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Trophic Interactions of Two Ponto-Caspian Gobies in the Turkish Part of Their Native Range

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Abstract

Several Ponto-Caspian gobiids have expanded from their native distribution ranges to Europe and North America. As knowledge on their bio-ecological features in their native range is still limited, the trophic ecology of monkey goby *Neogobius fluviatilis* and Western tubenose goby *Proterorhinus semilunaris* was studied in three natural lakes in the Marmara Region of NW Turkey using the stable isotopes of $\delta^{13}C$ and $\delta^{15}N$. In two of the lakes, the trophic niches (as the isotopic niche) of the gobies were highly divergent with co-existing native fishes, with no overlap. Moreover, mixing models suggests considerable inter-specific dietary differences. In all lakes, the trophic niches of gobies were never significantly larger than those of co-existing fishes. These results suggest that when introduced outside of their natural range, the gobies might integrate into new fish communities via exploiting resources that are underexploited by native fishes or will initially share resources with these species before their niches diverge, perhaps through competitive displacement.

Keywords: Monkey goby, tubenose goby, isotopic niche, niche plasticity, trophic relationships.

Introduction

The Gobiidae is one of the largest fish families, comprising more than 2,000 species in over 200 genera (Patzner, Van Tassell, Kovačić, & Kapoor, 2011). Two of these gobies from Black Sea Region of Turkey, Western tubenose goby *Proterorhinus semilunaris* (Heckel, 1837) and monkey goby *Neogobius fluviatilis* (Pallas, 1814), are an important component of the native fish communities of Turkish inland waters and locally marketed for food consumption (Özuluğ, Altın, & Meriç, 2005; Turan, Taş, Çilek, & Yılmaz, 2008; Çınar *et al.*, 2013). Whilst they are usually included within ichthyofaunal studies (Geldiay & Balık, 1988), there is only limited knowledge on their biology, such as length-weight relationships (Tarkan, Gaygusuz, Özuluğ, Gürsoy Gaygusuz, & Saç, 2009), reproductive parameters (Sasi & Berber, 2010) and diurnal feeding preferences (Gaygusuz, Gürsoy Gaygusuz, Tarkan, Acıpınar, & Türer, 2007). They are also considered potential invaders in Europe and North America. In North America, *N. fluviatilis* has been listed as one of five potential high-impact non-native species for the Great Lakes Basin (Pagnucco *et al.*, 2015), with *P. semilunaris* already recorded from the St. Clair River

(Dougherty, Moore, & Ram, 1996) and Lake Erie (Dillon & Stepien, 2001).

In both their native and invasive range, studies on their trophic relationships with other fishes remain limited (Grabowska, Grabowksi, & Kostecka, 2009; Vašek, Všetičková, Roche, & Jurajda, 2014; Mikl *et al.*, 2017). This is despite these data having fundamental importance for understanding their ecological interactions in their native range and for informing their ecological risk assessment in their non-native range (Copp, 2013). Correspondingly, to assess their trophic ecology and generate new knowledge on trophic relationships of invasive fishes in their native range for application to ecological risk assessments, the aim here was to assess the trophic ecology of native *P. semilunaris* and *N. fluviatilis* in relation to native species in three lakes that provided a gradient of environmental characters and fish assemblages. As these gobies have a generalist and highly flexible feeding strategies (Grabowska *et al.*, 2009; Adámek, Andreji, & Gallardo, 2007; Adámek, Jurajda, Prášek, & Sukop, 2010; Vašek *et al.*, 2014; Mikl *et al.*, 2017), it was predicted that compared with other fishes, they would have relatively large trophic niche sizes, with trophic overlap with other species that indicate high potential for competitive

interactions. Dietary analyses were completed using stable isotope analysis (SIA) that provides a temporally integrated assessment method of diet (Busst & Britton, 2016).

