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Differential habitat use of a notorious invasive fish, the round goby, in a translocation-relevant system **1**

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Abstract

Anthropogenic structures can form novel ecosystem niches. Invasive species are often particularly successful in occupying these habitats and utilize them as beachheads for further spread. The invasive round goby (Neogobius melanostomus, Pallas 1814), an inherently bottom-dwelling fish, uses vertical harbor walls as habitat, enabling them to reach boats (i.e., potential translocation vectors). To evaluate the relevance of vertical habitat use for population dynamics and translocation, we exemplary investigated a population of round gobies in a harbor ecosystem. Specifically, we investigated differences in trophic niche characteristics, individual trophic specialization, phenotypic traits, and breeding frequency in wall versus bottom-dwelling round gobies. Habitatcharacteristic dietary signatures indicated habitat partitioning during the breeding season. Trophic niches overlapped but were clearly distinguishable between the habitats: Walls were inhabited by 1.4 times more trophic generalists than specialists, while the bottom was inhabited by 2.1 times more trophic specialists. Breeding frequency was 24 times higher on the walls than on the bottom. After the reproductive season, we found a higher similarity in trophic ecology of gobies inhabiting the two habitats, and differences in abundance, size, and condition. These results are in line with winter migrations to deeper habitats, which are common in round gobies in lentic and marine ecosystems. Our results suggest a high potential for microgeographic adaptation to either horizontal or vertical habitat use in invasive round gobies. We demonstrated that male gobies using the walls during the breeding season are larger and heavier, suggesting that wall climbing may select for more competitive individuals. Additionally, the overall abundance of round gobies likely increases with the additional use of vertical habitat space, which may lead to higher propagule pressure. The ability to exploit anthropogenic habitats, and a higher translocation probability of competitive individuals, can contribute to the invasion success of round gobies in anthropogenically influenced aquatic systems.

KEYWORDS

aquatic invasive species, diet, *Neogobius melanostomus*, niche dynamics, niche partitioning, stable isotopes

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1 | INTRODUCTION

The ecological niche of a species can be described by multiple dimensions. Diet and distribution in space are among the most commonly investigated ones (Ingram et al., 2018). Flexibility in niche use is often considered a typical feature of successful invaders (Chuang & Peterson, 2016; Wright et al., 2010). Niche expansion, dispersal, and broadened resource utilization is common under conditions of increased intraspecific competition (Mateus et al., 2016; Mendes et al., 2019; Svanbäck & Bolnick, 2007), which are typical for invasive species populations. We recently observed that the round goby (Neogobius melanostomus, Pallas 1814), a benthic fish considered "one of Europe's 100 worst invasive species" (Vilà et al., 2009) and a species thriving in anthropogenic habitats (Cerwenka et al., 2018), uses vertical walls up to the water surface as habitat and foraging ground. The use of harbor walls can also facilitate contact to potential translocation vectors, that is, boats (Bussmann & Burkhardt-Holm, 2020). Because of their normally bottom-dwelling nature, and their preference for shallow slopes (Jakubčinová et al., 2018), this behavior can be considered an expansion of their traditional niche, enabled by man-made structures.

Population niche expansion can be driven by all/most individuals of a population, each utilizing both the traditional and the novel niche dimension (generalists), or can involve the partitioning of the population with some individuals specializing on the novel niche dimension and others utilizing their original niche (specialists; Bolnick et al., 2003). In the first case, the between-individual variation in resource use in a population will be low, while each individual will use a wide range of resources (high within-individual variation). In the latter case, the between-individual variation in resource use in a population will be high, while the within-individual variation will be low. These options represent extremes of a spectrum of different niche expansion scenarios, with the proportion of generalists and specialists within a population varying widely. In fact, generalist populations are commonly comprised of specialized individuals selecting different parts of a niche (Araújo et al., 2011). Which and how many individuals specialize may depend, for example, on the availability of different niches (Bolnick & Ballare, 2020), the amount of intra- and interspecific competition, or predation pressure (Araújo et al., 2011). For example, in racer gobies (Babka gymnotrachelus, Iljin 1927), spatial niche expansion of the population under conditions of high intraspecific competition is driven by subordinate individuals with lower growth rates, while dominant males occupy shelters in the original habitat (Grabowska et al., 2019).

The round goby's diet is typically broad (trophic generalists, Borcherding et al., 2013; Brandner, Auerswald, et al., 2013). The existence of anthropogenic vertical habitat, which likely harbors different benthic food organism communities compared with the bottom, may provide opportunities to specialize in either habitat use, diet, or both. Additionally, vertical habitat use might be correlated with phenotypic traits, as it is likely linked to higher energy expenditure (Bussmann & Burkhardt-Holm, 2020). There might also be seasonal differences in vertical habitat use. Round gobies occupy small home ranges ($5 \pm 1.2 \text{ m}^2$, Ray & Corkum, 2001) in shallow waters during the reproductive season (April–September in their native range, Kornis et al., 2012). In the winter months after the reproductive season, they migrate to deeper waters in lentic and marine systems (Behrens et al., 2021; Carlson et al., 2021).

A likely, yet not proven, translocation mode for round gobies is that they lay eggs on boat hulls, which are then moved to uncolonized waters (Adrian-Kalchhauser et al., 2017), from where they typically migrate rapidly into adjacent areas (Brandner, Cerwenka, et al., 2013). Based on the resulting assumption that gobies utilizing vertical habitats (or their eggs) are more likely to be translocated, we aimed to understand whether round gobies using walls are a distinguishable part of the population, and if that part is defined by specific ecological or phenotypic traits in- and outside of the reproductive season.

In this study, we investigated whether (1) the use of man-made vertical walls as habitat is a distinct trophic strategy of a round goby population (total or selection of it), whether (2) the level of individual trophic specialization differs between the habitats, and whether (3) habitat selection is correlated with phenotypic traits. We expected the results to be consistent with one of three hypothetical scenarios (Figure 1): In scenario A, all individuals in a population spend at least a part of their time using walls as habitat (= trophic niches not distinguishable; high within-individual and low between-individual differences in trophic resource use; no differences in phenotypic traits between the two habitats; Figure 1a). In this case, wall climbing behavior would indicate generalism in both diet and habitat use. In scenario B, only a fraction of the total population forages on walls on occasion, while the other portion of the population stays on the bottom (= overlap of niches with unique regions in wall-utilizing individuals; within-individual variation in resource use larger in individuals inhabiting walls; differences in phenotype possible; Figure 1b). In this scenario, wall climbing could be regarded as a niche expansion of a subset of the population, potentially linked to a demographic feature (e.g., sex), a certain phenotype (e.g., size, weight, and condition), or behavior (e.g., foraging strategy). In scenario C, wall climbing is exhibited by a completely separate part of the population (= no overlap of niches; low within-individual variation in both habitats; differences in phenotype; Figure 1c). In this case, wall climbing could be considered a specialization in habitat use, indicating the development of subpopulations or ecotypes.

