FISHERIES & AQUATIC LIFE (2022) 30: 1 - 13
Archives of Polish Fisheries
DOI 10.2478/aopf-2022-0001

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RESEARCH ARTICLE

Trophic niche overlap between non-indigenous round goby, *Neogobius melanostomus*, and native fishes in tributaries of the lower Danube River (Bulgaria)

Eliza Uzunova, Dimitriy Dashinov

Received – 31January 2022/Accepted – 29 March 2022. Published online: 31 March 2022; ©Inland Fisheries Institute in Olsztyn, Poland
Citation: Uzunova, E., Dashinov, D. (2022). Trophic niche overlap between non-indigenous round goby, *Neogobius melanostomus,* and native fishes in tributaries of the lower Danube River (Bulgaria). Fisheries & Aquatic Life 30, 1-13.

Abstract. The recent expansion of the round goby (*Neogobius melanostomus*) in tributaries of the lower Danube (Bulgaria) prompted assessing potential negative effects on local fish fauna such as competition for food resources. Diet overlap between the round goby and native fishes was analyzed using samples of fishes and macroinvertebrates collected monthly for a one-year period. Significant dietary overlap between *N. melanostomus* and *Barbus petenyi*, *Perca fluviatilis*, *Gobio gobio*, *Vimba vimba*, and *Neogobius fluviatilis* was observed. Dietary overlap in the Iskar River was found to be considerable mainly in the spring months. Conversely, overlap in the Vit River was observed during summer and late fall. The main reason for the overlap noted was the preference for Chironomidae and Hydropsychidae larvae and Gammaridae. The results suggest that there is potential for competition between the round goby and native fish species inhabiting the tributaries of the Danube. Potentially harmful effects on rare, protected fish species in the area are discussed.

Keywords: native fishes, invasive round goby, diet overlap, competition, Danube tributaries

E. Uzunova, D. Dashinov [✉]
Department of General and Applied Hydrobiology, Biological faculty, Sofia University "St. Kliment Ohridski"
E-mail: ddashinov@abv.bg

Introduction

Invasive fish species can have severe ecological impacts on freshwater ecosystems that result from competition, predation, hybridization, bio-fouling, etc. (Blackburn et al. 2014). The establishment success of invasive species and the occurrence and magnitude of their negative impacts are strongly case specific (Ricciardi and Kipp 2008, Zenni and Nuńez 2013). The Ponto-Caspian goby (*Neogobius melanostomus* Pallas) is among the most successful fish invaders in the inland waters of Europe and the Great Lakes of North America (Brownscombe and Fox 2012, Hirsch et al. 2016, Kornis et al. 2017), and there are many reports of negative effects throughout its invasive range (e.g., Kornis et al. 2012, Hirsch et al. 2016). Feeding plasticity accounts for the round goby's highly invasive potential (Brandner et al. 2013, Nurkse et al. 2016, Dashinov and Uzunova 2020) as does its ability to compete for various food resources with local fishes in different environments.

Dietary overlap is commonly used as an indirect metric to evaluate feeding competition among different species (e.g., Wallace 1981). Overlap in food niches has been observed in round goby-infested regions of the Great Lakes watershed, where the invader competes with native Percidae species (French and Jude 2001, Balshine et al. 2005, Bergstrom and Mensinger 2009, Duncan et al. 2011). In the mid Daube River (Central Europe), high diet overlap between the round goby and *Perca fluviatilis* L. and *Gymnocephalus baloni* Holcik and Hensel was detected (Copp et al. 2008). In the Baltic Sea region, the round goby competes for food with a local marine flatfish (Karlson et al. 2007, Schrandt et al. 2016, Ustups et al. 2016). In some brackish sections of the same region, *N. melanostomus* competes with *Gymnocephalus cernuus* L. (Rakauskas et al. 2013). Analysis of the round goby's feeding patterns suggests possible competition with the native Baltic Sea fish species *Vimba vimba* L. and *Rutilus rutilus* L., although diet overlap in this region has not yet been assessed (Oesterwind et al. 2017). Competition with the round goby resulting from food niche overlap is presumed to be the reason for decreases in the body sizes and growth rates of numerous native fish species (Crane et al. 2015, Van Kessel et al. 2016, Cerwenka et al. 2017, Jůza et al. 2018). Substantial negative effects, including local extinctions (e.g., *Cottus bairdii* Girard and *G. cernuus*; Janssen and Jude 2001, Jůza et al. 2018), shifts in the diets of local fishes (Firth et al. 2021), and restructured benthic habitats (Skabeikis et al. 2019), have been reported as being directly linked to invasions of *N. melanostomus*. The establishment of numbers of invasive gobies has coincided with a progressive decline in some native benthic fishes such as *Cottus gobio* L., *Romanogobio albipinnatus* Lukasch, and *Barbatula barbatula* L. (Jurajda et al. 2005). Significant reductions in macroinvertebrate density were observed in river sections invaded by *N. melanostomus* (Mikl et al. 2017).

