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Trophic consequences of introduced species: comparative impacts of increased inter-specific versus intra-specific competitive interactions

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Running title: Trophic niche variation

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Abstract

1. Invasive species can cause substantial ecological impacts on native biodiversity. Whilst ecological theory attempts to explain the processes involved in the trophic integration of invaders into native food webs and their competitive impacts on resident species, results are equivocal. In addition, quantifying the relative strength of impacts from non-native species (inter-specific competition) versus the release of native conspecifics (intra-specific competition) is important but rarely completed.

2. Two model non-native fishes, the globally invasive *Cyprinus carpio* and *Carassius auratus*, and the model native fish *Tinca tinca*, were used in a pond experiment to test how increased intra- and inter-specific competition influenced trophic niches and somatic growth rates. This was complemented by samples collected from three natural fish communities where the model fishes were present. The isotopic niche, calculated using stable isotope data, represented the trophic niche.

3. The pond experiment used additive and substitutive treatments to quantify the trophic niche variation that resulted from intra- and inter-specific competitive interactions. Although the trophic niche sizes of the model species were not significantly altered by any competitive treatment, they all resulted in patterns of inter-specific niche divergence. Increased inter-specific competition caused the trophic niche of *T. tinca* to shift to a significantly higher trophic position, whereas intra-specific competition caused its position to shift towards elevated δ¹³C. These patterns were independent of impacts on fish growth rates, which were only significantly altered when inter-specific competition was elevated.
4. In the natural fish communities, patterns of trophic niche partitioning between the model fishes was evident, with no niche sharing. Comparison of these results with those of the experiment revealed the most similar results between the two approaches were for the niche partitioning between sympatric *T. tinca* and *C. carpio*.

5. These results indicate that trophic niche divergence facilitates the integration of introduced species into food webs, but there are differences in how this manifests between introductions that increase inter- and intra-specific competition. In entirety, these results suggest that the initial ecological response to an introduction appears to be a trophic re-organisation of the food web that minimises the trophic interactions between competing species.

*Keywords*: Biological invasions; global change; isotopic niche; niche divergence.
Introduction

Biological invasions are a substantial driver of global environmental change that have major implications for native biodiversity (Simerloff et al. 2013). When an introduced species establishes a population then ecological impacts on native species can be incurred through competitive interactions (Gozlan et al. 2010). These impacts can be particularly strong where the invader and native species are closely related (Ricciardi & Atkinson 2004; Li et al. 2015) or functionally similar (Dick et al. 2016, 2017), as the species are more likely to be foraging on the same food resources (Buoro et al. 2016).

A number of niche-based hypotheses have been suggested to explain the processes that facilitate the development of successful invasions and enable native species to coexist trophically with the invader (Ricciardi et al. 2013). For example, the utilisation of unexploited resources by an invader would avoid competitive interactions with native species and so facilitate their integration into the food web (Okabe & Agetsuma 2007; Mason et al. 2008; Juncos et al. 2015). In situations where resources are fully exploited then niche theory predicts that competitive interactions between invasive and native species will result in both their niches being smaller than in allopatry (Bolnick et al. 2010; Tran et al. 2015; Jackson et al. 2016a). Conversely, increased inter-specific competition might result in species increasing their niche breadths to maintain their energy requirements (Svanbäck & Bolnick 2007). Moreover, where the non-native species is a superior competitor, they can compete for the same resources as used by native species and result in the competitive exclusion of that native species (Tran et al. 2015). This can cause niche shift or
replacement in the native species, leading to reduced food intake, slower growth rates and/or reduced population density (Bøhn, Amundsen & Sparrow 2008).

Ecological impacts resulting from increased competitive interactions due to introductions are, however, not limited to non-native species. Impacts can also develop when the population of a native species increases in abundance. Whilst this can occur naturally through strong recruitment, it also occurs when conspecifics are released to increase population size (‘stocking’) (Bašić & Britton 2016). In fishes of the Salmonidae family, ecological impacts from stocking with conspecifics are often stronger than those resulting from non-native fishes (Buoro et al. 2016). This is due to the ‘pre-adaptation hypothesis’, where the released fishes have almost identical functional traits as resident species and so have a similar ability to acquire resources (Ricciardi & Mottiar 2006). The subsequent increase in intra-specific competition then potentially results in similar ecological consequences to those resulting from inter-specific competition (Carey et al. 2012), and can be tested using the similar hypotheses (Svanbäck & Bolnick 2007; Bøhn, Amundsen & Sparrow 2008; Ricciardi et al. 2013). However, differences in the processes by which ecological impacts result from intra- versus inter-specific competitive interactions remain poorly understood for many taxa.