The hypotheses tested in this study (see below) are based on scenario B, which, leaning on previous work (Bussmann & Burkhardt-Holm, 2020),

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FIGURE 1 Schematic overview of expected results under different scenarios. (a) The whole population of round gobies uses both the bottom and the wall habitat flexibly. With regard to trophic (i.e., isotopic) niche space (1), there would be no difference between size and extension of the trophic niche of specimen caught on the bottom or the wall. In this case, all individuals show a generalist feeding strategy, with high variation between long-term diet (muscle δ^{13} C isotopes) and short-term diet (liver δ^{13} C isotopes, 2). Moreover, there would be no difference in body traits between specimens caught on the bottom or the wall (3). (b) The whole population uses the habitat on the bottom, but some specific individuals extend their niche to the vertical walls. In the isotope space, the trophic niche of gobies caught on the bottom, while at the same time, there would be a high overlap (1). Individuals utilizing the wall as their expanded habitat would exhibit larger variation between long-term diet than individuals using the bottom only (2). The part of the population utilizing the walls would exhibit different traits from the one on the bottom (3). (c) The population is separated in two portions: One utilizing exclusively the bottom, the other one using exclusively the walls as their habitat. In the isotopic space, the trophic niches would be relatively small and distinct (1). All individuals would show a low variance between long-term diet and short-term diet (2). The part of the population utilizing the walls is again expected to exhibit different traits from that on the bottom (3).

we consider most likely. In Bussmann and Burkhardt-Holm (2020), we demonstrate that some individuals move inbetween the habitats. The quantification of movement activities of the round gobies in the same work indicates that individuals utilizing walls require a higher amount of energy than those on the bottom, as round gobies do not possess a swim bladder and individuals on the walls exhibit higher fin beat frequencies and movement. Using walls for foraging might therefore be a behavior exhibited by only a subset of the population.

The hypotheses formulated in this study were as follows:

- **H1** The trophic niche of round gobies caught on the walls is distinguishable in size and position from the niche of gobies caught on the bottom.
- **H2** The number of individual diet specialists is lower in gobies using the walls as habitat than in those using the bottom.
- **H3** Round gobies using the walls are distinguishable from those using the bottom by their body size, weight, and body condition.

The hypotheses were tested on individuals caught both during and after the reproductive season, as round gobies show different movement and space-use patterns between the seasons, which in turn may influence their ecology and their translocation probabilities. Most round gobies exhibit site-fidelity during the reproductive season, with males either guarding nests or following a sneaker tactic, where they enter the nest of a different male while a female spawns and attempt to fertilize eggs without investing in nest-building themselves (Lynch & Mensinger, 2012; Ray & Corkum, 2001). The proportion of sneaker males in a population can vary widely, with values between 4% (Cerwenka et al., 2020) and 40% (Bleeker et al., 2017) reported in different ecosystems. Outside of the reproductive season, they show higher mobility, or even migration patterns to deeper waters (Behrens et al., 2021; Carlson et al., 2021).

To complement the niche-based hypotheses, we compared breeding frequencies of round gobies in vertical and horizontal habitats to find out whether an opportunity for microgeographic divergence based on habitat-related selective breeding exists (Maciejewski et al., 2020; Richardson et al., 2014).

2 | MATERIALS AND METHODS

2.1 | Trophic ecology and population traits

2.1.1 | Study site and sample collection

The study took place in the commercial harbor Kleinhüningen in the river Rhine in Basel, Switzerland (47°35′10″N 7°35′27″E). Monitoring of spawning activities in the population inhabiting this harbor showed that spawning activities cease during August (Hirsch et al., 2016). To investigate vertical habitat use in- and outside of the reproductive season, we sampled the population twice in the year 2020: between 19th August and 04th September (reproductive season); and between 28th September and 23rd October (postreproductive season). Sampling at the end of the reproductive season allowed us to catch gobies when most nesting activities had stopped, so that males could be caught independent of reproductive status, while the isotopic signatures should still reflect feeding patterns during the reproductive season.

We brought out minnow traps $(44 \times 23 \text{ cm}, \text{ openings: } 4.5 \text{ cm})$ baited with 2–3 pieces of dry Frolic® dogfood along a 150 m long stretch of harbor wall (depth 3–5 m, Figure S1). We installed minnow traps on the wall at <1 m depth (reproductive season: four traps, postreproductive season: eight traps). Others were placed on the bottom 3–8 m off the harbor wall (reproductive season: two traps, postreproductive season: four traps). We emptied the traps every second day, euthanized the caught gobies, and transported them back to the laboratory on ice. In August, we caught 46 gobies on the bottom and 49 on walls. In October, we caught 97 gobies on the bottom and 65 on walls.

In the laboratory, we determined the sex of all round gobies by the shape of their urogenital papilla (broad rectangular for females, triangular for males, not identifiable for juveniles, Marentette et al., 2009), measured standard length (SL \pm 1mm), and recorded wet weight of the blotted dry fish $(\pm 0.01g)$. Three gobies caught in August on the bottom were juveniles, and we did not use them for further analyses. All other gobies were used for the analysis of phenotypic traits. The condition factor Fulton's K was calculated with the formula $K = [100^*$ wet weight, g]/[standard length, cm]³. We tested for differences in the abundance of males and females using Chi-square tests. We tested the measured traits for differences between wall and bottom-caught individuals, and between sexes within each season using linear models (trait value as dependent variable, sampling period, habitat, and sex as fixed factors including all possible interactions) using the package lme4 version 1.1-27.1 (Bates et al., 2015; R Core Team, 2021). For Tukey's HSD post hoc comparisons between habitats within season, we used the package and emmeans version 1.6.3 (Lenth, 2021). We conducted these and all further statistical analyses in R version 4.0.5 (R Core Team, 2021).

After taking all measurements, we extracted the whole liver of each goby and removed a ~1 ×1 cm piece of skinless and boneless muscle tissue from the caudo-ventral region of the fish. The tissue samples were individually stored at -80°C until further processing for stable isotope analysis. For stable isotope analysis, we focused on a subset of the round goby population, which fell into a standardized range of size and condition. This way, we wanted to eliminate the influence of confounding factors, such as the well-documented dietary switch of round gobies from arthropods to mollusks around 10-13 cm total length (Brush et al., 2012; Miano et al., 2021), and body condition (Karlson et al., 2018). More specifically, we excluded juvenile individuals (n = 3), individuals with a standard length of \geq 10 cm (*n* = 9), and with a condition factor *K* \leq 1.7 or *K* \geq 2.4 (*n* = 11). Additionally, we excluded individuals with a visible parasite or skin infection (n = 5), or a notably receded liver (very small and dark red as opposed to large and pink, n = 2). From the remaining 230 round gobies, we selected those individuals that were closest to the mean condition factor and standard length of the entire population to end up with a sample size of ~30 individuals per habitat and sampling period. The standardized subset of the gobies used for the stable isotope analysis is presented in Table S1.

