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Coexisting with the alien: Evidence for environmental control on trophic interactions between a native (*Atherina boyeri*) and a non-indigenous fish species (*Gambusia holbrooki*) in a Mediterranean coastal ecosystem

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Biological invasions are a widespread problem worldwide, as invasive non-indigenous species (NIS) may affect native populations through direct (e.g., predation) or indirect (e.g., competition) trophic interactions, leading to changes in the food web structure. The trophic relationships of the invasive eastern mosquitofish *Gambusia holbrooki* and the native big-scale sand smelt *Atherina boyeri* coexisting in three Mediterranean coastal ponds characterized by different trophic statuses (from oligotrophic to hypereutrophic) were assessed in spring through isotopic niche analysis and Bayesian mixing models. The two fish relied on the distinctive trophic pathways in the different ponds, with the evidence of minimal interspecific niche overlap indicating site-specific niche divergence mechanisms. In more detail, under hypereutrophic and mesotrophic conditions, the two species occupied different trophic positions but relying on a single trophic pathway, whereas, under oligotrophic conditions, both occupied a similar trophic position but belonging to distinct trophic pathways. Furthermore, the invaders showed the widest niche breadth while the native species showed a niche compression and displacement in the ponds at a higher trophic status compared to the oligotrophic pond. We argue that this may be the result of an asymmetric competition arising between the two species because of the higher competitive ability of *G. holbrooki* and may have been further shaped by the trophic status of the ponds, through a conjoint effect of prey availability and habitat complexity. While the high trophic plasticity and adaptability of both species to different environmental

features and resource availability may have favored their coexistence through site-specific mechanisms of niche segregation, we provide also empirical evidence of the importance of environmental control in invaded food webs, calling for greater attention to this aspect in future studies.

KEYWORDS

biological invasion, alien species, stable isotopes, mosquitofish, sand smelt, coastal ponds

Introduction

The invasion of non-indigenous species (NIS) is considered among the major threats for marine ecosystem functioning and services worldwide. The consequences of species invasion and establishment in recipient ecosystems are complex and depend on the interaction between the ecological characteristics of the invader species and the environmental and biological attributes of the recipient ecosystem (Occhipinti-Ambrogi, 2007; Chan and Briski, 2017). In particular, while high dispersal ability and physiological plasticity, fast growth, high feeding rate, and generalist feeding strategies are among the key factors of success for invasive NIS (David et al., 2017 and reference therein), the establishment of self-sustaining NIS populations is further favored in disturbed habitats (Chan and Briski, 2017).

Change in trophic dynamics and structure with severe consequences for native species is one of the most common ecological processes that the establishment of invasive NIS may trigger in recipient ecosystems (Jackson et al., 2012; Britton et al., 2018; Costa-Pereira et al., 2019). Depending on the trophic position of the invader species, direct trophic interactions with native species can exert bottom-up or top-down cascading controls on local food webs (Vander Zanden et al., 1999; Gallardo et al., 2016). On the contrary, interspecific exploitative competition mechanisms may occur when invasive and native species are trophically analogous and may lead to competitive exclusion or coexistence through niche differentiation mechanisms, such as spatial segregation or resource partitioning (David et al., 2017 and references therein). As invasive NIS are known for their high competitive abilities, they may induce changes in habitat or resource use by natives, displacement and/or contraction of their trophic niche, and alteration of trophic interactions and food web structure (Vander Zanden et al., 1999; Carmona-Catot et al., 2013; Tran et al., 2015). Therefore, the study of trophic niche and food web structure in invaded ecosystems can help to reveal the ecological changes driven by the biological invasion, to understand how trophic relationships between invasive and native species promoted their coexistence, and to predict the future evolution of recently invaded ecosystems.

The eastern mosquitofish *Gambusia holbrooki* has been intentionally introduced from the southern USA to European

and Australian freshwaters since the early 1900s with the purpose to control mosquito populations and reduce the risk of spreading mosquito-related diseases. In addition to freshwaters, *G. holbrooki* thrives in a wide range of habitats, including estuaries and near-shore marine areas, and has been identified as one of the most widespread invasive fish worldwide (Lowe et al., 2000). It is a voracious predator with high feeding rates (Rehage et al., 2005), also known as an omnivore with opportunistic feeding strategies (Blanco et al., 2004; Kalogianni et al., 2014). A large array of terrestrial and aquatic organisms have been identified as common prey, along with macrophyte detritus (Blanco et al., 2004; Rehage et al., 2005; Remon et al., 2016). Besides high physiological adaptability and trophic plasticity and feeding rates, viviparity, high fecundity, and resistance to pollutants provide mosquitofish with a high competitive ability compared to native fish (Pyke, 2005).

Overall, a strong competitive impact of *G. holbrooki* on native species has been well documented in a variety of ecosystems (Alcaraz et al., 2008; MacDonald et al., 2012; Ruiz-Navarro et al., 2013). In particular, laboratory and field studies demonstrated the influence of abiotic factors in shaping the performance of mosquitofish, including the competitive effects on native species (Rincón et al., 2002; Blanco et al., 2004; Carmona-Catot et al., 2013; Ruiz-Navarro et al., 2013). High frequency of aggressive behaviors and higher feeding rate than native species have been observed at higher water temperature (Rincón et al., 2002; Carmona-Catot et al., 2013) and lower salinity (Alcaraz et al., 2008; Ruiz-Navarro et al., 2013). On the contrary, a limited influence of habitat features (e.g., size and complexity) and water quality (e.g., nutrient concentration and turbidity) on mosquitofish life-history traits and predation has been reported (Blanco et al., 2004; Cano-Rocabayera et al., 2019). Even with the large body of literature existing on the ecological effects of invasive fish, including mosquitofish, in aquatic systems, the trophic aspects have been scantily addressed especially in combination with environmental stressors.

Carbon and nitrogen stable isotope analysis (SIA, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$) is a powerful tool to investigate trophic interactions (e.g., Michener and Kaufman, 2008; Mancinelli and Vizzini, 2015; Nielsen et al., 2018) and has been widely used to describe trophic niche features within an isotopic framework (e.g., isotopic niche breadth and overlap) (Chen et al., 2011;

Abrantes et al., 2014), ontogenetic diet shifts (Layman et al., 2011; Andolina et al., 2020), and organic matter pathways (Vizzini et al., 2005; Signa et al., 2013b). Furthermore, SIA has the potential to infer the effects of invasive species on aquatic food webs, including resource shift deriving from intraspecific and interspecific competitions (Jackson et al., 2012; Mancinelli et al., 2017; Britton et al., 2018).

