PRIMARY RESEARCH PAPER

Trophic relationships of translocated and indigenous chub *Squalius cephalus* populations with trophically analogous fishes

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Abstract The translocation of non-indigenous fishes into lowland rives can result in invasive populations establishing and dispersing. Because non-indigenous fishes can cause ecological harm, it is important to understand their trophic relationships and the effects they may have on native fishes. We assessed the trophic ecology of a translocated chub *Squalius cephalus* population in the River Frome, a lowland chalk stream in Southern England, using bulk stable isotope ($\delta^{13}C$, $\delta^{15}N$) metrics, and compared the results with those derived from indigenous chub populations. The isotopic niche sizes of all fishes in the River Frome were substantially larger in the tidal versus non-tidal site, with the $\delta^{13}C$ values suggesting some fish were foraging further downstream of their sampling point in areas that had greater tidal and salinity influences. Inter-specific comparisons of isotopic niches revealed a consistent pattern of similar niche size and overlap between chub and the trophically analogous dace *Leuciscus leuciscus*. These isotopic relationships between chub and dace were then also apparent in the indigenous populations of these fishes. These results suggest that the colonisation of this lowland river by translocated chub is being facilitated by their isotopic relationships with other fishes that are similar to those observed in their native range.

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Introduction

When an alien fish species is introduced or translocated directly into a river catchment then the introduced propagules have the potential to establish a population which can disperse (Dominguez Almela et al., 2020, 2022). These fish populations can then have ecological impacts on native species, which develop through processes including predation and competition (Gozlan et al., 2010). Predicting which introduced fishes will establish, disperse and impact native species remains a major ecological challenge (Copp et al., 2009; Tran et al., 2015), especially given that species-rich riverine fish communities can inhibit

these processes through biological resistance (Britton, 2022).

The magnitude and direction of ecological impact that arises from competitive interactions depends on how the non-native and native fishes interact trophically, such as whether they exploit similar prey resources and so converge in resource use or largely exploit different resources and so diverge (Jackson et al., 2012; Tran et al., 2015). If there is convergence in resource use within the species, their trophic niche sizes and positions will be similar, with potential for the species to compete in the prey resources are limiting (Britton, 2022). If these competitive interactions are asymmetric then the consequences for the inferior competitor can include depressed growth rates, reduced body condition and trophic niche displacement (Cucherousset et al., 2012; Kakareko et al., 2013). The niche variation hypothesis posits that where increased competitive interactions develop then the competing species become more specialised in their resource use, resulting in smaller trophic niches that are increasingly diverged (Van Valen 1965; Thomson 2004; Olsson et al., 2009). Alternatively, the fishes can develop more generalist diets, characterised by larger trophic niches, with the competing species exploiting a wider dietary base to maintain their energetic requirements (Svanbäck & Bolnick, 2007). If the available prey resources are not being fully exploited by native species then these provides feeding opportunities for non-native species that minimises their inter-specific competitive interactions (Shea & Chesson, 2002; Tran et al., 2015).

Activities associated with recreational angling, such as fish stocking and live bait release, represent a major introduction pathway for freshwater fish (Cambray, 2003; Winfield & Durie, 2004). This introduction pathway includes the translocation of indigenous fishes into areas within regions where they are non-indigenous (Winfield et al., 2008; Britton et al., 2011). For example, the chub *Squalius cephalus* (Linnaeus, 1758) was translocated (most likely by anglers) into the River Frome, Southern England, in the late 2000s. Their presence in the river has increased the diversity of non-salmonid species available for angling exploitation, although other rivers in the region already have natural chub populations present (Warren et al., 2022). The natural range of chub is from the northern latitudes of Scandinavia to southern latitudes of the Mediterranean (Kottelat & Freyhof, 2007), but

with non-native populations in Italy (Haubrock et al., 2021) and Ireland (Caffrey et al., 2008), although the latter population is now eradicated (Caffrey et al., 2018). In the River Frome, angling reports indicated chub were present in catches from 2008 in the lower tidal river. Their upstream dispersal appears to be relatively slow, but their colonisation of the lower river has been facilitated by their exceptionally fast somatic growth rates (Warren et al., 2022).

Chub is considered indigenous to Britain, with a relatively widespread distribution in lowland rivers (Bolland et al., 2007), although their status in southwest England has been uncertain. This contrasts to the functionally analogous dace *Leuciscus leuciscus* (Linnaeus, 1758), known to be present in rivers such as the Dorset Stour in 500 BC, suggesting their colonisation of these rivers through connections with rivers of mainland Europe following the end of the last glacial maximum (Wheeler, 1977). Indeed, unlike chub, the dace population of the River Frome is considered as indigenous and has previously been the subject of considerable research attention (e.g. Mann, 1974; Mills & Mann, 1985; Mills et al., 1985). The translocation of chub into this river, a species with an omnivorous diet that, while primarily being invertebrate based, can include facultative piscivory (Caffrey et al., 2008), could result in inter-specific competition with trophically analogous species, including other species of the Cyprinidae family, including dace.

Our initial aim was to assess the trophic ecology of translocated chub in the River Frome in relation to the indigenous cyprinid species present [dace, roach *Rutilus rutilus* (Linnaeus, 1758), rudd *Scardinius erythrophthalmus* (Linnaeus, 1758)]. The inter-specific trophic relationships were assessed using a stable isotope analysis (SIA)-based approach in two areas of the river, including the tidal river where values of the carbon stable isotope (^{13}C) are often enriched (Winter et al., 2021). The stable isotope approach enabled comparisons of the isotopic niche sizes and extent of niche overlap between the cyprinid species. The isotopic niche is closely related to the trophic niche, but is also influenced by factors including growth rate and metabolism (Jackson et al., 2011; Hette-Tronquart, 2019). The SI approach was based on the analysis of scale tissue, which provides a longer term perspective on dietary resources when compared to other fish tissues, such as dorsal muscle and fin (e.g. Busst & Britton, 2018). We then identified whether

the inter-specific trophic relationships patterns apparent between chub and dace in the River Frome (as the two most functionally similar species present) were similar to those of their native populations. This step enabled us to test whether the interactions of the non-indigenous chub with native fishes were similar to their indigenous populations, as has been observed in other translocated fishes in England, including European barbel *Barbus barbus* (Linnaeus, 1758) (Gutmann Roberts & Britton, 2020).

