26	0.744	55	12	28	12	0	11	30	18	12
	T1	8	0.490	59	37	20	20	2	7	20	13	6
	T2	14	0.381	70	35	0	5	0	12	21	15	5
	T3	13	0.215	74	22	5	0	6	9	11	20	0

Table 3. Mean fractionation for each prey item between the fish species per treatment for the uncorrected (δSI_{actual}) and corrected ($\delta SI_{G0.95}$) stable isotope data (confidence limits are not shown for brevity); Δ are in ‰. Busst & Britton (2016) predicted for chub, $\Delta^{13}C$ on plant-based diets of 4.24 ± 0.13 ‰ and invertebrate diets of 2.74 ± 0.13 ‰, and $\Delta^{15}N$ on plant-based diets of 6.79 ± 0.10 ‰ and invertebrate diets of 4.59 ± 0.23 ‰; and for barbel $\Delta^{13}C$ on plant-based diets of 5.31 ± 0.09 ‰ and invertebrate diets of 3.97 ± 0.14 ‰, and $\Delta^{15}N$ on plant-based diets of 6.43 ± 0.13 ‰ and invertebrate diets of 5.00 ± 0.21 ‰ (see Fig. 4).

			Putative prey resource					
			Macro-invertebrate		Macrophyte		Terrestrial insects	
Species	SI data	Treatment	$\Delta^{13}C$	$\Delta^{15}N$	$\Delta^{13}C$	$\Delta^{15}N$	$\Delta^{13}C$	$\Delta^{15}N$
Chub	δSI_{actual}	C5	2.21	4.81	5.15	4.82	0.94	5.57
		C10	3.89	4.92	6.83	4.93	2.62	5.68
		T1	5.16	5.08	8.10	5.09	3.89	5.84
		T2	6.57	5.30	9.51	5.31	5.30	6.06
		T3	6.12	5.21	9.06	5.22	4.85	5.97
Chub	$\delta SI_{G0.95}$	C5	3.01	4.81	5.95	4.82	1.74	5.57
		C10	2.67	4.60	5.61	4.61	1.40	5.36
		T1	2.02	4.54	4.96	4.55	0.75	5.30
		T2	1.48	4.35	4.42	4.36	0.21	5.11
		T3	1.50	4.35	4.44	4.36	0.23	5.11
Barbel	δSI_{actual}	T1	7.30	5.83	10.24	5.84	6.03	6.59
		T2	8.72	6.09	11.66	6.10	7.45	6.85
		T3	8.74	6.14	11.68	6.15	7.47	6.90
Barbel	$\delta SI_{G0.95}$	T1	4.98	5.58	7.92	5.59	3.71	6.34
		T2	6.07	5.80	9.01	5.81	4.80	6.56
		T3	4.91	5.72	7.85	5.73	3.64	6.48

Table 4. Mean unconverted (δSI_{actual}) and converted ($\delta SI_{G0.95}$) stable isotope data (± 95 % confidence limits) per species treatment, and their mean standard ellipse areas as SEAc and SEA_B (95% credible intervals)

			δSI_{actual}				$\delta SI_{G0.95}$			
Species	Treatment	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	SEA_c	SEA_B	$\delta^{13}C_{G0.95}$	$\delta^{15}N_{G0.95}$	SEA_c	SEA_B
Chub	C5	10	-28.2 ± 0.4	9.9 ± 0.1	0.96	0.75 (0.42-1.62)	-27.4 ± 0.35	9.9 ± 0.08	0.87	0.73 (0.38-1.48)
	C10	15	-26.5 ± 0.4	10.0 ± 0.1	1.35	1.23 (0.70-2.11)	-27.8 ± 0.40	9.7 ± 0.12	2.17	1.96 (1.11-3.33)
	T1	8	-25.3 ± 0.3	10.2 ± 0.1	0.64	0.50 (0.26-1.11)	-28.4 ± 0.40	9.6 ± 0.09	0.55	0.45 (0.19-0.96)
	T2	13	-23.9 ± 0.3	10.4 ± 0.1	0.59	0.53 (0.28-0.97)	-29.0 ± 0.30	9.4 ± 0.06	0.55	0.51 (0.28-0.92)
	T3	13	-24.3 ± 0.2	10.3 ± 0.0	0.74	0.64 (0.37-1.14)	-28.9 ± 0.17	9.4 ± 0.04	0.79	0.68 (0.38-1.25)
Barbel	T1	12	-23.1 ± 0.3	10.9 ± 0.0	0.87	0.75 (0.40-1.46)	-25.5 ± 0.37	10.7 ± 0.04	1.08	0.96 (0.51-1.88)
	T2	15	-21.7 ± 0.2	11.2 ± 0.1	0.31	0.28 (0.16-0.48)	-24.4 ± 0.20	10.9 ± 0.06	0.34	0.29 (0.18-0.52)
	T3	15	-21.7 ± 0.2	11.2 ± 0.1	0.57	0.51 (0.28-0.87)	-25.5 ± 0.41	10.8 ± 0.04	0.60	0.53 (0.31-0.93)

Figure captions

Figure 1. Mean specific growth rates of chub (as estimated marginal means with the effect of fish starting weight controlled as a covariate) per treatment, where the error bars represent 95 % confidence limits.