Materials and Methods

Study Sites and Sample Collection

The study was conducted in three lakes (47 to 308 km^2) in the Marmara Region in the north-west of Anatolia, Turkey (Table 1, Figure 1). İznik and Sapanca are deep lakes that have suffered from ecological succession in recent decades and are now considered mesotrophic (Ustaoğlu, 1993; Akçaalan, Mazur-Marzec, Zalewska, & Albay, 2009). In contrast, Lake Uluabat is a shallow eutrophic lake with RAMSAR status (Magnin & Yarar, 1997). All of the lakes have a relatively diverse ichthyofauna, where species of Cyprinidae dominate, including invasive gibel carp *Carassius gibelio* in İznik and Uluabat (Geldiay & Balık, 1988; Aydın *et al*., 2011). With the exception of *N. fluviatilis* in Lake İznik, both gobies were present in all lakes.

Sampling of the fish communities was conducted in 06/01/16 and 08/01/2016 and was completed in littoral areas, with electric fishing (SAMUS-725G) in Sapanca and İznik lakes and seine netting in Lake Uluabat in depths of up to 1.5 m. The capture of these fishes in January enabled the stable isotope dietary assessments to reflect their feeding in the preceding months (Pond *et al*., 2015; Busst & Britton, 2018), thus were indicative of their diet in autumn when the fish would have still been active in the lakes. However, the January sampling resulted in a small sample size for *P. semilunaris* in Lake Uluabat (n = 3) due to cold temperatures, so it was removed from the dataset.

To better understand trophic relationships and position of the two gobiids, other abundant and co-existing fish species in the studied lakes were also sampled and assessed, *Salaria fluviatilis* in Lake İznik, *Rhodeus amarus* in Lake Sapanca, and *Alburnus alburnus* in Lake Uluabat (Table 2). These species were used as comparators due to their high abundance and likely co-habitation with the gobies, accepting that the cyprinids might utilize different food resources to gobiids through their functional differences.

In the field, all captured fishes were euthanized, measured for total length (nearest mm) and weight (nearest 0.1 g), and then dorsal muscle tissue samples was taken. For the purposes of SIA, the most important putative food resources of the fishes (periphyton, macrophytes, zooplankton, macrobenthos, detritus) that were previously detected by traditional stomach analyses in the lakes under study (unpublished data) were also collected from

Table 1. Latitude, longitude, surface area (km^2), mean and max depth (m) of three lakes in Marmara Region where *Proterorhinus semilunaris* and *Neogobius fluviatilis* were captured

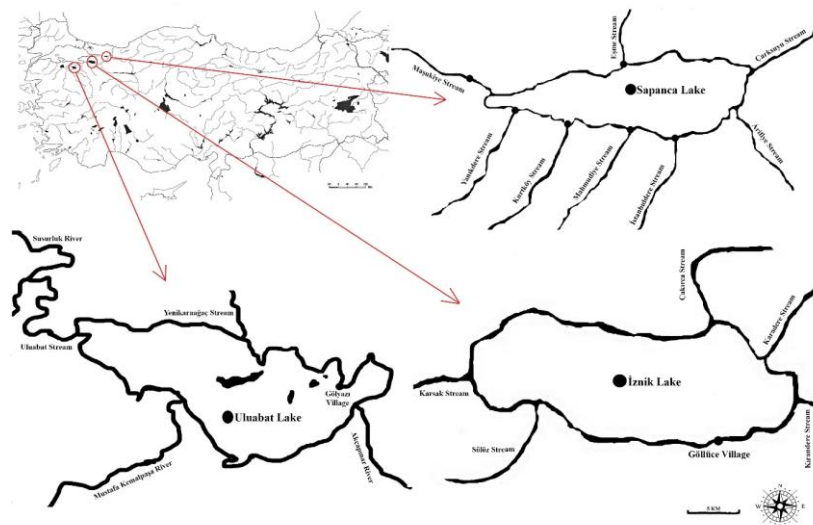| Lake	Latitude	Longitude	Area (km^2)	Mean depth (m)	Max depth (m)
İznik	40°27'	29°32'	308	30	65
Sapanca	40°42'	30°15'	47	36	53
Uluabat	40°10'	28°35'	136	3	10

Figure 1. Lakes where *Proterorhinus semilunaris* and *Neogobius fluviatilis* populations were sampled in Marmara Region.