Materials and methods

River Frome study site (non-indigenous chub)

The River Frome is a lowland chalk stream in southern England that flows for approximately 70 km from its source (50.50. 24°N; 02.36. 12°W) to its tidal limit (50.40. 38°N; 02.07. 30°W). It is characterised by pool-riffle habitats throughout much of its length, but transitions to being wider and deeper in its tidal reaches (Simmons et al., 2022). The fish assemblage is dominated numerically by European minnow *Phoxinus phoxinus* (Linnaeus, 1758), with the river renowned for its population of Atlantic salmon *Salmo salar* Linnaeus, 1758 and brown trout *Salmo trutta* Linnaeus, 1758 (Simmons et al., 2020). Indigenous species of the Cyprinidae family, including dace and roach, are found in the lower river. Even in the lower reaches, the river rarely exceeds 20 m width and 2 m depth.

Fish samples were collected in late August and early September 2021. Two areas of the lower river were sampled that were approximately 8 km apart. Site 1, the most upstream site, comprised of the main river channel, a connected millstream and a series of side-streams (Fig. 1). The river was not tidally influenced here. Site 2 was located downstream of the town of Wareham in the tidally influenced section of the river, approximately 4 km upstream of the river's confluence with Poole Harbour and the sea, meaning fish had the opportunity to forage in tidal areas of the river providing they could tolerate the salinity (Fig. 1). With increasing distance upstream from Site 1, the fish community is dominated by salmonid fishes, with few cyprinid species present in these areas. The physical characteristics of the river at both sites (as depth, flow and width) prevented the use of

sampling methods to derive population abundance estimates. Correspondingly, at Site 1, a combination of rod-and-line angling [using bait, usually maggots, *Calliphora vomitoria* (Linnaeus, 1758)] and electric fishing (Smith Root LR24 back-mounted equipment, pulsed DC, various voltage depending on capture efficiency, water depth and fish recovery rates) was used to sample all habitats, although all of the fish that were used in subsequent analyses were from the millstream and side-streams. At Site 2, fish were sampled by baited rod-and-line angling only due to the inefficiency of electric fishing in that area. Following their capture, all fish were identified to species, measured (fork length, nearest mm) and a sample of scales taken from below the dorsal fin but above the lateral line, where scales were originally taken for the purposes of age and growth analyses for management purposes (Warren et al., 2022). Scales were then stored in paper envelopes that enabled drying, which were then stored in dry, cool conditions (12 to 15 °C). All fish were then returned to the area of river where they were captured. The sample sizes used for stable isotope analysis are provided in Table 1.

Following fish sampling, a common macroinvertebrate fish prey resource was collected from both sites using a sweep net (250 mm wide; 0.3 m bag depth; 250 μm mesh). The most frequently occurring macroinvertebrate at both sites was *Gammarus* spp., with triplicate samples taken for stable isotope analysis, which were stored frozen before SIA preparation. These samples were used to identify the differences in the stable isotope values of fish putative prey resources between the two sites; if differences between the two sites were not statistically significant then comparisons of the fish stable isotope data could be made between the sites without any correction (Olsson et al., 2009; Britton et al. 2022).

Other sampled rivers (indigenous chub)