Figure 2. Non-metric multidimensional scaling (nMDS) plot showing the trophic niches as 40% standard deviation ellipses of chub (black) and barbel (grey) per treatment, where lines represent: solid = C5; dashed = C10; dotted = T1; dot-dashed = T2; and long-dashed = T3.

Figure 3A,B. Relationship of mean $\delta^{13}C$ (filled circle; solid line) and $\delta^{15}N$ (clear circle; dashed line) per replicate versus (A) chub specific growth rate (SGR) and (B) the predicted number of completed stable isotope half-lives (Busst & Britton 2018). Solid lines represent their significant relationships according to linear regression. (C): Relationship of chub SGR versus the predicted number of completed stable isotope half-lives per replicate, where the solid line represents the significant relationship according to polynomial regression.

Figure 4. Mean unconverted (δSI_{actual}; filled circle) and converted ($\delta SI_{G0.95}$; clear circles) (± 95 % confidence limits) stable isotope (SI) data for (A) chub and (B) barbel, where clear triangle: mean aquatic macro-invertebrate SI data (n = 15), grey triangle: mean macrophyte SI data (n = 3), and black triangle: mean terrestrial invertebrate SI data (n =3). Dashed lines represent the mean fractionation factors of each species with their prey types from Busst & Britton (2016).

Figure 5. Stable isotope plots for (A) unconverted (δSI_{actual}) and (B) converted ($\delta SI_{G0.95}$) showing the standard ellipse areas (SEA_c) for chub (filled circles; black ellipses) and barbel (clear circles;

grey ellipses) per treatment, where solid line: C5; solid = C5; dashed: C10; dotted: T1; dot-dashed: T2; and long-dashed: T3.