Table 2. Mean lengths, $\delta^{13}C$, $\delta^{15}N$, Trophic Position (TP), isotopic niche size (as 95% CL of standard ellipse area, SEA$_b$) of fish species in three studied lakes and the extent of their overlap between species, and the estimated contributions of putative foods to their diet (0–1 scale), as predicted in SIAR with ±95% CL. MB = Macrobenthos (mainly Insecta and Oligochaeta), PP = Periphyton, ZP = Zooplankton (mainly Rotifera and Cladocera), DT = Detritus, MP = Macrophytes

Lake	Species	n	Mean length (TL, mm)	Mean $\delta^{13}C$ (‰)	Mean $\delta^{15}N$ (‰)	Mean TP	SEA$_b$ (‰^{2})	MB	PP	ZP	DT	MP
İznik	*P. semilunaris*	12	41.9±1.39	-21.54±0.40	7.10±0.47	2.54± 0.39	2.53– 8.13	0.18 (0.07– 0.38)	0.39 (0.18– 0.61)	0.33 (0.18– 0.47)	0.10 (0.00– 0.25)	-
	S. fluviatilis	10	32.3±0.40	-21.02±0.16	6.86±0.27	2.47± 0.08	0.92– 3.27	0.21 (0.01– 0.39)	0.52 (0.33– 0.68)	0.22 (0.10– 0.34)	0.06 (0.02– 0.15)	-
	Macrobenthos			-20.25±0.10	5.25±0.10							
	Periphyton			-20.58±0.10	2.09±0.10							
	Zooplankton			-25.03±1.00	5.33±0.80							
	Detritus			-18.57±3.27	7.07±3.92							
Sapanca	*N. fluviatilis*	9	84.3±1.46	-30.32±0.68	11.28±0.19	4.20± 0.06	1.75– 6.77	0.33 (0.02– 0.62)	0.04 (0.00– 0.14)	0.29 (0.00– 0.56)	0.34 (0.01– 0.63)	-
	P. semilunaris	9	38.5±1.00	-29.09±0.62	10.10±0.26	3.85± 0.08	2.18– 8.54	0.40 (0.10– 0.72)	0.10 (0.03– 0.16)	0.21 (0.00- 0.42)	0.29 (0.01– 0.53)	-
	R. amarus	10	47.3±0.63	-25.29±0.31	8.67±0.25	3.43± 0.08	1.25– 4.61	0.33 (0.01– 0.61)	0.03 (0.00– 0.10)	0.28 (0.00– 0.59)	0.36 (0.01- 0.70)	-
	Macrobenthos			-26.83±0.09	3.80±0.05							
	Periphyton			-14.63±0.03	0.74±0.01							
	Zooplankton			-27.14±2.00	2.84±0.10							
	Detritus			-27.13±2.00	3.68±1.50							
Uluabat	*N. fluviatilis*	10	93.4±1.82	-28.29±0.20	14.37±0.20	2.97± 0.06	1.00– 3.58	-	-	0.31 (0.06– 0.53)	0.38 (0.06– 0.69)	0.31 (0.11– 0.49)
	A. alburnus	10	64.5±0.28	-28.28±0.20	12.52±0.23	2.42± 0.08	0.83– 3.00	-	-	0.55 (0.23– 0.86)	0.39 (0.03– 0.70)	0.07 (0.00– 0.17)
	Zooplankton			-23.06±2.24	11.08±1.30							
	Detritus			-25.41±3.00	9.49±2.00							
	Macrophyte			-23.43±1.84	6.59±0.47							

each sampling site (Table 2). Macrophytes with molluscs and insects from both bottom and surface were collected with a grab and a scoop while detritus was collected by hand, and zooplankton was collected with a zooplankton net. Macrophyte samples were washed with tap water and insects and/or larvae were removed and stored separately for SIA. Since the detritus did not include sediments, there was no requirement for an -acidified procedure to remove carbonates prior to SIA.