The round goby has recently been expanding upstream into the tributaries of the lower Danube River (Bulgaria) (Zarev et al. 2013, Dashinov et al. 2018). In these newly invaded river sections, the round goby diet is characterized by a greater taxonomic diversity compared to the diets of populations in other locations including approximately 76 taxa, most of which are macroinvertebrates (Dashinov and Uzunova 2020). Bulgarian Danube tributaries are inhabited by a number of rare and endangered fish species listed in Annex 2 of Council Directive 92/43/EEC, Bern Convention Annex 2, and Annex 4 of the Bulgarian Biodiversity Act. (Pehlivanov et al. 2009, Trichkova et al. 2009).

Assessing possible food niche overlap between the round goby and native fishes can facilitate targeted conservation actions to minimize the potential negative effects caused by this invader. Considering the available literature, we hypothesized that several local species (such as representatives of Percidae and benthophagic Cyprinidae) might potentially be affected by food competition with the round goby. The aim of the study was (i) to assess the diet overlap between the round goby and native fishes in the Bulgarian Danube tributaries and (ii) to analyze the potential risks of competitive food relationships between native species and the invasive *N. melanostomus*.

Material and methods

Study area

The study was performed at single sites from the two rivers Iskar and Vit (Danube River basin, Bulgaria). The Iskar River was sampled year round (April 2017 to May 2018), while the Vit River was sampled from March 2017 to December 2017. Dangerously high waters prevented sampling in January and February. The Iskar River is the longest internal river in Bulgaria with a length of 368 km and a discharge of 54.5 m^3 s^{-1} (Hristova 2012). The sampling area (N 43.4987; E 24.2424) was located 53 km upstream from the Iskar River mouth. The main bottom substrate included gravel and pebbles, with sections of silt and sand near the river bank. Water depth at the sampling site was between 0.5 and 1 m along the bank and more than 2 m in the main river current. The Vit River is 188.2 km long with a discharge of 14.3 m^3 s^{-1} (Hristova 2012). The sampling site (N 43.4079; E 24.5218) was located

41 km upstream from the Vit River mouth. The bottom substrate was comprised mostly of a shale rock bed and zones of gravel and pebbles. Water depth in the sampling area ranged from 0.3 m in the shoreline zone to 2 m in the main river current. Aquatic vegetation was present at all sites and was mostly *Myriophyllum* spp., *Potamogeton crispus*, *Najas minor*, and *Stuckenia pectinata*.

Fish and macroinvertebrate sampling and processing

Fish and macroinvertebrates were sampled monthly during the daytime (between 10:00–16:00). A portable electrofisher (model SAMUS, 200/350 V, 3/12 A, 45-50 Hz) was used to catch fishes. The study area of each river was about 0.5 km long. Monthly sampling at these sites covered an area of about 350 m^2 depending on hydrological conditions. All fishes caught were treated with an overdose of clove oil and preserved in 4% formalin. In the laboratory, the standard body length (SL) and total body length (TL) of each specimen was measured with an electronic calliper to the nearest 0.01 cm. The specimens were weighed to the nearest 0.1 g before (W*t*, body weight) and after (W*evs*, eviscerated body weight) dissection.

A total of 719 fish specimens (347 from the Iskar River and 372 from the Vit River) were analyzed for diet overlap (Tables 1 and 2) among the following fish species: *Neogobius fluviatilis* Pallas; *Alburnoides bipunctatus* Bloch; *Alburnus alburnus* L.; *Barbus petenyi* Heckel; *Gobio gobio* L.; *Pseudorasbora parva* Temminck and Schlegel; *Squalius cephalus* L.; *V. vimba*; *P. fluviatilis*; *Cobitis taenia* L.; *Cobitis albicoloris* Chichkoff.

The gut of each specimen was opened, and the content of the entire digestive tract was removed. Food items were separated and identified to the lowest possible level and counted using a stereo microscope (model Micros Austria) at ×10 and ×30 magnification.

