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Diet composition and trophic niche differentiation of *Neogobius melanostomus* along an invasion gradient in a large lowland river

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Abstract

The Ponto-Caspian goby *Neogobius melanostomus* (Pallas 1814) is a widespread non-native fish species in European rivers. It occurs in high abundance and can potentially alter the food webs of invaded water bodies profoundly. However, the invasion process usually goes unnoticed, and changes in food webs during the early stages of invasions are rarely described. Here, we studied populations of *N. melanostomus* along an invasion gradient in the Elbe River (Germany) and tested the effects of time since invasion on the diets and the associated trophic dynamics in two dominant habitats, i.e., rip rap and sand. Results showed that the abundance and biomass of *N. melanostomus* was lowest at the most recently invaded sites. The trophic niche space quantified by stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) showed that populations at earlier invaded sites exhibited a wider trophic niche than those at the most recently invaded sites. Diptera and Crustacea were the most abundant taxa in *N. melanostomus* guts at both habitat structures. At rip rap habitats, *N. melanostomus* showed a significant increasing preference for Crustacea and a decreasing one for Gastropoda along the invasion gradient. At sand habitats no significant relationships between the invasion gradient and electivity index for *N. melanostomus* were detected. We demonstrated that the invasion stage and habitat in which *N. melanostomus* occurs affect the feeding preference of this invasive fish and that these factors are therefore important for tracing changes in the macroinvertebrate communities of affected river systems.

KEY WORDS: Invasive fish, Round goby, Invasion front, Elbe River, feeding ecology, Stable isotopes

Introduction

The Ponto-Caspian round goby *Neogobius melanostomus* (Pallas 1814) is a benthic-feeding fish species with its origins in the Ponto-Caspian region. Since the 1990s, it has successfully spread and invaded rivers throughout Europe and North America (Borcherding et al., 2013; Hempel & Thiel, 2013; Kalchhauser et al., 2013).

The round goby has a suite of typical invader traits such as a high reproduction rate, tolerance of a wide range of environmental conditions, low food selectivity as well as species-typical behaviours like dispersal tendencies or aggressiveness (Church et al., 2017; Foley et al., 2017; Groen et al., 2012; Pothoven, 2018). These traits result in the ability to dominate even with a limited food supply and lead to an impact on the prey population (Krakowiak & Pennuto, 2008; Lederer et al., 2008), local food webs (Brush et al., 2012) and could ultimately even lead to the local extirpation of native benthic species and habitat destruction (Cooper et al., 2009; Pettitt-Wade et al., 2015). Therefore, this introduced fish species may significantly alter food webs and trophic structures by changing community composition and major nutrient fluxes (Herlevi et al., 2018). *N. melanostomus* prefers artificial shoreline structures commonly found in navigable rivers, i.e., rip rap structures (Borcherding et al., 2011) and feeds on a wide variety of macroinvertebrates. Its dominant prey throughout rivers in Europe and its home range are Crustacea (mainly amphipods) and Diptera (mainly chironomids) (Błońska et al., 2015; Borcherding et al., 2013; Raby et al., 2010).

Studies conducted in the Baltic Sea or the North American Great Lakes area have shown that round goby affects macroinvertebrate communities (Kipp & Ricciardi, 2012; Lederer et al., 2008) by decreasing their densities and changing taxonomic composition (Henseler et al., 2021; Krakowiak & Pennuto, 2008). The consequence is that *N. melanostomus* may have a crucial influence on the taxonomic composition of macroinvertebrate communities (Henseler et al., 2021) and may cause shifts in their assemblage structure with possible impacts such as the exploitation of prey resources and increased competition for native fish (Mikl et al., 2017). This is mainly due to the ability of *N. melanostomus* to exhibit an opportunistic feeding behaviour and an adaptive predation strategy that facilitates the successive establishment of new water bodies and contributes to its widespread distribution (Didenko et al., 2020; Perello et al., 2015). The prey selection of *N. melanostomus* depends on several factors, e.g., **(1)** the habitat structure in which they occur and, therefore, the availability of habitat-specific prey (Borcherding et al., 2013), **(2)** seasonal dynamics of macroinvertebrates abundance and thus varying availability (Didenko et al., 2020), **(3)** inter and intra-specific interactions, e. g. strong competition for food and high dietary overlaps (Borcherding et al., 2019), **(4)** mouth gaps in smaller fish may influence the feeding ability (Borcherding et al., 2013; Krebs & Turingan, 2003; Raby et al., 2010), **(5)** diel patterns as feeding can vary during day and night. The main feeding time of *N. melanostomus* was determined directly after sunrise whereby, especially juveniles may migrate into gravel and sand habitats during the night (Borcherding et al., 2013), or **(6)** body size, as *N. melanostomus* revealed an ontogenetic diet shift with a switch from preying upon insects and crustaceans to mainly molluscs with at a total length of 10 cm (Brandner et al., 2013b).

The resulting alterations in food webs (Brandner et al., 2013a; Brush et al., 2012) are expected to change with time since the invasion began (Herlevi et al., 2018). As invasion stages are correlated to the numerical abundance of *N. melanostomus* (Brandner et al., 2013b), knowledge of their ecology at all stages of the invasion is crucial to estimate associated ecosystem impacts and to quantify the potential impact on riverine food webs (Brandner et al., 2013b). However, determining the current invasion stage in invaded rivers is notoriously difficult, as the required field monitoring is often lacking or carried out after the invasion (Brandner et al., 2013b). Consequently, the invasion process usually goes unnoticed, and changes in food webs during the early stages of invasions are rarely described.

This knowledge about invasion dynamics and the factors that facilitate the adaption to the new ecosystem is necessary to understand the interactions between the invasive species and their prey and to help elucidate any associated impacts on the ecosystem (Taraborelli et al., 2010). In particular, an early-stage characterization of the 'invaders' impact can help to develop sufficient ecosystem conservation measures and preventative management actions (Henseler et al., 2020; Kornis et al., 2013).

In the Elbe River, *N. melanostomus* was detected for the first time in 2008 in the tidal reach (Hempel & Thiel, 2013) in Germany and 2015 in the upper Elbe River in the Czech Republic (Buřič et al., 2015; Roche et al., 2015). A previous study successfully traced the dispersal patterns and population structure of *N. melanostomus* in the Elbe River (Nogueira Tavares et al., 2020). There, we could demonstrate that *N. melanostomus* had not invaded the entire river course, as sites in the middle course showed a very low *N. melanostomus* abundance and had low environmental DNA (eDNA) concentrations (Nogueira Tavares et al., 2020). Based on these findings, the ongoing invasion process in the Elbe River was described as continuously increasing from upstream to downstream. This study took advantage of the unique opportunity to follow the invasion process and studied differences in diet composition and corresponding trophic dynamics along the invasion gradient. We hypothesized that macroinvertebrate abundance are influenced negatively by *N. melanostomus* abundance along the invasion gradient. We expected earlier invaded sites to have higher numbers of *N. melanostomus* individuals and therefore, lower numbers of macroinvertebrates than the recently invaded sites. Further, we expected gut contents of *N. melanostomus* caught in sand habitats to indicate different prey items than those caught in rip rap habitats.

