PRIMARY RESEARCH PAPER

Dietary contributions of the alien zebra mussel *Dreissena polymorpha* in British freshwater fish suggest low biological resistance to their invasion

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Abstract Native communities can resist the establishment and invasion of alien species through consumptive and/or competitive interactions. The extent of consumptive resistance from freshwater fish to the invasion of zebra mussels *Dreissena polymorpha*, a globally invasive Ponto-Caspian species, was assessed in two areas in Britain using stable isotope analysis, where mixing models predicted the contribution of putative prey resources (including zebra mussel) to fish diet. Across the sites and species, only roach *Rutilus rutilus* were predicted to have a diet where zebra mussels contributed highly (predicted contribution: 44%), with literature suggesting that their functional morphology would have facilitated their consumption of this prey item. Predicted contributions of zebra mussels to common bream *Abramis brama* diet was comparatively low (29%), despite them being present to much larger sizes than roach, and with pike *Esox lucius*, perch *Perca fluviatilis* and pikeperch *Sander lucioperca* also predicted to have low dietary contributions of zebra mussels (0.08%,

24% and 24%, respectively). These results suggest the consumptive resistance to its invasion in Britain has been low and, correspondingly, if there is a management desire to further limit the invasion of zebra mussels then relying on biological resistance to limit their invasion appears to be insufficient.

Keywords Bayesian mixing models · Consumptive resistance · Diet · *Rutilus rutilus* · Stable isotope analysis

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Introduction

Alien species are a pervasive agent of global change (Simberloff et al., 2013), with the consequences of invasions including both ecological and economic damage (Crystal-Ornelas & Lockwood, 2020; Diagne et al., 2021). Although the number of alien species introduced into new regions remains relatively high at global levels (Dawson et al., 2017; Turbelin et al., 2017), the proportion of these introductions that develop damaging invasions tends to be relatively low (Gozlan, 2008). Nevertheless, an area that has produced a relatively high number of globally invasive aquatic species is the Ponto-Caspian region (Ricciardi & MacIsaac, 2000), which comprises a large geographic extension around the Black, Azov and Caspian seas that is characterised by variable climatic and habitat conditions that provide its native species with relatively high adaptation capacity (Gallardo &

Aldridge, 2013). Invasive species that originate from this region include amphipods (e.g. killer shrimp *Dikerogammarus villosus* (Sowinsky, 1894)) and molluscs (e.g. zebra mussel *Dreissena polymorpha* (Pallas, 1771)) (Gallardo & Aldridge, 2015).

In the risk management of introduced alien species, an important component is understanding the processes by which introduced species are able to colonise novel ecosystems (Dominguez Almela et al., 2020), where the establishment of the population and its integration into the native food web are fundamentally important (Britton & Gozlan, 2013; Britton et al., 2019). There are a number of hypotheses on factors that influence the establishment of an introduced species and thus whether it will go on to develop an invasive population (Catford et al., 2009). These include the biological resistance hypothesis, which posits that communities of native species can mediate the establishment and performance of invasive species through competitive and/or consumptive interactions (Britton, 2012; Alofs & Jackson, 2014). In freshwaters ecosystems, evidence suggests that the mechanisms of biological resistance are stronger from consumptive resistance than competitive resistance (Alofs & Jackson, 2014), with functionally diverse communities having relatively high resistance against multiple invaders (Byun et al., 2020).

Zebra mussels are a global invader whose continued range expansion has been facilitated by their transport on boats (Robertson et al., 2020). Zebra mussels have been implicated in substantial changes in invaded freshwaters, including increased water clarity, modified nutrient cycling, altered composition of the benthic community and changes in the fish community (Hansen et al., 2020; Robertson et al., 2020). Furthermore, the ability of zebra mussels to efficiently filter water (4 L/day; Silverman et al., 1996) has been shown to impact dissolved oxygen concentrations (Strayer, 2009; Wong et al., 2021) and phytoplankton/zooplankton densities (Wong et al., 2021), resulting in cascading effects (Higgins & Vander Zanden, 2010; Rennie et al., 2013). However, zebra mussels can also be important dietary component of the resident fish community, with studies in North America suggesting that at least six fish species are potential predators of these mussels, with this facilitated by their mouth size, teeth and chewing abilities (French, 1993). Magoulick & Lewis (2002) reported that zebra mussels were an important part of the diet of blue catfish *Ictalurus furcatus* (Lesueur, 1840), freshwater drum *Aplodinotus grunniens* (Rafinesque, 1819) and redear sunfish *Lepomis microlophus* (Günther, 1859). Laboratory experiments on round goby *Neogobius melanostomus* (Pallas, 1814) suggested they preferred consuming zebra mussels over species of clams and snails (Ghedotti et al., 1995). Studies on roach *Rutilus rutilus* (Linnaeus, 1758) indicated that larger fish would consume more mussels than smaller individuals, with fish below 160 mm in length considered as unable to consume them (Prejs et al., 1990). In a Canadian study, Wong et al. (2021) argued that although some fish may feed on zebra mussels, they would still use other prey resources as their primary dietary items and so would not act as a biological control of these invaders. The bias of studies on zebra mussels to North America indicates there are considerable knowledge gaps on their invasion elsewhere in their range, especially in relation to their integration into invaded food webs and how they contribute to consumer diets, and thus what the extent of the biological resistance is to their invasion.

