

Trophic consequences of an invasive, small-bodied non-native fish, sunbleak *Leucaspis delineatus*, for native pond fishes

Tea Bašić · Gordon H. Copp  · V. Ronni Edmonds-Brown · Emre Keskin · Phillip I. Davison · J. Robert Britton

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Abstract Assessments of the trophic consequences of invasive fishes are important for quantifying their ecological impacts on native species more generally. A small-bodied cyprinid fish native to continental Europe and introduced in the 1970s to the U.K, the sunbleak *Leuciscus delineatus*, has been shown previously to establish closer social associations with native species of similar size than do native species amongst themselves. To assess the potential detrimental trophic consequences of native species associations with *L. delineatus*, a field-based experiment

was undertaken in summer 2015 in six outdoor, artificial ponds containing three native cyprinid species (rudd *Scardinius erthrophthalmus*, gudgeon *Gobio gobio*, tench *Tinca tinca*). Three ponds were controls (no *L. delineatus*) and three were treatments (*L. delineatus* present). The results of stable isotope analysis (SIA) of fish tissue samples provided strong evidence that the isotopic niches of both native benthic fishes were reduced in the presence of *L. delineatus*, although there were no significant effects on the trophic position, body size or condition of two of the three native fish species. Introduced *L. delineatus* maintained a core isotopic niche that was distinct from the two native benthic fishes, with no overlap detected between native and non-native fishes when including 40% and 95% of the data. These results indicate that the response of the native fishes to the introduction of *L. delineatus* was niche constriction via trophic specialisation, with this response sufficient to maintain their growth rates and condition. This result is similar to studies on a range of small-bodied invasive fishes, suggesting the trophic impacts of these invaders are relatively consistent across species and systems.

T. Bašić · G. H. Copp (✉) · E. Keskin · P. I. Davison
Centre for Environment, Fisheries and Aquaculture
Science, Salmon and Freshwater Team, Pakefield Road,
Lowestoft NR33 0HT, UK
e-mail: gordon.copp@cefas.co.uk

G. H. Copp · P. I. Davison · J. R. Britton
Centre for Conservation and Environmental Change,
School of Conservation Sciences, Bournemouth
University, Poole, Dorset BH12 5BB, UK

G. H. Copp
Environmental and Life Sciences Graduate Program,
Trent University, Peterborough, ON K9J 7B8, Canada

V. R. Edmonds-Brown
University of Hertfordshire, College Lane, Hatfield,
Hertfordshire AL10 9AB, UK

E. Keskin
Evolutionary Genetics Laboratory (eGL), Department of
Fisheries and Aquaculture, Faculty of Agriculture, Ankara
University, Diskapi, 06110 Ankara, Turkey

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Introduction

There is a plethora of research suggesting non-native freshwater fishes cause adverse ecological impacts on native species and ecosystems (e.g. Collier et al. 2017; Klop-Toker et al. 2017). However, with the exception of certain species, e.g. common carp *Cyprinus carpio* (e.g. Vilizzi et al. 2015) and topmouth gudgeon *Pseudorasbora parva* (e.g. Britton et al. 2010a), substantive evidence is often limited (e.g. Gozlan 2008; Gozlan et al. 2010).

A major challenge in invasion fish biology remains the assessment of impacts of introduced populations on the recipient communities and food webs (Copp et al. 2009; Tran et al. 2015). Trophic relationships that establish themselves between the introduced and native fishes can be an effective means for evaluating the potential consequences of a biological invasion for native species (Gozlan et al. 2010; Tran et al. 2015; Copp et al. 2017). The importance of these relationships is that, where they indicate dietary overlap between the species, they suggest competitive interactions could be occurring, especially in fishes of the same feeding guild and/or when food resources are limited (Griffen et al. 2008). Indeed, invasion theory predicts that following an introduction, the impact on the trophic niches of native species will vary temporally and spatially as a function of the intensity of the inter-specific feeding interactions and the availability of food resources (Tran et al. 2015). For example, where food resources are not limiting, then the introduced species can integrate into the food web with few competitive interactions with native species, enhancing their probability of establishment (Shea and Chesson 2002; Tran et al. 2015). However, where resources are more limiting, the diet of species can become specialised, as this restricts the extent of the inter-specific trophic interactions and results in the trophic niches of the species becoming constricted (Van Valen 1965; Thomson 2004; Olsson et al. 2009). Experimental field studies in support of the latter prediction are increasing (e.g. Fobert et al. 2011; Jackson and Britton 2014; Tran et al. 2015; Copp et al. 2017), and these approaches have tended to rely on stable isotope analysis (SIA) to evaluate the trophic consequences of invasion.

Amongst introduced fishes, some of the most concerning species are those introduced accidentally or illegally, as their release into the wild will have been

uncontrolled, lacking risk assessment and approval by regulatory authorities (Hickley and Chare 2004; Copp et al. 2009, 2016). These fishes are often small-bodied with *r*-selected traits, which can benefit their accidental transfers via aquaculture and fisheries without detection (Davies et al. 2013), especially in cases of strong physical resemblance to a native species. An example of such a species is sunbleak *Leucaspis delineatus*, which is used as a model organism in the present study due to its small body size, its ability to invade both lentic (Pinder and Gozlan 2003) and lotic systems (Farr-Cox et al. 1996; Copp et al. 2006; Pollux et al. 2006) and its ability to integrate rapidly into native fish assemblages (Beyer et al. 2010). While *L. delineatus* is considered rare and vulnerable across its native range under appendix III of the Bern convention (WCMC 1996), it was introduced to Great Britain in 1986 (Gozlan et al. 2003a, b), either for ornamental purposes or, more likely, as a contaminant of an ornamental fish consignment (Farr-Cox et al. 1996). Initially, *L. delineatus* was primarily limited in distribution to southwest England (Farr-Cox et al. 1996; Pinder and Gozlan 2003), but has recently spread into southeast England, probably as a contaminant of anglers' gear (Zięba et al. 2010). Given the paucity of extant information on the potential impacts of *L. delineatus* on native species (Farr-Cox et al. 1996), studies initially focused on the species' environmental biology (e.g. Gozlan et al. 2003a, b), its potential role as a vector of non-native parasites (Beyer et al. 2005), and its interactions and integration with similar-sized native fishes (Beyer et al. 2010). This ability to integrate into native fish shoals, coupled with the species' similarity in diet with young native cyprinids (Gozlan et al. 2003b), suggests a high probability of inter-specific trophic and competitive interactions, given that foraging in small cyprinid fishes is more efficient as part of a shoal (Pitcher 1986).

The aim of the present study was, therefore, to assess the trophic consequences of a non-native *L. delineatus* on temperate fish assemblage in experimental ponds of uniform size and physical character. Stable isotope analysis (SIA) was the primary assessment method due to its provision of a temporally integrated dietary analytical tool that has high applicability to assessing impacts of non-native fishes (Cucherousset et al. 2012). Specific objectives were to: (1) quantify how the trophic position, carbon

source and trophic niche size of native fishes were modified by introduced *L. delineatus*; and (2) identify any consequences of the trophic interactions for native fish growth rates and body condition. The hypothesis tested was that in *L. delineatus* presence, the trophic niche of native fishes will be significantly constricted, resulting in reduced growth rates and impaired body condition. Note that 'isotopic niche' in the present study refers to trophic niche, which was measured as the isotopic niche size. Whilst the isotopic niche is closely related to the trophic niche, which is a sub-component of the ecological 'niche' (Copp 2008), it is also influenced by factors including growth rate and metabolism (Busst and Britton 2018).