Table 1

Total number of specimens of round goby and other fish species processed from the Iskar River with mean standard length (SL, cm) and standard deviation (SD)

	Month							Total
Species	March	April	May	June	August	September	October	number
N. melanostomus (ind.)	12	27	27	14*	28	14	9	131
average SL ± SD (cm)	3.8 ± 0.66	4.5 ± 1.3	5.1 ± 0.8	5.7 ± 1.2	6 ± 1.5	7.5 ± 1.3	4.9 ± 2.3	
A. alburnus (ind.)		18					9	27
average SL ± SD (cm)		6.0 ± 1.3					6.9 ± 0.98	
A. bipunctatus (ind.)				6*	5**		6***	17
average SL ± SD (cm)				6.4 ± 0.63	6.6 ± 0.61		5.7 ± 0.74	
B. petenyi (ind)	12	38	8	8	11		7	84
average SL ± SD (cm)	4.0 ± 0.65	4.9 ± 0.54	4.6 ± 0.42	8.0 ± 2.6	6.5 ± 1.3		5.3 ± 2.2	
S. cephalus (ind.)	13	7	15	10	6	8	6	65
average SL ± SD (cm)	6.7± 3.1	5.0 ± 1.6	6.6 ± 0.42	8.0 ± 2.6	7.0 ± 1.9	6.8 ± 1.7	5.9 ± 2.1	
V. vimba (ind.)		6						6
average SL ± SD (cm)		5.6 ± 1.4						
N. fluviatilis (ind.)		10		7*				17
average SL ± SD (cm)		4.9 ± 0.9		5.1 ± 1.3				

*pooled sample from June and July
**pooled sample from August and September
***pooled sample from October and November

Table 2
Total number of specimens of round goby and other fish species processed from the Vit River with mean standard length (SL, cm) and standard deviation (SD)

	Month							
Species	March	May	June	July	September	October	November	Total number
N. melanostomus (ind.)	47	19	10	6	10	7	5	104
average SL ± SD (cm)	4.9 ± 0.8	5.6 ± 1.2	6.5 ± 1.1	6.2 ± 2	5.0 ± 0.8	4.2 ± 1.3	3.5 ± 0.5	
A. bipunctatus (ind.)	15	5	14		8		8	50
average SL ± SD (cm)	6.4 ± 0.8	5.8 ± 0.7	6.2 ± 1.3		5.2 ± 1.2		6.0 ± 0.9	
B. petenyi (ind.)	18	11	9	8	15	11	8	80
average SL ± SD (cm)	6.7 ± 1.9	6.4 ± 1.7	7.2 ± 2.7	10 ± 3.7	7.2 ± 2.0	6.8 ± 1.9	7.5 ± 2.7	
G. gobio (ind.)							6	6
average SL ± SD (cm)							8.7 ± 1.2	
P. parva (ind.)	24							
average SL ± SD (cm)	5.2 ± 1.4							24
S. cephalus (ind.)	13	9	11	10	7	11		61
average SL ± SD (cm)	7.2 ± 1.8	6.0 ± 1.4	7.0 ± 3.4	6.5 ± 1.7	6.2 ± 1.5	5.4 ± 2.1		
N. fluviatilis (ind.)					8*			8
average SL ± SD (cm)					5.0 ± 2.4			
P. fluviatilis (ind.)				5**				5
average SL ± SD (cm)				7.2 ± 1.4				
C. taenia (ind.)	8			10*				18
average SL ± SD (cm)	6.2 ± 3.6			6.0 ± 8.0				
C. albicoloris (ind.)	9	7						16
average SL ± SD (cm)	8.6 ± 1.4	8.2 ± 1.4						

*pooled sample from August and September
**pooled sample from May, August and September

Aquatic macroinvertebrates were collected from the fishing area immediately after the fish were sampled. Three to six samples of macroinvertebrates were collected from each study site using a Hess sampler (ISO 8265:1988) with a frame size of 0.3 × 0.3 m and a mesh size of 0.5 mm. The number of replicate samples from each location depended on the microhabitat diversity and the *in situ* preliminary rough estimation of macroinvertebrate abundance. For each site, all replicates were pooled together in a single sample, fixed, and preserved in 70% ethanol.

Gobies were identified using Vassilev et al. (2012), while the rest of the fish were identified according to Kottelat and Freyhof (2007). Macroinvertebrates were identified using Uzunov et al. (2010) and Moller Pillot (2009). Identification efforts were determined by the state of the prey items and the reliability of identification.

Data analysis

For each macroinvertebrate taxon identified in the benthic samples, density (*Di*) was calculated as individuals per square meter:

$$D_i = (\Lambda i / 0.9) \times Fr^{-1} (ind m^{-2}) \qquad (1)$$

where Ai is the abundance for taxon i in the sample, *Fr* is the number of collected frames that make up the sample, and the number 0.9 is the area in square meters outlined by the Hess sampler frame. The sum of *Di* gives the total macroinvertebrate density (*Dt*).

Pairwise comparisons between the two studied rivers both with respect to fish and macroinvertebrate taxonomic diversity were done with the Bray and Curtis similarity index (*BC*) (Bray and Curtis 1957):

$$BC = 2W / (a + b)\% \qquad (2)$$

where W is the count of fish/macroinvertebrate taxa found at both sites compared, while a and *b* are the counts of fish/macroinvertebrate taxa for each individual location.