Stable Isotope Analysis

All SIA samples (fish muscle and putative food resources) were dried for 24 h at 60 ºC and then homogenized with a pestle and mortar, with 1 mg for fish/invertebrate material and 2 mg for plant material then weighed accurately into 5 x 9 mm tin cups using an ultra-microbalance (Sartorius MSA3.6P-000-DM Cubis Micro Balance). Stable isotope analysis was then completed at Davis, University of California, using an elemental analyser (Flash EA, 1112 series, Thermo-Finnigang), coupled to a continuous flow isotope ratio mass spectrometer (Finnigan MAT DeltaPlus, Thermo-Finnigang). Stable carbon and nitrogen isotope ratios were expressed as per mille (‰) using the delta notation (δ). As reference materials, secondary standards with known relation to international standards (Pee Dee Belemnite for carbon; nitrogen in air for nitrogen) were used. Dried

and homogenized peach leaves with known isotopic composition were used as an internal standard and repeat analyses resulted in typical precision of less than 0.1 ‰ for $\delta^{13}C$ and less than 0.3 ‰ for $\delta^{15}N$. These were used as internal working standards for animal tissue and detritus/plant material respectively and inserted in each run. Since the C:N ratios indicated low lipid content (<3.5), the muscle $\delta^{13}C$ values were not lipid corrected (Post *et al*., 2007). The mean coefficient of variation and range of $\delta^{13}C$ and $\delta^{15}N$ were calculated per species. The trophic position (TP) of the fishes was calculated using the following equation:

$$TP_i = [(\delta^{15}N_i - \delta^{15}N_{base})/3.4] + 2$$

where TPi is the trophic position of the consumer, $\delta^{15}N_i$ is the isotopic ratio of fish species, $\delta^{15}N_{base}$ is the isotopic ratio of primary consumers, 3.4 is the fractionation between trophic levels and 2 is the trophic position of the baseline organism (Post, 2002). The mean $\delta^{15}N$ value of all macroinvertebrates was used as the baseline for each lake, which are usually preferred for baseline corrections (Cabana & Rasmussen, 1996; Post, 2002).

Data Analyses

Differences in total length, $\delta^{13}C$, $\delta^{15}N$ and TP between species and among lakes were tested with

permutational analysis of variance (PERANOVA, Anderson, Gorley, & Clarke, 2008) using the PERMANOVA 1.0.1. + add-in to PRIMER version 6.1.11 (PRIMER-E Ltd, Plymouth, UK). This was done with Type III sums of squares following normalization of the data and was based on a Euclidian distance matrix and 9999 permutations of the residuals under a reduced model including *a posteriori* pair-wise comparisons. The advantage of PERANOVA over traditional parametric analysis of variance is that the stringent assumptions of normality and homoscedasticity in the data, which prove very often unrealistic when dealing with ecological data sets, are relaxed considerably (Anderson, 2001).

The SIAR package in R (R Core Team, 2016) was also used to calculate the isotopic niche size of the two goby species at different lakes. This was done by using standard ellipse areas (SEA), a bivariate estimate of the core isotopic niche based on the measures of variability in mean δ^{13}C and δ^{15}N of all samples at each lake (Jackson, Inger, Parnell, & Bearshop, 2011; Jackson *et al.*, 2012). Each ellipse encompasses 40% of the data and thus represents the core dietary niche, indicating typical resource use within the population (Jackson *et al.*, 2011; 2012). A Bayesian estimate of SEA (SEAb) was used due to the small sample sizes; this utilises a Markov chain Monte Carlo simulation with 10^4 iterations for each group and provides 95% confidence limits of isotopic niche size (Jackson *et al.*, 2011; R Core Team, 2016). To quantify trophic niche overlap, the bivariate area shared by both species in isotopic space and percentage of overlap was also calculated using SEAc (Jackson *et al.*, 2011; 2012).

Bayesian mixing models then estimated the relative contribution of each resource to the fish diet using the SIAR package in R (R Core Team, 2016). Before performing the model, similar isotope values were combined to prevent using excessive putative food resources. The resources were then combined at each site (where available) as following: periphyton, macrophytes, zooplankton, macrobenthos and detritus. Correction for isotopic fractionation between resources and consumers used 3.4‰ (± 0.98‰) for δ^{15}N and 0.39‰ (± 1.3‰) for δ^{13}C (Post, 2002).