Materials and methods

The experiment was completed in six outdoor experimental ponds located in southern England and constructed for research on non-native species, specifically *Lepomis gibbosus* (Zięba et al. 2010, 2015; Fobert et al. 2011; Copp et al. 2017). Each pond enclosed an area of 5 × 5 m and consisted of a shallow 1-m wide area (0.2–0.5 m) on one side (see Fig. 1 in Copp et al. 2017), with the depth in the remaining area around 1.2 m. Following the experiments in early autumn 2014 described in Copp et al. (2017), the ponds were drained, excess silt removed and left over the winter of 2014–2015 to refill with rain water and be re-populated naturally by aquatic invertebrates. Once filled, the pond water was circulated through a fiberglass cistern (0.2 m³) containing Canterbury spar stone chips (to facilitate microbial filtration) via a fountain pump (P2500, Bladgon, UK; maximum flow-through discharge: 2400 L h⁻¹), discharging back into the pond through an overflow pipe. Any loss of water was substituted with the gravel-filtered water from a nearby pond. Each pond was lined with a plastic liner and enclosed with wooden planks raised around 30 cm above the ground and fitted with anti-bird netting. Temperature loggers (TinyTag Aquatic 2, Gemini Data Loggers Ltd, UK) were placed into each pond to monitor temperature changes during the experiment.

The experimental design, which effectively replicates that used by a previous study in the same ponds on the trophic consequences of *L. gibbosus* on native pond fishes (Copp et al. 2017), consisted of a control

and a treatment with three replicates of each, with the ponds selected randomly as controls and treatments. Both the control and treatment ponds were stocked to contain an assemblage of small-sized native fishes that are characteristic of pond fish assemblages, which are rarely of equal density (Table 1): rudd *Scardinius erythrophthalmus* ($n = 5$), gudgeon *Gobio gobio* ($n = 9$) and tench *Tinca tinca* ($n = 10$). The control ponds contained no *L. delineatus* (NLd), whereas treatment ponds (Ld) were stocked with *L. delineatus* at densities ($n = 24$; Table 1) that correspond with future invasion predictions under climate-change scenarios (Fobert et al. 2013).

To avoid handling stress and the resulting increased risk of mortality often observed with small fishes (Persson and Greenberg 1990), total fish mass (M) at the start of the experiment was estimated from total M determined using the volumetric method to: (1) ensure that similar-sized fish of each species were stocked across ponds; and (2) measure any change in total M over the course of the experiment, with any progeny produced during the study period ignored to avoid bias due to reproduction. All fishes used in the experiment were available from adjacent, large angling ponds on the site except for *T. tinca*, which were obtained from an aquaculture facility. The release of native fishes into the ponds was performed on 23 March 2015, allowing the native fish and pond communities to establish, followed by *L. delineatus* release into the ponds on 19 May 2015. The experiment ended on 19 August 2015, providing sufficient time (93 days for *L. delineatus* and 150 days for other fish species) for fish fin tissue to achieve isotopic equilibrium with their new diet at water temperatures between 19.7 and 20.5 °C (Thomas and Crowther 2015; Copp et al. 2017).

At the conclusion of the experiment, ponds were drained and fish recovered, counted, measured for total length (L_T , to 1 mm) and weighed for mass (M , to 0.1 g), with a sample of tissue (fin clip) for stable isotope analysis taken from a sub-sample of specimens of each species, which were under mild sedation (5 mL L⁻¹ of 2-phenoxyethanol) using a UK Home Office licensed procedure. The L_T and M of each fish were used to: 1) estimate body 'condition', using the Fulton condition index ($K = 100 \times M/L_T^3$), where M and L_T are given in g and cm, respectively; and 2) test the effect of *L. delineatus* on body size and condition of native fishes, excluding progeny. After

Table 1 Numbers of native (Gg = *Gobio gobio*, Se = *Scardinius erythrophthalmus*, Tt = *Tinca tinca*) and non-native fish (Ld = *Leucaspis delineatus*) stocked into the experimental ponds (NLd = no *L. delineatus*, Ld = *L. delineatus* present) in March and May (*L. delineatus*) 2015 and re-captured by electrofishing in August 2015

Pond	NLd1	Ld1	Ld2	NLd2	NLd3	Ld3	Total
Total no. of fish stocked in March and May 2015							
Gg	9	9	9	9	9	9	54
Ld	0	24	24	0	0	24	72
Se	10	10	10	10	10	10	60
Tt	5	5	5	5	5	5	30
No. of stocked fish recovered in August 2015							
Gg	4	6	7	5	3	5	30
Ld	0	3	11	0	0	11	25
Se	4	7	5	3	4	7	30
Tt	5	5	5	5	5	5	30
No. of fish gained in August 2015 (reproduction)							
Gg	65	40	61	12	0	35	213
Ld	0	287	0	0	0	0	287
Se	0	0	0	0	0	0	0
Tt	3	0	0	0	25	11	39
Total no. of fish recovered at the end of the experiment							
Gg	69	46	68	17	3	40	243
Ld	0	290	11	0	0	11	312
Se	4	7	5	3	4	7	30
Tt	8	5	5	5	30	16	69
Total no. of native fish recovered	81	58	78	25	37	63	342
NN-to-native-ratio excluding progeny	–	0.2	0.6	–	–	0.6	–
NN-to-native ratio including progeny	–	5	0.1	–	–	0.2	–

processing, *L. delineatus* specimens were euthanized and native fish species were released back to their angling ponds of origin, following recovery from anaesthesia. Water conductivity ($\mu\text{S cm}^{-1}$), dissolved oxygen (mg L^{-1}), total nitrogen (mg L^{-1}), total phosphorous (mg L^{-1}), pH and water temperature ($^{\circ}\text{C}$) were assessed one day prior to fish stocking at the start of the experiment and a day before the termination of the experiment. Semi-quantitative samples of macro-invertebrates were obtained with a Freshwater Biological Association (FBA) pond net (mesh size = $900 \mu\text{m}$) during a 5-min sweep, counted in the laboratory and their relative abundance was estimated.

Stable isotope analysis was performed on fish fin tissues and macro-invertebrate samples. Specifically, macro-invertebrate species used for the analysis were *Gerris lacustris*, *Baetis* spp. and Chironomidae, with each sample representing 3–9 individuals, depending on their size to ensure enough material for stable isotope analysis. Triplicate samples of each macro-invertebrate species were analysed for each pond,

with the exception of bloodworms in ponds 1 and 3, and mayfly nymphs in Pond 5 for which only two replicate samples were possible due to limited numbers of those macro-invertebrates in those ponds. Fish tissue and macro-invertebrate samples were then dried at 60°C for 24 h and analysed at the Cornell University Stable Isotope Laboratory (2015), New York, USA for their stable isotopes of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, expressed as isotope ratios per mille (‰). The C:N ratios indicated very low lipid content and thus lipid extraction or normalization would have little effect on $\delta^{13}\text{C}$ (Post et al. 2007), so no lipid correction was applied to the data. For detailed description of analytical procedures, see Copp et al. (2017).

Data analysis

Linear models were used to test for the difference in the initial M (estimated from volumetric mass) across treatments (NLd vs. Ld) for each species (as interaction between treatment and species). In addition,

differences in carbon and nitrogen isotopic values between macro-invertebrate baseline were tested across ponds by linear models. The relationship between *L. delineatus* L_T and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values at recovery was also assessed with linear models independently for each pond.

Linear, mixed-effects models (LMEMs) were used to test the impact of treatment on final L_T , M and Fulton index of each species at the end of the experiment. The differences in L_T between species at recovery were also assessed with the LMEM. In addition, LMEMs were used to investigate the impact of treatment on trophic positions and corrected carbon values, with L_T as a covariate as informed by the previous model. All LMEMs were fitted by maximum likelihood in the package lme4 (Bates et al. 2015), with interaction of treatment and species as a fixed effect. Each pond was assessed as a random effect on the intercept to account for spatial dependency of individual data points.