If a consumer acquires resources from both the littoral and the pelagic food web, organisms representing baseline δ^{15} N are necessary to calculate the trophic position and estimate baseline variation of the N and C isotopic composition (Post, 2002). To this end, we collected ten specimen each of the killer shrimp *Dikerogammarus villosus* (Sovinskij 1894) (as a representative of amphipods), and of the zebra mussel *Dreissena polymorpha* (Pallas 1771) per sampling season and habitat (August 27, 2020 and October 30, 2020). *D. villosus* is a known main food source for round gobies in the river Rhine (Borcherding et al., 2013) and incorporates microhabitat differences in δ^{13} C (Brandner et al., 2015). *D. polymorpha* is a filter feeder and hence provides a good baseline for pelagic food webs. We kept all baseline organisms in tap water for >24 h to empty their guts before processing them (*D. villosus* whole specimen, *D. polymorpha* soft tissue).

2.1.2 | Stable isotope analysis

We prepared tissue samples of round goby muscles and livers as well as *D. villosus* and *D. polymorpha* by drying all samples at 60°C for >48 h, and grinding dried samples to obtain a homogenous powder using mortar and pestle. We weighed 1 mg \pm 20µg of each sample into 5×9 mm tin cups, before analyzing the samples for δ^{13} C, δ^{15} N, C%, and N% using an isotope ratio mass spectrometer coupled to an elemental analyzer (EA-IRMS). Details on the mass spectrometric analyses, information on the EA-IRMS standards used for elemental and isotope analysis, instrumental precision, and definition of the delta notation are presented in Appendix S2.

Because lipids are ¹³C-depleted with respect to the bulk organic matter/tissue, a high content of lipids in animal tissue can influence the δ^{13} C values of that tissue (DeNiro & Epstein, 1978;

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Focken & Becker, 1998). Especially for tissues with high fat content, such as liver tissue, it is necessary to correct for the lipid content of a given sample prior to comparison with low-fat content tissues such as muscle (Logan et al., 2008; Post et al., 2007). We used a subsample of eight round goby livers to establish an equation for lipid δ^{13} C correction of the rest of the samples. We split the eight liver samples in two halves, and extracted lipids from one half based on a protocol of Bligh and Dyer (1959), modified by Turschak et al. (2014). Briefly, a 2:1 chloroform-methanol solution was added to the dried tissue homogenate, the mixture was periodically agitated for 30 min, and then centrifuged before decanting the supernatant. This procedure was repeated three times, before the residual sample was dried. The lipid-corrected $\delta^{13}C_1$ was then determined using the formula $\delta^{13}C_1 = \delta^{13}C_0 + \beta_1^*C:N_0 + \beta_0$, where $\delta^{13}C_0$ and C:N₀ are the $\delta^{13}C$ and C:N of the sample before lipid extraction, and β_1 and β_0 are the slope and intercept of the linear relationship between C:N₀ and $\Delta \delta^{13}$ C (i.e., δ^{13} C₁- δ^{13} C₀), respectively. Details of this approach and outcomes of lipid correction are presented in Appendix S3.

2.1.3 | Trophic niche: Size, overlap, and position

To compare the size and position of the trophic niche occupied by round gobies in both habitats during and after the reproductive season, we calculated the standard ellipse areas (SEA) based on muscle isotopic composition using the package SIBER version 2.6.1 (Jackson et al., 2011). The SEA describes the size of the core niche of a population in a biplot of δ^{13} C versus δ^{15} N, including 40% of the data points (Jackson et al., 2011). We corrected SEAs for small sample sizes by removing one degree of freedom (SEA_c). For statistical comparisons of niche size and overlap, we fit Bayesian multivariate normal distributions to each group in the dataset (10^5 posterior draws), and then calculated the SEA on the posterior distribution of covariance matrices, thereby yielding the Bayesian SEA_B. The SEA_B has been shown to reliably represent the niche size of the real population even when based on small sample sizes (Jackson et al., 2011).

Niche size

To compare the niche sizes between wall and bottom, we calculated the proportion of posterior ellipses in one habitat that were smaller than the posterior ellipses of the other habitat, both during and after the reproductive season. This proportion equates to the probability p of the bottom niche being smaller than the wall niche (Jackson et al., 2011). We assumed niche size to be significantly different at a probability of p > .95.

Niche overlap

We used the mean overlap of the first 1000 posterior ellipses (SEA_B) to calculate the proportion overlap between the niches of gobies in both habitats during and after the reproductive season. We assumed the overlap of two niches to be significant if it was >60% (Guzzo et al., 2013; Pettitt-Wade et al., 2015; Wallace, 1981). A significant

overlap between bottom ellipses and wall ellipses would support Scenario A; a significant overlap of the bottom niche with the wall niche, but not of the wall niche with the bottom niche, would support Scenario B; no (significant) overlap between the bottom and wall ellipses would support Scenario C (Figure 1).

Isotopic ranges

Apart from the SEA_B, we also calculated the δ^{15} N range and of δ^{13} C range of the gobies caught in both habitats. The isotopic ranges hereby represent the isotopic distance between the individuals with the highest and lowest δ^{15} N or δ^{13} C values in the population, as described in Layman et al. (2007). We bootstrapped the isotopic ranges (*n* = 10,000, indicated with a subscript "b") based on the minimum sample size in the dataset (*n* = 28) to allow for a comparison among populations (Jackson et al., 2012).

Niche position

To compare the position of the trophic niche of round gobies caught in both habitats, we calculated linear models using the mean δ^{13} C and $\delta^{15}N$ as dependent variables and habitat and sampling period (including their interaction) as fixed factors. We calculated the same models for the amphipods used as baseline organism and compared differences in round goby isotopes to differences in amphipod isotopes. The nitrogen isotope ratio of a species is representative of its trophic position, since its value increases stepwise with each trophic level by about 3.4‰ (Post, 2002). As the δ^{15} N of a consumer in a food chain is dependent on the $\delta^{15}N$ of the food at the base of the food chain, estimating the trophic position of the consumer requires that δ^{15} N of a system-specific baseline organism is known. Here, we calculated the trophic position (TP) of round gobies using the equation from Post (2002) for secondary consumers: $TP = \lambda + (\delta^{15}N_{round goby}^{-1})^{-1}$ $\delta^{15}N_{hase}/\Delta_n$, where λ is the trophic position of the baseline organisms (assumed to be 2 for the amphipod *D*. villosus), and Δ_n is the isotopic enrichment per trophic level (assumed to be 3.4‰). We did not calculate the trophic position based on both a littoral (D. villosus) and a pelagic baseline (D. polymorpha), because initial screening of data showed that the round gobies in our chosen subset almost exclusively acquired resources from the littoral food web.