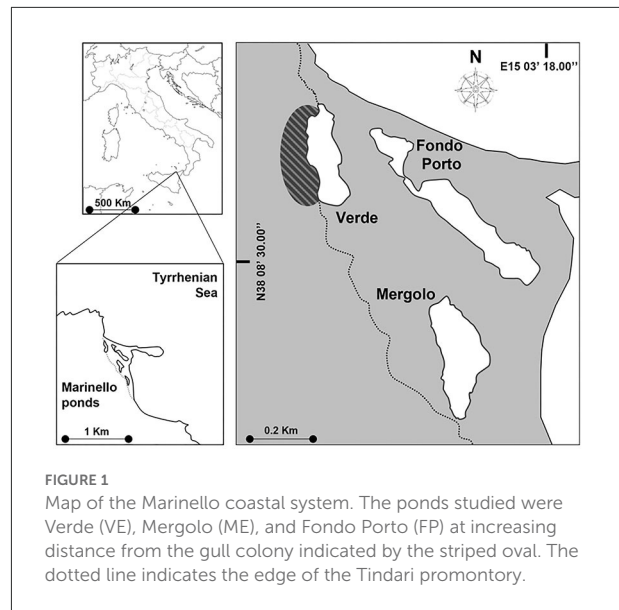
Here, we studied the isotopic niche as a proxy for the trophic niche (*sensu* Bearhop et al., 2004) of the invasive eastern mosquitofish *G. holbrooki* (Girard, 1859) and the native big-scale sand smelt *Atherina boyeri* (Risso, 1810), coexisting in a Mediterranean coastal system (Marinello ponds, Italy) featured by several small, shallow, and brackish ponds with different marine influences, geomorphological features, and trophic conditions (from oligotrophic to hypereutrophic) due to the external subsidies of gull guano (Signa et al., 2012). The big-scale sand smelt is a small euryhaline fish that inhabits the littoral zones of the Eastern Atlantic and the Mediterranean Sea, where it is frequently found in large schools both along the coasts and in lagoons and coastal lakes (Kara and Quignard, 2019). Alongside physiological adaptability, the big-scale sand smelt shows also high trophic plasticity and a generalist trophic behavior (Vizzini and Mazzola, 2002, 2005).

We hypothesize that generalist feeding behavior and trophic plasticity of both *G. holbrooki* and *A. boyeri* represent strategies which promote the coexistence of the two species through resource partitioning, therefore resulting in the separation of the trophic niche and different isotopic niche features (e.g., breadth, overlap). We also hypothesize that the contrasting trophic conditions may modulate this process by shaping the trophic niches according to prey availability and habitat complexity.

Materials and methods

Study area

The study was carried out in the coastal system of Marinello located along the north-eastern coast of Sicily (Italy, Mediterranean Sea) (Figure 1), consisting of five small (1–4 ha), shallow (max depth: 2–4 m), and brackish (mean salinity: 26–34 PSU) ponds (Verde, Fondo Porto, Porto Vecchio, Mergolo, and Marinello), separated from the adjacent sea by littoral bars and lacking direct freshwater input (Mazzola et al., 2010). The present research focused on three of the five ponds (i.e., VE, Verde; ME, Mergolo; and FP, Fondo Porto), on which several studies were conducted and found high inter-pond variability in terms of trophic status and primary production (Signa et al., 2012), contamination level (Signa et al., 2013a,b), macrobenthic communities (Signa et al., 2015), and trophic structure (Vizzini et al., 2016). These differences were attributed to the guano-derived fertilization induced by the colony of the



yellow-legged gull *Larus michahellis* (Naumann, 1840) resident in the cliff next to the pond VE alongside the variability in geomorphological features of the ponds. The deeper landward ponds, VE, and ME (max depth, respectively, 3 and 3.5 m, Mazzola et al., 2010), are characterized by seabird-induced hypereutrophication (i.e., guanotrophication) and mesotrophic conditions, respectively (mean Chl-*a*: 44.7 and 8.8 mg m⁻³; mean TSI_{CHL} (Trophic State Index, *sensu* Acquavita et al., 2015): 65 and 41 mg m⁻³), resulting in high (although seasonally fluctuating) phytoplanktonic production and high internal variability (littoral vs. deeper area) (Signa et al., 2012, 2015). In contrast, the smaller (1.3 ha) and shallower (1.2 m deep, Mazzola et al., 2010) seaward pond FP is oligotrophic (mean Chl-*a*: 3.3 mg m⁻³; mean TSI_{CHL}: 35 mg m⁻³) and features higher water transparency and a macrophyte-covered seabed (Signa et al., 2012, 2015). Accordingly, biotic communities and trophic pathways vary among ponds (Vizzini et al., 2016), with benthic assemblages partially mirroring the strong environmental gradients, namely showing not only decreasing abundances from FP to VE but also the highest structural and functional diversity in ME (Signa et al., 2015). As regards fish, comparable assemblages characterize the three Marinello ponds, with the native *A. boyeri* and the invasive *G. holbrooki* among the most abundant species in the three ponds, where they coexist throughout the year, with the highest abundance in spring (Vizzini et al., 2016).

Sample collection

Atherina boyeri and *Gambusia holbrooki* specimens were collected using a small beach seine (4 m length, 3 mm mesh) in the three ponds Verde (VE), Mergolo (ME), and Fondo

TABLE 1 Sample size (*N*), biometric measures (Mean \pm SD), and trophic position (TP) of small and large specimens of *A. boyeri* (ATE) and *G. holbrooki* (GAM) from the three ponds of the Marinello coastal system.