Zebra mussels were introduced into Great Britain in 1824 and have since spread to a number of discrete lakes, rivers and canals, with Aldridge et al. (2004) suggesting relatively recent increases in their abundance and distribution. However, there remains limited knowledge on their invasion in Britain, including to what extent they are dietary components of fish communities, where species present include roach (as per Prejs et al., 1990). Also present is the common bream *Abramis brama* (Linnaeus, 1758), which Nagelkerke & Sibbing (2011) considered to not be an efficient consumer of zebra mussels due to limitations set by the crushing power of their pharyngeal teeth. The objective of the current study was to assess the dietary contributions of zebra mussels to the fish community of two invaded and spatially discrete sites of Britain, where dietary contributions were predicted from the ecological application of stable isotope analysis. These dietary contributions were predicted and compared between omnivorous and piscivorous native and alien fishes, given these will differ in their putative prey resources, with the effect of body lengths of individual fish also tested on the dietary predictions. We posit that zebra mussels are an important component of the diet of at least some fish species in invaded waters in Britain and these fish

species would have thus provided some consumptive resistance to their invasion.

Materials and methods

Site details

Site 1 was the Gloucester–Sharpness Canal in western England that is connected to the River Severn estuary at its downstream end (51.7249 N, 2.4733 W). This canal was constructed to allow for navigation of goods from the sea to Gloucester docks; 25 m wide and 5 m deep (Nolan & Britton, 2018). Sampling was focused in the areas downstream of Gloucester, where the fish assemblage is dominated by the cyprinid species roach *Rutilus rutilus* and common bream *Abramis brama* ('bream' hereafter), with perch *Perca fluviatilis* (Linnaeus, 1758) and invasive pikeperch *Sander lucioperca* (Linnaeus, 1758) also present (J.R. Britton, personal observations). Site 2 was the River Bure in eastern England. This river is 87 km in length, flowing south-east towards Breydon Water estuary at Great Yarmouth. Along with its tributaries, the Rivers Ant and Thurne, it forms the northern area of the Broads National Park, a wetland of significant ecological importance (Natural England, 2020; Winter et al., 2021c). The River Ant is mentioned as being colonised by zebra mussels in Aldridge et al. (2004). The area is characterised by multiple small shallow lakes termed 'Broads' (mediaeval peat diggings) less than 40 ha in size. As the landscape is generally flat, the catchment is tidal for approximately 45 km inland. The focal area under study here was upstream of the upper limit of saline incursion at Horning (Winter et al., 2021c), where the river was approximately 25 m wide with depths to 1.5 m. Depths of the connected lakes were typically ~ 1.5 m and did not exceed 3 m. Across the two reaches, the fish assemblage has limited diversity, being dominated by roach and bream, with perch and pike *Esox lucius* (Linnaeus, 1758) also present (Table 1). Both sites had zebra mussels present in all sampled areas, being abundant on areas such as submerged boat moorings.

Sampling

The main sampling period for site 1 was April to June 2017 where, due to the habitat of the canal (depth, width, boat traffic), sampling by traditional capture methods (e.g. electric fishing, seine netting) was not feasible. Rod-and-line angling was used as an alternative, where scales were collected from captured fish and used subsequently in stable isotope analysis (SIA). This scale collection was facilitated by the Environment Agency, the inland fishery regulatory body of England, who established a network of anglers within the area, where the participating anglers recorded their catches and were trained in the collection of scale samples that were used initially in

Table 1 Species, number of individuals (n), tissue type, $\delta^{13}C$ and $\delta^{15}N$ stable isotope ratios (Mean ± SD) and range by fish site

Site	Species	n	Tissue	Mean $\delta^{13}C$ (‰)	$\delta^{13}C$ range (‰)	Mean $\delta^{15}N$ (‰)	$\delta^{15}N$ range (‰)
1	Bream	15	Scale	-26.42 ± 0.77	$(-27.66, -24.81)$	15.56 ± 0.72	(14.03, 16.44)
	Perch	10	Scale	-26.15 ± 0.66	$(-27.51, -25.13)$	15.82 ± 1.00	(14.64, 17.98)
	Pikeperch	14	Scale	-25.61 ± 0.59	$(-26.43, -24.40)$	17.31 ± 0.94	(15.64, 18.91)
	Zebra mussel	18	Muscle	-35.45 ± 1.42	$(-37.72, -33.56)$	11.86 ± 0.60	(10.81, 12.88)
	Chironomidae		Whole	-30.19 ± 1.26	$(-32.19, -28.27)$	11.06 ± 0.79	(10.13, 12.39)
	Gammaridae		Whole	-31.28 ± 1.27	$(-32.99, -29.13)$	12.14 ± 0.85	(10.32, 13.12)
	Macrophyte		Whole	-30.08 ± 0.88	$(-31.12, -28.83)$	9.28 ± 0.27	(9.05, 9.72)
2	Bream	28	Fin clip	-28.52 ± 1.30	$(-30.92, -25.57)$	17.07 ± 0.73	(15.69, 18.69)
	Perch	9	Fin clip	-28.77 ± 1.39	$(-31.90, -26.84)$	17.59 ± 1.77	(14.92, 20.73)
	Pike	15	Fin clip	-27.71 ± 1.24	$(-30.98, -25.69)$	19.35 ± 0.65	(18.19, 20.31)
	Roach	11	Fin clip	-30.04 ± 1.12	$(-31.98, -28.56)$	16.55 ± 1.25	(14.13, 18.16)
	Zebra mussel		Muscle	-34.26 ± 0.34	$(-34.69, -33.87)$	11.92 ± 0.14	(11.73, 12.06)
	Gammaridae		Whole	-36.11 ± 0.82	$(-36.86, -34.97)$	13.67 ± 0.11	(13.52, 13.77)
	Killer shrimp		Whole	-30.63 ± 0.33	$(-31.07, -30.27)$	15.36 ± 0.70	(14.44, 16.13)

age and growth analyses for fisheries management purposes (Nolan & Britton, 2019). Following their capture, the fish were identified to species, measured (fork length, nearest mm), and between 3 and 5 scales removed from the area below the dorsal fin and above the lateral line, and stored in a small paper envelope. In the same period, putative prey resources were sampled using long-handled sweep-nets, where macro-invertebrate samples were dominated by Gammaridae and Chironomidae. Also present in these samples were zebra mussels and macrophytes.