2.2 | Individual trophic specialization

We based our analyses of trophic specialization on temporal variation of carbon isotope composition. Carbon isotopes change little with trophic level of a consumer, but rather reflect their primary carbon source and are therefore suitable to investigate variation in diet choice (Layman et al., 2012). Muscle and liver tissues are frequently used to measure temporal variation in diet, because muscle tissue has a longer turnover time than the metabolically active liver tissue, and the two tissue types therefore integrate dietary information over different time frames (Boecklen et al., 2011; Bond et al., 2016). We therefore used the muscle C-isotopic composition as proxy for "long-term," and the liver C- isotopic composition as proxy for WILEV_Ecology and Evolution

"short-term" diet. For each individual, we calculated the difference between muscle and liver $\delta^{13}C (\Delta \delta^{13}C_{M-1} = \delta^{13}C_M - \delta^{13}C_1)$. We compared $\Delta \delta^{13}C_{M-1}$ values between habitats using paired *t*-tests.

Individual trophic specialization is measured by comparing the contributions of two components of the total niche width (TNW), the within-individual component (WIC) and the between-individual component (BIC, Roughgarden, 1972). The WIC describes the variance in resource use within individuals, while the BIC describes the variance in resource use among individuals (Roughgarden, 1972). To determine which individual was feeding on a more specialized vs. a more generalized diet, we determined the average Euclidean distance of the absolute values of $\Delta \delta^{13}C_{M-1}$ between all individuals per season. The resulting values represent the variation of diet shifts between individuals (BIC) of a population. $\Delta \delta^{13} C_{M-I}$ was considered the dietary variation of each individual (WIC). We therefore considered an individual a trophic specialist when WIC < BIC, and a trophic generalist when WIC ≥ BIC. After identifying which individuals were trophic specialists or generalists, we compared the proportion of specialists between habitats within seasons, and between seasons within habitats, using a generalized linear model including sex and standard length as covariates (binomial distribution and log link). Additionally, we investigated whether trophic specialists and generalists were distinguishable by their position within the niche. For this comparison, we used linear models with muscle δ^{13} C or $\delta^{15}N$ as dependent variable, and sampling period, habitat, and specialization as fixed factors, including all possible interactions using the packages Ime4 version 1.1-27.1 (Bates et al., 2015), and emmeans version 1.6.3 (Lenth, 2021) for Tukey's HSD post hoc comparisons.

2.3 Potential food organisms

We collected representative samples of the macroinvertebrate communities every second week from May to August 2020 on walls and bottom to characterize the habitats. The goal was not a thorough guantification of food resources in both habitats. Rather, we aimed for a qualitative assessment to support our understanding of the observed differences in trophic niches and habitat use of round gobies. Habitat differences and temporal dynamics in the composition of macroinvertebrate communities can help interpret differences in stable isotope analysis results in tissues integrating dietary signatures over different time frames. For example, macroinvertebrate community differences between habitats can provide information about how much isotopic difference in consumers are expectable and shifts in macroinvertebrate communities over time can be reflected in different isotopic compositions in muscle and liver tissues.

To sample the walls, we scraped off layers of biofouling at 2-4 locations ad libitum and collected them into a vial filled with 70% ethanol. To sample the bottom sediments, we used a Van Veen grab sampler. We then sampled the 2-3 cm of the sediment into vials filled with 70% ethanol.

In the laboratory, we identified the first 100 macroinvertebrates found in a sample under a dissecting microscope. To randomize the choice of organisms, we transferred the entire sample into a

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and randomly extracted material to be examined using a plastic pipette (3 ml). We identified the macroinvertebrates to the lowest practical taxonomical level (Order or Family). We did not quantify sessile macroinvertebrates (e.g., bivalves), as our methods were not suitable to sample organisms firmly attached to the substrate.

2.4 Use of nesting opportunities

To investigate whether gobies use nesting opportunities on vertical surfaces, we built five spawning traps (based on N'Guyen et al., 2018; Figure S2) that were deployed along vertical walls, as well as on the bottom underneath a wall. For each spawning trap, we attached two sets of five PVC tubes each (diameter: 4.5 cm, length: 20 cm) to a metal chain. The vertical distance between the two sets along the chain was 2.5 m. We deployed the traps at five sampling sites in the harbor Kleinhüningen by lowering the metal chain along the harbor wall until the lower trap reached the ground, while the other one was flush with the wall 2.5 m above the ground (Figure S2). Earlier studies confirmed that round gobies readily accept these traps as nesting opportunities (Hirsch et al., 2016; N'Guyen et al., 2018).

Between May 5 and August 24, 2020, we checked the traps for evidence of round goby spawning twice weekly. Such evidence included the presence of clutches with intact eggs, and/or traces of clutches (attachment filaments and empty eggshells). After having documented photographically any evidence for spawning, we removed the clutch or the clutch traces and redeployed the trap with cleaned tubes.

RESULTS 3

Trophic niche: Size, overlap, and position 3.1

3.1.1 | Niche size

The trophic niche size of gobies caught on the bottom was significantly larger than the one of gobies caught on the walls during the reproductive season (Figure 2, Table 1). The trophic niches were similar in size after the reproductive season (Figure 2, Table 1).

3.1.2 Niche overlap

The trophic niche of round gobies caught on walls overlapped significantly (>60%) with the trophic niche of the round gobies caught on the bottom during the reproductive season (Figure 2, Table 1). On the contrary, the trophic niche of round gobies caught on the bottom did not overlap significantly with the trophic niche on the wall during the reproductive season (Figure 2, Table 1). The trophic niches of round gobies overlapped significantly after the reproductive season (Figure 2, Table 1).

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FIGURE 2 Bi-plot of δ^{13} C and δ^{15} N with standard ellipses (enclosing 40% of the data) of bulk muscle from round gobies either caught during the reproductive season (left) or after the reproductive season (right). Light blue and light green colored triangles with solid ellipses represent gobies caught on the bottom, and dark blue or dark green points with dashed ellipses represent gobies caught on vertical harbor walls close to the surface. Outliers of >2 SD in δ^{15} N in the bottom data are marked in red. Mean ± SD of the baseline organisms killer shrimp and zebra mussel (indicated by species-symbols) are shown as black triangle (caught on bottom) or black circle (caught on wall).