Assessment of the isotopic relationships of indigenous chub and dace were through the use of stable isotope data collated from populations in studies completed in the last decade by the authors. Five comparative chub and dace populations were used; these were from the Rivers Avon, Stour and Teme, and two tributaries of the River Great Ouse ('Great Ouse T1', 'Great Ouse T2'; Table 1). These rivers were typically between 8 and 10 m in width, depths to 2 m and
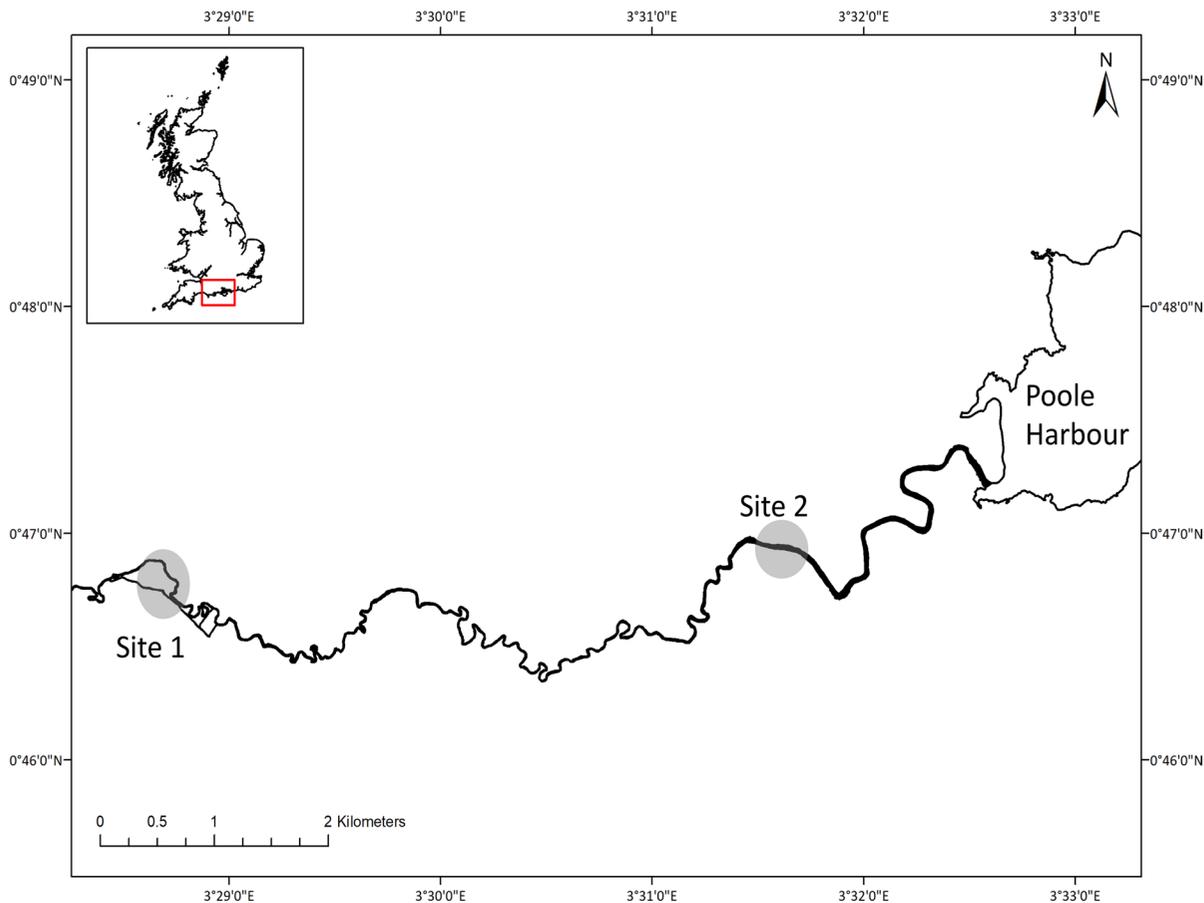
Fig. 1 Inset: location of the River Frome in Great Britain. Main map: The River Frome (solid line), showing the location of Site 1 and Site 2 (as grey shade), and their proximity to Poole Harbour. The direction of river flow is approximately west to east

were in lowland areas where their fish communities were dominated by cyprinid fishes. The fish samples were all collected in mid to late summer from these rivers. In the Rivers Avon, Teme and Stour, the fish samples were all collected by rod-and-line angling (given the relatively large width and depths of these rivers prevented the effective use of electric fishing), using the same equipment as the River Frome. The two tributaries of the Great Ouse were sampled by generator powered electric fishing (Electracatch; voltage dependent on capture efficiency and habitat sampled), as these were shallower and enabled fishing by wading. At all sites, the captured fish were identified to species, measured (fork length, nearest mm) and a sample of scales taken from below the dorsal fin and above the lateral line that were initially used for age and growth analysis before being used for stable

isotope analysis (as already described for the Frome samples above).

Stable isotope analysis

All of the fish scale material and the River Frome *Gammarus* spp. samples were dried to constant mass (approximately 5 mg) of at 60 °C before analysis at the Cornell University Stable Isotope Laboratory (New York, USA) for $\delta^{13}C$ and $\delta^{15}N$ in a Thermo Delta V isotope ratio mass spectrometer (Thermo Scientific, USA) interfaced to a NC2500 elemental analyser (CE Elantach Inc., USA). Analytical precision of the $\delta^{13}C$ and $\delta^{15}N$ sample runs was estimated against an internal standard sample of animal (deer) material every 10 samples, with the overall standard deviation estimated at 0.08 and 0.04 ‰, respectively.

Table 1 River locations (as latitude, longitude and approximate location in England) and for each species, their sample size (*n*), and mean lengths ± 95% confidence limits and length range

River	Latitude/longitude	Approximate location	Species	n	Mean length (range) (mm)	References
Frome, Site 1	50.680201/–2.183393	Southern England	Chub	19	233 ± 20 (148–295)	This study
			Dace	11	201 ± 25 (118–248)	
			Roach	10	117 ± 8 (99–149)	
Frome, Site 2	50.682880/–2.103091	Southern England	Chub	21	178 ± 16 (82–314)	This study
			Dace	14	154 ± 16 (188–264)	
			Roach	20	137 ± 7 (101–122)	
			Rudd	13	124 ± 6 (72–146)	
River Avon	52.096011/–2.073321	Central England	Chub	11	252 ± 55 (175–420)	Nolan et al. (2019)
			Dace	10	161 ± 14 (120–196	
Gt Ouse T1	52.321542/–0.072521	Eastern England	Chub	13	232 ± 13 (189–281)	Bašić & Britton (2016)
			Dace	12	150 ± 35 (62–205)	
Gt Ouse T2	52.328607/0.116417	Eastern England	Chub	12	186 ± 14 (150–225)	Bašić & Britton (2016)
			Dace	12	186 ± 8 (161–204)	
River Stour	50.765516/–1.863684	Southern England	Chub	12	166 ± 25 (76–243)	Parker et al. (2022)
			Dace	20	131 ± 15 (73–190)	
River Teme	52.315846/–2.488160	Western England	Chub	15	151 ± 15 (112–207)	Gutmann Roberts & Britton (2018)
			Dace	30	167 ± 11 (102–214)	

Ratios of C:N indicated no requirement for lipid normalisation (generally 3.5 to 3.9) (Winter & Britton, 2021).