Pond	Species	Size-class	N	Standard length (mm)	Wet weight (mg)	TP
Verde	ATE	Small	10	23.8 \pm 1.6	121.8 \pm 19.6	3.8 \pm 0.2
		Large	10	32.9 \pm 1.6	336.4 \pm 51.9	3.8 \pm 0.2
	GAM	Small	8	22.3 \pm 1.3	141.4 \pm 35.9	3.3 \pm 0.4
		Large	10	38.3 \pm 1.4	1,098.7 \pm 251.6	3.1 \pm 0.2
Mergolo	ATE	Small	10	24.9 \pm 1.2	120.9 \pm 15.5	3.1 \pm 0.2
		Large	9	35.1 \pm 6.5	423.7 \pm 282.1	3.1 \pm 0.1
	GAM	Small	9	21.8 \pm 1.1	139.9 \pm 36.7	3.7 \pm 0.4
		Large	8	35.2 \pm 4.6	517.2 \pm 314.6	3.3 \pm 0.2
Fondo	ATE	Large	9	35.7 \pm 1.7	343.6 \pm 65.0	2.8 \pm 0.4
Porto	GAM	Small	10	21.5 \pm 0.8	119.2 \pm 36.1	3.2 \pm 0.3
		Large	5	31.7 \pm 0.3	352.3 \pm 84.3	3.2 \pm 0.2

Porto (FP) in spring 2012 when the ponds host the highest diversity and abundances of fish, invertebrates, and organic matter sources (Signa et al., 2015). The hauls were performed at each pond in triplicate by dragging the seine on a perpendicular line (10 m) from a depth of about 1.5 m up to the shoreline. In addition, the percent coverage of macrophytes (macroalgae and seagrasses) was estimated by visual census along the shores and the central area of each pond and ranked according to the marine version of the Braun-Blanquet score developed by Kenworthy et al. (1993) as a proxy for habitat complexity.

After collection, fish were kept cool and in the dark upon arrival at the laboratory, where they were identified, subjected to biometric measurements (standard length SL, wet weight WW), and grouped per size class: small (SL < 30 mm) and large (SL > 30 mm), according to previous studies carried out in Mediterranean lagoons (Vizzini and Mazzola, 2002; Blanco et al., 2004). Both size classes of the two species were found in all the ponds, except for small *A. boyeri*, which was not found in FP. Total fish abundance was calculated for both species and size class by pooling data obtained from the three hauls and expressed as individuals per 100 m². Moreover, a minimum of five and a maximum of ten specimens per size class were randomly taken for each species from each pond (Table 1) and processed for isotopic analyses, with this sample size being sufficient to ensure reliable isotopic niche determination through the specific statistical package SIBER (Jackson et al., 2011; see Section Data analysis for details). Dorsal muscle was dissected, dried at 60°C to constant weight, and ground to a fine homogeneous powder using a mortar and pestle. Stable isotope analysis was performed through an isotope ratio mass spectrometer (Thermo Delta Plus XP) connected to

an elemental analyzer (Thermo Flash EA 1112). Stable isotopes were expressed in standard delta (δ) notation as parts per thousand (‰):

$$\delta^{13}\text{C or } \delta^{15}\text{N} = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) / R_{\text{standard}} \right] \times 1000,$$

where *R* is the ratio ¹³C:¹²C or ¹⁵N:¹⁴N. The results were reported relative to Vienna Pee Dee Belemnite (VPDB) for $\delta^{13}\text{C}$ and atmospheric air for $\delta^{15}\text{N}$. Analytical precision based on the standard deviation of replicates of internal standards (International Atomic Energy Agency IAEA-CH-6 for $\delta^{13}\text{C}$ and IAEA-NO-3 for $\delta^{15}\text{N}$) was 0.1‰ for $\delta^{13}\text{C}$ and 0.2‰ for $\delta^{15}\text{N}$.

Data analysis

To assess isotopic niche breadth and overlap of the populations of the two species (*Atherina boyeri* and *Gambusia holbrooki*) across size classes in the three ponds, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data were first corrected to avoid any potential bias given by differences in basal resources of the three ponds (Olsson et al., 2009).

The surface-grazing snail *Hydrobia ventrosa* was used as baseline, according to Vizzini et al. (2016), to correct both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. In more detail, the corrected $\delta^{13}\text{C}$ was calculated following the equation by Olsson et al. (2009):

$$\delta^{13}\text{C}_{\text{corr}} = \delta^{13}\text{C}_f - \delta^{13}\text{C}_{\text{bmean}} / \text{CR}_b,$$

where $\delta^{13}\text{C}_f$ is the carbon isotopic value of the fish, $\delta^{13}\text{C}_{\text{bmean}}$ is the mean carbon isotopic value of the baseline ($\delta^{13}\text{C}$ mean value \pm standard deviation: $-17.4 \pm 0.7\text{‰}$ in VE, $-16.4 \pm 0.5\text{‰}$ in ME, and $-17.4 \pm 0.2\text{‰}$ in FP), and CR_b is the carbon range ($\delta^{13}\text{C}_{\text{max}} - \delta^{13}\text{C}_{\text{min}}$) of the baseline (1.3‰ in VE, 1.0‰ in ME, and 0.3‰ in FP) (data from Vizzini et al., 2016).

The same baseline was used to correct $\delta^{15}\text{N}$, i.e., estimating the fish trophic position (TP) following the equation by Post (2002):

$$\text{TP} = \left[\left(\frac{\delta^{15}\text{N}_f - \delta^{15}\text{N}_b}{\Delta_n} \right) + \lambda, \right]$$

with $\delta^{15}\text{N}_f$ being the nitrogen isotopic value of the fish, $\delta^{15}\text{N}_b$ being the mean nitrogen isotopic value of the baseline ($\delta^{15}\text{N}$ mean value \pm standard deviation: $13.4 \pm 0.4\text{‰}$ in VE, $6.9 \pm 0.7\text{‰}$ in ME, and $10.9 \pm 0.1\text{‰}$ in FP), Δ_n being the expected enrichment in $\delta^{15}\text{N}$ per trophic level (3.4‰ according to Post, 2002), and λ being the trophic level of the species used as a baseline that was set as 2.

Corrected $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data were then used to estimate the standard ellipse area corrected for small sample size (SEAc), the SEAc overlap, and the Bayesian standard ellipse area (SEAb) (Jackson et al., 2011) using the SIBER package v 2.1.5 (Stable Isotope Bayesian Ellipses in R) (Jackson et al., 2011) in R v.

TABLE 2 Macroalgae and seagrass percentage cover of the three ponds of the Marinello coastal system (shores and central area) ranked according to the marine version of the Braun-Blanquet score (Kenworthy et al., 1993).