Results

Sample Sizes and Fish Lengths

Mean fish length (TL, mm) varied significantly amongst the species in Lake Sapanca (# = permutational, $F^{\#}_{2,26}$ = 38.22, P<0.01), with *N. fluviatilis* significantly larger than *P. semilunaris* ($t^{\#}$ = 6.76, $P < 0.01$) and *R. amarus* ($t^{\#}$ = 7.39, P<0.01); *P. semilunaris* was then significantly larger than *R. amarus* ($t^{\#}$ = 2.36, P = 0.03) (Table 2). In Lake Uluabat, *N. fluviatilis* was significantly larger than *A. alburnus* ($F^{\#}_{1,18}$ = 28.82, P<0.01) and in Lake İznik, *P. semilunaris* was significantly larger than *S.*

fluviatilis ($F^{\#}_{1,21}$ = 3.71, P = 0.05), although the actual differences in length were relatively minor (Table 2).

Stable Isotope Analysis

In Lake Sapanca, the relationship between δ^{13}C and total length of the gobies was significant (*N. fluviatilis*: r^2=0.47; F=6.23, P=0.04; *P. semilunaris*: r^2 = 0.82; F=31.23, P<0.01), but was not for δ^{15}N (*N. fluviatilis*: r^2=0.05; F=0.40, P=0.06; *P. semilunaris*: r^2 = 0.001; F=0.01, P=0.92). For *R. amarus*, the relationships between length and both stable isotopes were not significant (δ^{13}C: r^2=0.002; F=0.01, P=0.91; δ^{15}N: r^2=0.32; F=3.69, P=0.09). In Lake İznik, total lengths of both sampled fishes were only significantly related to δ^{15}N (*P. semilunaris*: r^2=0.48; F=9.29, P=0.01; *S. fluviatilis*: r^2=0.65; F=15.00, P = 0.005). In Lake Uluabat, the relationship between length and both isotopes were not significant for any species (*N. fluviatilis*; δ^{13}C: r^2=0.21; F=0.40, P=0.18; δ^{15}N: r^2 = 0.27; F=2.99, P=0.12; *A. alburnus*; δ^{13}C: r^2 = 0.39; F=4.54, P=0.07; δ^{15}N: r^2=0.18; F=1.52, P=0.26).

Regarding the stable isotope data between species in the lakes, in Lake İznik, where *P. semilunaris* and *S. fluviatilis* were relatively abundant, differences in their stable isotope data and trophic position were not significant (# = permutational; δ^{13}C: $F^{\#}_{1,20}$ =1.39, P=0.28; δ^{15}N: $F^{\#}_{1,20}$ = 0.18, P=0.673; TP: $F^{\#}_{1,20}$=0.18, P=0.674) (Table 2). In Lake Sapanca, the mean δ^{13}C and TP values were significantly higher for both gobiid species than *R. amarus* (δ^{13}C: $F^{\#}_{2,25}$=23.86, P<0.01); TP:$F^{\#}_{2,25}$=30.20, P<0.01) (Table 2). In Lake Uluabat, *N. fluviatilis* had a significantly higher mean TP than the abundant *A. alburnus* ($t^{\#}$ = 5.45, P<0.01) (Table 2).

Isotopic Niche Size and Predicted Diet Composition

The 95% confidence limits of the estimates of isotopic niche size (SEAb) indicated that the niche sizes of all co-existing species in the studied lakes were not significantly different (Table 2). In Lakes Sapanca and Uluabat, the isotopic niches of the gobies and other fishes did not overlap, being distinct in isotopic space (Figure 2). In Lake İznik, however, the niches of the two analysed fishes overlapped considerably, with the niche of *Salaria fluviatilis* being sat almost entirely within the niche of *P. semilunaris* (Figure 2).