The $\delta^{13}C$ and $\delta^{15}N$ data for each species and site (Tables 1, 2) were initially tested for the influence of fish length using linear regression, followed by testing for differences in both $\delta^{13}C$ and $\delta^{15}N$ between the species at each site using a generalised linear model (GLM), where species was the response variable, $\delta^{13}C$ or $\delta^{15}N$ was the fixed factor and fish length was the covariate. Reported test results were the significance of the model and of the differences in mean $\delta^{13}C$ and $\delta^{15}N$ between the species (from linearly independent pairwise comparisons, with Bonferroni correction for multiple comparisons). The $\delta^{13}C$ and $\delta^{15}N$ data were then used to calculate the isotopic niche of each fish species at the sites using standard ellipse areas (SEA) in the SIBER package in R (Jackson et al., 2011, 2012), where SEAs are a bivariate measure of the distribution of individuals in isotopic space. As the ellipses enclose the core 40% of data, they represent the typical resource use of the analysed population (Jackson et al., 2011). The Bayesian estimate of SEA (SEA$_B$) was used to test differences in isotopic niche sizes between the species at both sites, calculated using a Markov chain Monte Carlo simulation (10^4 iterations per category) (Jackson et al., 2011, 2012). Differences in the sizes of isotopic niches (as SEA$_B$) of the species were evaluated in SIBER by calculating the probability that the relative posterior distributions of the niche sizes were significantly smaller or larger between the species ($\alpha = 0.05$) (Jackson et al., 2011, 2012). Thus, no overlap in the 95% credible intervals of SEA$_B$ indicated whether chub had a significantly smaller or larger niche size versus the comparator species. The extent of overlap in the isotopic niches (as SEA$_B$) between species at each site were then determined as 95% credible intervals, also calculated in SIBER. For presenting standard ellipse areas on plots, values of SEA$_c$ were calculated in SIBER (subscript '$_c$'

Table 2 Mean $\delta^{13}C$ and $\delta^{15}N$ ($\pm 95\%$ CI) the results of linear regression testing the relationship between fish fork length and their stable isotope values (fork length/ $\delta^{13}C$; fork length/ $\delta^{15}N$)

Site	Species	Mean $\delta^{13}C$ (‰)	Mean $\delta^{15}N$ (‰)	Fork length/ $\delta^{13}C$	Fork length/ $\delta^{15}N$
Frome 1	Chub	-27.67 ± 0.30	11.16 ± 0.19	$R^2 = 0.01$, $F_{1,17} = 0.14$, $P = 0.72$	$R^2 = 0.02$, $F_{1,17} = 0.29$, $P = 0.60$
	Dace	-27.36 ± 0.39	10.91 ± 0.30	$R^2 = 0.19$, $F_{1,9} = 2.09$, $P = 0.18$	$R^2 = 0.09$, $F_{1,9} = 0.81$, $P = 0.39$
	Roach	-27.62 ± 0.17	11.25 ± 0.37	$R^2 = 0.01$, $F_{1,8} = 0.11$, $P = 0.75$	$R^2 = 0.05$, $F_{1,8} = 0.39$, $P = 0.55$
Frome 2	Chub	-25.62 ± 0.33	11.20 ± 0.11	$R^2 = 0.16$, $F_{1,19} = 3.51$, $P = 0.08$	$R^2 = 0.11$, $F_{1,19} = 2.30$, $P = 0.15$
	Dace	-26.86 ± 0.34	10.98 ± 0.15	$R^2 = 0.25$, $F_{1,12} = 4.00$, $P = 0.07$	$R^2 = 0.17$, $F_{1,12} = 2.38$, $P = 0.15$
	Roach	-27.51 ± 0.58	12.40 ± 0.23	$R^2 = 0.02$, $F_{1,18} = 0.38$, $P = 0.55$	$R^2 = 0.49$, $F_{1,18} = 17.54$, ***P < 0.01***
	Rudd	-27.46 ± 0.68	12.82 ± 0.21	$R^2 = 0.02$, $F_{1,11} = 0.23$, $P = 0.64$	$R^2 = 0.06$, $F_{1,12} = 0.73$, $P = 0.41$
Avon	Chub	-25.02 ± 0.75	13.58 ± 0.51	$R^2 = 0.03$, $F_{1,9} = 0.26$, $P = 0.62$	$R^2 = 0.08$, $F_{1,9} = 0.81$, $P = 0.39$
	Dace	-24.09 ± 0.86	14.51 ± 0.50	$R^2 = 0.01$, $F_{1,8} = 0.04$, $P = 0.86$	$R^2 = 0.07$, $F_{1,8} = 0.59$, $P = 0.46$
Gt Ouse T1	Chub	-26.62 ± 0.56	15.48 ± 0.67	$R^2 = 0.02$, $F_{1,11} = 0.20$, $P = 0.66$	$R^2 = 0.25$, $F_{1,11} = 3.60$, $P = 0.08$
	Dace	-27.40 ± 0.84	16.12 ± 0.97	$R^2 = 0.32$, $F_{1,10} = 4.78$, ***P = 0.05***	$R^2 = 0.50$, $F_{1,10} = 10.06$, ***P < 0.01***
Gt Ouse T2	Chub	-27.11 ± 1.03	16.53 ± 0.84	$R^2 = 0.01$, $F_{1,10} = 0.10$, $P = 0.77$	$R^2 = 0.01$, $F_{1,10} = 0.13$, $P = 0.72$
	Dace	-28.40 ± 0.36	17.95 ± 0.36	$R^2 = 0.33$, $F_{1,10} = 5.03$, ***P = 0.05***	$R^2 = 0.45$, $F_{1,10} = 8.13$, ***P < 0.02***
Stour	Chub	-28.81 ± 1.49	14.55 ± 0.87	$R^2 = 0.12$, $F_{1,10} = 1.30$, $P = 0.28$	$R^2 = 0.38$, $F_{1,10} = 6.04$, ***P = 0.03***
	Dace	-29.26 ± 0.52	13.87 ± 0.68	$R^2 = 0.03$, $F_{1,18} = 0.57$, $P = 0.46$	$R^2 = 0.01$, $F_{1,18} = 0.15$, $P = 0.70$
Teme	Chub	-25.24 ± 0.25	11.75 ± 0.23	$R^2 = 0.01$, $F_{1,13} = 0.12$, $P = 0.74$	$R^2 = 0.53$, $F_{1,13} = 14.72$, ***P < 0.01***
	Dace	-26.07 ± 0.32	12.25 ± 0.15	$R^2 = 0.11$, $F_{1,28} = 3.35$, $P = 0.08$	$R^2 = 0.11$, $F_{1,28} = 3.57$, $P = 0.07$