In order to account for both the prey's individual volume and its contribution to the overall gut content, the relative volume of each taxon in the diet was calculated using the visual method. The gut content was spread in a Petri dish with a grid millimetre paper placed underneath. The number of grid cells obscured by individuals of each prey taxon were counted. This permitted calculating the relative percentage volume (*Vi*):

$$Vi = Ci / Ct \times 100\% \qquad (3)$$

where *Ci* is the number of cells obscured by taxon I in all the full guts of samples examined and *Ct* is the number of cells obscured by all the prey taxa in all the full guts examined within a sample.

Schoener's index was calculated (O_D) (Schoener 1970, Wallace 1981) to assess the diet overlap between the round goby and native fish species from each river as follows:

$$O_D = 1 - \left(0.5 \sum_{i=1}^{\pi} \left|p(A)_i - p(Nm)_i\right|\right) \qquad (4)$$

where $p(A)_i$ and $p(Nm)_i$ are the relative volumes of prey taxa i for an analyzed native species and a round goby, respectively. The values of the index vary between 0 (no overlap) and 1 (complete overlap), where a value of 0.5 indicates substantial overlap between the diets of the two species.

Using the O_D index, diet overlap is assessed with respect to the overall similarity between all taxa present in the guts examined from each sample. In order to explore which food resource contributes the most to the food niche of a particular fish species, the index of relative food importance (I_{FI}) (Herder and Freyhof 2006) was calculated for the round goby and all other fishes with which it had a significant food niche overlap:

$$I_{FI} = (OiVi) / \left(\sum_{i=1}^{\pi} OiVi\right) \times 100\% \qquad (5)$$

where *Oi* is the frequency of occurrence of prey taxa i (% of guts, in which the prey is present) and *Vi* is from equation [3]. I_{FI} ranges from 0 to 100 with high values corresponding to the relative importance of a prey taxon in the diet.

Diet overlap and the food importance of the consumed prey were assessed separately for all fish individuals captured during a certain month. In cases when specimens of a given fish species were < 5 in a month, neighboring months were pooled in a single sample. Species for which bottom invertebrates are not a major food resource, such as *Chondrostoma nasus* L. and *Rhodeus amarus* Bloch, were excluded from the analysis. Fish with low representativeness in respective sections of a river (catches < 5 annually) were also omitted.

Results

A total of 17 fish species were recorded in the sampled river sections –14 in Iskar River and 14 in the Vit River, and 11 species were found in both rivers (Table 3). Three of the species are included in Annex 2 of Council Directive 92/43/EEC. Four of the species caught were non-native – *P. parva*; *Lepomis gibbosus* L.; *Carassius gibelio*; round goby, *N. melanostomus.*

The similarity of fish species composition between the sections of the Iskar and Vit rivers sampled was very high (BC > 93%). Differences in the macroinvertebrate community structure was moderate (BC = 57%). The mean total density of the macroinvertebrates in Iskar River was 447 ind. m^{-2} ± 266 SD with a maximum density in August (1053 ind. m^{-2}) and a minimum in December (130 ind. m^{-2}). The mean macroinvertebrate density in the Vit River was 501 ind. m^{-2} ± 250 SD, and the highest values were observed in October and the lowest in May (944 and 218 ind. m^{-2}, respectively).

High diet overlap ($O_D \geq 0.5$) was observed between *N. melanostsomus* and the following fish species: *B. petenyi*; *V. vimba*; *N. fluviatilis*; *P. fluviatilis*; *G. gobio* (Tables 4 and 5).

Table 3
Taxonomic list of the fish species collected in the study areas in the Iskar and Vit rivers, with IUCN status (LC – least concern); HD – species included in Annex 2 of the Council Directive 92/43/EEC of habitats in Natura 2000

Species/Family	Iskar	Vit	IUCN status	HD	Non-native
Centrarchidae					
Lepomis gibbosus (Linnaeus, 1758)	+	+	LC		+
Cobitidae					
Cobitis albicoloris Chichkoff, 1932		+			
Cobitis taenia Linnaeus, 1758		+	LC	+	
Cyprinidae					
Alburnoides bipunctatus (Bloch, 1782)	+	+			
Alburnus alburnus (Linnaeus, 1758)	+	+	LC		
Barbus petenyi Heckel, 1852	+	+	LC	+	
Carassius gibelio (Bloch, 1782)	+	+			+
Chondrostoma nasus (Linnaeus, 1758)	+		LC		
Gobio gobio (Linnaeus, 1758)	+	+	LC		
Pseudorasbora parva Temminck and Schlegel, 1846	+	+			+
Rhodeus amarus (Bloch, 1782)	+	+	LC	+	
Squalius cephalus (Linnaeus, 1758)	+	+	LC		
Vimba vimba (Linnaeus, 1758)	+		LC		
Gobiidae					
Neogobius melanostomus (Pallas, 1814)	+	+			+
Neogobius fluviatilis (Pallas, 1814)	+	+	LC		
Percidae					
Perca fluviatilis (Linnaeus, 1758)		+	LC		
Siluridae					
Silurus glanis (Linnaeus, 1758)	+		LC		