Pond	Site	Seagrass cover %		Macroalgae cover %		BB score range
		Min	Max	Min	Max	
Verde	Shore	0	25–100	0	75–50	0–5
	Center	0	0	0	0	0
Mergolo	Shore	0	25–50	0	50–75	0–4
	Center	25–50	100	0	25–50	0–5
Fondo Porto	Shore	5–25	50–75	5–25	50–75	2–4
	Center	25–50	25–50	25–50	75–100	3–5

4.0.2 (R Core Team, 2020). The SEAc encompassed 40% of the data and was expressed as single values; the SEAb was derived from 100,000 posterior iterations and was expressed as a range of probable values reported in posterior density plots reflecting estimation uncertainty (Jackson et al., 2011). Differences in TP and SEAb among ponds, fish species, and size classes were tested, respectively, with non-parametric permutational analysis of variance (PERMANOVA, PRIMER-E Ltd., Plymouth, UK; Anderson, 2017) and ANOVA (R v. 4.0.2; R Core Team, 2020) followed by pairwise comparisons.

In addition, to estimate the main trophic pathways sustaining the two species' population in the ponds, Bayesian mixing models were run using MixSIAR v 3.1.11 (Stock et al., 2018) in R (R Core Team, 2020). Carbon and nitrogen stable isotope data (not corrected) of all possible basal sources of organic matter in each pond were taken from Vizzini et al. (2016). Sources included in the models were seagrasses, macroalgae, suspended particulate organic matter (SPOM), and sedimentary organic matter (SOM) (see Supplementary Table S1 for species list and isotopic values). Trophic enrichment factors (TEFs) used in the model were, respectively, $0.4 \pm 1.3\text{‰}$ for $\delta^{13}\text{C}$ and $3.4 \pm 1.0\text{‰}$ for $\delta^{15}\text{N}$, according to Post (2002), which were doubled as these fish are secondary consumers/omnivores. Whenever more than one species belonged to the seagrass or macroalgae source categories, the mixing model output was reported as the a posteriori sum of the contribution of each species (see Supplementary Table S1).

Results

The three ponds were characterized by different habitat complexity in terms of macrophyte cover and marine Braun-Blanquet score. High internal variability characterized both ponds at a higher trophic status, Verde (VE) and Mergolo (ME), in contrast to what was observed in the oligotrophic pond Fondo Porto (FP). In particular, the shores of both VE and ME were covered with patches of seagrasses and macroalgae interspersed with bare bottom ranging from 0 to 50% for seagrasses in

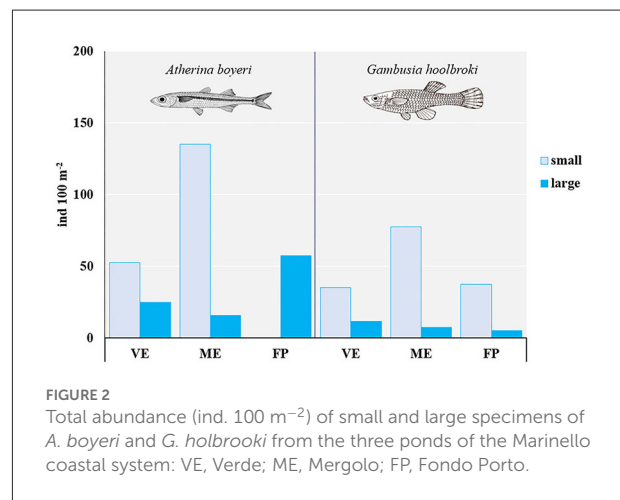


FIGURE 2 Total abundance (ind. 100 m⁻²) of small and large specimens of *A. boyeri* and *G. holbrooki* from the three ponds of the Marinello coastal system: VE, Verde; ME, Mergolo; FP, Fondo Porto.

both ponds and from 0 to 100% and to 75% for macroalgae in VE and ME, respectively (Table 2). In contrast, the shores of FP were entirely covered by macrophytes with the percentage ranging between 5 and 75% for both seagrasses and macroalgae (Table 2). The greatest variability among the ponds was found in the central areas, with no macrophytes recorded in VE, patchy macrophyte cover in ME (25–100 and 0–50% for seagrasses and macroalgae, respectively), and higher and more homogeneous coverage of both seagrasses (25–50%) and macroalgae (25–100%) in FP. Accordingly, the marine Braun-Blanquet score varied from 0 to 5 in both VE and ME and only from 2 to 5 in FP (Table 2).

Overall, the native big-scale sand smelt *A. boyeri* outnumbered the invasive eastern mosquitofish *G. holbrooki* (Figure 2). Small specimens predominated over large specimens in each pond, except for *A. boyeri* in FP where no small fish were found and were more abundant in ME than in the other ponds.

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of fish varied among the three ponds according to their trophic status: values were overall more depleted in both carbon and nitrogen in the mesotrophic ME, intermediate in carbon and most enriched in nitrogen in the guanotrophic VE, and most enriched in carbon and intermediate

in nitrogen in the oligotrophic FP (Figure 3A). Similarly, the standard ellipse area (SEAc), representing the “core” isotopic niche of the two fish species, showed a clear grouping by pond within the corrected isotopic space ($\delta^{13}\text{C}_{\text{corr}}$ -TP bi-plot) (Figure 3B). The niche of both species collected in ME showed a more carbon-depleted position, followed by the niches in VE and FP, which showed the most carbon-enriched position. Within each pond, the niches of the two fish across size classes were positioned in different ways: both in VE and ME, the niches of the two species showed a similar positioning along the $\delta^{13}\text{C}_{\text{corr}}$ axis, while they clustered apart along the TP axis, with *A. boyeri* at higher TP values than *G. holbrooki* in VE and conversely in ME (Figure 3B). In contrast, in FP, the niches of the two species differed mainly in $\delta^{13}\text{C}_{\text{corr}}$ values, with more $^{13}\text{C}_{\text{corr}}$ -enriched values for *G. holbrooki* than *A. boyeri* (Figure 3B). In line with these patterns, the niche overlap between the two species at all size classes was negligible in all the ponds (range: 0–12%, Table 3). High intraspecific SEAc overlap was observed for *A. boyeri*: 87 and 98% of the niche of large specimens overlapped with that of small specimens in VE and ME, respectively (Figure 3B, Table 3). In contrast, only a partial overlap was observed between the two size classes of *G. holbrooki* in all the ponds (38, 33, and 24%, respectively, in VE, ME, and FP; Figure 3B, Table 3).