Moreover, we hypothesised that more established populations at the longer invaded sites would face stronger intraspecific competition, leading to a broader trophic niche.

Materials and methods

Study area and invasion gradient

The study area was located at the Elbe River, from river km (rkm) 66 downstream to rkm 268, covering a free-flowing river section in Germany. We took samples from six sites placed approximately every 60 rkm along the river course. Based on a previous study (Nogueira Tavares et al., 2020), the river course between rkm 66 and 217.4 can be characterised as being invaded earlier (i.e., before 2018). In contrast, rkm 259.1 and 268 can be characterised as the most recently invaded sites at sampling times (i.e., after 2019, Fig. 1).

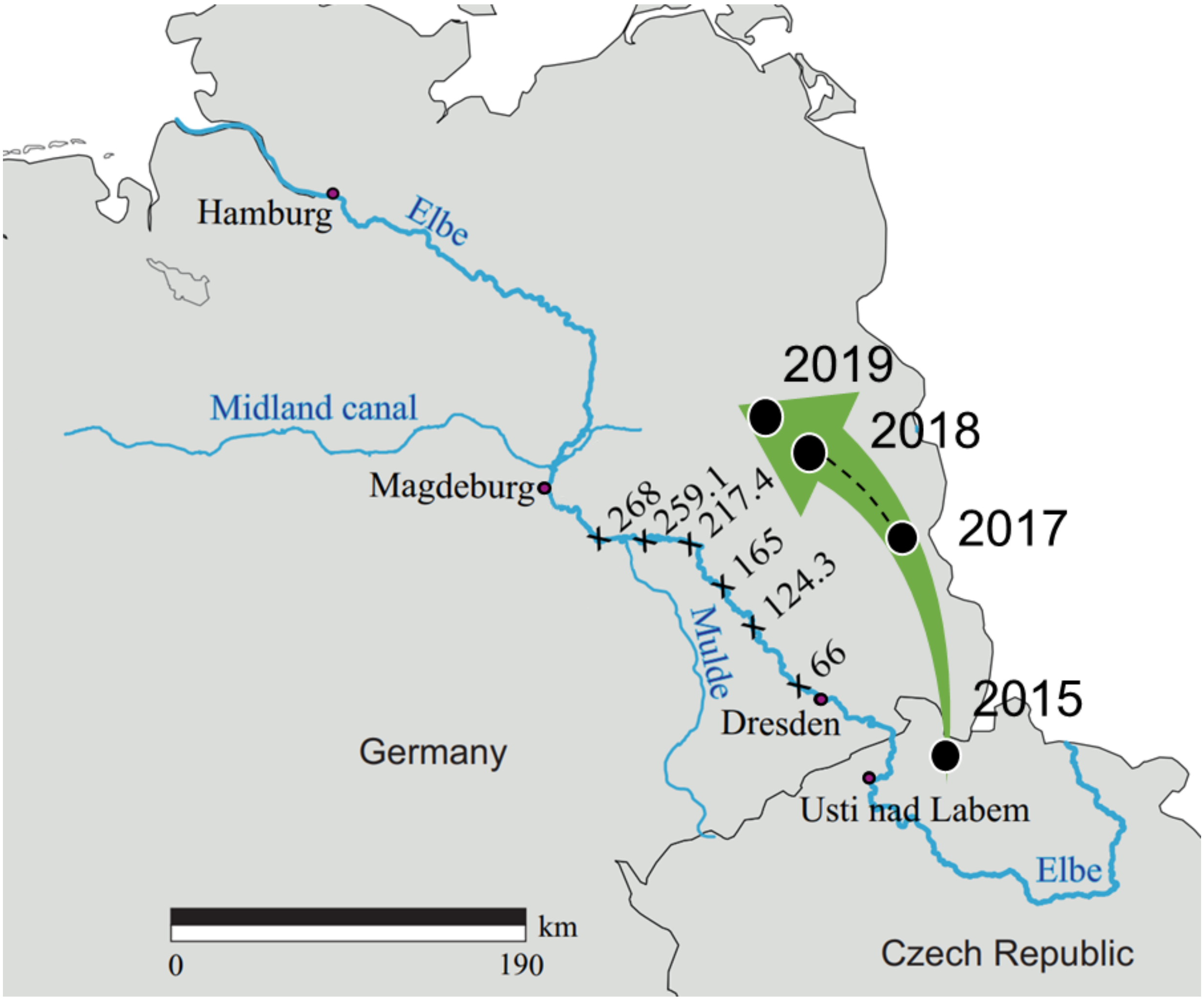


Fig. 1. Invasion history of *N. melanostomus* in the Elbe River and its main tributaries (blue lines). The invasion gradient is marked with an arrow, indicating the invasion progress and the corresponding year of round goby detection.

Sampling and sample analysis

Fish

Fish sampling was carried out in May 2019 by point abundance electrofishing (electrofishing generator DEKA 7000, DEKA Gerätebau Marsberg, Germany) in the morning during daylight hours. Sampling was carried out along 400 m near-shore stretches consisting of two groynes and two habitat structures: sand and rip rap (Fig. 2). The rocks of the rip rap varied from 5 to 50 cm in diameter. Similarly, structured sampling sites were chosen to minimize habitat effects on *N. melanostomus* consumption patterns. Caught fishes were identified to the species level, counted, and total body length was measured to the nearest 1 mm. Gobies were additionally weighed to the nearest 0.01 g and frozen at -20°C. Up to 30 individuals per rip rap and sandy habitat from each site were randomly subsampled for gut content and stable isotope analyses in the laboratory.