Table 4
Results of food niche overlap index (O_D) between the round goby and fish species from the Vit River; values indicating significant overlap (<0.5) are in bold

Species	Month							Total
O_D index	March	May	June	July	Sepember	Octtober	November	number
A. bipunctatus	0.25	0.35	0.22		0.31		0.05	
B. petenyi	0.29	0.39	0.21	0.18	0.13	0.17	0.20	
G. gobio							**0.53**	
P. parva	0.28							
S. cephalus	0.10	0.14	0.15	0.18	0.23	0.17		
N. fluviatilis					0.36			
P. fluviatilis				**0.50**				
C. taenia	0.24			0.38				
C. albicoloris	0.25	0.31						

Table 5
Results of the food niche overlap index (O_D) between the round goby and fish species from the Iskar River; values indicating significant overlap (<0.5) are in bold.

Species	Month							Total
O_D index	March	April	May	June	August	Sepember	October	number
A. alburnus		0.23					0.06	
A. bipunctatus				0.33	0.29		0.01	
B. petenyi	**0.64**	**0.70**	0.40	0.20	0.22		0.38	
S. cephalus	0.09	0.21	0.10	0.01	0.13	0.10	0.14	
V. vimba		**0.56**						
N. fluviatilis		**0.50**		**0.51**				

In the Iskar River, food overlap between the round goby and native species was observed in March, April, and June/July. The round goby and *B. petenyi* food overlapped with respect to Chironomidae larvae (*Orthocladius/Cricotopus* spp., Tanytarsini g. sp., *Eukiefferiella* spp.) and mayfly nymphs (*Baetis* spp.) (Fig. 1a). The overall diet overlap between the round goby and *V. vimba* was found to be considerable in spring and included mostly larvae of *Orthocladius/Cricotopus* spp. and Tanytarsini g. sp. (Fig. 2a). Overlap between *N. fluviatilis* and the round goby was observed during April and June/July when both fishes consumed *Orthocladius/Cricotopus* spp., Tanytarsini g. sp., and Psychomyiidae larvae (Fig. 3a).

In the Iskar River the density of *Baetis* spp. in the bottom substrate was 74 ind. m^{-2} during March (Fig. 1b). The abundance of *Orthocladius/Cricotopus* spp. increased from 2 ind. m^{-2} in March to 52 ind. m^{-2} in April. The same was observed for other Chironomid larvae such as Tanytarsini g. sp. (from 2 ind. m^{-2} to 20 ind. m^{-2}), while the number of *Eukiefferiella* spp. decreased in April to 13 ind. m^{-2} from 43 ind. m^{-2} in March. In June and July, the mean abundance of *Orthocladius/Cricotopus* spp. was 42 ind. m^{-2} and that of Tanytarsini g. sp. was 23 ind. m^{-2}. Trichoptera larvae such as those from the Psychomyiidae family were represented by a mean of 20 ind. m^{-2} in April, June, and July (Fig. 3b).

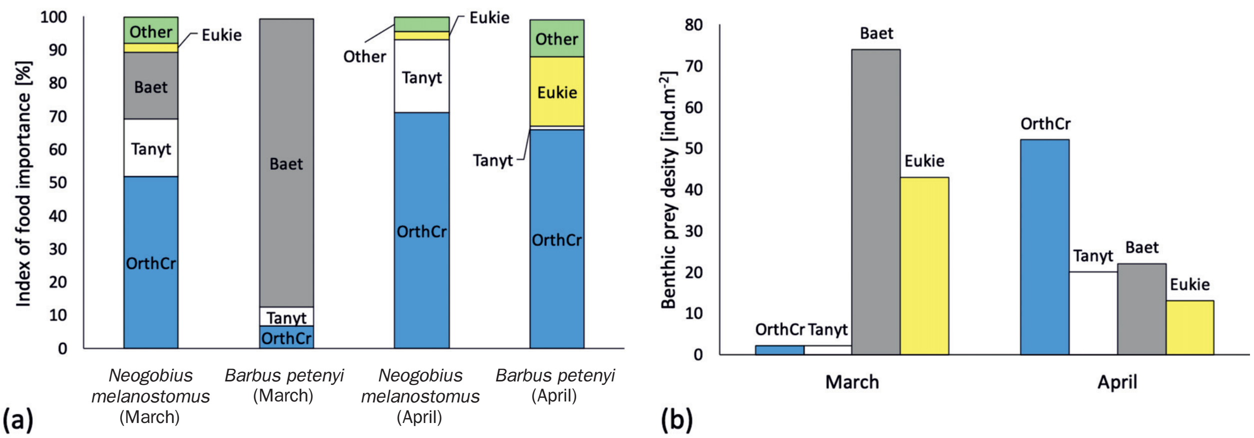

Figure 1. Index of food importance (IFI) of significant prey of *Neogobius melanostomus* and *Barbus petenyi* in the Iskar River (March and April) (a) and benthic prey density for the same period (b). For (a) and (b): OrthCr – *Orthocladius/Cricotopus* spp. (Diptera); Tanyt – Tanytarsini g. sp. (Diptera); Eukie – *Eukiefferiella* sp. (Diptera); Baet – *Baetis* spp. (Ephemeroptera).