The aim of this study was therefore to test the trophic consequences for populations of a model native species from increased intra- and inter-specific competitive interactions that result from introductions. The model species were freshwater fishes, as they are adaptable and tractable experimental animals that provide excellent model systems for competitive studies (Ward, Webster & Hart 2006). For example, their
indeterminate nature of growth enables correlation with competitive success (Ward, Webster & Hart 2006). The model native species was tench *Tinca tinca*, a fish of the Cyprinidae family with a native range across most of Eurasia (Fishbase 2017). Its rationale for use as a model was that its omnivory and broad environmental tolerances potentially provide highly plastic responses to increased competition (Guo et al. 2016). As the drivers of invasion success of non-native fishes include their functional similarities to many native fishes, then the model species used to increase interspecific competition were the global cyprinid invaders carp *Cyprinus carpio* and goldfish *Carassius auratus*. Both fishes are primarily benthic foragers that exploit a wide range of trophic resources and so they are trophic analogues of *T. tinca* (Weber & Brown 2011; Guo et al. 2016). All of the model fishes are exploitative competitors and thus were assumed to overlap in their diet in situations where food resources are limited.

Understanding the trophic interactions of invasive and native fishes is enhanced when experimental approaches are coupled with studies of invaded natural communities as, in combination, they enable ecological patterns and processes to be understood over a range of temporal and spatial scales (Tran et al. 2015). Consequently, using stable isotope analysis (SIA; $\delta^{13}C$, $\delta^{15}N$) to determine trophic relationships, the model species were initially used in a pond experiment based on additive and substitutive treatments. This experiment tested the following predictions: (1) competitive interactions between the model fishes significantly alters the size of their trophic niches and reduces their somatic growth rates; and (2) impacts from inter- and intra-specific competition are similar on the size and position of the trophic niche of the native species. Then, samples of the model fishes collected from invaded natural
communities tested the prediction that: (3) the trophic relationships of the model species are similar in the experimental simulations and their populations in the wild.

**Materials and Methods**

*Experimental design*

The experimental design (hereafter referred to as the ‘experiment’) used 10 additive and substitutive treatments across a combination of allopatric and sympatric contexts, with each treatment replicated three times (Table 1). Three control treatments used each species in allopatry (‘Allopatry’; N = 8; Table 1). Three substitutive treatments then paired the native and non-native species in their different sympatric combinations (‘Sympatry’; n = 4+4, N = 8; Table 1). It was these three treatments whose isotopic niches of the sympatric fishes were compared to those from the invaded natural communities (Table 1, 2). Three additive treatments then used all three species in sympatry across three different abundances (‘Inter-specific competition’; n = 4+4+4, 8+8+8, 12+12+12; N = 12, 24, 36; Table 1). Finally, a single treatment used the native species in higher abundance (‘Intra-specific competition’; N = 12). All the fish used in the treatments were juveniles and had been hatchery reared. As their starting lengths were 45 to 60 mm and starting weights < 10 g, the predicted stable isotope half-life for their dorsal muscle was 36 and 38 days for δ13C and δ15N respectively (Thomas & Crowther 2015).

The experiment was completed using the treatments within enclosures that sat within a larger, man-made pond (30 x 30 m; 1 m consistent depth) that was located in Southern England. Following Bašić & Britton (2016), the enclosures comprised of an
aluminium frame (length 1.66 m; width: 1.05 m; height: 1.2 m) within a net (mesh: 7 mm²) 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 able to grow within each of them (primarily *Elodea* spp.). Bird predation was prevented via netting over the enclosures (15 mm mesh). The experiment ran for 150 days from April 2016. This duration enabled fish dorsal muscle to undergo approximately 4 half-lives and so by its conclusion, the δ¹³C and δ¹⁵N data of the experimental fish would represent their diet in the ponds (Thomas & Crowther 2015). All fish were weighed (nearest 0.1g) prior to release into the enclosures. Temperature loggers (TinyTag TGP-4017) in the larger pond revealed the mean water temperature was 18.1 ± 0.6 °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. For the purpose of stable isotope analysis, macroinvertebrate and macrophyte samples were taken from the larger pond, sorted into samples (one sample = 3 to 9 individuals per species), with triplicate samples taken.