The main sampling period for site 2 was September 2018. Roach and perch were also sampled from the main river by angling, as sampling by typical fish capture methods (e.g. electric fishing, seine netting) was not feasible for the same reasons as per site 1. The captured fish were identified to species, measured for fork length (nearest mm) and a pelvic fin biopsy taken and frozen. Concomitantly, samples of amphipods were collected using a sweep net, with subsequent identification in the laboratory indicating the presence of species of Gammaridae and the invasive killer shrimp. This method was also used to collect samples of zebra mussels from colonised areas (e.g. boat moorings). There were few areas of macrophyte in the main river and so samples were not collected for subsequent analysis as per site 1. For bream, samples were also collected in the vicinity of the areas where the putative prey resources were collected using rod-and-line angling as per the roach and perch, with the same biometric data recorded and fin biopsy taken, but with the samples collected in April 2018. Only bream that subsequently foraged in the areas around the tagging site were used in analyses, as other individuals present were identified as spawning migrants from reaches further downstream and the River Thurne (Winter et al., 2021a, b, c).

Stable isotope analysis

For the fish at site 1, scales were the tissue used for SIA, with scale decalcification not performed prior to their analysis. This was because although comparisons of acidified versus non-acidified scales have revealed some differences in their isotopic data, the actual changes tend to be minor and not considered biologically relevant. For example, Ventura & Jeppesen (2010) demonstrated that the method resulted in mean changes in $\delta^{13}C$ ($\pm SD$) of

0.18 ± 0.12 and in $\delta^{15}N$ of -0.21 ± 0.24. The preparation of the scales thus comprised of cleaning with distilled water and then the removal of the outer portion of the scale for analysis. The latter process was completed to ensure the analysed tissue represented only the most recent growth of the fish (generally, the last full year of growth; Hutchinson & Trueman, 2006; Bašić & Britton, 2015). For the fish analysed, only one scale was used per individual, as this provided enough material for analysis. For fish at site 2, the fin biopsies were used for SIA. For the analyses of macro-invertebrates at both sites, three replicate samples were used per family, where a sample comprised of between one and three individuals (dependent on their body sizes). Zebra mussels were used individually, with preparation involving removal of their shell and a sample of soft tissue taken for SIA. All samples were dried to constant mass at 60 °C prior to their analysis at the Cornell Isotope Laboratory, New York, U.S.A. Stable isotope analytical details were as per Busst & Britton (2017), with lipid correction not necessary as C:N ratios indicated very low lipid content (< 4.0; Post et al., 2007).

Data analyses and mixing models

Differences in the stable isotope data between the omnivorous fish species per site were initially tested in ANCOVA, with fish fork length included as a covariate, with differences in length within the piscivorous fish then were tested in one-way ANOVA per site ($\alpha = 0.05$ in all cases). For visual representation of the stable isotopes data per site, the isotopic niches of each fish species, expressed as standard ellipse areas (SEA_c), were calculated in the R package 'SIBER' (Jackson et al., 2011), where the ellipses enclose 40% of the stable isotopes data and so represent the core niche of the species (Jackson et al., 2012).

The stable isotopes data for both sites were then applied to Bayesian mixing models which allowed for diet predictions based on posterior probability distributions of the contribution of relative proportions of the putative prey resources per fish species. For both sites, two models were run in the package MixSIAR (Stock et al., 2018) to predict the dietary contributions of the putative prey resources: (1) omnivorous fishes; and (2) piscivorous fishes. Separate models were run as these species required the use of different putative prey resources, with Carassou et al.

(2008) suggesting that mixing models for piscivorous fish should use specific fractionation factors in models rather than use those used for dietary predictions of fishes that tend to feed primarily on invertebrates (Table 2). For the omnivores, bream and perch were the consumer species used in the models for site 1, and bream, perch and roach for site 2. For the piscivorous fishes, pikeperch were the consumer species used for site 1 and pike for site 2. Each model was then run at two levels: (1) at the population/community level ('population' hereafter; providing mean predicted dietary contributions per species); and (2) at the individual level (predicted diet composition per individual fish). All models were run using normal run length (chain length: 100,000 iterations with burn-in of 50,000, with posterior thinning (thin: 50) and 3 chains). Model diagnostics were based on Gelman-Rubin and Geweke (Stock & Semmens, 2016) to track algorithm convergence.