In each case, model assumptions were validated using standard graphical validation for linear models and LMEMs in R (Zuur et al. 2009). Fitted linear models and LMEMs were evaluated by F -test and Wald test, respectively, with the ANOVA function in the car package in R (Fox and Weisberg 2011). Following a significant effect in each model, pair-wise comparisons of covariate-adjusted means (Students' t tests and Wilcoxon z tests) were conducted with single-step adjustment for P values in the package multcomp in R (Hothorn et al. 2008). All the analyses were performed in R version 3.4.2. (R Development Core Team 2010). Complete dataset and R code used for the analysis and creation of figures can be found at doi: <https://doi.org/10.14466/CefasDataHub.48>.

Primary analysis of stable isotopes consisted of exploring biplots of $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ of fish individual data and mean macro-invertebrates values for each pond (Fig. 2). While nitrogen values of macro-invertebrates did not vary significantly among ponds (LM; $F_{1,37} = 0.01$, $P > 0.05$), differences in carbon values were significant (LM; $F_{1,37} = 9.70$, $P < 0.01$). Consequently, fish isotopic values were corrected prior to further comparisons between treatments. Specifically, $\delta^{15}\text{N}$ data were transformed to trophic position (TP) using the equation $\text{TP}_i = [(\delta^{15}\text{N}_i - \delta^{15}\text{N}_{\text{base}}) / 3.4] + 2$, where TP_i is the trophic position of the individual fish, $\delta^{15}\text{N}_i$ is the isotopic ratio of that fish, $\delta^{15}\text{N}_{\text{base}}$ is the isotopic ratio of the primary consumers

(mean $\delta^{15}\text{N}$ of all macro-invertebrates), 3.4 is the fractionation between trophic levels and 2.0 is the trophic position of the baseline organism (Post 2002). Correction of $\delta^{13}\text{C}$ was as follows: $\delta^{13}\text{C}_{\text{corr}} = \delta^{13}\text{C}_i - \delta^{13}\text{C}_{\text{meaninv}} / \text{CR}_{\text{inv}}$, where $\delta^{13}\text{C}_{\text{corr}}$ is the corrected carbon isotope ratio of the individual fish, $\delta^{13}\text{C}_i$ is the uncorrected isotope ratio of that fish, $\delta^{13}\text{C}_{\text{meaninv}}$ is the mean invertebrate isotope ratio and CR_{inv} is the invertebrate carbon range across all macro-invertebrate species ($\delta^{13}\text{C}_{\text{max}} - \delta^{13}\text{C}_{\text{min}}$; Olsson et al. 2009). Furthermore, fractionation factors were assumed to be equal for all fish species due to lack of data on species-specific values.

To avoid a significant impact of fish L_T on trophic position due to significant variations in L_T s between species detected at the end of the experiment (LMEM; $\chi^2_{(3)} = 41.44$, $P < 0.01$), fishes of different L_T s were excluded from the standard ellipse area calculations. Specifically, fish with $L_T > 7$ cm were omitted from further analysis to comply with the sizes of *L. delineatus* present in the experiment due to potential ontogenetic diet shifts, which could have compromised the analysis (Hyslop 1980; Bašić and Britton 2015). In the case of progeny of three species, those of *G. gobio* and *T. tinca* were included in the analysis because they were of similar L_T as adult *L. delineatus*, whereas *L. delineatus* progeny were excluded from further analysis due to significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of those obtained for adult *L. delineatus*.

Corrected stable isotope data were then used to calculate the standard ellipse area (SEA) for remaining species per treatment by using the SIAR package in R (Jackson et al. 2011). SEA represents distribution of individuals in the isotopic space as a bivariate measure, with the ellipses encompassing 40% of data, representing the core dietary isotopic niche, hereafter referred to as 'isotopic niche' (Jackson et al. 2011, 2012, 2016). Owing to limited numbers of fish per species after L_T correction, data from different ponds were pooled by treatment, and a Bayesian estimate of SEA (SEAb) was calculated using a Markov chain Monte Carlo simulation with 20,000 iterations. This generated estimates of SEAb modes and 95% credible intervals, which were used in the subsequent comparisons of SEAb size and calculation of SEAb overlap (shared isotopic resources, including 40 and 95% of the data) between species and treatments.

Results

Pond water chemistry variables showed little change during the course of the experiment, with minor differences found in nitrogen levels (Table 2). Macro-invertebrate species found across all experimental ponds included: *Asellus aquaticus*, *Gerris lacustris*, *Lymnaea peregra*, *Baetis* spp. Chironomidae, Corixidae juveniles, Oligochaeta, *Pisidium* sp., Simuliidae, and Tipulidae. Their relative abundance increased throughout the experiment, by 4 ± 2.2 (SE) ind. per sweep in the control ponds (from 5.7 ± 0.7 to 9.7 ± 2.9) and by 11 ± 6.8 ind. per sweep in the treatment ponds (from 4.3 ± 1.7 to 15.0 ± 8.5).

At the start of the experiment, there were no significant differences (Students' *t* test) in the mean *M* of *G. gobio* ($t = -0.63$, $P > 0.05$), *T. tinca* ($t = -0.93$, $P > 0.05$), or *S. erythrophthalmus* ($t = 0.43$, $P > 0.05$), within species between treatment and control ponds (Table 3). However, there were significant differences in initial *M* (linear model; $F_{5,12} = 150.1$, $P < 0.01$) among species within treatments (Table 3). At the end of the experiment, the total number of fishes recovered (excluding progeny) was generally reduced due most likely to fish mortality (and possibly sampling error), with the exception of *T. tinca*, which remained unchanged (Table 1). Regarding reproduction, progeny were observed for three species: *G. gobio* in all ponds except Pond NLd3; *T. tinca* in ponds NLd1, NLd3, Ld3; and *L. delineatus* in Pond Ld1 only. Mean *M* (g), *Lt* (cm) and Fulton index (all \pm SE), respectively, of the recovered progeny (all ponds combined) were: *G. gobio* (0.7 ± 0.0 ,

3.8 ± 0.1 , and 0.8 ± 0.0), *T. tinca* (0.4 ± 0.1 , 2.9 ± 0.1 , and 1.1 ± 0.1) and *L. delineatus* (0.2 ± 0.0 , 2.6 ± 0.1 , and 0.6 ± 0.0). All species except *L. delineatus* underwent an increase in *M* over the course of the experiment (Table 3).

For recovered fishes excluding progeny, the LMEMs revealed a significant effect of the interaction of treatment and species on their L_T ($\chi^2_{(5)} = 140.32$; $P < 0.01$), *M* ($\chi^2_{(5)} = 156.56$; $P < 0.01$) and condition ($\chi^2_{(5)} = 148.23$; $P < 0.01$). While native species decreased in L_T , *M* and condition index in the presence of *L. delineatus*, pair-wise comparisons (Wilcoxon *z* tests) did not reveal any significant differences between control and treatment individuals of *G. gobio* (L_T : $z = 1.30$; $P > 0.05$; *M*: $z = 0.91$; $P > 0.05$; Fulton: $z = 1.19$; $P > 0.05$) and *T. tinca* (L_T : $z = 1.47$; $P > 0.05$; *M*: $z = 2.10$; $P > 0.05$; Fulton: $z = 1.30$; $P > 0.05$) (Table 3; Fig. 1). The only significant differences observed were between L_T of control and treatment *S. erythrophthalmus* (L_T : $z = 3.54$; $P < 0.01$), but this was not reflected in their *M* ($z = 2.33$; $P > 0.05$) or condition index ($z = 1.19$; $P > 0.05$) (Table 3; Fig. 1).