**Invaded wild fish communities**

Three wild pond fish communities (hereafter referred to as the ‘invaded communities’) were used, with each having two of the model species present within a mixed community of other fishes (Table 2). At each site, the model fishes had been present for at least five years prior to sampling. Although data from each of these fish communities have been reported previously, the data used in this paper have not previously been compared (Table 2). Note that replicates of each combination of
species were not used due to inherent logistical difficulties of locating sites where each species was present and sufficiently abundant to provide adequate sample sizes. Also, at Site 2, whilst \textit{C. carpio} was present in sympatry with a \textit{Carassius} species, this was identified in the field as \textit{Carassius gibelio}. However, Busst & Britton (2017) indicated that \textit{Carassius} species generally have high trophic similarity due to their similar functional traits (Busst & Britton 2017) and thus \textit{C. gibelio} was used as a surrogate of \textit{C. auratus} at Site 2. Site 1 and 2 was sampled in spring 2013, whereas Site 3 was sampled in March 2008. At each site, fish sampling incorporated electric fishing, seine nets, fish traps and fyke nets. Following their capture, the fish were euthanized and returned to the laboratory for processing. The sample size for stable isotope analysis was a minimum of 10 individuals per species, with individuals randomly selected across the length range sampled (Table 2). As these were fish sampled from the wild then this random selection resulted in a wider length range of fish being used than was the case in the experiment (Table 2).

\textit{Stable isotope analysis}

In the laboratory, fish from the experiment and the invaded communities were measured and weighed, and a dorsal muscle sample taken for stable isotope analysis (SIA). SIA sample sizes were 10 fish per species for Sites 1 and 2, and 15 per species for Site 3. Along with the macro-invertebrate samples, all samples were dried at 60°C to constant mass before stable isotope analysis (SIA) ($\delta^{13}$C, $\delta^{15}$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). 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 the format of delta (δ) isotope ratios expressed per mille (‰).

There was no lipid correction applied to the data as C:N ratios indicated very low lipid content (Post et al. 2007).

**Data analysis**

The SIA data from the experiment and invaded communities were used to calculate the trophic niche size of each fish species 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). It was calculated using standard ellipse areas (SEA) in SIBER (Jackson et al. 2012), 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. 2011). The generally small sample sizes used in both study components (i.e. <30) meant 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. 2012). 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).

For the invaded communities, SEA_B and SEA_c was calculated for each model species and compared between the species within each site, but not between sites due to the multiple context dependencies that can influence niche sizes between wild populations (Tran et al. 2015). For the experiment, 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 correction. Data per species were combined from replicates for each treatment to provide representative sample sizes sufficient for analyses of SEA_B and SEA_c. A minimum of four randomly chosen individuals was used from each replicate to provide both a balanced dataset across the experiment and a minimum sample size per treatment of 12 fish per species (Table 1; Appendix S1 in Supporting information, Fig. S1 to S3).

In the experiment, to then test differences in the SI data between species and treatments, δ^{15}N was converted to trophic position (TP) from $TP_i = \left[ (\delta^{15}N_i - \delta^{15}N_{base}) / 3.4 \right] + 2$, where $TP_i =$ trophic position of the individual fish, $\delta^{15}N_i =$ fish isotopic ratio, $\delta^{15}N_{base} =$ macro-invertebrate isotopic ratio, 3.4 = discrimination between trophic levels, and 2 = trophic position of baseline macro-invertebrates (Jackson and Britton 2014). TP and δ^{13}C data were used in linear mixed effects models (LMEM) to test differences between treatments per species, with enclosure 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). The
starting mass of fish in the enclosure was also initially used as a covariate, but was
removed from all final models due to its effects not being significant (P > 0.05 in all
cases). For each model, differences between species and treatment were assessed
using estimated marginal means and linearly independent pairwise comparisons with
Bonferroni adjustment for multiple comparisons.