Bold P values signify $P \leq 0.05$

indicates a small sample size correction was used; Jackson et al., 2012), as this provided a simpler visual representation of the data distribution and niche positions. However, all evaluations were based on the Bayesian estimates.

All data analyses were completed in R version 4.0.5 (R Development Core Team, 2021). Where error is provided around mean values, it represents 95% confidence limits unless otherwise stated.

Results

Fish length–stable isotope relationships

In the River Frome sites, the lengths of chub and dace in Site 1 and Site 2 were substantially larger than roach (and of rudd in Site 2) (Table 1). The relationships of fork length versus both $\delta^{13}C$ and $\delta^{15}N$ of all species in both sites were not statistically significant, with the exception of roach $\delta^{15}N$ in Site 2, where increased length resulted in more enriched ^{15}N (Table 2). In Site 1, the GLM indicated that $\delta^{13}C$ and $\delta^{15}N$ did not differ significantly among the species (Table 3). In contrast, the GLM indicated that

in site 2, there were significant differences in both $\delta^{13}C$ and $\delta^{15}N$ of the species (Table 3), where pairwise comparisons revealed that both $\delta^{13}C$ and $\delta^{15}N$ of chub differed significantly from roach and rudd ($P < 0.01$ in all cases), but not with dace ($P = 0.19$, 0.99, respectively).

In the other rivers, the effect of fish length on $\delta^{13}C$ was only significant for chub in Great Ouse T1; for $\delta^{15}N$, it was significant for dace in Great Ouse T1 and chub in both the River Stour and Teme (Table 2). The GLMs indicated that differences in $\delta^{13}C$ and $\delta^{15}N$ between the two species were significant in the Great Ouse T1 and T2, and the River Teme, where length was a significant covariate in Great Ouse T1 ($\delta^{13}C$, $\delta^{15}N$), and the Teme ($\delta^{13}C$ only) (Table 3).

Stable isotopic niche sizes and overlap

In the two River Frome sites, the differences in the SI values of the *Gammarus* spp. did not overlap in their 95% CI (Site 1 vs Site 2: $\delta^{13}C$: -31.90 ± 0.96 vs -30.57 ± 0.27; $\delta^{15}N$: vs 7.67 ± 0.19 vs 7.92 ± 0.44 ‰). Correspondingly, the fish isotopic niches could be compared across the two sites without correction. The isotopic niches (as SEA_B) of the fishes in

Table 3 Results of GLMs testing significance of difference between δ13C and δ15N of the species in each river (Ch: chub; Da: dace; Ro: roach; Ru: rudd)

River	Relationship	Species	Test result (Wald χ^2)	P
Frome, S1	Species vs $\delta^{13}C$	Ch, Da, Ro	3.32	0.19, 0.18
	Species vs $\delta^{15}N$	Ch, Da, Ro	3.71	0.16, 0.41
Frome S2	Species vs $\delta^{13}C$	Ch, Da, Ro, Ru	15.27	**< 0.01**, 0.07
	Species vs $\delta^{15}N$	Ch, Da, Ro, Ru	69.87	**< 0.01**, 0.37
Avon	Species vs $\delta^{13}C$	Ch, Da	1.19	0.28, 0.57
	Species vs $\delta^{15}N$	Ch, Da	2.74	0.10, 0.24
Gt Ouse T1	Species vs $\delta^{13}C$	Ch, Da	10.59	**< 0.01**, **0.01**
	Species vs $\delta^{15}N$	Ch, Da	17.16	**< 0.01**, **< 0.01**
Gt Ouse T2	Species vs $\delta^{13}C$	Ch, Da	6.05	**0.01**, 0.38
	Species vs $\delta^{15}N$	Ch, Da	10.11	**< 0.01**, 0.83
River Stour	Species vs $\delta^{13}C$	Ch, Da	2.66	0.10, 0.17
	Species vs $\delta^{15}N$	Ch, Da	0.44	0.51, 0.25
River Teme	Species vs $\delta^{13}C$	Ch, Da	9.91	**0.02**, **0.05**
	Species vs $\delta^{15}N$	Ch, Da	17.17	**< 0.01**, 0.67

In the table, the first P value shows species effects and the second shows the effects of length as a covariate in the models. Bold P values signify $P \leq 0.05$