The isotopic niche breadth of the two species, expressed as SEAb, showed overall wider niches for small mosquitofish than all the other fish and wider niche in FP than in the other ponds (Figure 4). Moreover, in the three ponds, the niche breadth of both mosquitofish and sand smelt was significantly wider in small than large specimens ($p < 0.001$), but only in VE and ME, the niche breadth of sand smelt was smaller than that of both mosquitofish size classes (Figure 4). At the same time, comparing ponds, both small

and large *G. holbrooki* showed significant decreasing niche breadth from FP to ME and VE, while large *A. boyeri* showed larger niche in FP than both ME and VE ($p < 0.001$). Similar to the large specimens, the niche of small *A. boyeri* was rather narrow and comparable between VE and ME (Figure 4).

The trophic position (TP) estimated showed that the two species broadly occupy a trophic level comprised between 3 and 4 while varying among ponds, species, and size classes (Table 1, Supplementary Table S2). Moreover, in the eutrophic pond VE, *A. boyeri* showed a significantly higher TP than *G. holbrooki*, while the opposite trend emerged in the other two ponds, where *G. holbrooki* showed higher TP than *A. boyeri*. Moreover, *A. boyeri* showed significantly increasing TP values from FP to ME and VE and *G. holbrooki* showed higher TP only in ME than in the other ponds. As regards size classes, only mosquitofish showed significant differences with higher values in small than large specimens (Table 1, Supplementary Table S2).

Mixing models revealed that the basal organic matter sources provided a different proportional contribution to the trophic pathways sustaining the two fish species in the different ponds (Figure 5, Supplementary Table S1 for details). In VE, sedimentary organic matter (SOM) was the dominant basal source underlying the diet of all fish, with the exception of large mosquitofish, for which the proportional contribution of SOM decreased in favor of macroalgae. A clear different pattern was found in ME, where the suspended particulate organic matter (SPOM) was the prevailing basal resource in the trophic pathways supporting the diet of all fish (Figure 5, Supplementary Table S1). Lastly, in FP, all the basal resources contributed in similar proportions to the pathways underlying the diet of large sand smelts, while macroalgae and seagrasses prevailed for mosquitofish (Figure 5, Supplementary Table S1).