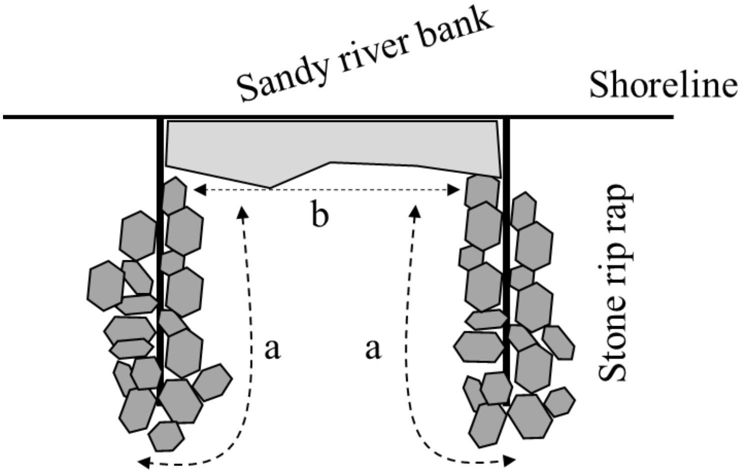


Fig. 2 Scheme of analysed groyne structure, divided into a rip rap (a) and a sandy river bank (b).

Macroinvertebrates

To characterise potential food resources of *N. melanostomus*, macroinvertebrates were collected along with the fish sampling from rip rap and sandy groyne fields (Fig. 2) with four replicates each. Stones were sampled by brushing off macroinvertebrates. To collect macroinvertebrates from sandy groyne fields, kick sampling was applied. Therefore handheld kick nets (0.25 mm mesh size) were placed vertically to the water bottom. The sampled area was calculated by measuring each boulder's length, height, and width under the assumption of a cuboid shape (sampled area 0.063 m²) following (Brabender et al., 2016). Samples were preserved in 70% ethanol. Macroinvertebrates were sorted, counted and identified to the species or genus level, except for Oligochaeta (order level) and Diptera (family level), excluding Chironomidae (species or genus level).

Gut content analysis

To determine the feeding activity and prey selection of *N. melanostomus,* the entire gastrointestinal tract was removed and preserved in 70% ethanol. Each gut was weighed both full and empty to the nearest 0.1 mg. Gut contents were preserved in 70% ethanol and later sorted under a dissecting microscope. The prey items were identified to the lowest possible taxonomic level and counted. Body lengths of all individuals or a random subsample (if more than ten individuals of one taxon were counted) were measured to the nearest 0.1 mm using an object micrometre. Shell length was measured for Bivalvia, shell height in Gastropoda, head capsule width in Trichoptera, carapax width in *Eriocheir sinensis* and length and width in Oligochaeta. The dry body mass of each prey individual was calculated using length–dry mass regressions (Baumgärtner & Rothhaupt, 2003; Benke et al., 1999; Burgherr & Meyer, 1997; Hellmann et al., 2015; Mährlein et al., 2016; Meyer, 1989; Normant et al., 2000). As shells are not digested but expelled from the gut, shell-free dry mass was calculated for molluscs.

Average dry masses were calculated for zooplankton prey, mostly Cladocera, cyclopoid copepods and copepodites. For Bryozoa, no length-dry mass relationship was available, and an average individual dry mass of 0.001 mg was assumed based on the biovolume of a typical zooid. The dry mass for all ingested individuals was calculated and summed up, and dry mass proportions in the diet were calculated for each fish. The dry mass proportions of the non-countable food sources detritus and filamentous algae were estimated visually and included in the diet calculation.

Stable isotope analyses

We conducted stable isotope analyses to determine the trophic niche space of *N. melanostomus*. Samples for stable isotopes were obtained by dissecting a small part of the dorsal muscle. The tissues were oven-dried for 48 hours at 40 °C and grounded with an oscillating mill (MM400, Retsch, Germany) to a fine homogeneous powder. Samples were weighed into tin cups (5 x 9 mm, IVA Analysentechnik GmbH & Co. KG, Germany). Carbon and nitrogen elemental concentration and $\delta^{13}C$ and $\delta^{15}N$ ratios were determined on a Thermo-Finnigan Flash 2000 elemental analyser connected to a Delta V Advantage mass spectrometer (Thermo Fisher

Scientific, Waltham, Massachusetts). Stable isotope data were expressed as the relative difference between the ratios of samples and international standards (PeeDee Belemnite for δ13C, atmospheric N for δ15N)

δ (‰) = (R_{sample}/ $R_{standard}$)*1000, and R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively.

Statistical analysis

The change of round goby abundance and biomass along the invasion gradient was tested with linear models of abundance and biomass and river km in Sigma Plot (v 13, Systat Software GmbH, Düsseldorf, Germany). We conducted separate models for the sand and rip rap habitats as differences between both habitats have been described previously (Borcherding et al., 2013). We visualised differences in prey communities along the invasion gradient by conducting non-metric multidimensional scaling on 4th root transformed macroinvertebrate abundances (Primer v7, PRIMER-E Ltd., Plymouth, UK). The analysis was supplemented by linear models of total macroinvertebrate abundance, taxa richness and abundance of macroinvertebrate major groups against river km. We also tested if macroinvertebrate abundance was related to goby abundance at rip rap and sand habitats by conducting Pearson correlation analysis in Sigma Plot.

To show the role of prey availability and goby dietary preferences, we calculated the gut fullness index (GFI, in %) to describe the feeding activity and estimated ingested food (Borcherding et al., 2013; Didenko et al., 2020; Všetičková et al., 2015):

$$GFI = (\text{gut content weight})/ (\text{fish weight}) * 100 \qquad \text{eq. 1}$$

where gut content weight is the sum of the wet weights of individual prey items. We quantified the diversity of ingested prey items as the Shannon Wiener Index calculated from prey composition (online Resource, Figure A.1).

The relative abundance of macroinvertebrates in *N. melanostomus* guts was compared to the relative abundance sampled in the Elbe River by calculating the electivity-index (E) (Lechowicz, 1982; Stauffer et al., 2016; Wiegleb et al., 2018):

$$E = (r_i - p_i) / (r_i + p_i) \qquad \text{eq. 2}$$

where r_i is the relative abundance of prey item i (percentage of total gut contents) in the gut and p_i is the relative abundance of the same item found in the environment.

Values range from −1 to +1. Negative values indicate the rejection or inaccessibility of the prey, zero = random feeding, and positive values indicate an active selection (Piria et al., 2016).

We used linear models to test for relationships of proportion of major macroinvertebrate groups in guts and electivity-index for macroinvertebrate major groups with river km in R (R Core Team, 2020). Prior to linear modelling, we tested for normality and constant variance.

Trophic niche width was calculated as the standard ellipse area corrected for small sample sizes (SEAc) using the SIBER package (Jackson et al., 2011) in R. Sample sizes were highly uneven for *N. melanostomus* caught in sand habitats (Table 1). To preclude that larger ellipse areas are simply the result of larger sample sizes, we randomly selected with replacement five isotope values (lowest sample size at rkm 66) out of the initial sample and repeated this procedure 100 times for rkm 124.3, 165 and 217. From the resulting 100 vectors for each rkm, we calculated the mean and 95% highest posterior density (HPD) intervals of SEAc for sand habitats. The HPD interval is the Bayesian counterpart to the frequentist's confidence intervals for posterior probability distributions (Marin & Robert, 2008).