Fig. 2 Distribution of the isotopic niche size for each sampled species (chub *Squalius cephalus*, dace *Leuciscus leuciscus*, roach *Rutilus rutilus* and rudd *Scardinius erythrophthalmus*) in Site 1 and Site 2 of the River Frome, where for each species and site, the horizontal lines represent the credible intervals of the niches (10th, 25th, 75th, 90th), the blue circle represents the mean and the red cross the modal value
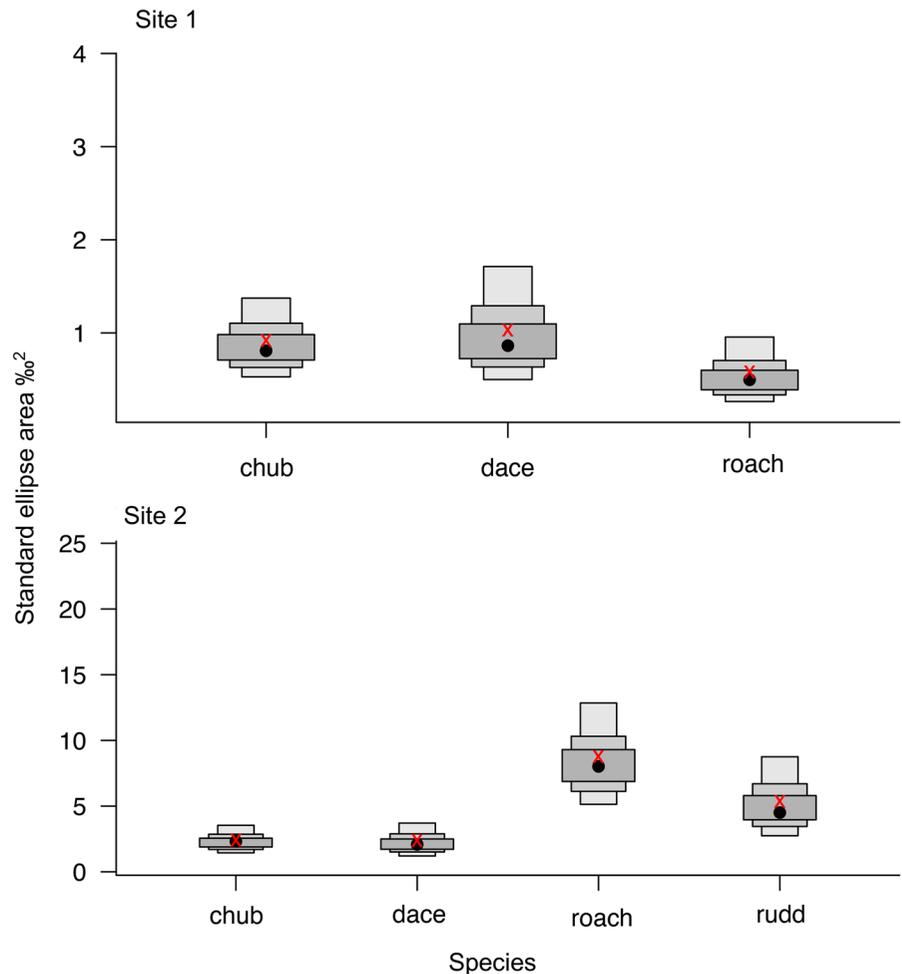
Table 4 Isotopic niche sizes [as standard ellipse area, SEA$_B$, with 95% credible intervals (CI)] of chub and other cyprinid fishes, and the extent of their isotopic niche overlap (as 95% CI from SEA$_B$), across all of the study rivers

River	Species	SEA$_B$ (95% CI) (‰^{2})	% isotopic niche overlap with chub (as 95% CI)
Frome Site 1	Chub	0.84 (0.51–1.35)	–
	Dace	0.89 (0.46–1.73)	52–97
	Roach	0.47 (0.24–0.96)	61–99
Frome Site 2	Chub	2.19 (1.42–3.55)	–
	Dace	2.00 (1.22–3.67)	59–98
	Roach	7.90 (4.98–12.76)	11–44
	Rudd	4.67 (2.74–8.51)	0–22
River Avon	Chub	3.08 (1.66–5.91)	–
	Dace	2.53 (1.28–5.04)	23–96
Gt Ouse T1	Chub	2.92 (1.65–5.72)	–
	Dace	6.06 (3.37–11.53)	39–90
Gt Ouse T2	Chub	5.30 (2.88–9.92)	–
	Dace	1.21 (0.64–2.25)	69–100
River Stour	Chub	3.49 (2.06–6.79)	–
	Dace	5.37 (3.28–8.31)	41–89
River Teme	Chub	0.65 (0.39–1.14)	–
	Dace	1.11 (0.76–1.58)	23–74

Site 1 were all relatively similar in size (Fig. 2), with substantial overlaps in their 95% credible intervals (Table 4). The positions of these niches in isotopic space were also similar between the species, with chub predicted to be sharing 52 to 97% of their niche with dace, and 61 to 99% of their niche with roach (Table 4; Fig. 3). In Site 2, roach had significantly larger isotopic niches than both chub and dace, but not rudd (Fig. 2). The isotopic niche of chub overlapped substantially with dace (up to 98%), but the overlap between chub and the other species was much lower (Table 4; Fig. 3). It was apparent that the species in Site 2 were had greater variability in their SI data than in Site 1, with some fish in Site 2 having enriched values of both ^{13}C and ^{15}N (Figs. 2, 3), resulting in substantially larger isotopic niches (Table 4).

As with chub and dace in the River Frome, comparisons of the isotopic niche sizes between the native populations of dace and chub revealed these were not significantly different within each river (Table 1; Figs. 2, 4). Similarly, there was overlap in the isotopic niches of the two species, although this varied between the rivers, with 95% credible intervals ranging from 23 to 74% in the River Teme to 69 to 100% in the Great Ouse T2 (Table 4; Fig. 4).

Discussion

The trophic relationships of translocated chub in the River Frome revealed that there were considerable overlaps in their isotopic niches with all species in Site 1, but only with dace in Site 2. This niche overlap with dace was consistent with the two species being trophically analogous. The overlapping isotopic niches of chub and dace also had some consistency with sympatric native populations from other rivers in England, where similar relationships were generally evident in the 95% credible intervals around the standard ellipse areas. In general, length was not always a strong predictor of δ^{13}C and δ^{15}N values of either species, but with evidence that in some rivers, enriched values of these isotopes were significantly correlated with fish length (e.g. roach in Site 2 of the River Frome), suggesting some ontogenetic dietary shifts and that overlaps in isotopic niches might have only been apparent for specific length ranges of the species.