## Mixing model prey resources and fractionation factors

For site 1, the putative prey resources used in the mixing models for the omnivorous fish species were macrophytes ('plants'), macro-invertebrates ('invertebrates') and zebra mussel. For the 'invertebrates' prey resource, the stable isotopes data of Gammaridae and Chironomidae were tested for the significance of their differences using t-tests. Differences in $\delta^{13}C$ were not significant (t-test: $t_{12.8} = -1.58$,

$p = 0.14$), but were significant for $\delta^{15}N$ ($t_{12.4} = 2.41$, $p = 0.03$) (Fig. 1a). However, the mean difference in $\delta^{15}N$ between the groups of 0.73 ‰ was not considered biologically significant (Fig. 1a). Correspondingly, for their use as putative prey resources in the mixing model, their data were combined into a single resource. In these models, the isotopic fractionation values between the prey resources and consumers used were initially based on values generated from laboratory feeding trials of Busst & Britton (2017) (Table 2). However, it was decided these fractionation factors should use higher standard deviation values around the mean values in order to account for uncertainties relating to the different consumer species, and scales being used as the analysed tissue rather than fin tissue (Table 2). Moreover, different fractionation factors were used for the different prey resources, where the factors used were highest for plants and reduced for invertebrates and then zebra mussels/fish (Table 2). This was to account for patterns in the fractionation factors detected between prey resources of that differ in their protein content, where prey items of lower protein content tend to have higher fractionation factors (Busst & Britton, 2017; Table 2). For site 2, the putative prey resources differed to site 1, comprising Gammaridae, killer shrimp and zebra mussel, where the stable isotope values per resources were sufficiently different to enable them to be entered separately into models (Table 2). While the basis of the fractionation factors used at site 2 was as per site 1, the values used were different to site 1 as fin tissue was now the analysed tissue, which tends to have more depleted $\delta^{13}C$ and enriched $\delta^{15}N$ values than scales (Busst et al., 2015). For the piscivorous fish models, the same fractionation factors were used as per the omnivore models (Table 2). In site 1, the putative prey resources for pikeperch were freshwater prey fishes (common bream, perch and roach of lengths ≤ 170 mm), 'invertebrates' and zebra mussel. In site 2, the putative prey resources for pike were also freshwater prey fishes (common bream, perch and roach with length ≤ 170 mm), and then Gammaridae, killer shrimp and zebra mussel.

All statistical analysis and graphical outputs were performed using R version 4.0.5 (R Core Team, 2021).

Table 2 Diet fractionation factors (± SD) between the consumers and the putative prey resources used in the stable isotope mixing models for site 1 (consumer tissue analysed: scale) and site 2 (consumer tissue analysed: fin)

Putative prey resources	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Site 1		
Zebra mussels, fish	2.05 ± 0.50	3.95 ± 0.50
Invertebrates (Gammaridae/Chironomidae/killer shrimp)	2.55 ± 0.50	5.70 ± 0.50
Plants	3.55 ± 0.50	5.95 ± 0.50
Site 2		
Zebra mussels, fish	3.00 ± 0.50	3.00 ± 0.50
Invertebrates (Gammaridae/Chironomidae/killer shrimp)	3.50 ± 0.50	4.80 ± 0.50

(a)

(b)
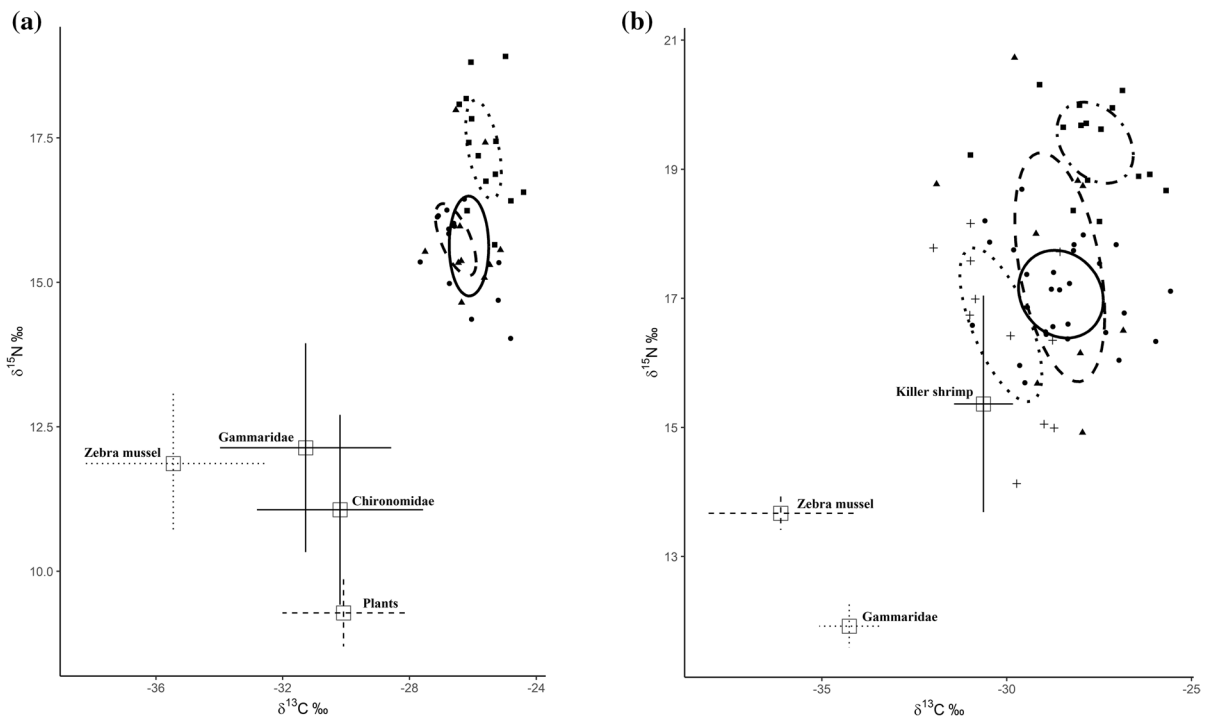
Fig. 1 Stable isotope data and isotopic niches (as standard ellipse areas; SEA$_c$) for the consumer species used in the stable isotope mixing models, where **a** site 1 (circle point, dashed ellipse: bream; triangle point, continuous ellipse: perch; square point, dotted ellipse: pikeperch); and **b** site 2 (circle point, con-tinuous ellipse: bream; triangle point, dashed ellipse: perch; cross point, dotted ellipse: roach; square point, dashed-dotted ellipse: pike). Mean values are displayed for the putative prey resources ($\pm$ SD)