We graphically analysed the relationship between trophic niche width and prey diversity but refrained from linear model analysis. Only four out of the six sites had sufficient goby abundances (Table 1) to calculate trophic niche width.

Results

Abundance of *N. melanostomus* along the invasion gradient

A total of 318 individuals of *N. melanostomus* were caught at the six study sites, and round goby were overall more abundant in rip rap than in sand habitats (Table 1). The two most recently invaded sites (rkm 259.1 and 268) had the lowest number of individuals (< 1% of total catch) and biomass (Table 1). However, abundance and biomass did not significantly change along the invasion gradient in rip rap and sand habitats (all models P > 0.05).

Table 1 Overview of total abundance (ind./ 100m²) and biomass (g/ 100m²) of *N. melanostomus* and the number of individuals subjected to gut content and stable isotope analysis per sampling site and habitat structure.

	Elbe km					
	66	**124.3**	**165**	**217.4**	**259.1**	**268**

Rip rap	Total abundance	12.5	30.5	51.5	35	0.5	1
	Total biomass	42.6	244.25	334.8	165.65	0.75	2.5
	No. individuals	23	27	28	25	1	2
Sand	Total abundance	2.5	9.5	6	10	0	0
	Total biomass	2.65	28.2	25.4	50.95	0	0
	No. individuals	5	13	12	15	0	0

Macroinvertebrate composition in river and guts

A total of 121,065 macroinvertebrates belonging to 10 major taxonomic groups were recorded (online Resource, Figure A.2). Macroinvertebrate community composition differed between habitat structures and along the invasion gradient. The downstream communities (at rkm 217.4 and 268) were more dissimilar between the habitats than the upstream communities, as indicated by the larger distance in the NMS ordination (Fig. 3).

We found a significant relationship between rkm and abundances of Crustacea (mostly non-native *Dikerogammarus villosus*) (Linear model, $R^2 = 0.82$, $P = 0.035$) and Bivalvia (Linear model, $R^2 = 0.79$, $P = 0.018$) in rip rap habitats that increases from upstream to downstream (online Resource, Figure A.2). Conversely, total macroinvertebrate abundance and taxa richness was not significantly related with river km for sand and rip rap habitat (all linear models $P > 0.05$).

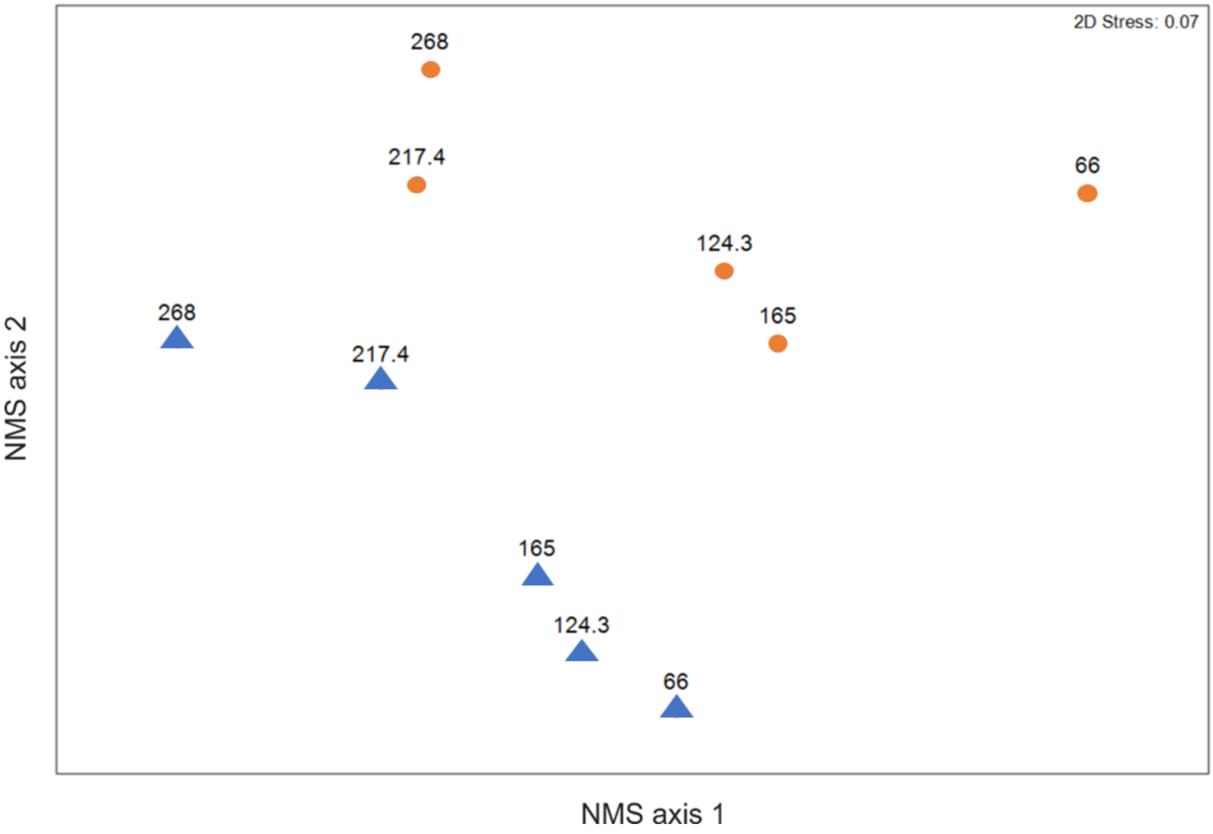


Fig. 3. Non-metric multidimensional scaling ordination of macroinvertebrate communities collected from sand (circles) and rip rap habitats (triangles) at the Elbe River. Numbers refer to river km and depict the invasion gradient of *N. melanostomus*. Due to low sample sizes, rkm 259.1 is not shown.

Within the rip rap habitats, total macroinvertebrate abundance decreased with increasing goby abundance (Pearson correlation r = 0.67; P = 0.049, n = 6). Within sand habitats, total macroinvertebrate abundance was not significantly correlated to goby abundance (r = 0.02, P = 0.777, n = 6).