To determine fish growth rates in the experiment, the mean specific growth rate
(SGR) per model species and replicate was determined from: 
\[ \frac{(\ln W_{t+1}) - (\ln W_t)}{n/\text{t}}, \]
where \( W_t \) = total starting weight, \( W_{t+1} \) = total end weight, \( n \) = the number of fish used
to determine \( W \), and \( t \) = the duration of the experiment (days). A generalized linear
model (GLM) tested the differences in SGR between treatments for each species. In
each GLM, SGR was the dependent variable and treatment was the independent
variable; total starting mass of fish per replicate initially used as a covariate and was
retained in the final model when its effect on SGR was significant.

Results

Trophic impacts of inter-specific competition from non-native species
Across all of the experimental treatments, the isotopic niche sizes (as \( \text{SEA}_B \)) of each
species varied, but their 95% confidence intervals always overlapped between
allopatri and sympatry, indicating no significant differences in isotopic niche size
caused by the competition scenarios (Table 3). The LMEMs testing differences in TP
and \( \delta^{13}\text{C} \) between treatments for each species were significant (P < 0.01), except TP
in \( C. \text{carpio} \) (P = 0.47) (Table S1 to S3). Pairwise comparisons revealed the
significant shifts TP and \( \delta^{13}\text{C} \) were mainly between the allopatric treatments and the
inter-specific competition (8) and (12) treatments (P < 0.05; Fig. 1; Tables S1 to S3).

In the inter-specific competition treatments, the pattern for T. tinca was a shift to significantly higher TP and higher $\delta^{13}$C, for C. carpio, the only shift was to significantly higher $\delta^{13}$C, and for C. auratus, the significant shifts were to lower TP and higher $\delta^{13}$C (Fig. 1). Where shifts were to elevated values of $\delta^{13}$C, the fish were moving towards using macrophyte as an energy source (mean $\delta^{13}$C: -24.37 ± 0.88 ‰), away from macro-invertebrate prey resources (Chironomid larvae, Corixidae, Odonata and Ephemeroptera: mean $\delta^{13}$C: -30.57 ± 1.28 ‰).

These shifts in isotopic positions resulted in some divergence in the isotopic niches of each species between allopatry and sympatry. In allopatry, T. tinca shared 39 % of their isotopic niche with C. auratus, but this reduced to 14 % in sympathy; T. tinca only shared 2 % of their isotopic niche with C. carpio in allopatry, but this reduced to 0.3 % in sympathy (Fig. S4). In contrast, C. carpio shared 74 % of their niche with C. auratus, and only reduced to 52 % in sympathy (Fig. S4). In the three inter-specific competition treatments (Table 1), T. tinca no longer shared any of their isotopic niche with either non-native species, whereas the extent of shared C. carpio niche with C. auratus was reduced to between 15 and 26 % (Fig. S5).

*Trophic impacts of intra- versus inter-specific competition*

The LMEMs testing differences in T. tinca $\delta^{13}$C and TP between allopatry and the intra- and inter-specific competition experimental treatments were significant (P < 0.01; Table S4). For $\delta^{13}$C, there was a significant difference between allopatry and the intra-specific competition treatment (allopatry: -27.72 ± 0.51 ‰; intra-specific competition: -26.25 ± 0.54 ‰; P < 0.01), and between allopatry and the inter-specific
competition (12) treatment (-25.82 ± 0.38 ‰, P < 0.01) (Table S4; Fig. 1). Their TP was significantly higher in allopatry than the intra-specific competition treatment (allopatry: 3.21 ± 0.06 ‰; intra-specific competition: 3.10 ± 0.05; P = 0.05; Table S4; Fig. 2). In the inter-specific competition treatments, the *T. tinca* niche shift was to higher trophic positions compared to allopatry, with these differences significant in the (8) and (12) treatments (P ≤ 0.05; Table S4; Fig. 2). In the treatments when numbers of fish were equal (N = 12), allopatric TP (3.10 ± 0.05) was significantly lower than when in competition with the two non-native fishes (3.33 ± 0.07) (P < 0.01; Fig. 2). Regarding overlaps in isotopic niches (as SEA₆), the intra-specific competition treatment shared 15 % of their niche with the allopatric treatment and 3 % with the inter-specific competition (4) treatment (Fig. 2); this reduced to 0 % for inter-specific competition (12) treatment.