	Reproductive seaso	n	Postreproductive se	Postreproductive season	
	Wall	Bottom	Wall	Bottom	
δ ¹³ C	-26.92 ± 0.34	-27.07 ± 0.55	-26.92 ± 0.32	-26.82±0.39	
$\delta^{15}N$	10.06 ± 0.73	9.74±0.67	10.23 ± 0.63	9.91±0.48	
SEA _C	0.56	1.18	0.66	0.72	
p	.002	.998	.347	.653	
Overlap of SEA with the other habitat, respectively	82.05%	50.20%	62.39%	73.04%	
Unique area of SEA	17.95%	49.80%	37.61%	27.96%	

TABLE 1 Niche characteristics of round gobies caught during and after the reproductive season on either the wall or the bottom.

Note: δ^{13} C and δ^{15} N (mean ± SD, ‰), SEA_C, *p* = probability of SEA of one habitat being larger than the respective other, overlap of SEA with the other habitat = mean proportion of the area of SEA_B per habitat that overlapped with the respective other habitat, unique area of SEA = mean proportion of the area of SEA_B per habitat that did not overlap with the respective other habitat.



FIGURE 3 Bootstrapped δ^{15} N and δ^{13} C range (n = 10,000, sample size per draw = 28) of round gobies caught on the harbor bottom or the harbor wall during and after the reproductive season based on muscle tissue.

3.1.3 | Isotopic ranges

Round gobies caught on the bottom displayed a wider δ^{13} C and δ^{15} N range during the reproductive season (Figures 2 and 3). The δ^{13} C range of gobies caught on the bottom was greater than on the wall, while there were no differences in the δ^{15} N range between the two habitats after the reproductive season (Figure 3).

3.1.4 | Niche position

The trophic position was higher on the wall than on the bottom in both seasons; however, this difference was only significant after the reproductive season (Table 2, Figure 4). Amphipods did not differ in their δ^{15} N signature between the habitats. In both habitats, the trophic position of round gobies was significantly lower after than during the reproductive season (Table 2, Figure 4), driven by a significant increase in δ^{15} N in amphipods (Table 2, Figure 2). Round gobies did not differ significantly in δ^{13} C between habitats during both seasons (Table 2, Figure 2). Amphipods differed significantly in δ^{13} C after the round goby reproductive season (Table 2, Figure 2). The δ^{13} C shift between the reproductive and postreproductive season in round gobies is consistent with the concomitant δ^{13} C shift for amphipods (Figure 2).

It is noteworthy that the results of δ^{15} N analyses are strongly influenced by outliers of >2 standard deviations among the gobies caught on the bottom (two outliers during the reproductive season, one outlier after the reproductive season, Figures 2 and 4). There were no technical or biological reasons to exclude these outliers. However, as these outliers are conspicuous, we conducted the same isotopic analyses that are presented here without the outliers. In summary, without including the outliers, the SEA of the bottom niche was similar in size to the SEA of the wall niche during the reproductive season and smaller after the reproductive season. The wall niche did not significantly overlap with the bottom niche and therefore displayed a larger unique area in both sampling seasons. The bootstrapped range of δ^{15} N on the bottom was smaller than the δ^{15} N range on the walls. All figures and results of the analyses without outliers are presented in Appendix S4.

3.2 | Individual specialization

The proportion of trophic specialists in the population was significantly lower on the wall (specialists: generalists = 15:21 (42% of total), Figure 5a) than on the bottom (specialists: generalists = 19:9 (68% of total), Figure 5b) during the reproductive season (odds ratio = 0.35, CI = 0.12-0.96, p = .046). The proportion of specialists was similar on the wall (specialists: generalists = 20:10 (67% of total), Figure 5c) and on the bottom (specialists: generalists = 20:16 (56% of total)) after the reproductive season (odds ratio = 0.87, CI = 0.27-2.77, p = .817). On the walls, the proportion of specialists was significantly higher after the reproductive season than during the reproductive season (odds ratio 4.52, CI = 1.13-21.17, p = .041). On the bottom, generalists were slightly but significantly larger than on the wall during the reproductive season (odds ratio = 0.48, CI = 0.23-0.91, p = .034). The covariates sex and standard length did not influence trophic specialization in any of the other models.

On the bottom, δ^{13} C was significantly lower in specialists than in generalists during the reproductive season (estimate = -0.43, Cl = -0.12 to (-0.75), t_{122} = 2.71, p =.008). On the wall, δ^{15} N was significantly higher in generalists than in specialists during the

TABLE 2 Results of linear models for δ^{13} C and δ^{13} N of round gobies and amphipods, and round goby trophic position including habitat,				
reproductive season and their interaction as fixed factors.				

Contrast: Bottom	Reproductive season					Postreproductive season			
- Wall		Est	CI	t _{126/52}	р	Est	CI	t _{126/52}	р
$\delta^{13}C$	Round goby	-0.15	-0.35 to 0.05	-1.50	.136	0.10	-0.09 to 0.30	1.05	.296
	Amphipods	-0.46	-1.10 to 0.18	-1.44	.157	1.87	1.02 to 2.72	4.41	.0001
$\delta^{15}N$	Round goby	-0.32	-0.63 to -0.01	2.02	.046	0.32	0.01 to 0.63	2.06	.042
	Amphipods	-0.17	-0.27 to -0.63	0.78	.438	0.12	-0.47 to 0.71	0.41	.687
Trophic position	Round goby	-0.06	-0.16 to -0.03	-1.39	.168	-0.12	-0.22 to -0.03	-2.70	.008
	Bottom					Wall			
Contrast: Rep. seas – post-rep. Season	ion	Est	CI	t _{126/52}	р	Est	CI	t _{126/52}	р
$\delta^{13}C$	Round g	oby 0.25	5 0.05 to 0.45	2.49	.014	-0.003	-0.35 to 0.05	-1.50	.136
	Amphip	ods 1.53	3 0.76 to 2.29	3.99	<.001	-0.80	–1.54 to –0.07	2.19	.033
$\delta^{15}N$	Round g	oby 0.17	-0.14 to 0.48	1.11	.280	0.17	-0.14 to 0.48	1.11	.269
	Amphip	ods 1.02	2 0.49 to 1.55	3.85	<.001	0.73	0.22 to 1.24	2.86	.006
Trophic position	Round g	oby 0.15	5 0.06 to 0.25	3.32	.001	0.10	0.01 to 0.19	2.09	.038

Note: Pairwise comparisons of contrasts were calculated using Tukey HSD post hoc tests.

Abbreviations: CI, 95% confidence interval; Est, estimate; p, p-value (printed in bold if p < .05); t_n , t ratio_{degrees of freedom (round goby/amphipods)};

FIGURE 4 Trophic position of round gobies caught on the harbor bottom or the harbor wall during and after the reproductive season based on muscle tissue. To calculate the trophic position, δ^{15} N values of Dikerogammarus villosus were used as a baseline.