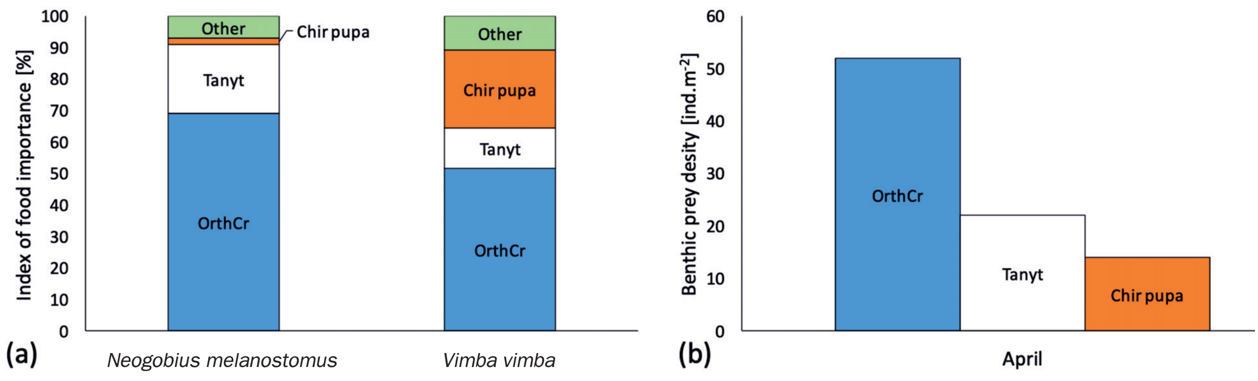

Figure 2. Index of food importance (IFI) of significant prey of *Neogobius melanostomus* and *Vimba vimba* in the Iskar River (April) (a) and benthic prey density for the same period (b). For (a) and (b): OrthCr – *Orthocladius/Cricotopus* spp. (Diptera); Tanyt – Tanytarsini g. sp. (Diptera); Chir pupa – Chironomidae pupae (Diptera).

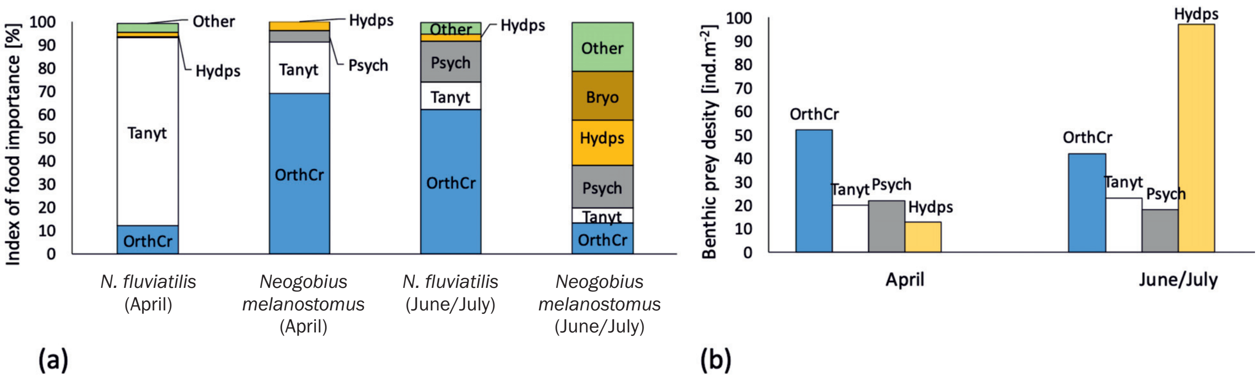

Figure 3. Index of food importance (IFI) of significant prey of *Neogobius melanostomus* and *Neogobius fluviatilis* in the Iskar River (April and June/July, pooled sample) (a) and benthic prey density for the same period (b). For (a) and (b): OrthCr – *Orthocladius/Cricotopus* spp. (Diptera); Tanyt – Tanytarsini g. sp. (Diptera); Eukie – *Eukiefferiella* sp. (Diptera); Hydps – *Hydropsyche* spp. (Trichoptera); Psych – Psychomyiidae g. sp. (Trichoptera); Bryo – Bryozoa g. sp.