**Impacts of intra- and inter-specific competition on specific growth rates**

In each experimental treatment, there was an increase in total fish mass in each species across the 150 days and thus all mean SGRs were positive (Fig. 3). The GLM testing the effect of treatment on SGR was significant for each species (*T. tinca*: Wald $\chi^2 = 139.39$, d.f. = 6, P < 0.01; *C. carpio*: Wald $\chi^2 = 35.50$, d.f. = 5, P < 0.01; *C. carassius*: Wald $\chi^2 = 13.73$, d.f. = 5, P = 0.02). The effect of starting mass as a covariate on SGR was significant for *T. tinca* (P < 0.01) and *C. carpio* (P = 0.02) and so it was retained in their final models. It was not significant for *C. auratus* (P = 0.48) and so it was removed from their final model. Pairwise comparisons of differences in mean SGR between allopatry and the other treatments revealed that for each species, significantly decreased SGR was only apparent in the inter-specific competition (8) and (12) treatments (P < 0.01) (Fig. 3).
Trophic relationships in the invaded communities versus the experiment

In the invaded communities of Sites 1 and 3, there were no significant differences in the isotopic niche sizes of the sympatric model fishes (Site 1: T. tinca: 1.92 to 3.01‰, C. auratus: 1.64 to 2.41‰; Site 3: T. tinca: 3.04 to 4.27‰, C. carpio: 2.97 to 4.03‰) (Fig. 5). In Site 2, however, the isotopic niche of C. carpio (3.12 to 4.70‰) was significantly higher than the Carassius spp. (0.94 to 1.89‰) (Fig. 4). In the invaded communities, the isotopic niches of fishes were highly divergent with no overlap. Whilst they showed some consistency with the patterns identified within the experiment, their niches were also more divergent (Fig. 4). There were also similarities in the relative positions of their niches in isotopic space between the experiment and invaded communities (Fig. 4). For example, in both the invaded communities and the experiment, the isotopic niche of T. tinca was at a higher trophic position than C. carpio, but had similar values of δ13C (Fig. 4). The sympatric treatment of T. tinca and C. auratus was the least similar to their pattern in the invaded communities, although the T. tinca niche was at the higher trophic position in both contexts (Fig. 4).

Discussion

The results of the experiment revealed that increased competition did not cause any significant shifts in isotopic niche sizes, contrary to Prediction 1. Instead, divergence in the isotopic niches of the fishes occurred, with this independent of shifts in somatic growth rates. Both increased intra- and inter-specific competition impacted the isotopic niche of the model native species, as per Prediction 2. However, the impact on the isotopic niche differed between the competition types. Increased inter-specific
competition resulted in the niche shifting to a significantly higher trophic position, whereas increased intra-specific competition caused the niche to shift towards elevated $\delta^{13}$C. Finally, there were similar patterns of trophic niche divergence between the model species in the experiment and invaded communities, as per Prediction 3. This suggests that experimental predictions can help the understanding of how trophic relationships develop in invaded communities in the wild.

Ecological theory relating to invasions posits that invaders can out-compete similar native competitors by occupying a broader niche (Elton 1958). This has been supported empirically in studies involving non-native taxa such as invasive crayfish (e.g. Olsson et al. 2009; Ercoli et al. 2014). By occupying a broad niche, theory suggests that the invader suppresses the niche size of trophically analogous native species (Thomson 2004; Jackson et al. 2016a). Conversely, other studies have suggested that when in sympatry, the trophic niche of both the invader and native species will constrict due to dietary specialisations (Tran et al. 2015; Jackson et al. 2016a). In the experiment, there were no significant shifts in the trophic niche sizes of each species between their treatments. This finding is, therefore, contrary to these aspects of invasion theory. Where an invader competes for the same resources as being used by a native species then, if that invader is a superior competitor, it has been predicted that their interactions will competitively exclude the native species. The can result in a shift in the position of the niche of the native species, potentially resulting in reduced food intake and suppressing growth rates (Bøhn, Amundsen & Sparrow 2008). The experiment results had some consistency with this prediction, as all treatments resulted in a change in the position of the trophic niche of *T. tinca*. As invader abundance increased in the inter-specific competition treatments, the extent of
niche divergence also increased. Whilst this suggests some competitive exclusion driven by *C. carpio* and *C. auratus*, some niche shifts were also apparent in the intra-specific competition treatment, suggesting the presence of some density dependent effects. However, the divergent niches developed independently of changes in fish growth rates, suggesting they enabled the fishes to maintain their food intake rates.