We examined the gut contents of 151 individuals of *N. melanostomus* (Table 1). The most abundant prey at both habitat structures were Crustacea (mostly *Dikerogammarus villosus*) and Diptera (mostly Chironomidae Gen. sp.) (online Resource, Figure A.1). Gut fullness was affected by habitat structure, and scores were overall lower for *N. melanostomus* caught at sand habitats. The lowest gut fullness was detected at rkm 268 (number of examined guts = 2) for rip rap habitats and at rkm 66 (number of examined guts = 5) for sand habitats (Fig. 4). Gut fullness was highest for both habitats at rkm124.3. There were no significant relationships with river km neither for sand (linear model, $R^2 = 0.43$, P = 0.160) nor the rip rap habitat ($R^2 = 0.19$, P = 0.387) (Fig. 4).

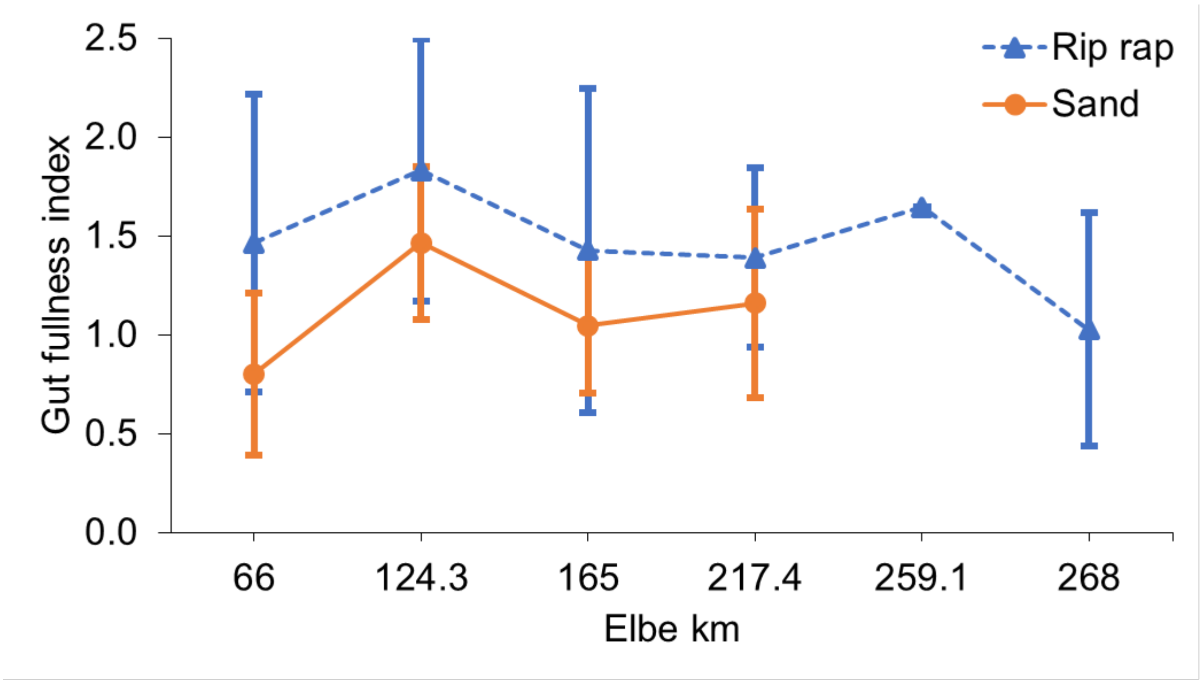
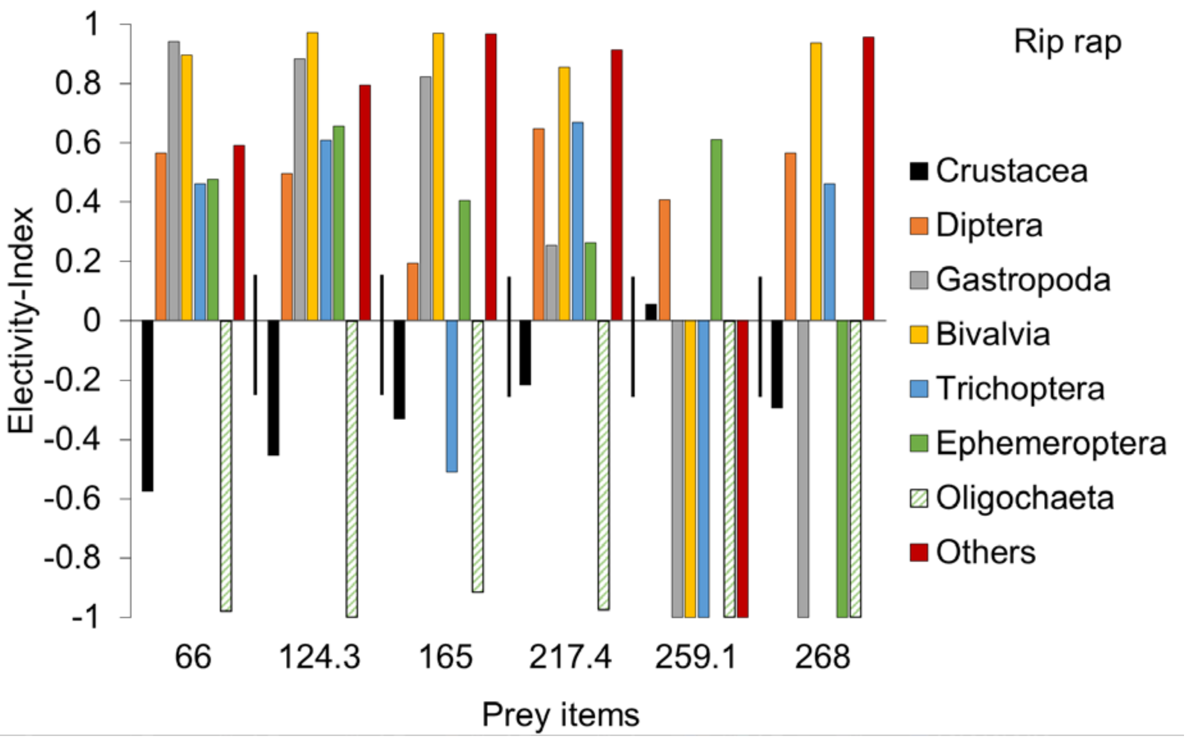


Fig. 4 Mean (± SD) gut fullness index (GFI) of *N. melanostomus* caught on sand and rip rap habitats along the invasion gradient. No data were available for sand habitats at rkm 259.1 and 268 as no gobies were present.