Results

Stable isotope data

For the analysed omnivorous fish species in site 1, perch were between 121 and 217 mm (mean $\pm$ SD: 172 ± 28 mm), whereas common bream were 127 to 476 mm (mean $\pm$ SD: 297 ± 126 mm). Differences in their stable isotope data were not significant between species (δ^{13}C: $F_{1,22} = 1.56$, $p = 0.22$; δ^{15}N: $F_{1,22} = 0.46$, $p = 0.50$; Fig. 1a), where the effect of fish length as a covariate was also not significant (δ^{13}C: $p = 0.33$; δ^{15}N: $p = 0.87$). For the piscivorous pikeperch in site 1, their lengths were 345 to 780 mm (mean 573 ± 107 mm), which had a significant effect on δ^{13}C ($F_{1,12} = 16.87$, $p < 0.01$) but not δ^{15}N ($F_{1,12} = 0.08$, $p = 0.77$).

For site 2, common bream lengths were 286 to 491 mm (mean: 414 ± 50 mm), perch were 121 to 224 mm (mean: 151 ± 32) and roach were 98 to 217 mm (143 ± 35). Differences between the species in their stable isotope data were not significant between species (δ^{13}C: $F_{1, 44} = 2.22$, $p = 0.12$; δ^{15}N: $F_{1, 44} = 2.23$, $p = 0.12$) (Fig. 1b), with the effect of fish length as a covariate also not significant (δ^{13}C: $p = 0.62$; δ^{15}N: $p = 0.44$). For the piscivorous pike, their lengths were 471 to 1014 mm (mean: 754 ± 147 mm), and length had a significant effect on δ^{13}C ($F_{1,13} = 5.39$, $p = 0.04$), but not δ^{15}N ($F_{1,13} = 2.71, p = 0.12$).

Stable isotope mixing models—omnivorous fishes

In site 1, the mixing models for bream and perch at both population and individual levels predicted that the 'invertebrates' prey resource had the highest contribution to the diets of both fish species, whereas zebra mussels were predicted to have a low dietary contribution irrespective of fish size ($<16\%$; Table 3, Fig. 2). In site 2, population models predicted killer shrimp was prey resource with the highest contribution to the diets of for bream and perch, followed by
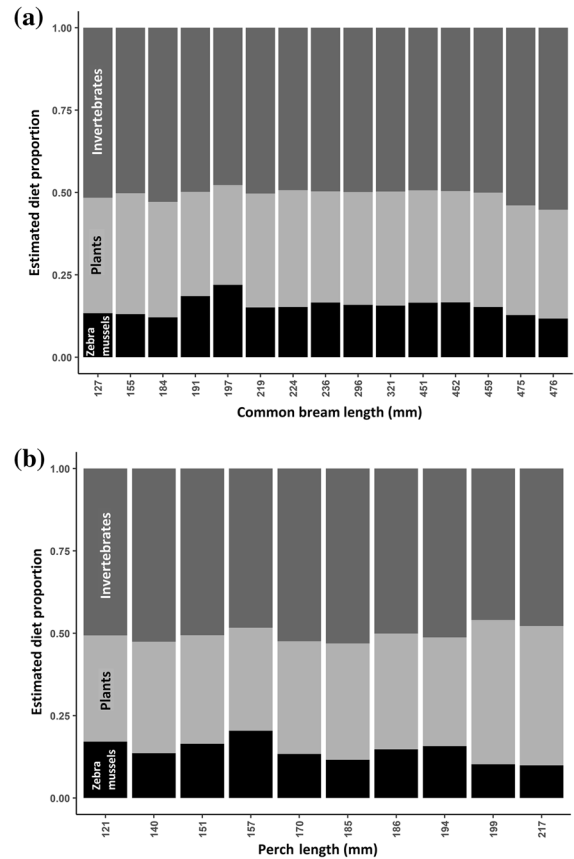
Table 3 Mean predicted dietary contributions, and their standard deviation and 5 and 95% credible intervals, from Bayesian mixing models (population and individual level models) of the prey resources of invertebrates (Gammaridae and Chironomidae), macrophytes ('plants') and zebra mussels to the diet of common bream and perch at site 1

Species	Source	Mean	SD	5%	95%
Population level					
Bream	Invertebrates	0.69	0.30	0.06	0.96
	Plants	0.19	0.26	0.00	0.77
	Zebra mussels	0.12	0.08	0.01	0.28
Perch	Invertebrates	0.73	0.34	0.03	0.99
	Plants	0.19	0.31	0.00	0.89
	Zebra mussels	0.08	0.08	0.00	0.24
Individual level					
Bream	Invertebrates	0.54	0.02	0.07	0.96
	Plants	0.31	0.01	0.00	0.81
	Zebra mussels	0.15	0.03	0.01	0.41
Perch	Invertebrates	0.54	0.02	0.06	0.96
	Plants	0.32	0.04	0.00	0.83
	Zebra mussels	0.14	0.03	0.01	0.39