FIGURE 5 Individual trophic specialization of round gobies based on the δ^{13} C difference between muscle (longer turnover time) and liver (shorter turnover time) tissues. A specialist is an individual, for which the difference between muscle and liver δ^{13} C ($\Delta\delta^{13}$ C_{M-I} = within-individual component, WIC) is smaller than the average Euclidean distance of the absolute values of $\Delta \delta^{13} C_{M-L}$ between all individuals per season (= betweenindividual component, BIC). A generalist is an individual for which WIC is equal or larger than BIC. Reproductive season: a: Bottom: nine generalists, 19 specialists. b: Wall: 21 generalists, 15 specialists. Postreproductive season: c: Bottom: 16 generalists, 20 specialists. d: Wall: 10 generalists, 20 specialists.

-25

-26

-27

-28

-25

-26

-27

-28

-29

Muscle

Liver

(b) Wall

δ¹³C (‰) -29



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reproductive season (estimate = 0.78, CI = 0.38-1.17, t_{122} = 3.89, p = .0002). This means that the unique region of the trophic niche of the bottom was dominated by trophic specialists, while the unique region of the trophic niche of the wall was dominated by trophic generalists (Figure 2). In contrast to that, δ^{15} N was marginally higher in specialists than in generalists on walls after the reproductive season (estimate = -0.43, CI = -0.89 to 0.02, t_{122} = -1.89, p = .061). On the bottom, no significant difference in δ^{15} N between specialists and generalists could be discerned in either season.

For gobies caught on the wall, we observed a significant difference between long-term (muscle) and short-term (liver) δ^{13} C (on average - 0.40‰) during the reproductive season (paired t-test, t_{35} =6.1, CI = 0.27-0.53, p <.0001, Figure 5a), whereas no δ^{13} C difference was observed on the bottom (paired *t*-test, $t_{27} = 0.35$,

CI = -0.14 to 0.19, p = .73, Figure 5b). This difference between longand short-term $\delta^{13}C$ was caused by a uniform shift toward more negative values in most of the generalist individuals, instead of random differences between liver and muscle shift of varying amounts δ^{13} C values as observed in the generalist individuals on the bottom (Figure 5b lines).

Live

Muscle

3.3 Composition of potential food organism communities

The interpretation of data in the context of niche dynamics and trophic specialization in round gobies was guided by the analyses of the macroinvertebrate communities on bottom and wall as potential

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food organisms. The composition of macroinvertebrates differed between the habitats throughout the reproductive season of round gobies. On the wall, chironomid larvae and oligochaetes dominated the communities between May and June, while amphipods increased in relative abundance from end of June on, and constituted a major part of the community in July and August (Figure 6). On the bottom, we found that oligochaetes and tipulid larvae dominated the communities, with the relative abundance of oligochaetes increasing in late summer (Figure 6).

3.4 | Population characteristics

Females were caught in equal numbers in both habitats and in both seasons (reproductive season: $X^2 = 0.08$, df = 1, p = .777, postreproductive season: $X^2 = 0.05$, df = 1, p = .829). The number of males caught was only slightly (not significantly) higher on the wall during the reproductive season ($X^2 = 1.52$, df = 1, p = .217), while it was significantly lower on the wall after the reproductive season ($X^2 = 11.84$, df = 1, p = .001). Results for the abundance of sexes are shown in Figure 7a,b.

Females were similar in size and weight in both habitats, while males were significantly larger and heavier on the wall during the reproductive season (Figure 7c, Table 2). In contrast, both females and males caught on the walls were significantly smaller and lighter than those caught on the bottom after the reproductive season (Figure 7d, Table 2; differences in standard length for females only marginally significant). Because of the correlation between standard length and log-transformed weight and the resulting similarities of the plots, we only graphically present the results for standard length in Figure 7c,d.

There were no differences in condition between individuals of both habitats, or between female and male gobies during the reproductive season (Figure 7e, Table 3). After the reproductive season, the condition factor of males caught on the bottom was higher than the condition factor of males caught on the walls (Figure 7f, Table 3). Females did not differ in condition factor in either season.

3.5 | Use of nesting opportunities

We found signs of spawning (clutches or traces of clutches) exclusively between May 29, 2020 and July 14, 2020. We did not find any signs of clutches in any of the traps before or after these dates. Round gobies spawned 48 times on the traps on the walls, and only twice in the traps on the harbor bottom (Figure 8).

4 | DISCUSSION

In this study, we use several indicators for differential habitat use to demonstrate how a subset of an invasive fish population might utilize a previously undescribed anthropogenic niche. Specifically, we compared population trophic niches, individual trophic specialization, phenotypic traits, and breeding frequencies between invasive round gobies using either the bottom or the vertical walls as habitat in a harbor ecosystem to find out whether vertical walls represent a niche extension of the whole population, or a specific subset of the population. Vertical walls as habitat for this bottom-dwelling fish have received little attention, despite their role in facilitating contact to potential translocation vectors, that is, boats. Our results indicate that use of vertical walls can be regarded as a specialization in habitat use of a population subset, which is characterized by greater trophic generalism and larger, heavier males during the breeding season. Additionally, breeding frequencies on walls were higher than on the bottom. After the breeding season, our results show less signs for habitat partitioning, as indicated by a greater similarity in trophic ecology. Additionally, our findings suggest that larger round gobies, which preferentially used the harbor walls during breeding season, migrate to the bottom toward the winter. While limited to one population, the results of this study help elucidate round goby ecology in comparable harbor ecosystems, their typical point of introduction into new areas.



FIGURE 6 Relative abundance of macroinvertebrates on the harbor walls and the habour bottom. Communities differed between the habitats and changed between early and late summer. Especially on the walls, there was a notable increase in amphipod abundance starting in July. FIGURE 7 Population characteristics of round gobies caught on the harbor bottom or the harbor wall. a, b: Abundance c, d: Standard length, and e, f: Condition factor (Fulton's *K*) of female and male gobies in both habitats in the reproductive season and after the reproductive season. Dots show original data, boxplots show the median (middle line), interquartile range (IQR, box) and values within 1.5 IQR (whiskers). Density plots show the distribution of data.



TABLE 3 Results of linear models for phenotypic traits of round gobies including habitat, reproductive season, sex, and all interactions as fixed factors.