There was an unexpected and substantial difference in the size of the isotopic niches of all of the analysed species between Sites 1 and 2 of the River Frome, where the niches at Site 2 were consistently larger and had a much greater range of values of δ^{13}C
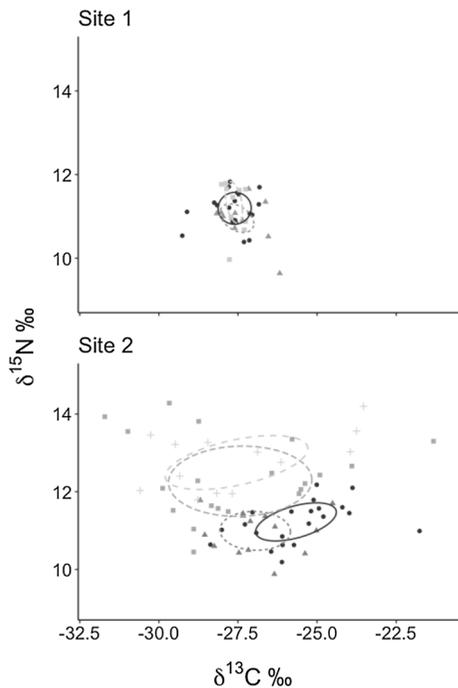
Fig. 3 Relationships of $\delta^{13}C$ and $\delta^{15}N$ and core isotopic niches of chub *Squalius cephalus* (black circle/ solid line), dace *Leuciscus leuciscus* (black triangle/ small dashed line), roach *Rutilus rutilus* (black square/medium dashed line) and rudd *Scardinius erythrophthalmus* (black cross/ largest dashed line) at Site 1 (top) and Site 2 (bottom). Note that the core isotopic niches of each species were calculated by SEA_C rather than SEA_B (both for presentation purposes only; see Table 4 for 95% credible intervals of the core niches)

and $\delta^{15}N$, especially in roach. This was despite the SI values of the *Gammarus* spp. (as a putative fish prey resource) being similar in the areas where fish were sampled. Thus, some of the fish in Site 2 were feeding on prey resources that were substantially enriched in ^{13}C and which did not include the *Gammarus* spp. sampled in the fish sampling area.

It was considered likely that these ^{13}C-enriched fish in Site 2 of the River Frome were feeding in areas downstream of the sampling area, i.e. towards Poole Harbour. This salinity of this area of the tidal river varies semi-diurnally (according to the tidal cycle) (Humphreys, 2005). This salinity variation becomes more pronounced as it nears Poole Harbour, with the river close to the confluence with the harbour being partially mixed but with harbour itself being vertically homogenous and considered an estuary (Humphreys, 2005). While this means the fish were unlikely to be

able to enter the harbour itself due to excessive salinity levels, they were considered as most likely moving into the lower river to forage, perhaps to exploit relatively rich prey resources that were not being fully exploited by other fishes. Indeed, in tidal rivers generally, SI values of macroinvertebrates (e.g. *Gammarus* spp.) and cyprinid fishes (e.g. roach) tend to be increasingly enriched in both ^{13}C and ^{15}N, but especially ^{13}C that can help discriminate between foraging areas of fresh water (depleted ^{13}C, e.g. < -26.0 ‰) and those further downstream where salinity is higher (enriched ^{13}C; e.g. > -22.0 ‰; Nolan et al., 2019; Winter et al., 2021). With scales providing a longer temporal dietary perspective than muscle and fin tissues (Busst & Britton, 2018), then it can be assumed that the fish feeding in these downstream tidal areas were doing so over extended time periods, i.e. as part of a foraging strategy. Despite their apparent foraging in the tidal reaches, the evidence from Site 2 SI data was that these fish were also mixing with individuals that primarily forage in fresh waters (the individuals with relatively depleted ^{13}C). These results suggest considerable individual specialisation in the foraging of these fishes (Araújo et al., 2011).

The suggestion that some individual cyprinid fishes were foraging in lower tidal river could not be tested further here. The only cyprinid species in the River Frome where tracking studies have been completed is dace, where the fish were sampled, tagged and released at Site 1 in work completed in the 1990s. The results of these studies indicated that the dace had clearly defined daytime and night-time habitats, but with the distances moved between these being only up to 680 m (i.e. these movements did not involve movements between Site 1 and Site 2 across 24 h periods) (Clough & Ladle, 1997). Conversely, a further study indicated that although the downstream distance moved by most dace was rarely > 1 km, a small proportion of individuals did move as far as 9 km downstream (i.e. from Site 1 to Site 2) (Clough & Beaumont, 1998), indicating that at least some dace in the river are capable of making relatively large movements from the non-tidal to tidal areas of the river (and to presumably forage there). Notwithstanding, all of the fish analysed at Site 1 had strong freshwater SI values and, in contrast, the values and range of $\delta^{13}C$ of the fishes at Site 2 have already been suggested as indicating some individuals move into the lower tidal river to forage. However, in the