Fig. 2 Estimated dietary prey contributions (0–1) to the diet of individual fish by body length for site 1: **a** common bream and **b** perch. Prey sources as overall means: invertebrates (dark grey), plants (light grey) and zebra mussels (black)

Gammaridae, and with zebra mussels again predicted to have a low dietary contribution. The individual models provided similar predictions, with Gammaridae predicted as the main prey resource for these two fish species, followed by killer shrimp (Table 4). Predictions for roach at the population level suggested a greater contribution of zebra mussels to diet than the other fish species (44%), but with the individual models suggesting Gammaridae was their main dietary source (Table 4). For roach, predictions suggested zebra mussels had relatively high dietary contributions for all fish sizes (Fig. 3).

Stable isotope mixing models—piscivorous fishes

For pikeperch in site 1, the mixing models predicted that prey fish resources were had the highest dietary contributions at both the population level and individual level (Table 5), with this evident for all fish lengths present (Fig. 4a). Predictions for zebra mussel were 24% at the population level and 17% at the individual level (Table 5, Fig. 4a). For site 2, the predictions for pike also indicated that prey fish resources also contributed highly to their diet, irrespective of fish size, followed by killer shrimp (Table 5, Fig. 4b). Both zebra mussels and Gammaridae were predicted

to contribute less than 18% of dietary resources (Table 5, Fig. 4b).

Discussion

The increased distribution of zebra mussels in British freshwaters (Aldridge et al., 2004) has presented a novel prey resource for the native fish communities. Across the five fish species analysed here, only roach, a fish ubiquitous to British lowland freshwaters (Britton, 2007), had predictions that zebra mussels were contributing strongly to their diet. The population level model prediction of 44% is consistent with Prejs et al. (1990), who suggested a predator–prey interaction between the two species, and Millane et al. (2012) who detected roach consumed them in

Table 4 Mean predicted dietary contributions, and their standard deviation and 5 and 95% credible intervals, from Bayesian mixing models (population and individual level models) of the prey resources of Gammaridae, killer shrimp and zebra mussels to the diet of common bream ('bream'), perch and roach at site 2

| Species	Source	Mean	SD	5%	95%
Population-level					
Bream	Gammaridae	0.28	0.17	0.05	0.59
	Killer shrimp	0.43	0.09	0.26	0.55
	Zebra mussels	0.29	0.12	0.09	0.47
Perch	Gammaridae	0.34	0.22	0.03	0.71
	Killer shrimp	0.44	0.12	0.21	0.61
	Zebra mussels	0.22	0.14	0.03	0.46
Roach	Gammaridae	0.35	0.24	0.03	0.79
	Killer shrimp	0.20	0.09	0.05	0.35
	Zebra mussels	0.44	0.18	0.09	0.70
Individual level					
Bream	Gammaridae	0.45	0.09	0.07	0.88
	Killer shrimp	0.37	0.11	0.05	0.70
	Zebra mussels	0.19	0.05	0.01	0.57
Perch	Gammaridae	0.43	0.11	0.05	0.88
	Killer shrimp	0.39	0.11	0.04	0.76
	Zebra mussels	0.18	0.06	0.01	0.62
Roach	Gammaridae	0.51	0.12	0.08	0.91
	Killer shrimp	0.25	0.08	0.03	0.58
	Zebra mussels	0.24	0.07	0.01	0.71

Lough Sheelin, Ireland, in all seasons. For the other fish species, zebra mussels were never predicted to comprise of more than 29% of their diets at the population level.

Common bream of over 400 mm were present in samples taken from both sites, with their diets predicted to primarily consist of 'invertebrate' prey resources (covering Gammaridae, Chironomidae and killer shrimp, depending on the site). These results are consistent with other dietary studies on common bream that have highlighted the importance of macro-invertebrates as prey resources for the species, with Chironomidae often being a dominant prey source (e.g. Giles et al., 1990; Kakareko, 2001). Although not sampled here, zooplankton can also be important in their diet, although usually only in fish of below 200 mm and in situations where there is a lack of macro-invertebrates available (Kakareko, 2001). Zebra mussels were predicted to be only a minor component of their diet in both sites here, with this

potentially related to their extensive branchial sieve which could make difficult to for them to ingest molluscs and limitations on their ability to crush the mussels imposed by the architecture of their pharyngeal teeth (Nagelkerke & Sibbing, 2011). Similarly, the analysed perch primarily consumed the 'invertebrates' prey resource at the population level, with this also consistent with other studies suggesting the importance of similar prey resources in their diet (Giles et al., 1990; Dörner et al., 2003). While there were no studies suggesting perch regularly prey upon zebra mussel, Mavrin & Strel'nikova (2011) suggested that when the species are in sympatry, with zebra mussels feeding extensively on zooplankton, juvenile perch might have to switch to macro-invertebrate prey from their early stages. This was also noted by Shcherbina & Bezmaternykh (2019), who also suggested that zebra mussel colonies also provide refuge for macro-invertebrates, increasing the difficulty of prey detection for perch during foraging. Roach were suggested in the literature as an important consumer of zebra mussels (Prejs et al., 1990; Millane et al., 2012) and, while they were only analysed in one site, the diet predictions here suggested all of the analysed fish had consumed some zebra mussels in recent months. However, this included individuals of below 160 mm length, whereas empirical studies have suggested a clear size threshold where these fish would not generally prey upon them (Prejs et al., 1990). Nagelkerke & Sibbing (2011) suggested that, functionally, roach are 'superior' in static crushing hard and brittle materials (like the shells of zebra mussels), compared to species such as common bream, emphasising those functional attributes such as gape size, strong pharyngeal teeth and the ability of chew prey items could be important for efficient feeding on these bivalves (French, 1993).