The mixing models indicated that according to food proportions of *P. semilunaris* in Lake İznik, periphyton and zooplankton were the most abundant food components. The other dominant fish species in Lake İznik, *S. fluviatilis* preferred mainly periphtyton (Table 2). In Lake Sapanca, three main food sources (detritus, macrobenthos and zooplankton) were dominant groups for all species examined (i.e. *N. fluviatilis*, *P. semilunaris*, *R. amarus*) (Table 2). Finally, in Lake Uluabat, zooplankton, detritus and

macrophytes were almost equally important food resources for *N. fluviatilis,* whereas for the other abundant co-existing species *A. alburnus*, zooplankton was by far the most important food resource followed by detritus (Table 2).

Discussions

The application of stable isotope analysis to populations of two gobiids in their native range enabled comparison of their trophic ecology with some co-existing fishes in the littoral areas of three lakes. Whilst the results indicated that the tropic positions of *P. semilunaris* and *N. fluviatilis* were variable between the lakes, suggesting some context dependency, their isotopic niches were never significantly larger than co-existing fishes and, in two lakes (Sapanca and Uluabat), they were highly divergent with no overlap. These results were contrary to predictions that the gobies would have relatively large niches with high overlap, with the prediction based on the generalist feeding strategy of the gobies that was inferred from stomach contents analysis of the fishes in both their native and invasive
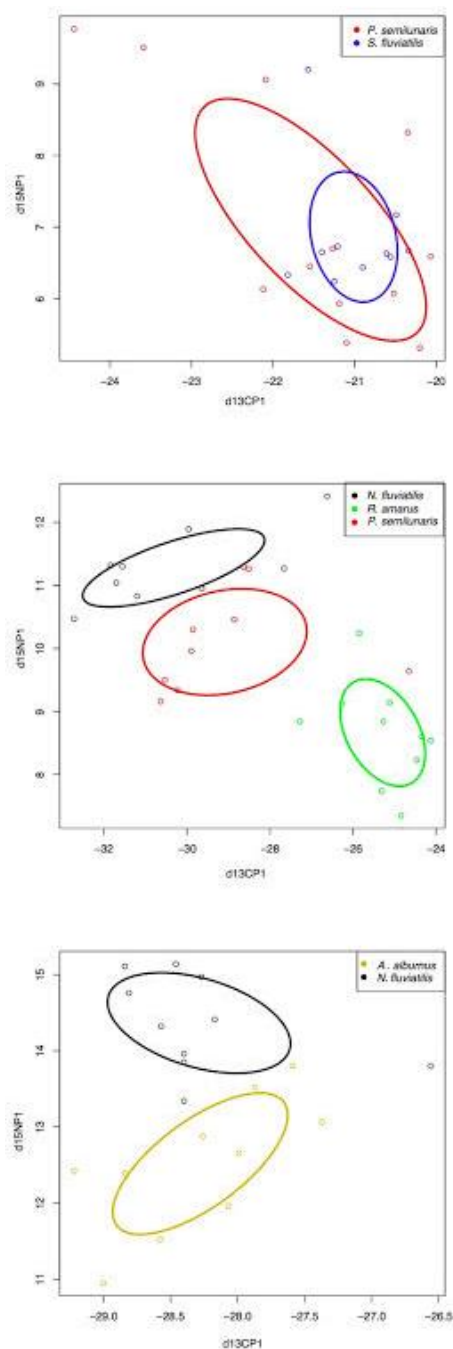

Figure 2. Bi-plot of δ^{13}C and δ^{15}N with Standard Ellipse Areas (SEA$_C$) of bulk muscle of *Proterorhinus semilunaris* and *Neogobius fluviatilis* from three natural lakes (upper; Lake İznik, middle; Lake Sapanca, lower; Lake Uluabat) in Marmara Region.

range (Grabowska *et al.*, 2009; Adámek *et al.* 2010; Vašek *et al.*, 2014; Mikl *et al.*, 2017). It was only in Lake İznik where the analysed fishes showed some overlap in their isotopic niches and thus the potential for competitive interactions.