In the Vit River food overlap was observed between the round goby and *G. gobio* in November and between the round goby and *P. fluviatilis* in May, August, and September (pooled sample). The overlap between *P. fluviatilis* and the round goby included *Gammarus* spp. and *Baetis* spp. (Fig. 4a). The diet overlap between *G. gobio* and the round goby included *Gammarus* spp. and *Hydropsyche* spp. and *Orthocladius/Cricotopus* spp. (Fig. 5a).

In the Vit River the abundance of *Gammarus* spp. was between 100 ind. m^{-2} (in May–August) and 260 ind. m^{-2} in November (Fig. 4b). In May, August, and September the mean abundance of both *Orthocladius/Cricotopus* spp. and *Hydropsyche* spp. was 30 ind. m^{-2} while the abundance of *Baetis* spp. was 48 ind. m^{-2}. In November the abundance of *Hydropsyche* spp. was 108 ind. m^{-2} and that of *Orthocladius/Cricotopus* spp. was 55 ind. m^{-2} (Fig. 5b).

Discussion

Feeding plasticity is considered to be one of the key traits that facilitates the invasive spread of the round goby (Brandner et al. 2013, Nurkse et al. 2016). The newly spreading populations in the tributaries of the Lower Danube River are no exception (Dashinov and Uzunova 2020). *N. melanostomus* coexists in these rivers with many fish species of similar feeding profiles, such as *Gymnocephalus baloni*, *G. schraetser*, *Zingel streber* Siebold and *Z. zingel* L., *G. gobio*, *Romanogobio* spp., *Barbus barbus* L., *B. petenyi*, and

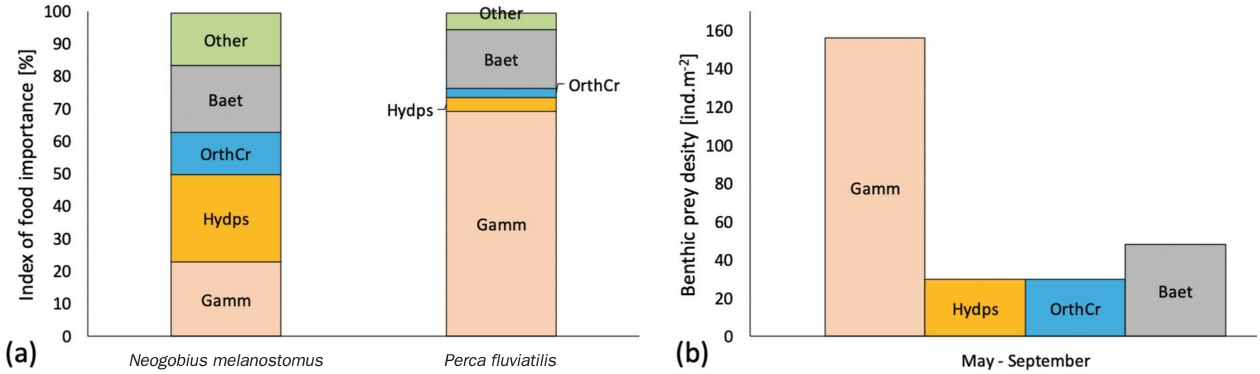

Figure 4. Index of food importance (IFI) of significant prey of *Neogobius melanostomus* and *Perca fluviatilis* in the Vit River (May, August and September (pooled sample) (a) and benthic prey density for the same period (b). For (a) and (b): OrthCr – *Orthocladius/Cricotopus* spp. (Diptera); Hydps – *Hydropsyche* spp. (Trichoptera); Baet – *Baetis* spp. (Ephemeroptera); Gamm – *Gammarus* spp. (Amphipoda).