Stable isotope analysis

The differences in N stable isotope values between the fishes and their putative prey resources (as mean macro-invertebrates SI values) were 2–5‰, except for the differences between *L. delineatus* and prey items, which were 3–7‰ (Fig. 2). Furthermore, *L. delineatus* had higher trophic position than other fish species, with differences in their SI data of 1.5–3.0‰ (Fig. 2).

Table 2 Mean water chemistry variables (all pond data combined) measured at the start (March 2015) and the end (August 2015) of the pond experiment (NLd = no *L. delineatus*, Ld = *L. delineatus* present)

	Start		End	
	NLd	Ld	NLd	Ld
Dissolved oxygen (mg L ⁻¹)	6.9 \pm 0.10	7.0 \pm 0.20	8.1 \pm 0.10	7.6 \pm 0.20
pH	7.7 \pm 0.20	7.7 \pm 0.10	7.8 \pm 0.20	7.8 \pm 0.20
Conductivity (μ S cm ⁻¹)	365.0 \pm 17.30	378.7 \pm 31.00	401.7 \pm 48.30	375.0 \pm 32.10
Temperature (°C)	20.4 \pm 0.10	20.5 \pm 0.10	19.7 \pm -0.20	19.9 \pm 0.20
Nitrogen (mg L ⁻¹)	14.6 \pm 7.40	5.6 \pm 0.30	20.9 \pm 9.40	12.3 \pm 2.20
Phosphorus (mg L ⁻¹)	0.1 \pm 0.10	0.1 \pm 0.00	0.1 \pm 0.00	0.1 \pm 0.03

The error around the mean is the standard error

Table 3 Estimated mean mass (M , in g; from volumetric mass) of non-native (Ld = *Leucaspius delineatus*) and native fishes (Gg = *Gobio gobio*, Se = *Scardinius erythrophthalmus*, Tt = *Tinca tinca*) (\dagger = Ld present) at stocking and measuredmean M (g), L_T (cm) and Fulton index at recovery (\pm SE) without progeny, including mean difference in M during the experiment and means of all the variables across ponds (n/a = not applicable)

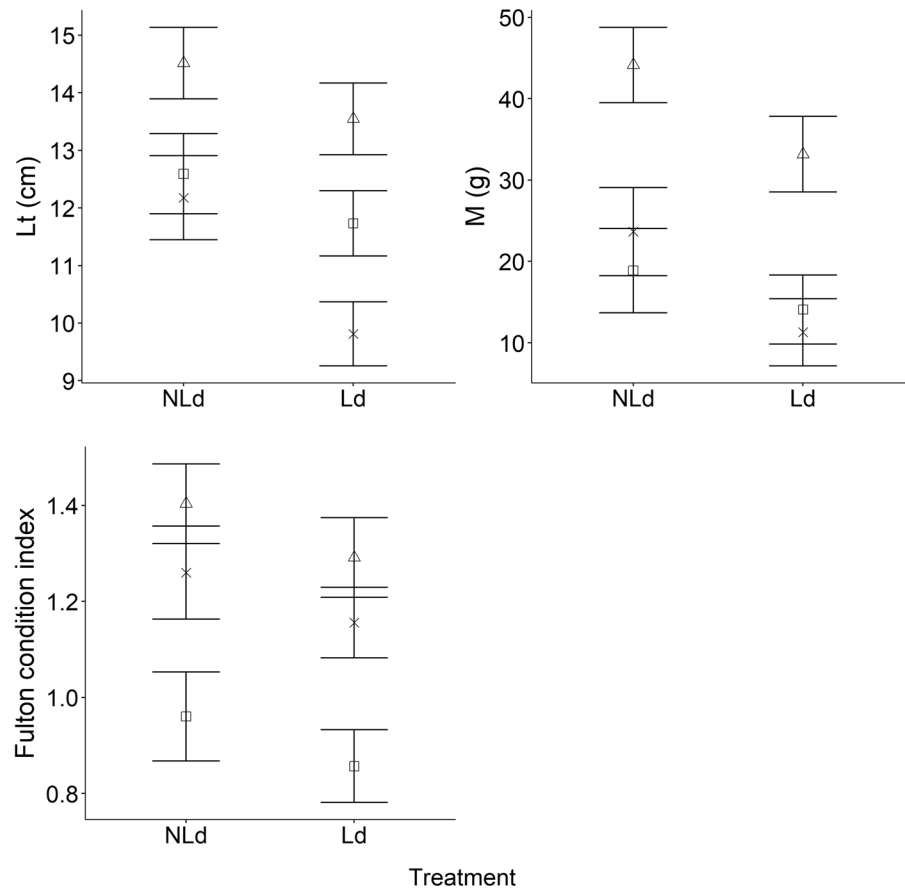
Pond	1	2 \dagger	3 \dagger	4	5	6 \dagger	Means
Mean estimated M of fish prior to stocking							
Gg	10.8	10.8	10.4	9.8	9.5	10.8	10.4
Ld	n/a	1.5	1.4	n/a	n/a	1.4	1.4
Se	7.4	6.6	6.6	7.1	6.4	6.4	6.8
Tt	21.7	26.2	24.4	26.0	26.6	26.5	25.2
Mean measured M of fish at recovery (\pm SE)							
Gg	17.7 \pm 1.1	15.9 \pm 0.6	10.6 \pm 0.6	22.0 \pm 1.7	18.7 \pm 1.5	15.7 \pm 1.7	16.8
Ld	n/a	0.9 \pm 0.03	1.4 \pm 0.1	n/a	n/a	1.4 \pm 0.1	1.2
Se	16.4 \pm 2.3	9.3 \pm 1.9	9.9 \pm 1.6	29.5 \pm 4.8	24.7 \pm 2.2	15.2 \pm 0.9	17.5
Tt	37.9 \pm 3.1	39.2 \pm 8.6	26.8 \pm 3.0	60.0 \pm 7.5	34.5 \pm 2.7	33.5 \pm 2.5	38.7
Mean measured L_T of fish at recovery (\pm SE)							
Gg	12.2 \pm 0.3	12.1 \pm 0.1	11.1 \pm 0.1	13.0 \pm 0.3	12.7 \pm 0.3	12.1 \pm 0.5	12.2
Ld	n/a	5.2 \pm 0.1	5.3 \pm 0.1	n/a	n/a	5.5 \pm 0.3	5.4
Se	10.9 \pm 0.6	9.4 \pm 0.6	9.7 \pm 0.6	13.2 \pm 0.8	12.5 \pm 0.3	10.4 \pm 0.2	11.0
Tt	14.0 \pm 0.3	14.4 \pm 0.7	13.0 \pm 0.5	16.1 \pm 0.7	13.4 \pm 0.5	13.2 \pm 0.4	14.0
Mean Fulton index of fish at recovery (\pm SE)							
Gg	1.0 \pm 0.02	0.9 \pm 0.02	0.8 \pm 0.03	1.0 \pm 0.04	0.9 \pm 0.03	0.9 \pm 0.03	0.9
Ld	n/a	0.7 \pm 0.01	0.9 \pm 0.04	n/a	n/a	0.9 \pm 0.1	0.8
Se	1.3 \pm 0.1	1.0 \pm 0.04	1.1 \pm 0.03	1.3 \pm 0.1	1.3 \pm 0.1	1.4 \pm 0.1	1.3
Tt	1.4 \pm 0.04	1.2 \pm 0.1	1.2 \pm 0.02	1.4 \pm 0.03	1.4 \pm 0.1	1.5 \pm 0.01	1.4
Difference in mean M over the course of the experiment							
Gg	6.9	6.1	0.2	12.8	9.2	4.9	6.7
Ld	n/a	- 0.6	0.0	n/a	n/a	0.0	- 0.2
Se	9.0	2.7	3.3	22.4	18.3	8.8	10.8
Tt	16.2	13.0	2.4	34.0	7.9	7.0	13.4

In general, there was no significant effect of *L. delineatus* L_T on carbon or nitrogen isotopic values, with the exception of the Pond Ld1, where individuals of short L_T had quite low nitrogen values (Fig. 3).