Traditional diet studies of *N. fluviatilis* and *P. semilunaris* in both native and non-native regions (Sindilariu & Freyhof, 2003; Kakareko, Zbikowski, & Zytkowicz, 2005; Adámek *et al.*, 2007; Gaygusuz *et al.*, 2007; Grabowska *et al.*, 2009; Gürsoy Gaygusuz, Tarkan, & Gaygusuz, 2010; Adámek *et al.* 2010; Piria *et al.*, 2011; Piria, Jakšić, Jakovlić, & Treer, 2016; Mikl *et al.*, 2017) are in accordance with SIA results in the present study, with the exception of plant material that was one of the important groups found in SIA for both gobiids in İznik and Uluabat lakes (Table 2). However, the dominance of food groups was represented differently between stomach contents and SIA, which might relate to the latter providing a time-integrated analysis of assimilated diet sources (Thomas & Crowther, 2015), whereas stomach content analysis provides only a snapshot of ingested food resources at the time of sampling (Cucherousset, Bouletreau, Martino, Roussel, & Santoul, 2012).

These results revealed that, despite the prediction and information from previous studies based on stomach contents (Grabowska *et al.*, 2009; Vašek *et al.*, 2014; Mikl *et al.*, 2017), the isotopic niches of the gobies and co-existing fishes were divergent. They add to an increasing literature base over how introduced and invasive freshwater fishes can trophically integrate into new communities. As with these native gobies, strong patterns of isotopic niche divergence have been recorded between the Asian invader *Pseudorasbora parva* and native fishes in Europe (Tran *et al.*, 2015). Similarly, reductions in the isotopic niche size in three native pond fishes were observed when they co-existed with the North American pumpkinseed *Lepomis gibbosus* (Copp *et al.*, 2017). However, they are also some context dependency within this, as for riverine *L. gibbosus* populations, they had minimal trophic interactions with native brown trout *Salmo trutta* (Jackson *et al.*, 2016). Similarly, there was also some context dependency in the present study as considerable niche overlap was detected between *P. semilunaris* and the co-existing *S. fluviatilis* in Lake İznik. Indeed, recent isotopic and traditional stomach analyses of *P. semilunaris* and another invasive gobiid *N. melanostomus* in their non-native range showed a consistent pattern that *N. melanostomus* had broader trophic niche and position than *P. semilunaris* (Vašek *et al.*, 2014; Pettitt-Wade, Wellband, Heath, & Fisk, 2015).

The apparent partitioning in the isotopic niche of the fishes in two of the lakes might also relate to issues of fish size and ontogenetic diet shifts. Differences in the lengths of gobiid species and the other fishes were significant in some cases, allied to the fishes having some functional differences. Thus, the patterns of niche partitioning between the fishes in the lakes might have been related more to their ontogenetic differences, with the potential for different patterns to have been detected had fish of similar lengths been used. Another potential issue in the study was the relatively low sample sizes used, although use of SEAb for the isotopic niche analysis helped overcome this (e.g. Jackson *et al.*, 2011; Tran *et al.*, 2015). Consequently, future studies should focus on completing longer-term field studies, coupled with experimental studies on competition and displacement to identify potential impact mechanisms of these gobiids. This might be done by validating experimental outputs by field studies that aim to identify if the impact mechanisms apparent in experimental studies are then evident in patterns detected in field data.

In summary, in these three native populations of Ponto-Caspian (PC) gobies, a general pattern was divergence in their isotopic niches with co-existing fishes that suggests that in their invasive range, they might integrate into new fish communities via exploiting resources that are either underexploited by other species or will initially share resources with these species before their niches diverge. Although not reported yet for *N. fluviatilis* or *P. semilunaris* expect for significant negative impact on aquatic invertebrate density and community composition of latter (Mikl *et al.*, 2017), invasion of another PC, *N. melanostomus* have been associated with sharp decline in abundance of native species through niche competition in their non-native range (Corkum, Sapota, & Skorá, 2004; Balshine, Verma, Chant, & Theysmeyer, 2005; Kornis, Mercado-Silva, & Vander Zanden, 2012). This suggests the latter mechanism might be important as it could potentially lead to competitive displacement. Irrespective, this study provides important baseline information on the trophic interactions of PC gobies in their native range that can be useful for understanding their consequences in their non-native range.

Acknowledgements

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