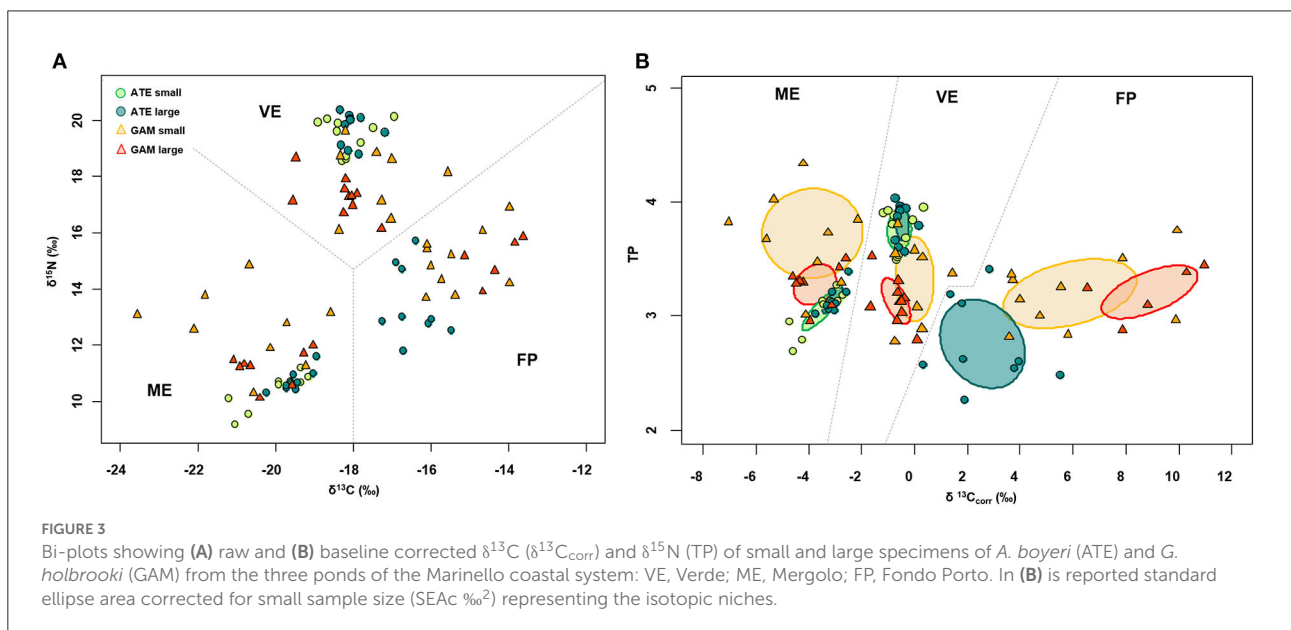
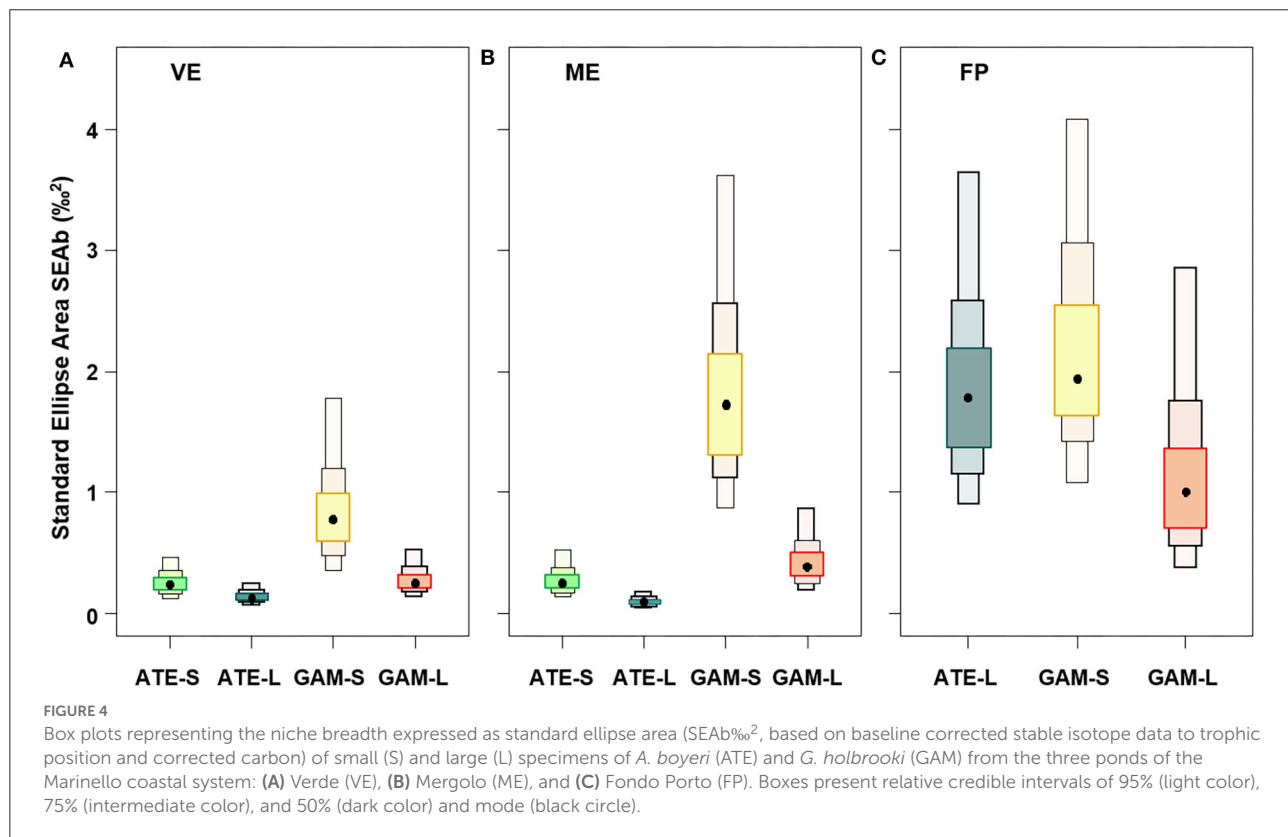
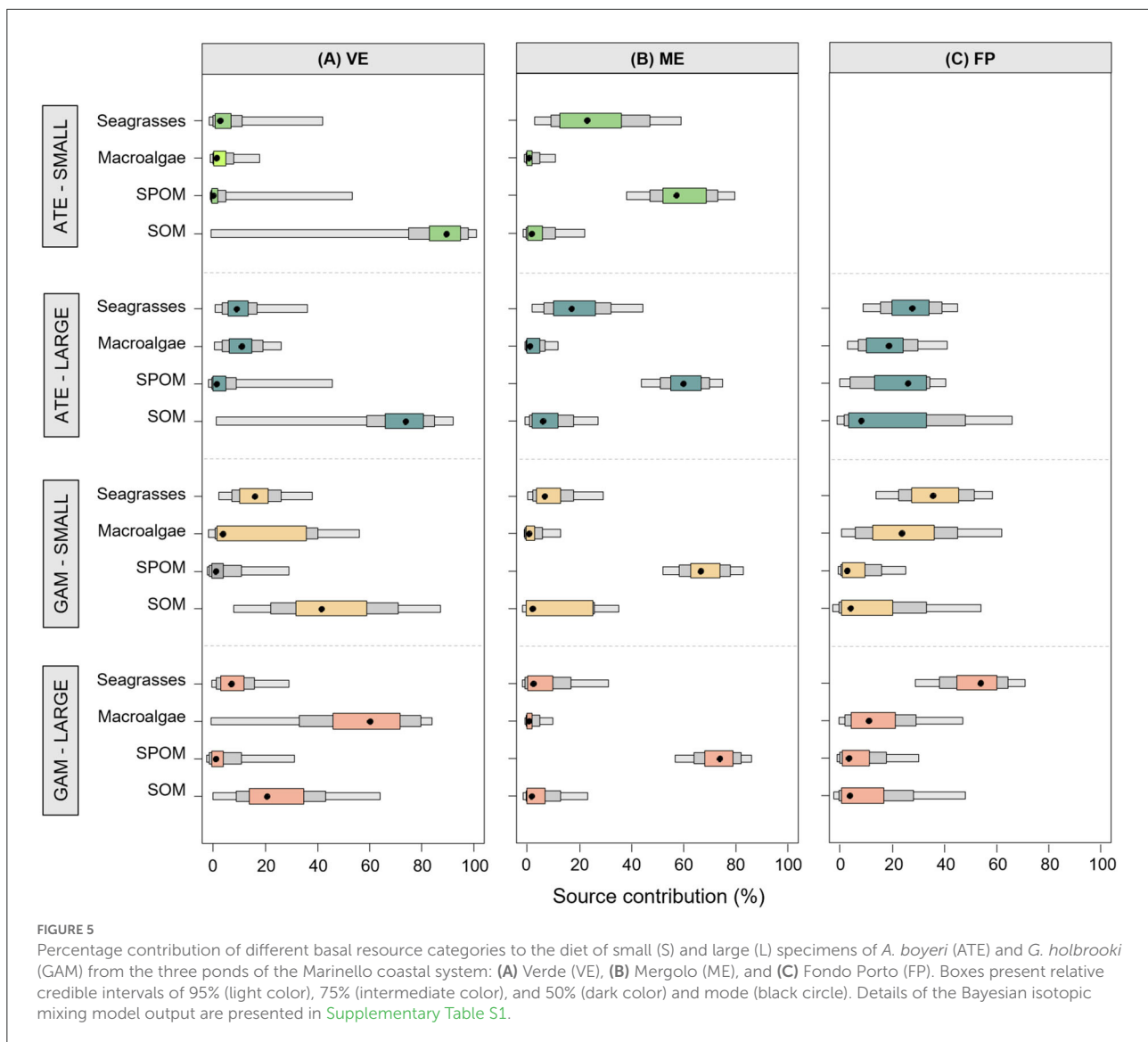


TABLE 3 Pairwise comparisons of SEAc (based on corrected stable isotope data) and overlap values (%²) estimated between small (S) and large (L) specimens of *A. boyeri* (ATE) and *G. holbrooki* (GAM) from the three ponds of the Marinello coastal system.