Prey selectivity

The analysis of the electivity index of *N. melanostomus* indicated differences in feeding preferences of *N. melanostomus* along the invasion gradient and between habitat structures (Fig. 5). At rip rap habitats, preference was significant for two taxa along the invasion gradient. Linear models between rkm and the electivity index were significant for Crustacea ($R^2 = 0.70$, $P = 0.037$) and Gastropoda ($R^2 = 0.80$, $P = 0.016$) at rip rap habitats, indicating that feeding preferences of *N. melanostomus* for both taxa changed along the invasion gradient (Fig. 5). Whereas the electivity index indicated mainly negative values for Crustacea, with the exception of rkm 259.1, and positive values for Gastropoda, with an exception at rkm 259.1 and 268 indicating negative ones. At the more recently invaded sites *N. melanostomus* from the rip rap habitat had a negative electivity index for Gastropoda, Bivalvia, Trichoptera and Oligochaeta at rkm 259.1(n = 1) and for Crustacea, Gastropoda, Ephemeroptera and Oligochaeta at rkm 268 (n = 2) (Fig. 5). There were no significant relationships between invasion history and electivity index for *N. melanostomus* caught in the sand habitat ($P < 0.05$ for all linear models). There was a tendency for an active selection of Crustacea and avoidance of Gastropoda (Fig. 5).



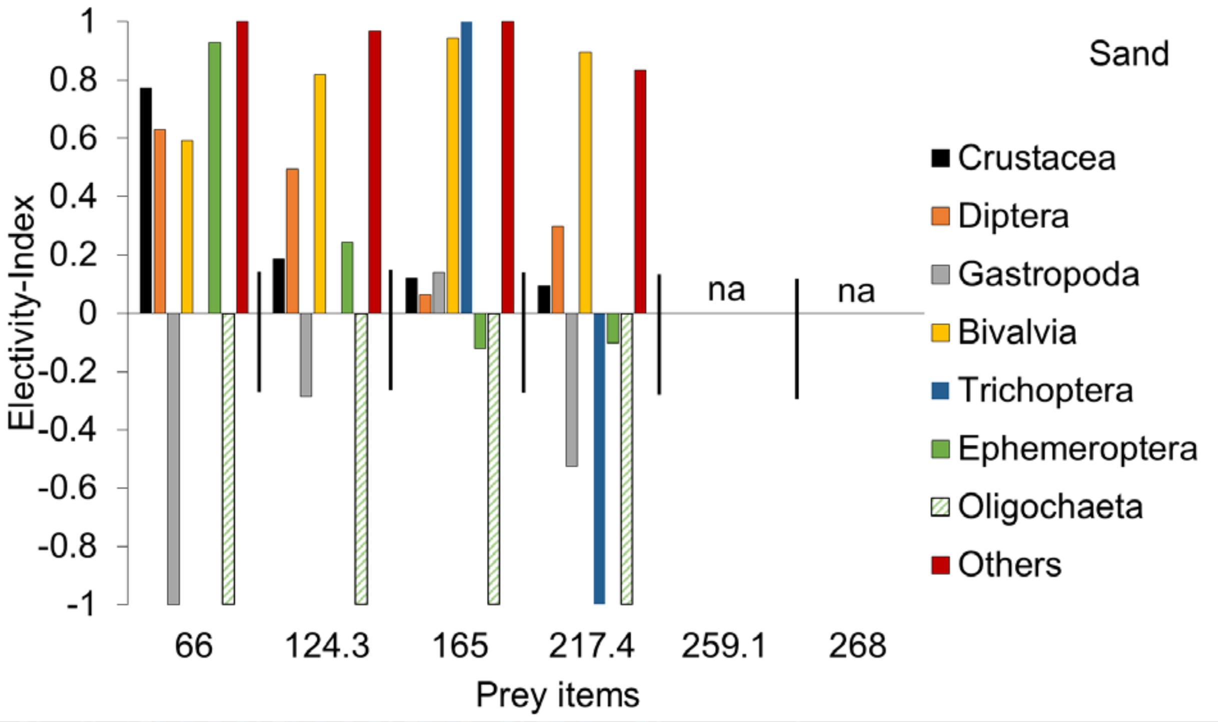


Fig. 5 Prey preferences of *N. melanostomus* quantified by the electivity-index along the invasion gradient for rip rap and sand habitats. Positive values indicate a preference, and negative values indicate avoidance of the respective macroinvertebrate major group. "na" indicates sites with no gut available.

Trophic niche size of *N. melanostomus* along the invasion gradient

In general, δ^{15}N values ranged between 15‰ and 17‰, indicating that *N. melanostomus* fed at similar trophic positions regardless of invasion stage or habitat structure (online Resource, Figure A.3). A decreasing trophic niche size was detected along the invasion gradient for *N. melanostomus* caught in the rip rap habitats. With increasing invasion progress, the trophic niche width decreased and most recently invaded sites had smaller niches than those invaded earlier (Fig. 6).

For *N. melanostomus* caught in sand habitats, trophic niche size showed a unimodal pattern along the invasion gradient, and the largest trophic niche was found at rkm 124.3 (Fig. 6).

The size of the trophic niche decreased with increasing prey diversity in the guts in the rip rap but did not show a relationship in the sand habitats (Fig. 7).