For piscivorous fish species, only fish above 170 mm were analysed, a size when the species tends to be an obligate piscivore (e.g. Nolan & Britton, 2018). Pikeperch primarily fed upon freshwater fish (70% at both population and individual levels), with a low contribution of the 'invertebrates' resource (< 10% both levels). Pike also had freshwater fish as their most important dietary resource, but with the alien killer shrimp also important in their diet. Recent studies on pike diet based on stable isotope data have indicated that macro-invertebrates can comprise a key component of their diet, irrespective of their body
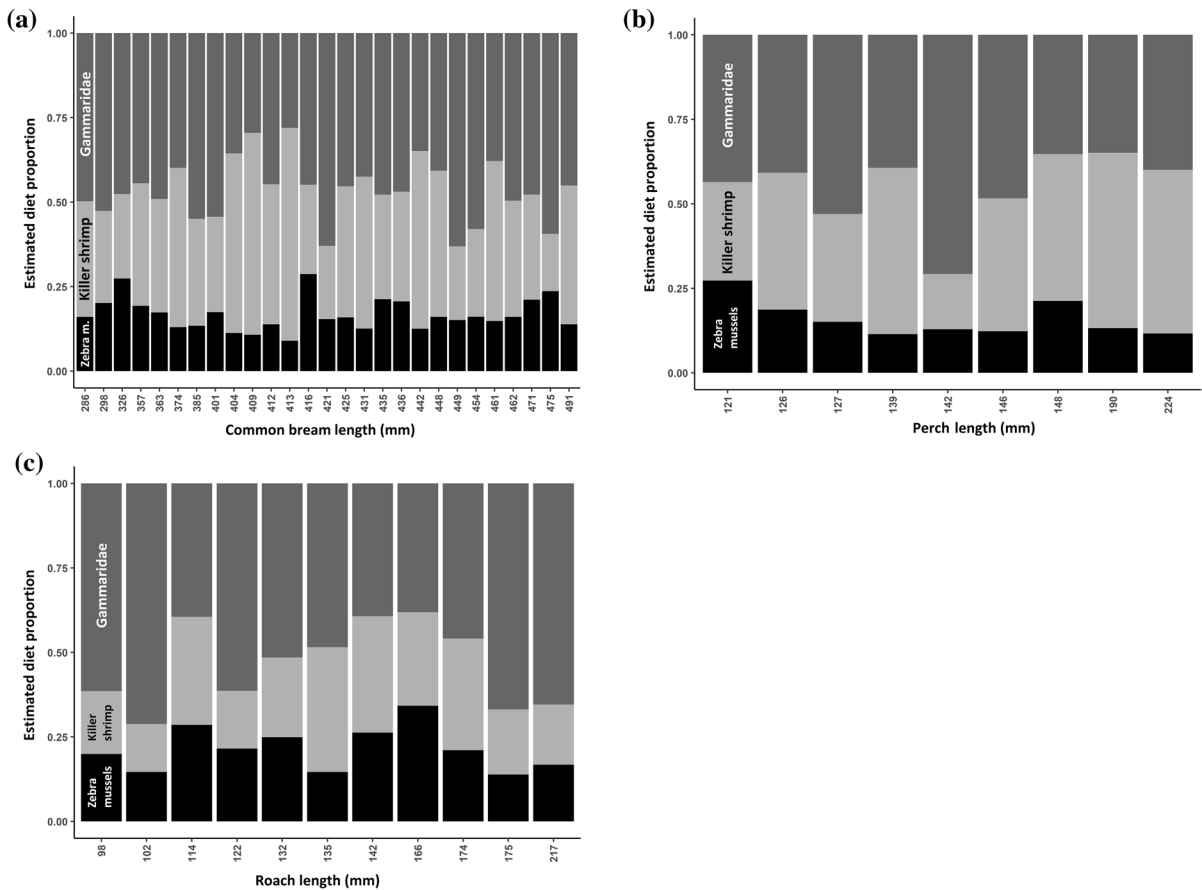
Fig. 3 Estimated dietary prey contributions (0—1) to the diet of individual fish by body length for site 2: **a** common bream, **b** perch and **c** roach. Prey sources as overall means: Gammaridae (dark grey), killer shrimp (light grey) and zebra mussels (black)

length, indicating some functional plasticity (Chapman & Mackay, 1990; Pedreschi et al., 2015; Nolan et al., 2019). Moreover, invasive killer shrimp have been detected as displacing native amphipod species in invaded waters, with fish species (including perch) responding by replacing native amphipods in their diet with the invasive ones (Eckmann et al., 2008). In site 2, invasive killer shrimp were in sympatry with native amphipods and with both making substantial contributions to fish diet generally. Correspondingly, the influence of this Ponto-Caspian amphipod on the diet of freshwater fish in Britain might prove to be higher than zebra mussel.