The results of the experiment were also consistent with patterns of inter-specific trophic niche divergence detected in other invasive fishes. For example, Tran et al. (2015) revealed that in allopatry, the diet of the Asian invasive fish *Pseudorasbora parva* had the potential to overlap with some native fishes, but this never occurred in sympatry. Niche divergence was also apparent between non-native pumpkinseed *Lepomis gibbosus* and native fishes in both rivers and ponds (Jackson et al. 2016b; Copp et al. 2017). Jackson and Britton (2014) detected partitioning between the trophic niches of sympatric *P. parva*, *C. carpio* and signal crayfish *Pacifastacus leniusculus* in ponds. In entirety, these results suggest that, in freshwater fishes at least, the initial response to an invasion is trophic niche divergence, leading to niche partitioning. This response reduces the strength of the competitive interactions, and can occur independently of shifts in niche size and growth rates. This response also occurs despite the high functional similarity of many of these fishes. Their traits must thus be sufficiently different or plastic between the species to enable these dietary specialisations to develop in sympathy (Jackson & Britton 2014; Tran et al. 2015; Jackson et al. 2016b).

Recently, studies have suggested that ‘native invasions’, such as where wild populations are supplemented by hatchery-reared conspecifics, can result in similar,
and sometimes stronger, ecological impacts than those caused by non-native invasions (Carey et al. 2012; Buoro et al. 2016). In the experiment, the comparison of intra- versus inter-specific competition across the treatments was also a simulation of a ‘native’ versus ‘non-native’ invasion, where the driver of impact was from increased competitive interactions. Whilst increased intra- and inter-specific competition both impacted the isotopic niche of *T. tinca*, there were differences in how these impacts manifested. Increased intra-specific competition caused the isotopic niche of *T. tinca* to shift to a significantly lower trophic position that was significantly carbon enriched. In contrast, increased inter-specific competition resulted in the isotopic niche of *T. tinca* shifting to a significantly higher trophic position, with this also apparent in the invaded communities. Whilst these results suggest that both ‘native’ and ‘non-native’ invasions can indeed impact native species (*cf.* Buoro et al. 2016), they indicate that the impacts might differ between the invasion types (i.e. native vs. non-native). This finding has potential implications for understanding how the model fishes can be better used in fishery enhancement schemes, particularly regarding the numbers being introduced in relation to the ecological effects they might incur (Bašić & Britton 2016). The experiment could not, however, determine how these differences between intra- and inter-specific competitive differences developed temporally. It is therefore recommended that this is explored in future work, such as through more controlled experiments using a wider range of model species (*e.g.* Dick et al. 2017).