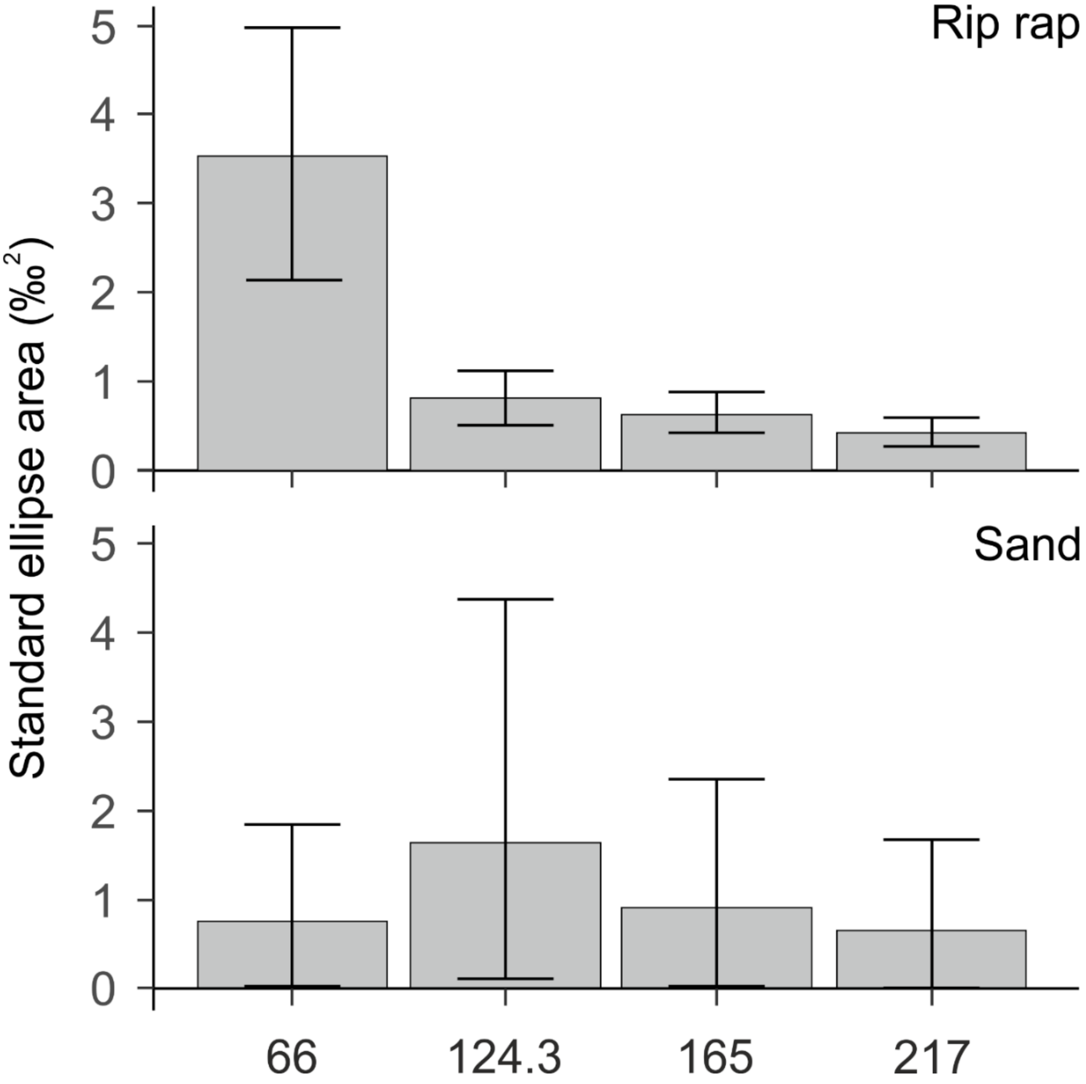


Fig. 6 Size of the trophic niche of *N. melanostomus* along the invasion gradient for rip rap and sand habitats expressed as the mean (± 95% highest posterior density intervals) standard ellipse area corrected for small sample sizes (SEAc).

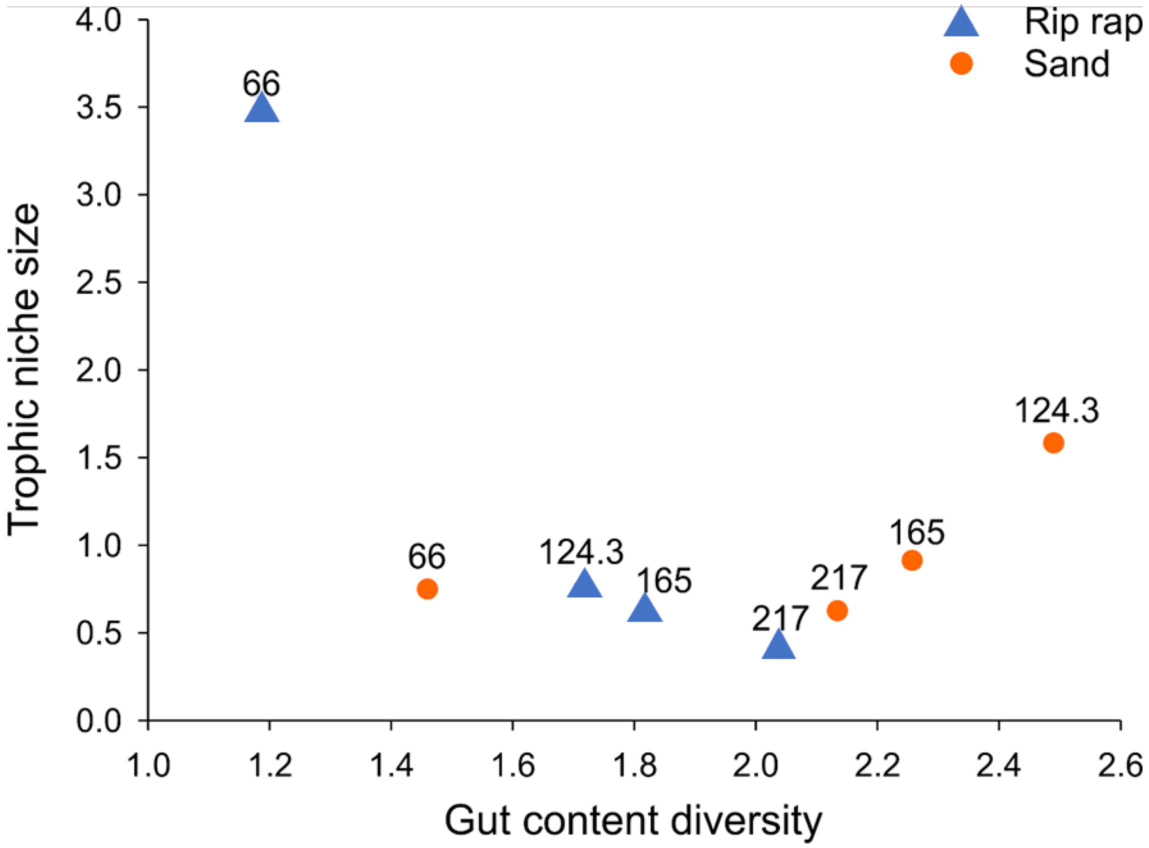


Fig. 7. Relationship between Shannon-Wiener diversity index of prey in guts and size of the trophic niche expressed as the mean of the standard ellipse area corrected for small sample sizes. Due to low sample sizes, rkm 259.1 and 268 are not shown.

Discussion

To predict future impacts for riverine ecosystems to be invaded by *N. melanostomus*, knowledge about the invasion process, habitat use and the factors that may influence the feeding behaviour and consequently trophic relationships of the invaded ecosystem remain essential. Here we were able to relate *N. melanostomus* feeding behaviour and trophic relationships to its respective invasion stage based on the known invasion pathways of *N. melanostomus* in the Elbe River (Nogueira Tavares et al., 2020).