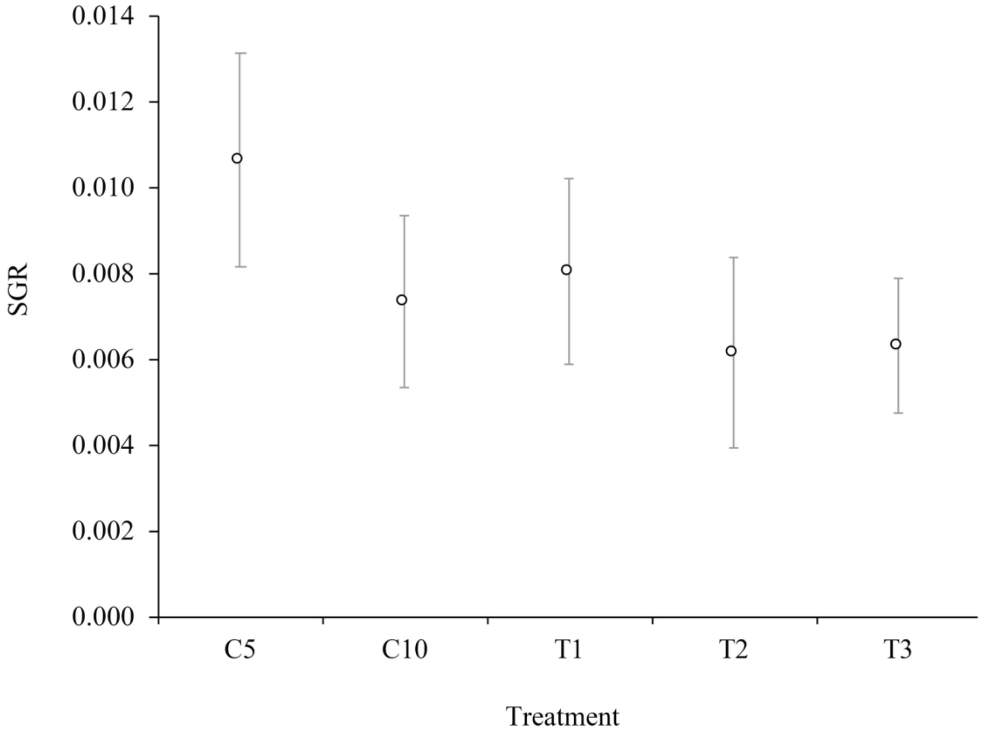

Figure 1

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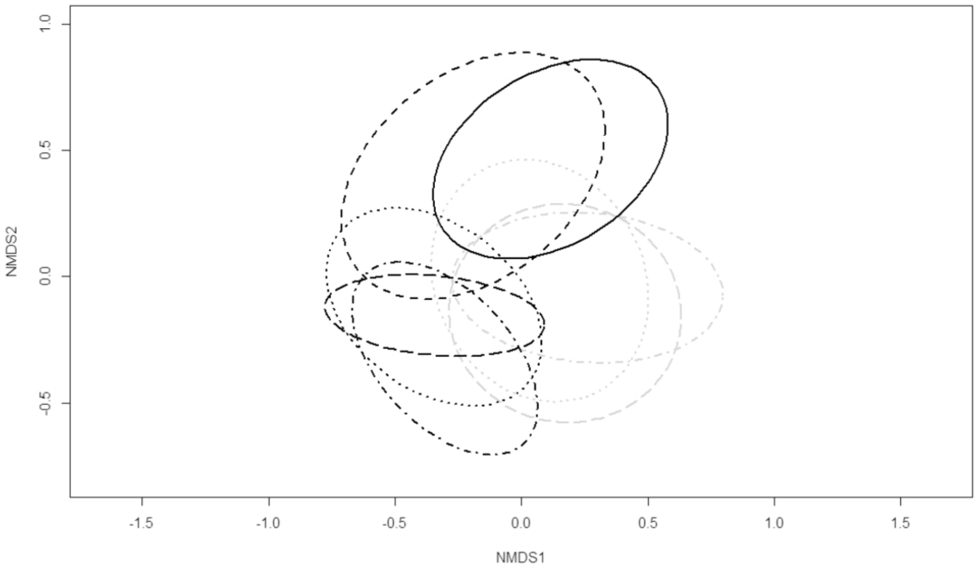

Figure 2

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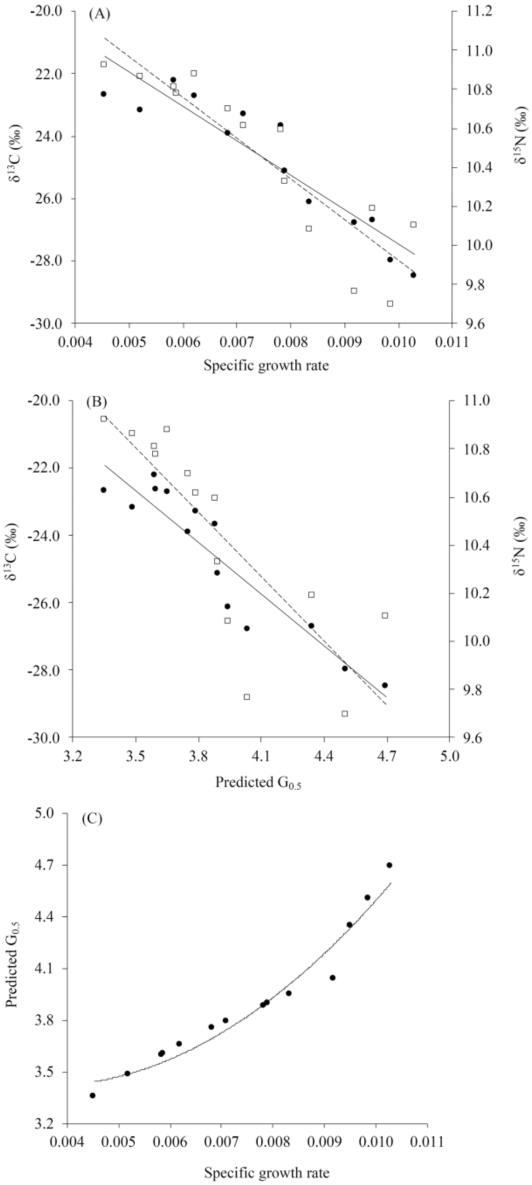