Fig. 4 Relationships of $\delta^{13}C$ and $\delta^{15}N$ and core isotopic niches of chub *Squalius cephalus* (black square/ solid line) and dace *Leuciscus leuciscus* (black triangle/dashed line) from five rivers where both species are native. Note differences in axes values and that the core isotopic niches of each species were calculated by SEA_C rather than SEA_B (both for presentation purposes only; see Table 4 for 95% credible intervals of the core niches)
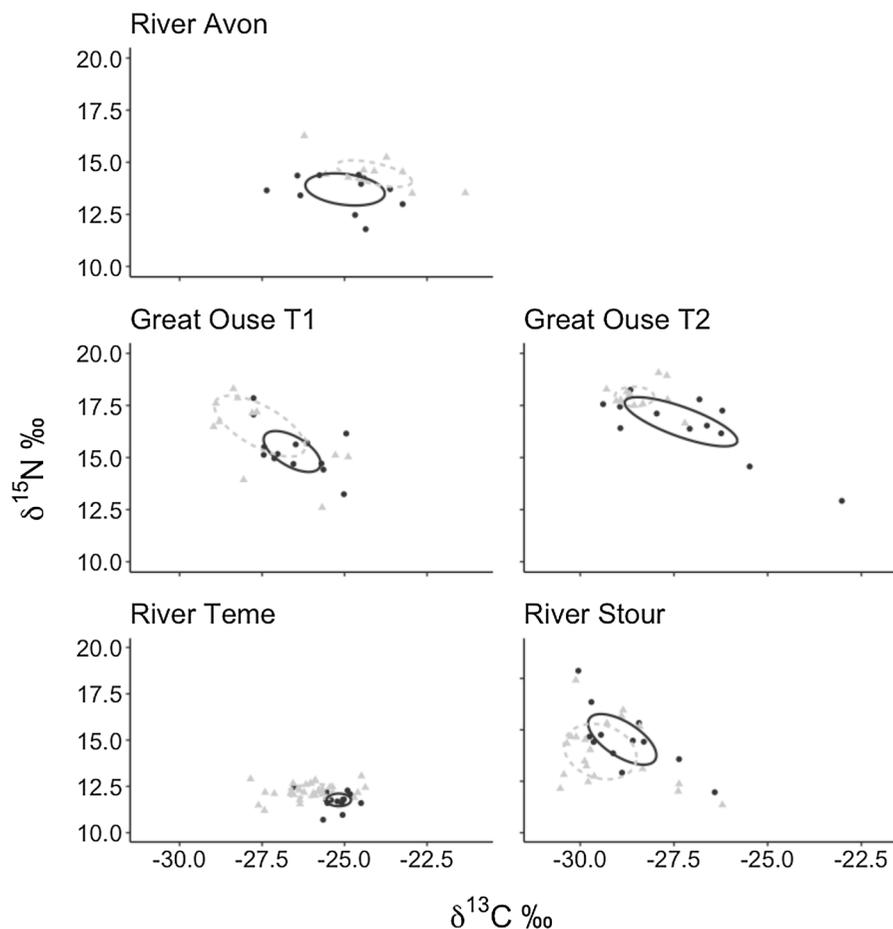
absence of data on both the fish putative prey items further downstream of Site 2 (an area which is largely inaccessible) and on fish movements in the lower river, this remains speculative but worthy of further work.

The isotopic niche overlap between the translocated chub and the indigenous fishes of the River Frome raises questions over their potential ecological impacts. Any overlap in isotopic niches between species suggests some sharing of prey resources (or at least prey with similar isotopic signatures) and thus the potential for competitive interactions, especially where these prey resources are limiting, with potential for inferior competitors to be displaced from their niche (Cucherousset et al., 2012; Kakareko et al., 2013). There is, however, no evidence to suggest that the native cyprinid fishes have been displaced by chub in the river, given the niche overlaps and isotopic relationships were relatively consistent

with other rivers where the species are naturally sympatric. Moreover, the river is considered to be highly productive in the context of fish growth. For examples, juvenile Atlantic salmon *Salmo salar* tend to emigrate to sea as smolts are age 1+, whereas in most rivers of similar latitude, smoltification occurs at least age 2+ years (Simmons et al., 2021). Indeed, the colonisation of the River Frome by chub has already been described by Warren et al. (2022) as being facilitated by their very fast growth. The results here on the isotopic ecology also suggest that their invasiveness might be further facilitated by their stable isotope ecology (and by extension, their feeding ecology) being similar in this translocated population and some of their native populations, i.e. they are expressing similar traits in both their invasive and natural range. The expression of these 'pre-adapted' traits is considered to generally facilitate the invasion of introduced species as there is no requirement to

adapt to the novel conditions (Catford et al., 2009), and has been observed ißn translocated fishes in England, including European barbel in western England (Gutmann Roberts & Britton, 2020). Notwithstanding these inferences, it is acknowledged that they are based on two sites that were sampled in one summer only. Moreover, greater insights into the trophic ecology of the fishes would have been gained through comprehensive SIA of putative prey items and their application to mixing models to predict diet composition (Nolan et al. 2019). However, the application of stable isotope mixing models can be problematic where the SI data are similar between putative prey resources (as the model cannot easily separate their dietary contributions). Moreover, while collecting putative prey resources in the vicinity of Sites 1 and 2 was possible, the areas downstream to Site 2 (where the fish with enriched ^{13}C values were considered to be foraging) were inherently difficult to sample due to its tidal nature and general inaccessibility from the riparian zone, and so could not be completed here. Rather than using SI mixing models to predict data composition, short-term dietary analyses based on stomach contents analyses could have been used, including DNA barcoding approaches. However, short- versus long-term dietary studies do not always correlate strongly (e.g. Locke et al., 2013) and, when coupled with stomach contents analyses generally being a destructive sampling method, it is not clear how much added value this method would have added to the stable isotope approach that was used.

In summary, the translocation of this chub population into the lower reaches of this productive chalk stream has resulted in the establishment of a population that is dispersing upstream. The results revealed that foraging by the fish populations in the lower river resulted in high dietary specialisations, where individuals differed in the extent of their ^{13}C enrichment and so in the extent of their non-tidal versus tidal foraging. Some overlaps in isotopic niches were evident between chub and the other fishes in the River Frome (as was also detected in naturally sympatric populations), but with the very fast growth of these species in the river (Warren et al., 2022) suggesting that the fish were not competing for limited resources.

Declarations

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