Pond	Group 1 vs. group 2	SEAc group 1 (% ²)	SEAc group 2 (% ²)	Overlap (% ²)	% of SEAc 1 overlapped by SEAc 2	% of SEAc 2 overlapped by SEAc 1
Verde	ATE-S-ATE-L	0.3	0.2	0.1	46	87
	ATE-S-GAM-S	0.3	1.0	0.03	12	4
	ATE-S-GAM-L	0.3	0.3	-	-	-
	ATE-L-GAM-S	0.2	1.0	0.003	2	0.3
	ATE-L-GAM-L	0.2	0.3	-	-	-
	GAM-S-GAM-L	1.0	0.3	0.1	12	38
Mergolo	ATE-S-ATE-L	0.3	0.1	0.1	36	98
	ATE-S-GAM-S	0.3	2.1	-	-	-
	ATE-S-GAM-L	0.3	0.5	0.02	10	5
	ATE-L-GAM-S	0.1	2.1	-	-	-
	ATE-L-GAM-L	0.1	0.5	-	-	-
	GAM-S-GAM-L	2.1	0.5	0.2	8	33
Fondo Porto	ATE-L-GAM-S	2.1	2.5	0.05	2	2
	ATE-L-GAM-L	2.1	1.4	-	-	-
	GAM-S-GAM-L	2.5	1.4	0.3	13	24





Discussion

This study analyses the trophic relationships between the invasive eastern mosquitofish *G. holbrooki* and the native big-scale sand smelt *A. boyeri* co-occurring in the brackish ponds of the Marinello coastal system at the beginning of the productive season (i.e., spring). The results obtained highlighted a clear differentiation in the fish isotopic values and niche position, consistent with the distinct trophic background of the coastal ponds and the reliance of fish on different trophic pathways.

The nutrient subsidies arising from the gull colony (*L. michahellis*) strongly influence the neighboring Verde pond (VE), but only to a limited extent the nearby Fondo Porto pond (FP) and the farthest Mergolo pond (ME) (Signa et al., 2012; Vizzini et al., 2016). $\delta^{15}\text{N}$ is acknowledged as a powerful

proxy for the ornithogenic input in terrestrial and coastal areas, due to the high values of bird guano (Mizutani and Wada, 1988; Signa et al., 2021 and references therein). Accordingly, the different positioning of the fish populations along the vertical axis of the isotopic bi-plot found in this study mirrors the gradual decrease in the incorporation of guano-derived nutrients into local food webs (Vizzini et al., 2016), with the highest influence in the closest pond VE and the lowest in the farthest ME. On the contrary, we found that the different positioning along the $\delta^{13}\text{C}$ axis mirrors the reliance of fish on distinct organic matter pathways. The overlap between the $\delta^{13}\text{C}$ values of guano ($\delta^{13}\text{C} = -20.2 \pm 2.5\text{‰}$, Vizzini et al., 2016) and those of both sedimentary organic matter (SOM) ($-19.5 \pm 1.1\text{‰}$, Supplementary Table S1) and macroalgae ($-20.0 \pm 0.8\text{‰}$, Supplementary Table S1) in VE indicates, in fact, a

clear accumulation of seabird-derived organic matter in the system and its incorporation in basal sources (Vizzini et al., 2016). Moreover, Bayesian mixing models revealed that the avian-subsidized SOM and the opportunistic macroalgae (e.g., *Cladophora* sp.), which are particularly abundant in the littoral zone forming dense patches, were the main basal sources supporting the trophic pathway leading to both fish species in VE. In contrast, we observed a single planktonic pathway based on suspended particulate organic matter (SPOM) dominating in the mesotrophic ME and a mixed benthic pathway characterized by a high contribution of macrophytes dominating in the seaward and oligotrophic FP. This is consistent with previous findings that showed a dominance of benthic pathways in VE in spring (Vizzini et al., 2016) and inter-pond variability in resource availability and habitat complexity that reflects on benthic abundance and diversity (Signa et al., 2015), confirming, therefore, that the observed differences in trophic pathways are driven in part by the differing seabird subsidies. Alongside the reliance on different organic matter pathways in the three ponds, the patterns observed within each pond in terms of isotopic niche positioning and trophic level suggest that the coexistence of the two species may have been promoted by site-specific mechanisms of niche differentiation. First, the negligible niche overlap between the two species throughout the ponds indicates a clear resource partitioning, consistent with previous studies of invasive fish, including mosquitofish. In particular, both in Australian wetlands and Mediterranean lagoons, mosquitofish coexists with other endemic fish, including sand smelt, with limited niche sharing (Gisbert et al., 1996; Stoffels and Humphries, 2003; MacDonald et al., 2012). Niche divergence, rather than convergence, is suggested, indeed, as a general pattern within invaded fish communities, except in cases of high invader abundance (Tran et al., 2015; Britton et al., 2018), a condition not observed in this study. Second, the different niche positioning in the three ponds indicated that the two species have developed different trophic, and maybe behavioral, strategies to coexist, depending on different trophic conditions, presumably mediated by different resource availability (e.g., habitat complexity and prey diversity and abundance). It is not new that resource availability can change significantly over space and time due to the presence of ecological gradients, such as seasonality and productivity, and that, in turn, this can affect species niches and competitive interactions (Abbey-Lee et al., 2013; Costa-Pereira et al., 2019). Here, we found that, in the oligotrophic shallow pond (FP), characterized by mixed macrophyte-covered bottoms and a high abundance of deposit feeders, the two fish species belonged to distinct benthic pathways, with SOM as the main basal carbon source for sand smelts, and macroalgae and seagrasses for mosquitofish. While habitat complexity would provide different niches that are partitioned by the two species (Beisel et al., 2000; MacDonald et al., 2012), the high abundance of food resources throughout the pond may have facilitated the integration of

mosquitofish into the native food web by being able to exploit unused resources and thus avoid competitive interactions (Britton et al., 2018).