Each natural fish community had been invaded by either *C. carpio* or *Carassius* spp. Within these multi-species communities, each sympatric combination of the model species had isotopic niches that were divergent, with no sharing of isotopic space between them. This might have been the result of differences in the length ranges of
the fishes in each site, especially in Site 3, resulting in the different size classes of fish
exploiting different food resources. However, these patterns were relatively consistent
with those detected in the experiment. They were also consistent with other studies on
invasive fishes that suggest isotopic niche partitioning, rather than niche convergence,
is the general pattern within invaded fish communities (e.g. Jackson & Britton 2014;
Tran et al. 2015; Basic & Britton 2016), except perhaps where the invader has
attained high population abundances (e.g. Britton, Davies & Harrod 2010). The data
from the invaded communities have the caveat that they were non-replicated wild
samples that were only sampled once, and were subject to uncontrolled environmental
conditions. Comparison of the results between the experiment and the invaded
communities did, however, indicate that when the model fishes were in the wild they
exhibited complete partitioning in their isotopic niches, a contrast to the experiment..
This might relate to the experiment being completed in relatively enclosed spaces,
resulting in reduced opportunities for exploiting different food resources. The invaded
communities were also more complex with higher species richness and so might have
contributed to their niche partitioning through stronger inter-specific competition.
Also, the experimental data were collated in relatively controlled conditions and over
shorter timeframes than the invaded communities. Indeed, ecological experiments
often have ‘scaling-up’ issues that arise from their limited timeframes (Korsu, Huusko
& Muotka 2009; Spivak, Vanni & Mette 2011). Mesocosm approaches have,
however, been used successfully for understanding the trophic relationships of
freshwater fishes (e.g. Jackson et al. 2013; Bašić and Britton 2016), with these
successfully extrapolated to wild populations to help explain ecological patterns (e.g.
Tran et al. 2015; Copp et al. 2017).
In summary, the experiment revealed isotopic niche divergence developed between the model fishes when intra- and inter-specific competition was elevated. The magnitude and direction of niche divergence in the model native fish did, however, differ between intra- and inter-specific competition. Patterns of trophic niche partitioning were also strongly apparent between the model fishes in the invaded communities. As isotopic niche divergence occurred in the experiment in isolation from niche constriction and impacts on growth rates, this suggests the initial ecological response to an introduction is the trophic re-organisation of the food web to minimise the interactions between the competing species.

**Author contributions**

RB and AN conceived the ideas and designed methodology; RB and HV collected the data; RB and FAT analysed the data; RB and FAT led the writing of the manuscript. All authors contributed critically to the manuscript and gave final approval for publication.

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Data accessibility

Data available from the Dryad Digital Repository: Experiment: http://dx.doi.org/10.5061/dryad.qn186 (Britton et al. 2017); Invaded communities: http://dx.doi.org/10.5061/dryad.12344 (Tran et al. 2015).

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Supporting information

Additional supporting information can be found in the online version of this article.

Appendix S1: Supplementary tables and figures

Table S1, S2, S3: Linear mixed model results for δ¹³C and trophic position in the experiment for *Tinca tinca*, *Cyprinus carpio*, and *Carassius auratus* respectively.

Table S4: Linear mixed model results for δ¹³C and trophic position in the experiment for intra- versus inter-specific experimental treatments of *Tinca tinca*.

Figures S1, S2, S3: Stable isotope bi-plots of the distribution of δ¹³C and δ¹⁵N per replicate and treatment for *Tinca tinca*, *Cyprinus carpio*, and *Carassius auratus* respectively.

Figure S4: Isotopic niche and niche overlap of the model species between allopatry and sympatry in the experiment.

Figure S5: Isotopic niche and niche overlap of *Tinca tinca* between allopatry and the competitive treatments in the experiment.

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Table 1. Structure of the treatments used in the experiment, showing the number of fish per species per treatment (n), and the total number of fish per treatment (N).

| Treatment                        | T. tinca (n) | C. carpio (n) | C. auratus (n) | N  |
|----------------------------------|--------------|---------------|----------------|----|
| Allopatry (T. tinca)             | 8            | 0             | 0              | 8  |
| Allopatry (C. carpio)            | 0            | 8             | 0              | 8  |
| Allopatry (C. auratus)           | 0            | 0             | 8              | 8  |
| Sympatry (T. tinca + C. carpio)  | 4            | 4             | 0              | 8  |
| Sympatry (T. tinca + C. auratus) | 4            | 0             | 4              | 8  |
| Sympatry (C. carpio + C. auratus)| 0            | 4             | 4              | 8  |
| Intra-specific competition       | 12           | 0             | 0              | 12 |
| Inter-specific competition (4)   | 4            | 4             | 4              | 12 |
| Inter-specific competition (8)   | 8            | 8             | 8              | 24 |
| Inter-specific competition (12)  | 12           | 12            | 12             | 36 |
Table 2. Details of the invaded communities (Sites 1 to 3), including their locations, sizes and information on the fish populations present.