Figure 3

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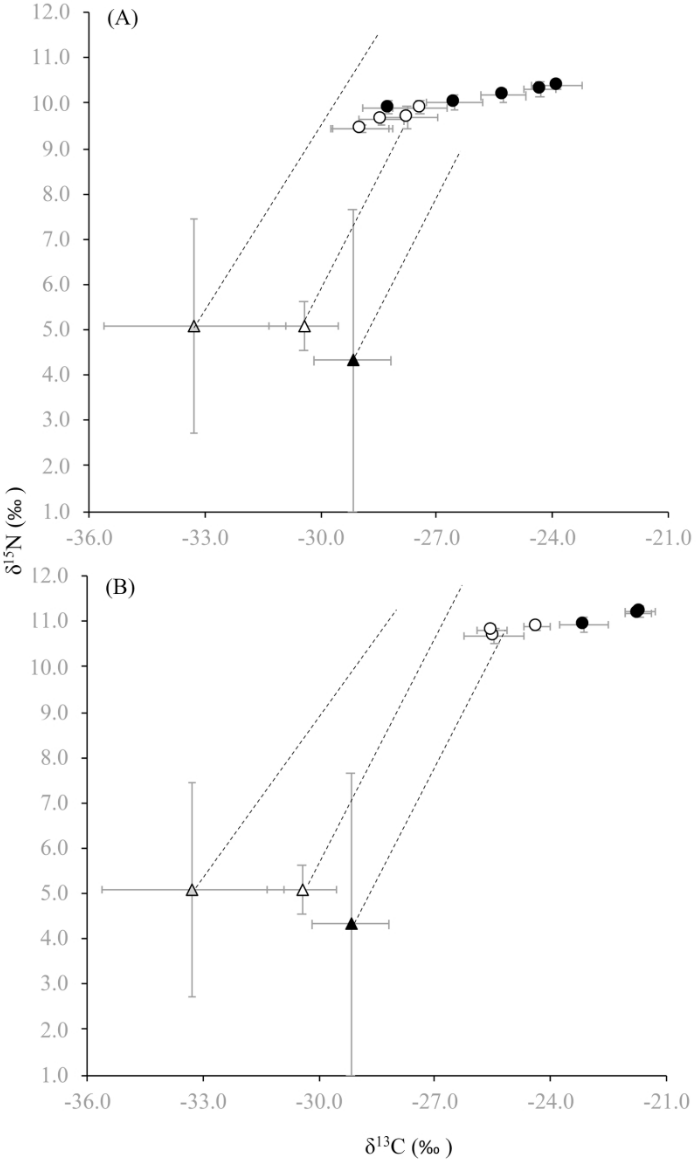

Figure 4

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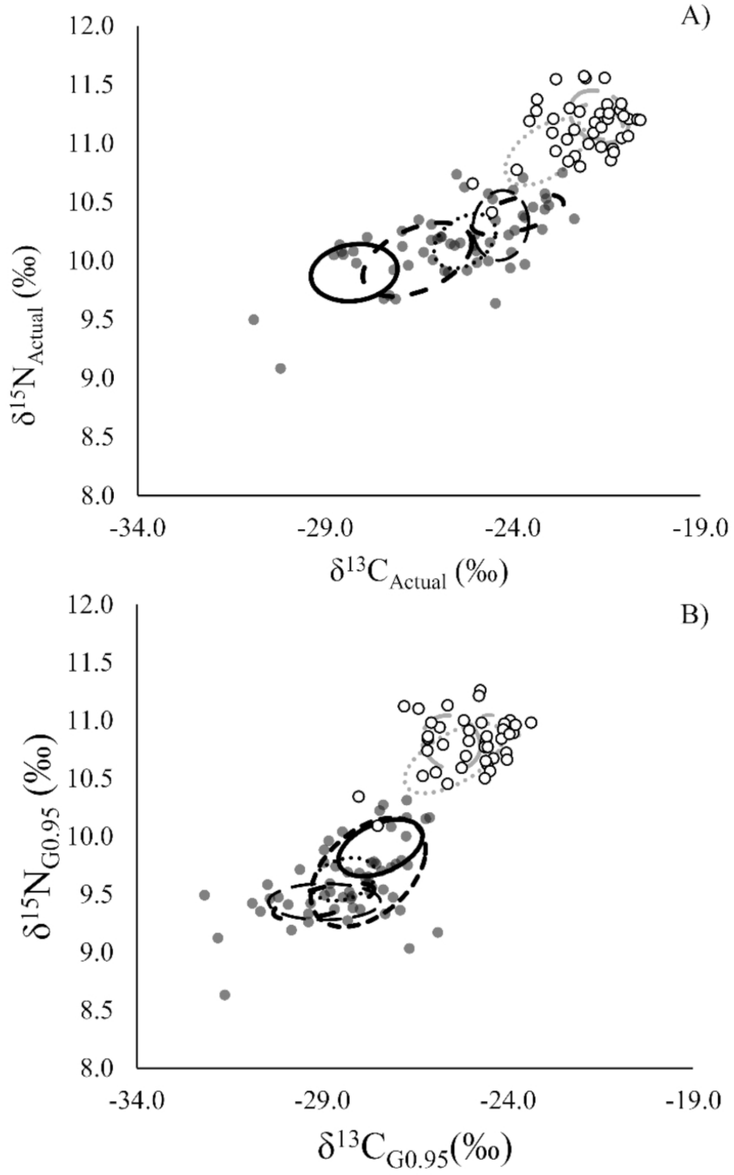

Figure 5

136x217mm (300 x 300 DPI)