Comparisons of stable isotope values between treatments used the corrected data (Table 4). Linear mixed-effect models indicated a significant impact of treatment and species interaction (LMEMs; Wald χ^2) on both corrected carbon ($\chi^2_{(5)} = 35.10$; $P < 0.01$) and trophic position ($\chi^2_{(5)} = 41.56$; $P < 0.01$), with the covariate of L_T significant only for trophic position ($\chi^2_{(1)} = 39.58$; $P < 0.01$). Multiple comparisons (Wilcoxon z tests) of estimated marginal means (with Tukey contrasts) revealed no significant differences between control (NLd) and treatment (Ld) corrected carbon values of *G. gobio* ($z = 0.65$, $P > 0.05$), *T. tinca* ($z = 0.41$, $P > 0.05$) or *S. erythrophthalmus*

($z = 1.04$, $P > 0.05$) (Fig. 4). Equally, trophic position between control and treatment (Fig. 4) did not differ significantly in *G. gobio* ($z = 0.41$, $P > 0.05$), *T. tinca* ($z = 1.81$, $P > 0.05$) or *S. erythrophthalmus* ($z = - 0.88$, $P > 0.05$). Significant differences in corrected carbon values were evident between *G. gobio* and *S. erythrophthalmus* in the presence of *L. delineatus* ($z = 4.66$, $P < 0.01$), with no differences observed in the control ($z = - 2.44$, $P > 0.05$). Similarly, differences in corrected carbon values between *G. gobio* and *T. tinca* were significant in the control ($z = 3.70$, $P < 0.01$), whereas no significant change between them was evident in the presence of *L. delineatus* ($z = 2.64$, $P > 0.05$). Trophic position differed significantly between *G. gobio* and *S. erythrophthalmus* ($z = - 5.30$, $P < 0.01$) and *T. tinca* and *S. erythrophthalmus* ($z = - 5.24$, $P < 0.01$) in the

Fig. 1 Mean marginal effects and 95% confidence intervals estimated from mixed-effects models testing the impact of Treatment (NLd = no *L. delineatus*, Ld = *L. delineatus* present) on the total length, mass and Fulton body index of native fishes at recovery, where: clear triangle = *T. tinca*, clear square = *G. gobio*, × = *S. erythrophthalmus*



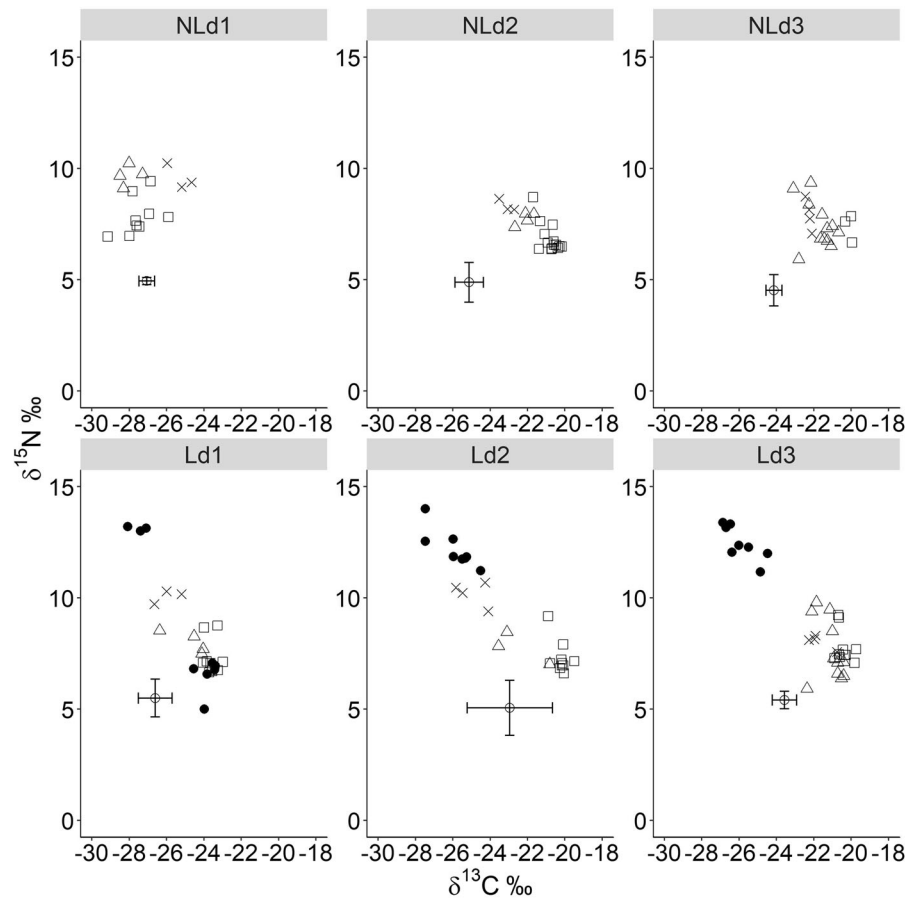
presence of *L. delineatus*. However, in the absence of *L. delineatus*, no significant differences were evident between *G. gobio* and *S. erythrophthalmus* ($z = -2.56$, $P > 0.05$) or *T. tinca* and *S. erythrophthalmus* ($z = -0.47$, $P > 0.05$).

Stable isotope analysis of fish of similar L_T demonstrated no overlap between core isotopic niches of *L. delineatus* and *G. gobio* or *T. tinca* (Table 5; Fig. 5). Equally, no overlaps in isotopic niches (comprising 95% the data) were evident between *L. delineatus* and *G. gobio*, nor between *L. delineatus* and *T. tinca* (Table 5; Fig. 5). Size of the core Bayesian ellipse areas differed significantly between fish species (100% probability), with *L. delineatus* utilising the largest isotopic niche ($0.27\% ^2$; Table 5; Fig. 5). Also, Bayesian ellipse areas of *G. gobio* and *T. tinca* were significantly decreased in the presence of *L. delineatus*, with high probabilities (100%) that ellipse sizes of both *G. gobio* and *T. tinca* were smaller than in the control ponds (Table 5; Fig. 5).

Discussion

In the experimental ponds with *L. delineatus* present, there were significant reductions in trophic niches of *G. gobio* and *T. tinca*, suggesting that the response in native fishes was niche constriction via trophic specialisation when co-habiting with *L. delineatus*. This result is consistent with similar studies on the trophic impacts on native fishes of non-native *L. gibbosus* (Copp et al. 2017) and invasive *P. parva* (Jackson and Britton 2014; Tran et al. 2015). It also aligns with the niche variation hypothesis, which predicts less-generalised diet of subordinate competitors under increased inter-specific competition with other species (Van Valen 1965; Thomson 2004; Olsson et al. 2009; Jackson et al. 2012). However, the presence of introduced *L. delineatus* had no negative consequences for the growth or body condition for two of the three native species in this experiment.