Contrast: Bottom – Wall	Reproductive season					Postrep	Postreproductive season			
		Est	CI	t ₂₄₆	р	Est	CI	t ₂₄₆	р	
Standard length (cm)	Female	0.07	-0.61 to 0.75	0.19	.848	0.47	-0.05 to 0.99	1.74	.074	
	Male	-0.93	–1-68 to (–0.17)	-2.42	.016	1.17	0.57 to 1.77	3.84	<.001	
Condition (Fulton's K)	Female	0.01	-0.08 to 0.09	0.17	.868	0.03	-0.04 to 0.09	0.91	.366	
	Male	-0.03	-0.13 to 0.06	-0.69	.488	0.11	0.03 to 0.18	2.76	.006	
Weight (g)	Female	1.03	0.77 to 1.38	0.18	.856	1.26	1.01 to 1.58	2.04	.043	
	Male	0.67	0.48 to 0.93	-2.42	.016	1.65	1.27 to 2.14	3.78	<.001	

Note: Pairwise comparisons of contrasts were calculated using Tukey HSD post hoc tests. Weight was modeled using a log-transformation, but results are given on the original scale.

Abbreviations: CI, 95% confidence interval; Est, estimate; p, p-value; t_n , t ratio_{degrees of freedom}.

4.1 | Trophic niches are distinguishable between vertical and horizontal habitats

The niche size of round gobies on the walls was smaller than that of round gobies on the bottom, yet with a significant overlap. In our

conceptual framework (Figure 1), these results would indicate that all individuals forage on the walls, but only a subset of the population uses the bottom as a niche extension (reversed scenario B, Figure 1). However, there are parts of the isotope-space that are unique to each of the two habitats, and the bottom niche area does



FIGURE 8 Spawning activities of round gobies in spawning traps deployed on the harbor walls (top panel) or the harbor bottom (lower panel).

not overlap significantly with the wall niche area. Additionally, the bottom niche area decreases in size, and there is no significant overlap of the wall niche with the bottom niche, if the two outliers in $\delta^{15}N$ on the bottom are not considered (Appendix S4). There is no obvious biological or technical reason to exclude the observed outliers. However, already in previous work, we sporadically observed movement between the wall and the bottom (Bussmann & Burkhardt-Holm, 2020), indicating that at least some individuals change their habitat occasionally. Based on this observation and given that it hardly makes sense to interpret the well-described use of the bottom substrate as a niche extension for a population that primarily utilizes vertical habitat, we argue that the outlier gobies might have been caught in the "wrong habitat." Alternatively, these individuals could be bottom-dwellers, whose feeding habits are outliers to those of the main population. Outliers in biological traits regularly occur in round goby populations and might even disproportionally influence the invasion success of round gobies by driving population expansions ("individual trait utility hypothesis," Cerwenka et al., 2017). If we disregard the outliers-either because of their potentially misleading catch location or because of their disproportionate influence on the population average-the trophic niches would be of similar size with nonsignificant overlaps (Figure S3, Table S2). To interpret the patterns in niche differentiation found, we used the composition of macroinvertebrate communities in both habitats. As the available food resources (i.e., macroinvertebrate communities) were distinguishable, the position and overlap of the trophic niches either indicate consistently differential habitat use, or specialized habitat use by some, and flexible habitat use by other individuals, supporting a scenario between B and C (Figure 1).

After the breeding season, the overlap of trophic niches of gobies caught on the walls and the bottom was higher than during the breeding season, and the level of individual specialization did not differ between the habitats. Increasing similarity of the respective trophic niches may be due to a higher mobility of round gobies, and migration from the walls to the bottom after the reproductive season. In lake and sea habitats, round gobies migrate to deeper waters (up to >70m) in winter (Behrens et al., 2021; Carlson et al., 2021). While we do not know if comparable processes happen in rivers of relatively shallow depths (river Rhine in Basel, Switzerland: ca. 5–8m depth), our results presented here point toward a seasonally different habitat use, of which we probably only observed the beginning.

4.2 | Individual specialization is habitat-related

The uniform shift toward more negative δ^{13} C values from long- to short-term diets, which was exclusively observed in generalist gobies caught on walls, indicates that many round gobies use the walls as habitat consistently over a long time. If generalist feeding in wall-using individuals was caused by moving and feeding in-between both habitats, we would expect the variation between long- and short-term diet of individuals to be random, not uniform (Matthews & Mazumder, 2004). The uniform shift in isotopic composition could be explained if these gobies all started feeding on a different food resource during the time period that liver tissue integrates over, or if the C isotopic composition in the whole food web shifted. The shift of the relative abundances of food organisms approximately 1-2 months before we sampled the gobies during the reproductive season supports the interpretation that the shift in δ^{13} C in gobies caught on the walls is related to a change in their diet. More specifically, both chironomid larvae and amphipods are common prey organisms for round gobies in European rivers (Borcherding et al., 2013; Brandner, Auerswald, et al., 2013). It is therefore likely that a change in relative abundance of the two taxa would cause a dietary shift in round gobies, which is well reflected by the isotopic composition. As we did not observe a similar diet shift in round gobies caught on the bottom, we conclude that round gobies exhibited habitat partitioning during the breeding season. This finding is highly plausible considering that round gobies are generally territorial during the breeding season, with home ranges of ca. 5 ± 1.2 m² (Ray & Corkum, 2001). In particular, males defend their nests and reportedly remain stationary during courtship and nest guarding (Corkum et al., 1998). Although there is less reason for females and non-nestguarding sneaker males to remain in a constrained home range, we did not observe differences in the isotopic composition that could

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be related to the sex of the gobies. Other studies support that males and females have similar home ranges during the reproductive season (Marentette et al., 2009). We therefore conclude that specialized habitat use is not limited to nest-guarding males.

We found a correlation between $\delta^{15}N$ and generalist feeding on the walls during the reproductive season. In males, higher values of δ^{15} N and a more generalist diet could be correlated to mating strategy: Nest-guarding males may feed more opportunistically because of spatial constraints during nesting (McCallum et al., 2018). Additionally, reproductive males may forage less during the breeding season than nonreproductive males, which in turn can lead to higher δ^{15} N through starvation stress (Bowes et al., 2014; McCallum et al., 2018). If the higher nesting frequencies we found for individuals dwelling on the walls indicate that more reproductive males are present there, this could explain the correlation between $\delta^{15}N$ and generalist feeding in males. Similarly, in females, energy allocation toward gametes or hormonal state during the reproductive season might lead to higher δ^{15} N in muscle tissue due to fractionation of internal energy reserves (Shipley & Matich, 2020). The higher reproductive activity on walls might therefore lead to more enriched δ^{15} N in both sexes. After the reproductive season, a higher δ^{15} N in observed for gobies caught on the walls (i.e., the isotopic region unique to the walls) correlated with more specialist feeding. This supports the onset of a winter migration dominated by reproductive males: If reproductive males with a more generalist feeding strategy start migrating to the bottom, they leave behind nonreproductive individuals with a more specialist feeding strategy.