| Site | Country | Location | Size (m²) | Comparator model species (mean length ± 95% CI, mm) | Other fishes present | Reference |
|------|---------|----------|-----------|---------------------------------------------------|----------------------|-----------|
| 1    | Wales   | N: 51°41’10.0” W: 4°12’06.00” | 3000 | *T. tinca (96 ± 20)* | *Scardinius erythrophthalmus;* | | Tran et al. 2015 |
|      |         |          |           | *C. auratus (60 ± 4)* | *Pseudorasbora parva* |           |
| 2    | Belgium | N:50°2’59’3.35” E: 5°20’10.52” | 1900 | *C. carpio (70 ± 6)* | *S. erythrophthalmus; Blicca bjoerka;* | | Tran et al. 2015 |
|      |         |          |           | *Carassius spp. (86 ± 10)* | *Rutilus rutilus Leucaspius delineates;* |           |
|      |         |          |           |                                                     | *Rhodeus amarus* |           |
| 3    | England | *N: 51°12” W: 0°34” | 3000 | *T. tinca (174 ± 20)* | *P. parva* | | Jackson & Britton (2013) |
|      |         |          |           | *C. carpio (218 ± 72)* | |           |

*Approximate location as exact location unable to be provided for business confidentially reasons relating to *P. parva* invasion and subsequent eradication.
Table 3. Isotopic niche size (as lower and upper 95% confidence intervals of \( SE_{AB} \)) of each species per treatment in the experiment.

| Treatment | Species | Tinca tinca | Cyprinus carpio | Carassius auratus |
|-----------|---------|-------------|-----------------|------------------|
| Allopatry |         | 0.63 - 1.48 | 0.42 - 1.19     | 0.74 - 1.84      |
| Sympathy (T. tinca + C. carpio) | | 0.50 - 1.40 | 1.08 - 3.88     | -                |
| Sympathy (T. tinca + C. auratus) | | 0.29 - 1.06 | -               | 0.32 - 1.07      |
| Sympathy (C. carpio + C. auratus) | | -          | 0.73 - 2.96     | 0.69 - 2.33      |
| Inter-specific competition (4) | | 0.41 - 1.37 | 0.39 - 1.33     | 0.29 - 1.01      |
| Inter-specific competition (8) | | 0.47 - 1.46 | 0.73 - 2.10     | 0.48 - 1.35      |
| Inter-specific competition (12) | | 0.51 - 1.22 | 1.01 - 2.48     | 0.50 - 1.23      |
Figure captions

Figure 1. Mean δ^{13}C (clear circle) and trophic position (filled circle) per experimental treatment for *Tinca tinca* (A), *Cyprinus carpio* (B) and *Carassius auratus* (C). *differenc* between Allopatry and the treatment is significant at P < 0.05. Error bars represent 95 % confidence limits.

Figure 2. Comparison of the isotopic niche (as SEA_{c}) of *Tinca tinca* in the experiment according to: Allopatry (black circles, solid black line), intra-specific competition (grey circles, dashed black line), inter-specific competition (4) (clear circles, grey line), and inter-specific competition (8) (grey triangles, grey dashed line) (*cf.* Table 1).

Figure 3. Mean specific growth rate by experimental treatment (adjusted for the effect of starting mass in *T. tinca* and *C. carpio* in the generalised linear models) for: (A) *Tinca tinca*, (B) *Cyprinus carpio* and (C) *Carassius auratus*. The treatments on the X axis are as per Table 1. *differenc* between Allopatry and the treatment is significant at P < 0.01. Error bars represent 95% confidence limits.

Figure 4. Stable isotope bi-plots comparing the isotopic niches (as SEA_{c}) of sympatric *T. tinca*, *C. carpio* and *Carassius* spp. between the invaded communities and the experiment. Plots A, C, E are Field sites 1, 2 and 3 respectively. Plots B, D and F are the comparator sympatric treatments from the field experiment. *T. tinca*: clear circles and solid black line; *C. carpio*: grey circles, grey line; and *C. auratus*: black circles, black dashed line. Error bars represent 95% confidence limits. Note differences in the X and Y axes in A, C and E.
Figure 1.
Figure 2.
Figure 3.
Figure 4.