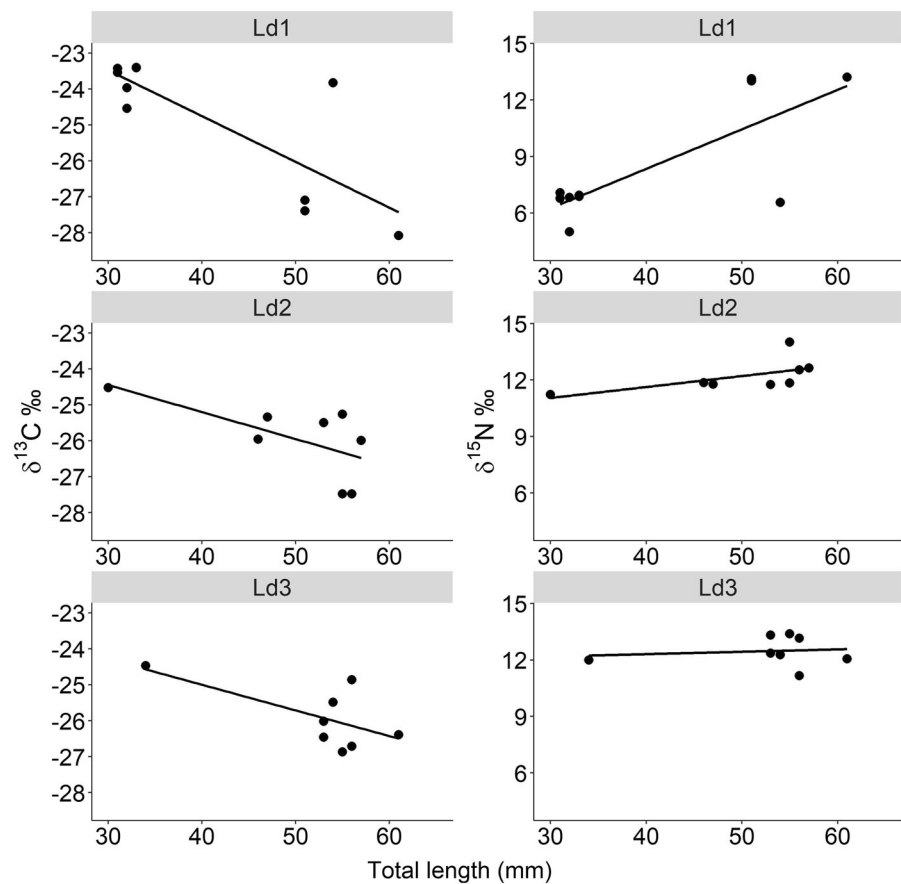
Fig. 2 Stable isotope biplots per pond with control NLd = no *L. delineatus* and treatment Ld = *L. delineatus* present, where clear triangle = *T. tinca*, clear square = *G. gobio*, × = *S. erythrophthalmus*, filled circle = *L. delineatus* and clear circle = mean macro-invertebrate stable isotope data (\pm SE)



The isotopic niches of *L. delineatus* and native fishes did not overlap when 40% or 95% of the data were included. Furthermore, no effects of *L. delineatus* on size (L_T , M) or body plumpness (Fulton condition index) of two of the three native fish species were detected. This could imply that interactions between non-native and native species were not strong enough to cause perceivable detrimental effects on fish growth and well-being, such as was reported in similar experiments in these same ponds between native fishes and introduced *L. gibbosus* (Copp et al. 2017). This could be a result of limited numbers of fish utilised in this experiment, which could have prevented density-dependent effects, specifically growth impairment to occur (Britton et al. 2017). Alternatively, this could suggest that food resources were not limiting, as observed in Bašić and Britton (2016), enabling native fishes to reduce their isotopic niche in the presence of elevated numbers of non-native species, whilst maintaining their energy requirements.

Climate-change predictions could favour fast colonisation of non-native species introduced outside their native range (Rahel and Olden 2008; Fobert et al. 2013). Mitigation efforts will include fast detection of non-native species in the environment, prediction of their potential for successful establishment and spread, as well as the evaluation of the risk they pose to the receiving environment and adjacent biota (Copp et al. 2016). Consequently, with the paucity of knowledge on some non-native freshwater fishes and their ecological impacts in the UK, there is a requirement for further studies to prioritise high-risk species and develop adequate mitigation measures. Of the extant non-native fishes in the UK, *L. delineatus* had been previously been found to create closer social interactions with young-of-the-year cyprinid fish species of similar size than did native species amongst themselves (Beyer et al. (2010), but information on the ecological consequences of those relationships are limited to the species' potential host of an existing

Fig. 3 Scatter plots of $\delta^{13}\text{C}$ (left side) and $\delta^{15}\text{N}$ (right side) as a function of total length of *L. delineatus* in each experimental pond. Effect of *L. delineatus* total length on stable isotope values was tested with ANOVA for each pond; Ld1 ($\delta^{13}\text{C}$: $F_{1,8} = 14.43$, $P < 0.01$; $\delta^{15}\text{N}$: $F_{1,8} = 11.86$, $P < 0.01$), Ld2 ($\delta^{13}\text{C}$: $F_{1,6} = 4.20$, $P > 0.05$; $\delta^{15}\text{N}$: $F_{1,6} = 3.57$, $P > 0.05$), Ld3 ($\delta^{13}\text{C}$: $F_{1,6} = 4.31$, $P > 0.05$; $\delta^{15}\text{N}$: $F_{1,6} = 0.10$, $P > 0.05$)



non-native fish parasite (Beyer et al. 2005) and the present results. Earlier studies on *L. delineatus* examined their potential for invading British rivers, including the species' reproductive strategy (Gozlan et al. 2003a), small body size (Gozlan et al. 2003b), morphology, growth and life-history traits, natural dispersal, dietary overlap, and the aforementioned parasite fauna (Beyer 2008). However, in a modelling study of climate compatibility under future climate conditions, *L. delineatus* was one of two species (the other being *Leuciscus idus*) predicted to be less compatible with warmer conditions (Britton et al. 2010b), indicating that climate change might diminish their impact on native species. However, one could argue that temperature conditions in this study could represent future climate conditions of large ponds and small lakes, as the water temperatures were $\approx 2^\circ\text{C}$ higher than the mean summer water temperatures recorded for a 0.8 ha ornamental pond ≈ 100 km to the north (based on data derived by Tarkan et al. 2011). This would suggest that the trophic impacts of *L.*

delineatus observed in the present experimental study, under elevated temperatures than normally observed in larger ponds, may be indicative of the species' response to future climate conditions. This could be interpreted as contradicting the predictions of Britton et al. (2010b), but the short-term study results presented here must be viewed within their context: the size of the experimental ponds was $\approx 13\%$ of the mean surface area (i.e. 0.019 ha) of garden ponds in the UK (Davies et al., 2009), the latter mean area being $\approx 7\%$ of landscape-situated ponds (Davies et al., 2008). As such, the present results may be suggestive of future impacts, but climate change is also likely to affect the abundance and growth rates of native fish populations (Ruiz-Navarro et al. 2016) as well as the strength of some of trophic interactions (e.g. Van der Putten et al. 2010). So, the extent and magnitude of future impacts by *L. delineatus* on biotic interactions across trophic levels remain unclear.