Prey preference along the invasion gradient

Our results show differences in *N. melanostomus* gut contents depending on habitat structure and invasion stage. Hence, they agree with Raby et al. (2010), who demonstrated that the type and quantity of prey consumed by round goby could vary along its invasion pathway with different consumption at the area near their original introduction and those occupying the edges of range expansion. As expected, round gobies were more abundant in rip rap than in sand habitats, which goes in line with previous studies indicating that the rip rap structure is more frequently selected by round goby (Ray & Corkum, 2001). Further, we observed differences in gut fullness among the different habitat structures. The overall lower gut fullness at sand habitats compared to rip rap habitats could be the result of rip rap structures generally providing a higher species richness and abundance of invertebrates (Brunke et al., 2002; Ray & Corkum, 2001) and therefore a higher resource availability.

Prey abundance is crucial when analysing *N. melanostomus* dietary progress at the different invasion stages along a river course. The higher macroinvertebrate abundance at the latest invaded sites compared to the longer invaded ones (online Resource, Figure A.2) can be explained by lower predation pressure (Brandner et al., 2013b), whereas the reduced macroinvertebrate abundance at the earlier invaded sites could be a result of an earlier sustained high consumption rate by invasive round gobies. However, the lower *N. melanostomus* abundance and biomass detected at sampling site rkm 66 could indicate a previous high intra- and interspecific competition for food and shelter at this sampling site (McCallum et al., 2014). The results of our study at the two most recently invaded sites are based on a small number of analysed *N. melanostomus* individuals ($n \leq 2$). Therefore, round goby predation may not have affected macroinvertebrate abundance that strong by now.

At sand habitats, we observed a tendency of *N. melanostomus* to feed preferentially on Crustacea with the preference continuously decreasing from upstream to downstream. Nevertheless, a significant change in feeding preference along the invasion gradient was only observed at rip rap habitats. Here, the preference increased for Crustacea and decreased for Gastropoda from upstream to downstream. Different than at sand habitats, the electivity index values for Crustacea at rip rap habitats were mainly negative along the invasion gradient, indicating a general avoidance of this item at rip rap habitats in opposite to the general preference at sand habitats. However, specific for Crustacea at rip rap habitats, a trend of increasing electivity index values from upstream to downstream were observed. Here, the longer invaded sites indicate a stronger avoidance (index value close to -1), while the latest invaded sites show a trend of a more random feeding (electivity index value close to 0). Such effects of negative electivity values but dominant predation were also observed by Piria et al. (2016) in the Sava and Kupa Rivers, who found Gastropoda being the main prey category for *N. melanostomus* and also the most abundant item in the environment, even though electivity index indicate avoidance of this item. Another example is the study by Polačik et al. (2009) in the Danube River, who hypothesized that Mollusca are an unavoidable alternative, rather than the most preferred prey.

Our study indicated, that the preference for Gastropoda decreases along the invasion gradient, with an avoidance of these taxa (with index values of -1) at the more recent invaded sites.

Previous studies reported, that crustaceans are preferred prey items in goby diet (Mikl et al., 2017; Vašek et al., 2014), while Gastropoda are avoided (Brandner et al., 2013a; Piria et al., 2016; Vašek et al., 2014).

Henseler et al. (2021) described that the effects of *N. melanostomus* might be stronger for dominant taxa and also Borcherding et al. (2013) show that round gobies prey on available organisms in high abundances, which fits the results of our study, as Crustacea were a frequently represented macroinvertebrate taxa in the Elbe River. In particular the Ponto-Caspian amphipod *Dikerogammarus villosus*, an invasive species to the Elbe River (Worischka et al., 2018) provides an abundant food source for *N. melanostomus*.

Not represented with significant values, but worth discussing, is the trend of *N. melanostomus* consumption preferences on Bivalvia along the invasion gradient in both habitats, as indicated by the mainly positive electivity index values (Fig. 5). However, compared to the other available taxa present at both habitats, Bivalvia were much less abundant (online Resource, Figure A.2). Nevertheless, the mainly positive values correspond with the preferred diet of *N. melanostomus* in their native ranges, where molluscs, mainly bivalves, represent the most important food items (Brandner et al., 2013a). Other studies even determined *N. melanostomus* as a distinct molluscivore (French & Jude, 2001; Ray & Corkum, 1997).

However, it remains difficult to determine why round gobies select a particular prey type, and several factors like prey size, mobility, fixing to substratum, activity and digestibility may influence the choice (Roşca et al., 2010). Henseler et al. (2020) further state that prey taxa might depend on the study area and, most likely, on the prey items that are available in a specific environment.

Trophic niche size of *N. melanostomus* along the invasion gradient

Our results indicate a decreasing trophic niche coinciding with an increasing prey diversity in the guts of *N. melanostomus* caught at rip rap habitats (Fig. 7). This result seems counterintuitive as one would expect an increasing trophic niche width with an increasing diversity of ingested prey. Nevertheless, stable isotopes capture the middle-to-long-term patterns of assimilation while gut content analyses provide information on short-term feeding patterns of ingested prey (Brandner et al., 2013a). Hence, our results suggest that both techniques should be used in conjunction to quantify trophic dynamics of invasive fish.

We found differences in trophic niche width depending on the invasion gradient and habitat structure (Fig. 6). Smaller niche width in both habitat structures were detected for *N. melanostomus* caught at the latest invaded river sites and were widest at the earlier invaded sites and, therefore, more established populations (online Resource, Figure A.3). This is consistent with other studies, e.g. (Brandner et al., 2013b; Herlevi et al., 2018; Pettitt-Wade et al., 2015) that indicated a broader niche for round goby in longer-established populations compared to a newly established round goby population. Brandner et al. (2013b) observed that a less intraspecific competition exhibited a more homogenous diet than an established population, where a pronounced ontogenetic diet shift was visible, mirrored in a broad dietary niche. We assume that the invasion process of *N. melanostomus* at those sites that were invaded earlier is still ongoing and that competitive interactions have still not decreased *N. melanostomus* trophic niche to minimize competition (Rakauskas et al., 2020). But we expected, that the trophic niche may change with time as interspecific competition increases. For the least invaded sites, we can assume that *N. melanostomus* occupies previously vacant dietary niches, which has facilitated further colonization (de Carvalho et al., 2019).

Differences in trophic niche width show that *N. melanostomus* feeding behaviour change with the invasion stage and are thus dynamic. Therefore, knowledge on the invasion stage is of high importance, not only for understanding further dispersal performance but also for predicting upcoming impacts on the food web structure as a result of abundance and habitat occupancy and upcoming trophic links within the food web (Rakauskas et al., 2020). Such knowledge can help develop appropriate ecosystem conservation and preventative management actions. Taken together, our results show that diet composition and prey preferences of *N. melanostomus* differ depending on the invasion stage and habitat structure. Future studies should follow the invasion process and quantify trophic dynamics as it is likely to change over time with consequences for prey communities.

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