In contrast, in the ponds characterized by higher trophic state and lower habitat complexity (VE and ME), the Bayesian mixing models revealed that the two coexisting fish tended to rely on the same trophic pathway, rather than exploit distinct pathways, probably as an effect of the limited range of exploitable pathways under mesotrophic and hypereutrophic conditions. At the same time, the change in trophic positions and the reduction of the niche breadth (SEAb) observed for the native big-scale sand smelt from the oligotrophic to both mesotrophic and hypereutrophic ponds helped to recall the classical niche theories and found confirmation in several empirical studies. It has been postulated, indeed, that invasive species tend to out-compete native species through asymmetrical competitive mechanisms, such as by contracting and/or displacing the native's niche to lower or higher trophic positions due to their higher competitive ability (Vander Zanden et al., 1999; Jackson et al., 2012; Britton et al., 2018). Under this framework, the different patterns observed in the two ponds at higher trophic state may find justification in the different levels of biodiversity that characterizes them. While the harsh conditions of the guano-trophic VE support low-diversity communities featured by only benthic deposit feeders (i.e., chironomids, amphipods, and small gastropods) and epifaunal carnivorous palaemonid shrimps, the mesotrophic ME is characterized by high-diversity communities with benthic filter feeders and deposit feeders, as well as carnivorous polychaetes (Signa et al., 2015). Therefore, we suggest that, in the less diverse and harsher pond VE, the mosquitofish included plant materials in their diet to balance the decrease in animal prey abundance (Blanco et al., 2004; Kalogianni et al., 2014) and restricted the access of sand smelts to only a few high-order consumers, such as the juveniles of the palaemonid shrimps that thrive in the pond. In contrast, the higher structural and functional biodiversity of ME favored the invader's niche expansion, particularly evident in small specimens, consistent with the "resource diversity hypothesis" (MacArthur, 1969), and constrained the native species' diet to a few low-order consumers, such as epifaunal filter/deposit feeders.

Looking at the intraspecific level, the narrow niche breadth (SEAb), together with the high niche overlap (>90%) of small- and big-sized sand smelts from ME and VE, indicates a specialist diet across size classes, contrary to what was observed in FP where the wider niche of large specimens may indicate release from intraspecific competition (Britton et al., 2018). This clear spatial pattern of SEAb also indicates lower trophic diversity and variety of both exploited resources and trophic levels for both small and large specimens from VE and ME than FP. *A. boyeri* can shift between small hyperbenthic and epifaunal prey (e.g., isopods, amphipods, mysids, polychaetes, gastropods, and bivalves) in shallow vegetated coastal areas, because of its

high trophic plasticity (Vizzini and Mazzola, 2002, 2005; Chrisafi et al., 2007). Such flexible feeding habits, coupled with the high abundance, may have represented the winning strategy that allowed the sand smelt to accommodate a certain degree of niche contraction rather than its suppression.

In contrast, the mosquitofish *G. holbrooki* exhibited a low intraspecific niche overlap (~20%) between size classes in all ponds and a clear niche narrowing from small to larger specimens, suggesting ontogenetic dietary specialization. The high niche breadth of the small mosquitofish indicated a clear generalist feeding behavior, as well as high trophic diversity and omnivory degree. Omnivory plays an important stabilizing role in spatially compressed food webs, alleviating the strong destabilizing force of top-down pressures potentially exerted by top predators (McCann et al., 2005). At the same time, the large among-individual variability may also indicate that individual mosquitofish use different foraging tactics feeding on only a subset of the available resources (Abbey-Lee et al., 2013) from different trophic pathways and levels, as a mechanism for reducing intraspecific competition (Matthews and Mazumder, 2004; Abbey-Lee et al., 2013) and ensuring population growth (Blanco et al., 2004). The eastern mosquitofish is acknowledged, indeed, not only as an opportunistic predator with a very wide prey spectrum, including zooplankton, insects, benthic invertebrates, fish, and amphibian larvae and eggs (Specziár, 2004; Pyke, 2005), but also as an omnivore able to ingest large amounts of algae and vegetal detritus in turbid and shallow estuaries and lakes (Blanco et al., 2004; Franco et al., 2008).

Lastly, assuming that the two species occupy a similar fundamental niche (i.e., the multidimensional environmental conditions within which a species can live in the absence of competitors, *sensu* Hutchinson, 1957), we infer that the native sand smelt has been induced to undergo different mechanisms of trophic displacement and/or contraction of its realized niche to coexist with the invasive mosquitofish. While this is consistent with the general adaptive response of native species subject to asymmetric competition with invaders exhibiting superior competitive abilities (Byers, 2000; Carey and Wahl, 2010; Tran et al., 2015), we provided evidence for the occurrence of site-specific environmental control on invaded trophic niches as a result of the combined effect of differing resource availability and habitat complexity.

Conclusion

We used a combination of isotopic niche analysis and Bayesian mixing models to reveal complex site-specific trophic relationships between the invasive eastern mosquitofish *Gambusia holbrooki* and the sympatric big-scale sand smelt *Atherina boyeri* co-occurring in shallow coastal ponds with

different environmental features. The interplay of the trophic status and geomorphological features of the ponds influenced the availability of resources, in terms of prey diversity and habitat complexity, leading to site-specific mechanisms of trophic niche divergence. Moreover, under oligotrophic conditions, the high habitat complexity and abundances of benthic prey provided different niches that were partitioned by the two species. In contrast, under higher trophic state and lower habitat complexity, an asymmetric competition between the two species might have arisen due to the competitive superiority of mosquitofish, leading to a clear displacement and contraction of the sand smelt niche. At the same time, the broadening of the invader's niche, especially marked for small specimens, may have been driven by a high prey diversity level, consistent with the "resource diversity hypothesis" (MacArthur, 1969). Although a large body of literature exists on the ecological effects of invasive fish in coastal systems, trophic aspects have been scantily addressed especially in combination with environmental stressors. This research gives new insights into the mechanisms that promote the coexistence of invasive and native species in shallow and highly variable marine coastal systems. However, our study is limited by the short temporal context serving as a snapshot of the trophic relationships between invasive and native species under the trophic gradient that occurs during the productive spring season. Given the highly variable nature of coastal ponds, we did not exclude seasonal changes of the trophic relationships of the two fish species according to resource availability, which ensures the success of their long coexistence. Lastly, while we demonstrated the great potential of isotopic niche analysis for detecting complex ecosystem responses to invasion by NIS, additional studies are advocated to further understand the interaction between environmental stressors and fish resource partitioning on a larger temporal scale.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

Ethical review and approval was not required for the animal study because Samplings were conducted with permits from the Authority of the Laghetti di Marinello Nature Reserve (permit # 28599). No other permits were needed. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Author contributions

GS, SV conceived, designed, and supervised data integration and interpretation. CA performed the mixing models and isotopic niche analysis. CA, GS, and GC performed data analysis and interpretation. AM, SV funded the study. All authors contributed to the manuscript drafting and revision. All authors read and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2022.958467/full#supplementary-material>

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