The present study encountered a few limitations. Firstly, identical fractionation factors between fin

Table 4 Sample sizes (n), mean total lengths (L_T) and SE in cm of non-native (Ld = *Leucaspius delineatus*) and native fish (Gg = *Gobio gobio*, Se = *Scardinius erythrophthalmus*, Tt = *Tinca tinca*) species (Sp.) used in the stable isotopes analysisand their corresponding means and SE (in ‰) for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, corrected values (Ccor and TP) and ranges of $\delta^{13}\text{C}$ (CR) and $\delta^{15}\text{N}$ (NR) per experimental pond (NLd = no *L. delineatus*, Ld = *L. delineatus* present)

Pond	Sp.	n	Mean L_T	Mean $\delta^{13}\text{C}$	Mean Ccor	CR	Mean $\delta^{15}\text{N}$	Mean TP	NR
NLd1	Gg	9	48.3 ± 15.0	- 27.5 ± 0.3	- 0.1 ± 0.1	3.2	7.8 ± 0.3	2.9 ± 0.1	2.5
	Se	3	112.0 ± 10.8	- 25.3 ± 0.4	0.5 ± 0.1	1.3	9.6 ± 0.3	3.4 ± 0.1	1.1
	Tt	4	112.3 ± 23.1	- 28.0 ± 0.3	- 0.3 ± 0.1	1.2	9.7 ± 0.2	3.4 ± 0.1	1.1
NLd2	Gg	14	72.1 ± 10.4	- 20.8 ± 0.1	0.9 ± 0.0	1.5	6.9 ± 0.2	2.6 ± 0.1	2.3
	Se	3	142.7 ± 10.9	- 23.1 ± 0.2	0.4 ± 0.0	0.8	8.3 ± 0.2	3.0 ± 0.0	0.5
	Tt	4	135.8 ± 6.7	- 22.1 ± 0.2	0.6 ± 0.0	1.0	7.7 ± 0.1	2.8 ± 0.0	0.6
NLd3	Gg	3	129.0 ± 2.5	- 20.1 ± 0.1	1.7 ± 0.0	0.4	7.4 ± 0.4	2.8 ± 0.1	1.2
	Se	4	104.5 ± 21.0	- 22.3 ± 0.1	0.8 ± 0.0	0.3	7.9 ± 0.3	3.0 ± 0.1	1.7
	Tt	12	70.3 ± 12.7	- 21.7 ± 0.2	1.1 ± 0.1	2.4	7.5 ± 0.3	2.9 ± 0.1	3.4
Ld1	Ld	10	40.9 ± 3.7	- 24.9 ± 0.6	0.4 ± 0.1	4.7	8.5 ± 1.0	2.9 ± 0.3	8.2
	Gg	10	58.0 ± 3.3	- 23.6 ± 0.1	0.6 ± 0.0	1.1	7.3 ± 0.2	2.5 ± 0.1	2.1
	Se	3	96.3 ± 13.8	- 26.0 ± 0.4	0.1 ± 0.1	1.5	10.1 ± 0.2	3.3 ± 0.1	0.6
	Tt	4	144.3 ± 9.7	- 24.8 ± 0.5	0.4 ± 0.1	2.3	8.0 ± 0.2	2.7 ± 0.1	1.0
Ld2	Ld	8	49.9 ± 3.2	- 25.9 ± 0.4	- 0.3 ± 0.0	3.0	12.2 ± 0.3	4.1 ± 0.1	2.8
	Gg	10	67.1 ± 11.1	- 20.2 ± 0.1	0.2 ± 0.0	1.4	7.3 ± 0.2	2.7 ± 0.1	2.6
	Se	4	102.3 ± 13.5	- 24.9 ± 0.4	- 0.2 ± 0.0	1.7	10.2 ± 0.3	3.5 ± 0.1	1.3
	Tt	3	116.3 ± 5.5	- 22.5 ± 0.8	0.0 ± 0.1	2.7	7.8 ± 0.4	2.8 ± 0.1	1.4
Ld3	Ld	8	52.8 ± 2.8	- 25.9 ± 0.3	- 0.5 ± 0.1	2.4	12.5 ± 0.3	4.1 ± 0.1	2.2
	Gg	10	57.4 ± 9.0	- 20.4 ± 0.1	0.7 ± 0.0	1.1	7.8 ± 0.2	2.7 ± 0.1	2.2
	Se	4	95.0 ± 20.6	- 21.7 ± 0.3	0.4 ± 0.1	1.5	8.0 ± 0.2	2.8 ± 0.0	0.7
	Tt	11	61.1 ± 14.6	- 21.1 ± 0.2	0.5 ± 0.0	2.0	7.6 ± 0.4	2.7 ± 0.1	3.9

tissues and prey items were used due to limited information on species-specific values, despite the existence of variable fractionation factors for different fish species and prey items (Tronquart et al. 2012; Busst et al. 2015; Busst and Britton 2016). Secondly, observed niche constrictions of native species could be argued to be a result of density-dependent processes due to the elevated densities of stocked *L. delineatus*, i.e. irrespective of their non-native status. However, elevated densities of a non-native species during initial establishment is characteristic of many biological invasions, leading to exacerbated trophic pressures on the native species (e.g. Britton et al. 2010b; Britton and Gozlan 2013; Britton et al. 2017; Copp et al. 2017), which the present study endeavoured to evaluate. Thirdly, and ignoring the presence of progeny of three species (c.f. Results), low recovery rates for *G. gobio* and *S. erythrophthalmus* in several ponds could have affected the results. Finally, the present study was simplistic in design and therefore difficult to scale

up, both temporally and spatially, to ‘natural’ ecosystems (Korsu et al. 2009; Spivak et al. 2011; Vilizzi et al. 2015). However, mesocosm experiments have been successfully used in SIA studies to underline complex large-scale processes with the benefit of having more control and greater replication (Spivak et al. 2011; Copp et al. 2017), such as demonstrated in studies with stocked native *Barbus barbus* (Bašić and Britton 2016) and invasive *P. parva* (Tran et al. 2015).

Despite some constraints in the experimental design, the present study provided the first evidence of the trophic consequences of invasive *L. delineatus* on native pond fishes, with increased dietary specialisation detected in their presence but with no shift in their growth rates or condition. As this result is consistent with a number of studies on small-bodied invasive fishes (Tran et al. 2015; Copp et al. 2017), these findings suggest that following the introduction of small-bodied, non-native fishes, constrictions in the niche size of native fishes is a mechanism that enables

Fig. 4 Mean marginal effects and 95% confidence intervals estimated from mixed-effects models testing the impact of Treatment (NLd = no *L. delineatus*, Ld = *L. delineatus* present) on the corrected carbon and trophic position of native fishes at recovery, where: clear triangle = *T. tinca*, clear square = *G. gobio*, × = *S. erythrophthalmus*