These results suggest that roach was the only analysed fish species that used zebra mussels within their diet in more than a minor way, suggesting that the extent of consumptive resistance on zebra mussel colonisation and spread was likely to be limited

in the analysed sites. While roach are ubiquitous in most regions of lowland Britain, populations tend to be dominated by fish below 200 mm (Britton, 2007), which are unlikely to be capable of consuming large quantities of bivalves. Similarly, while French & Bur (1992) revealed that although freshwater drum *Aplodinotus grunniens* predated heavily on these molluscs, they suggested this predation pressure would have little effect on the invasion of zebra mussels due to their rapid reproduction and growth. This suggests that where there is a management requirement to prevent the establishment of zebra mussel populations, or reduce their abundance, then reliance on biological resistance and/or biological control methods is not recommended. Thus, the application of chemical control methods would be required instead, but while these can substantially reduce their populations, these methods tend to also cause a high degree of collateral

Table 5 Mean predicted dietary contributions from Bayesian mixing models of fish, invertebrates, and zebra mussels to the diet of piscivorous fishes (pikeperch and pike) at population and individual levels, showing their standard deviation and 5 and 95% credible intervals

Species	Source	Mean	SD	5%	95%
Population level					
Pikeperch	Fish	0.68	0.07	0.565	0.784
	Invertebrates	0.07	0.07	0.004	0.214
	Zebra mussels	0.24	0.06	0.138	0.335
Pike	Fish	0.53	0.21	0.09	0.79
	Gammaridae	0.14	0.09	0.02	0.29
	Killer shrimp	0.25	0.20	0.02	0.65
	Zebra mussels	0.08	0.06	0.01	0.20
Individual level					
Pikeperch	Fish	0.73	0.06	0.47	0.95
	Invertebrates	0.09	0.02	0.00	0.34
	Zebra mussels	0.17	0.04	0.02	0.39
Pike	Fish	0.36	0.03	0.04	0.78
	Gammaridae	0.18	0.07	0.02	0.42
	Killer shrimp	0.42	0.08	0.07	0.80
	Zebra mussels	0.05	0.01	0.00	0.18

damage in non-target species (Aldridge et al., 2004; Lydeard et al., 2004). Awareness on the use of biosecurity (i.e. cleaning and drying techniques) to prevent introductions, rather than manage new and on-going invasions, tends to be used more, but clearly does not reduce their impacts in already invaded waters (Melly & Hanrahan, 2020; Smith et al., 2020).

Conclusion

The results from two invaded sites in England, one in the east and one in the west, suggest that in lowland areas at least, the consumptive resistance to the invasion of zebra mussels in Britain appears to have been limited. While it is acknowledged that the spatial and temporal extent of our sampling was limited, the results were largely consistent with studies elsewhere in the invaded range of zebra mussels, suggesting that while more in-depth studies might be interesting, they are unlikely to provide any novel insights. However, our analyses were based only on stable isotope analysis, with other methods of dietary analyses not being used, such as stomach contents analyses, which can provide both consistent (e.g. Nolan & Britton, 2018)
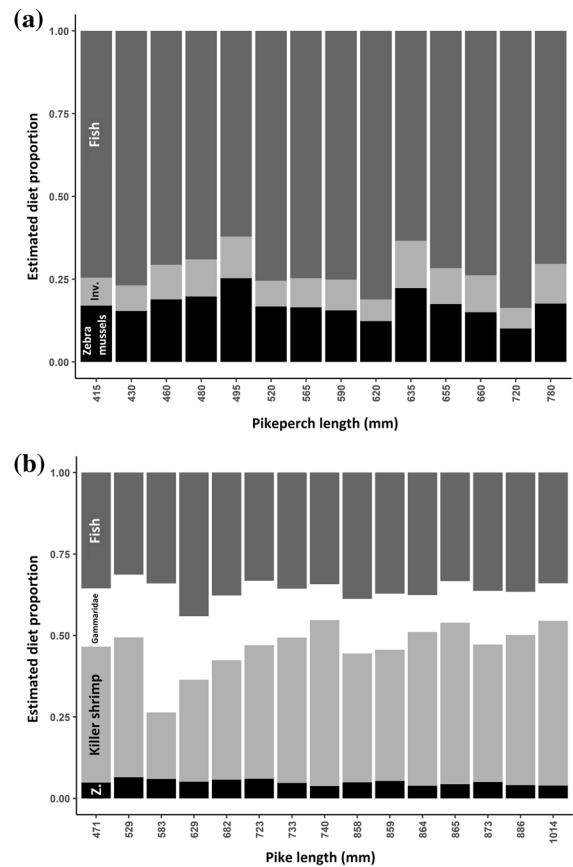
Fig. 4 Estimated dietary prey contributions to the diet of individual piscivorous fishes by body length (0–1): **a** pikeperch with fish (dark grey), invertebrates (light grey) and zebra mussel (black), and **b** pike with fish (dark grey), Gammaridae (white), killer shrimp (light grey) and zebra mussels (black). Prey source shown as overall mean per individual

and contrary results (e.g. Locke et al., 2013; Hamidan et al., 2016). Nevertheless, these results do suggest that if the invasion of Britain by zebra mussels is going to be contained, a more active management approach must be taken to prevent spread of existing populations and the establishment of new ones.

Data availability The datasets generated and analysed here are available from the corresponding author at reasonable request.

Declarations

Conflict of interest None of the co-authors have a conflict/competing interest.

Ethical approval All regulated procedures were completed under UK Home Office project licence 70/8063 and after ethical review.

Informed consent All authors reviewed the manuscript and agreed with its contents.

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