4.3 | Population characteristics and use of nesting opportunities indicate preferential use of vertical habitat

One explanation for habitat partitioning during the breeding season could be elevated competition between gobies for energy-rich resources and/or nesting opportunities. Some invasion fronts of round gobies are dominated by larger, more competitive individuals (Brandner, Cerwenka, et al., 2013; Gutowsky & Fox, 2011), indicating that those individuals might drive the colonization of new habitat under conditions of increasing competition. Considering that there was much more breeding activity on the wall than on the bottom, and that males caught on the wall were larger during the reproductive season, we conclude that the wall habitat is the preferred one, even though using it might be energetically more costly (Bussmann & Burkhardt-Holm, 2020). Advantages in terms of breeding could arise from the quantity or from the quality of nesting opportunities. For example, a greater availability of larger nesting sites in one habitat can lead to a preferential occupation of that habitat by larger males, as observed for sand gobies (Lehtonen & Lindström, 2004). Nesting opportunities and conditions might be generally more favorable on the walls, explaining the larger size of males on the wall during the reproductive season. The higher breeding frequency on the wall may also indicate that there are fewer naturally available options for breeding on the walls than on the bottom, so that more gobies use

the artificial spawning traps. In this case, larger males might prefer the walls primarily because of other reasons than nesting opportunities (e.g., higher density of zebra mussels, lower turbidity, and higher oxygen concentration), and once they encounter nesting opportunities, they readily use them. Larger males are more competitive in nest-holding potential, and they are preferred mating partners for females among ecologically similar goby species (Lehtonen et al., 2007; Lindström & Pampoulie, 2004; Marconato et al., 1989). A preferential use of nesting opportunities off the ground by the larger individuals would make boat hulls, therefore, even more prone to become breeding grounds, and eventually translocation vectors, for a particularly competitive subset of round gobies.

After the reproductive season, the population characteristics calculated from the total catch of all individuals support our interpretation of the presented trophic niche data as sign of a beginning migration to deeper habitats (see above). For both sexes, round goby specimens were smaller and lighter on the walls than on the bottom. Moreover, the number of males on the bottom was higher than on the wall, and males caught on the walls had a lower condition. The lower condition factor of males on the walls could be explained by a higher energy requirement during the reproductive season due to nesting activities. These results indicate that after the end of breeding activities, formerly reproductive individuals (particularly nest-guarding males) with small home ranges and generalist feeding strategies leave the walls and start inhabiting the bottom, while the smaller individuals with a more specialist feeding strategy remain on the walls. Marentette et al. (2011) also observed a higher mobility of males than females after the reproductive season. A greater dietary similarity after the reproductive season between alternative reproductive tactics in males could partly explain why the population niche size on the bottom decreases, despite the migrations of some individuals from the wall to the bottom (McCallum et al., 2018). A decreasing variability of available food resources, as was observed over the course of the summer, especially on the bottom, could provide an additional explanation for the smaller niche size, as well as for the high level of specialization in general.

4.4 | Implications for invasion success and translocation

Our results indicate the possibility of the development of resource polymorphisms (habitat-related differential phenotypes), that can develop under conditions of high gene flow, or by habitatrelated assortative mating (Lang et al., 2020). The latter could promote microgeographic adaptations to either horizontal or vertical habitat use in round gobies. While in theory, gene flow should prevent population differentiation over small spatial scales, nonrandom dispersal and habitat choice allow for the development of microgeographic divergence within populations (Edelaar et al., 2008; Mortier et al., 2019). This divergence can lead to local adaptations, if mating happens selectively in the chosen habitat (Edelaar et al., 2008; Richardson et al., 2014). Indeed our data point toward a nonrandom habitat use, especially during the breeding season. If assortative mating happens between wall and bottom inhabiting gobies, there should be genetic or morphological differences detectable. For example, in sticklebacks, consistent habitat choice leads to phenotypic divergence in populations of stream- and lake inhabiting individuals (Bolnick et al., 2009), and differences in substrate and depth correlated with morphological variance as well as genotype on some loci even within one population over negligible spatial scales (Maciejewski et al., 2020). On the contrary, resource polymorphisms related to habitat use can also develop under conditions of high gene flow, and morphological differentiation between habitats is not necessarily linked to genetic differentiation (Lang et al., 2020). In round gobies, Cerwenka et al. (2013) documented morphological differences correlating with substrate type, which developed within <15 generations, yet the role of adaptive processes in this case remains unclear. Interestingly, these results were also generated in a context of natural versus man-made habitat, demonstrating the potential of anthropogenic alterations to affect the phenotype of fish. Understanding the use of different habitats and within-population resource polymorphisms is important to estimate the impacts of invasive species on food chains and ecosystems (Lang et al., 2020).

As male gobies using the walls in the breeding season are larger and heavier, we hypothesize that wall climbing behavior could select for more competitive individuals. Because the use of walls as habitat can lead to contact with boat hulls (Bussmann & Burkhardt-Holm, 2020), new founding populations might be dominated by this more competitive subset of the round goby population in harbors. Going one step further, this aspect may in turn, at least partly, explain why round gobies are so successful in translocating from their native habitats and in invading new territories. Adaptive use of anthropogenic habitat such as harbor walls might promote invasions (Hufbauer et al., 2012). Anthropogenic ecosystems such as harbors strongly resemble each other at different locations, so that adaptations of a source population to the original habitat would benefit the founder population after translocation to a new, but similar habitat (Hufbauer et al., 2012). In the case of the round goby, the translocated portion of a population might additionally be an above-average competitive subset of the species. Apart from adaptive processes, vertical habitat use could allow populations in harbors to be larger than previously estimated in studies only considering the bottom as habitat (N'Guyen et al., 2018; Young et al., 2010). This higher population size could lead to an increased uptake of propagules, and therefore a higher probability of successful translocation (Cassey et al., 2018). Our results thus not only help to explain why round gobies are such successful invaders. They will be highly relevant for future models estimating population densities and invasion potentials of round gobies in harbors, their typical point of entry into novel ecosystems.

AUTHOR CONTRIBUTIONS

Karen Bussmann: Conceptualization (lead); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); project administration (lead); visualization (lead); writing – original draft (lead); writing – review and editing (equal). **Philipp Emanuel Hirsch:** Conceptualization (equal); formal analysis (supporting); methodology (supporting); supervision (supporting); validation (equal); visualization (supporting); writing – review and editing (equal). **Moritz F. Lehmann:** Investigation (supporting); methodology (supporting); resources (supporting); validation (equal); writing – review and editing (equal). **Patricia Burkhardt-Holm:** Conceptualization (supporting); funding acquisition (lead); investigation (supporting); methodology (supporting); project administration (supporting); resources (lead); supervision (lead); validation (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Dryad at https://doi.org/10.5061/dryad.05qfttf4z.

OPEN RESEARCH BADGES

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This article has earned Open Data and Open Materials badges. Data and materials are available at https://doi.org/10.5061/dryad.05qft tf4z.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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