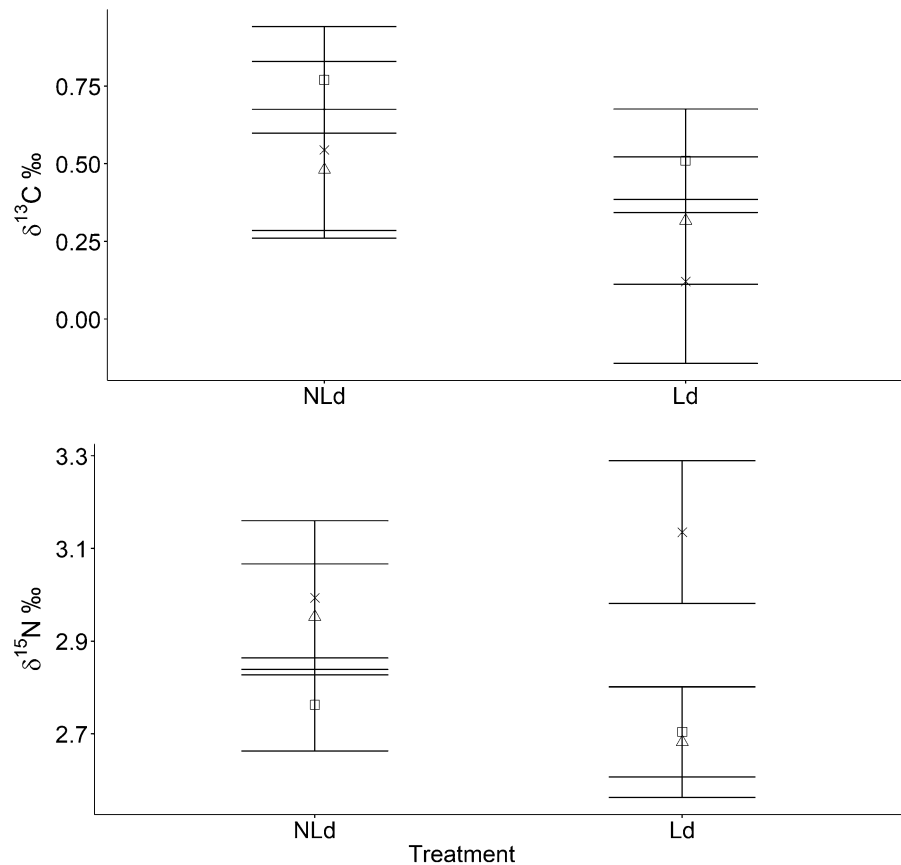


Table 5 Sample sizes (n) and mean initial total length (L_T) in mm of non-native (Ld = *Leucaspius delineatus*) and native fish (Gg = *Gobio gobio*, Tt = *Tinca tinca*) species (Sp.) used for the analysis of the standard ellipse areas and their means (\pm SE) of corrected $\delta^{13}\text{C}$ (Ccorr) and $\delta^{15}\text{N}$ (trophic position = TP) in ‰, as well as the species' trophic niche sizes (mode of the core Bayesian standard ellipse areas; SEAb in

‰², with 95% credible intervals) at the conclusion of the pond mesocosm experiment (NLd = no *L. delineatus*, Ld = *L. delineatus* present), and the trophic niche overlaps (as % SEAb with 95% credible intervals) between Ld and Gg and Ld and Tt, including 40% of data (core trophic niche; Ov^{40%}) and 95% of the data (Ov^{95%})

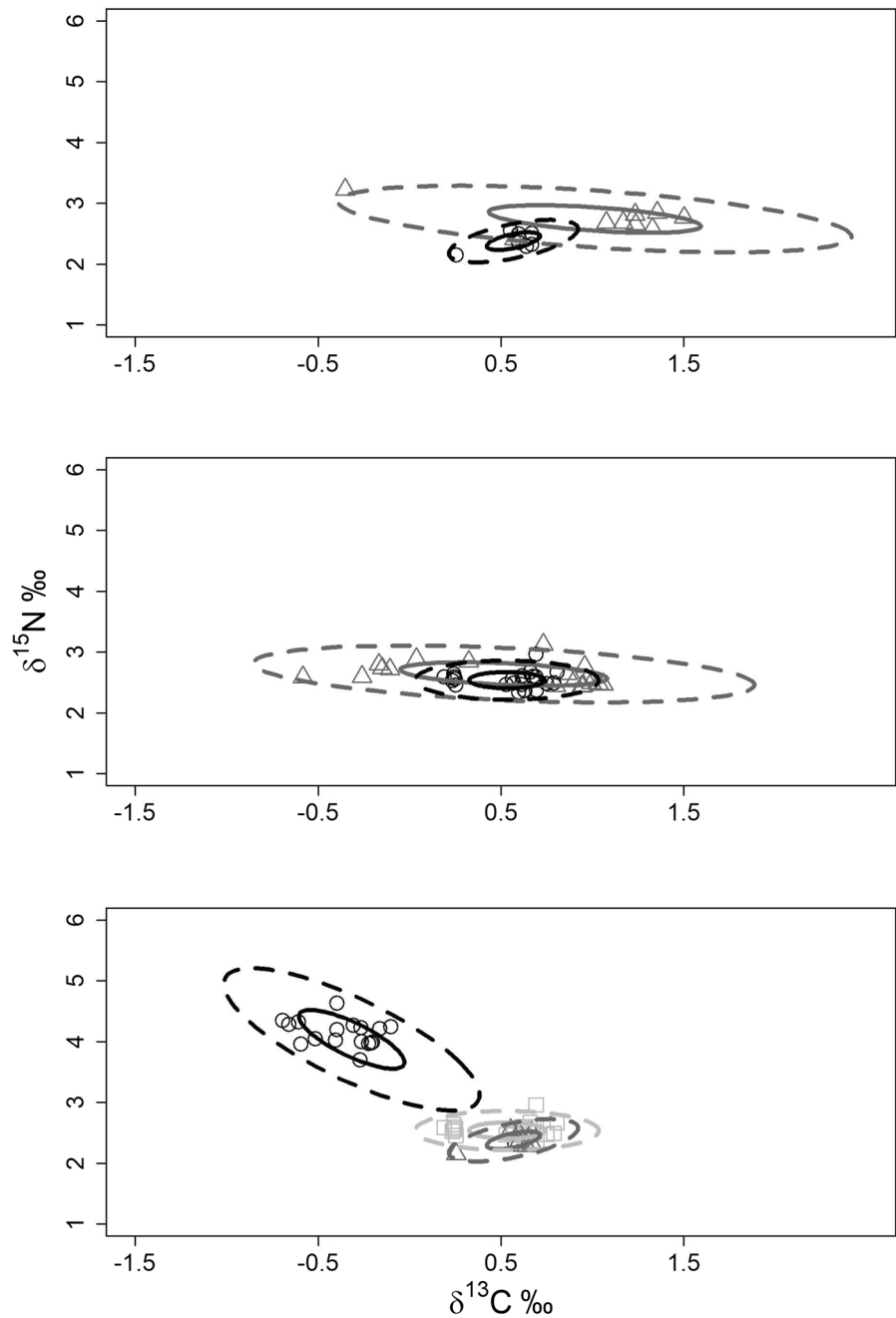
Treatment	Sp.	n	L_T	Ccorr	TP	SEAb	Ov ^{40%}	Ov ^{95%}
NLd	Gg	18	4.3 \pm 0.4	0.5 \pm 0.1	2.6 \pm 0.0	0.28 (0.18–0.46)		
	Tt	9	4.1 \pm 0.3	1.0 \pm 0.2	2.7 \pm 0.1	0.32 (0.17–0.69)		
Ld	Ld	18	5.4 \pm 0.1	– 0.3 \pm 0.1	4.0 \pm 0.1	0.27 (0.16–0.43)		
	Gg	24	4.8 \pm 0.1	0.5 \pm 0.0	2.5 \pm 0.0	0.09 (0.06–0.13)	0	0
	Tt	7	2.7 \pm 0.2	0.6 \pm 0.1	2.4 \pm 0.1	0.05 (0.02–0.12)	0	0

them to maintain their growth rates and condition in the face of possible invasion.

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Fig. 5 Stable isotope bi-plots for each treatment with individual data points and solid and dashed lines enclosing 40% and 95% of the standard ellipse areas for each species, respectively, where: Upper graph—*T. tinca* in presence (clear black circles and black line) and absence (clear dark grey triangles and dark grey line) of *L. delineatus*; Middle graph—*G. gobio* in presence (clear black circles and black line) and absence (clear dark grey triangles and dark grey line) of *L. delineatus*; Lower graph—*L. delineatus* (clear black circles and black line), *G. gobio* (clear grey triangles and grey line) and *T. tinca* (clear dark grey squares and dark grey line) in the ponds with *L. delineatus*



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