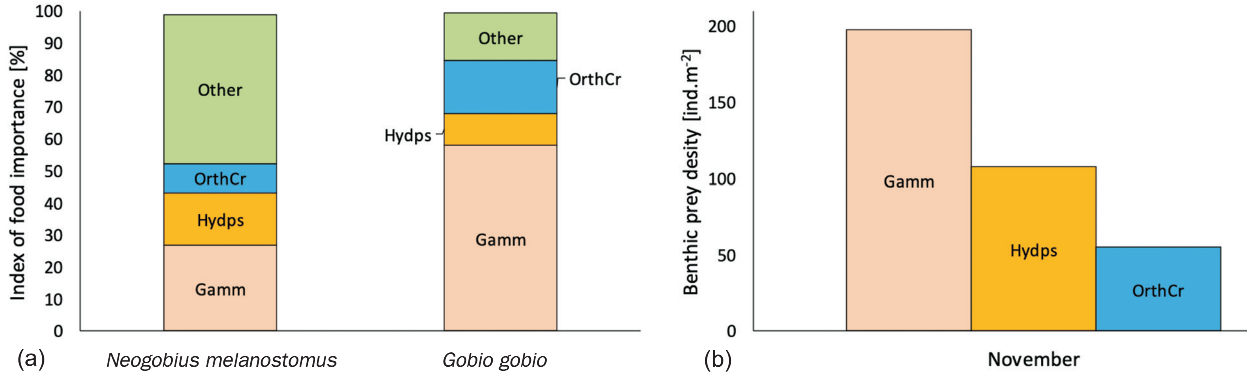

Figure 5. Index of food importance (IFI) of significant prey of *Neogobius melanostomus* and *Gobio gobio* in the Vit River (November) (a) and benthic prey density for the same period (b). For (a) and (b): OrthCr – *Orthocladius/Cricotopus* spp. (Diptera); Hydps – *Hydropsyche* spp. (Trichoptera); Gamm – *Gammarus* spp. (Amphipoda).

V. vimba (Pehlivanov et al. 2009). Generally, dietary overlap is greatest among ecologically similar species (e.g., Nagelkerke et al. 2018). Therefore, there is a potential for competitive relationships between these native fishes and the round goby, especially in cases when prey availability is low.

The existence of competitive food relationships between *N. melanostomus* and local fish species varies at the ecosystem level (Ricciardi and Cohen 2007, Hirsch et al. 2016). For example, we reported high food overlap between *B. petenyi* and the round goby in the Iskar River, but not in the Vit River. The food niche overlap between *B. petenyi* and the round goby also varied in intensity throughout the year. The most significant overlap was observed in the spring months (March and April). One possible reason is the intensive feeding of *B. petenyi* before the start of its breeding season. Competition for food resources might be the reason for the negative correlation between the abundance of the round goby and *Barbus barbus* L. that was recorded in the upper sections of the Danube (Cerwenka et al. 2017).

Overlap between the food niches of *V. vimba* and the round goby was also observed during the spring months; again, this coincides with the period when *V. vimba* begins to feed intensively after its winter dormancy and prior to its breading season (Okgerman et al. 2011). Oesterwind et al. (2017) suggest that *N. melanostomus* and *V. vimba* compete for resources such as crustaceans and molluscs in the near shore habitats of the Baltic Sea, but this was not confirmed by overlap analysis.

It is important to note that high overlap between the round goby and *N. fluviatilis*, *V. vimba*, and *B.*

petenyi was observed during periods with relatively low macroinvertebrate fauna abundance. This is especially true for larval Chironomidae (*Orthocladius/Cricotopus* spp., *Eukiefferiella* spp., and Tanytarsini g. sp.), which are highly important benthic groups for all of these fish species.

In contrast, food niche overlap between the round goby and *P. fluviatilis* and also *G. gobio* was registered in periods with relatively high food item abundance, which generally excludes the possibility of significant competition for resources. The dietary overlap with *P. fluviatilis* and with native Percidae in general, has been confirmed in many parts of the round goby's invasive range (French and Jude 2001, Balshine et al. 2005, Copp et al. 2008, Krakowiak and Pennuto 2008, Bergstrom and Mensinger 2009, Crane et al. 2015), but in the Danube tributaries, Percidae species are poorly represented. The *P. fluviatilis* specimens caught probably entered the river from adjacent reservoirs and/or artificial ponds.

While we confirmed dietary overlap between the invasive round goby and *G. gobio*, this was not observed in other regions where the latter feeds mainly on phytoplankton and sessile algae (Nagelkerke et al. 2018). The river sections sampled in our study are inhabited by a number of species that are ecologically similar to *G. gobio,* such as *Romanogobio kessleri* Dybowski*, R. uranoscopus* Agassiz, and *R. albipinnatus* (Pehlivanov et al. 2009, Stefanov and Trichkova 2012a). In contrast to *G. gobio* species from the *Romanogobio* genus are endangered or vulnerable in Bulgaria and could potentially be affected by the presence of the round goby. For example, the competitive relationship between *R. albipinnatus* and the round goby is the probable cause of the observed population decline of the former in the near shore habitats in the mid Danube (Jurajda et al. 2005).

Not surprisingly, the round goby had a high food niche overlap with the closely related *N. fluviatilis*. This overlap was also observed in other Danube tributaries, such as the Sava River in Serbia (Piria et al. 2016). There are reports of competition for food between the round goby and other invasive Gobiidae, such as *Ponticola kessleri* Günther and *Proterorhinus marmoratus* Pallas (Copp et al. 2008, Brandner et al. 2013, Nagelkerke et al. 2018), but these gobies were not registered in our survey, although they are self-introduced in Danube tributaries (Zarev et al. 2013).

The round goby can be especially harmful to native Cottidae species, since it is known to compete with them not only for food, but also for benthic refuges and nesting substrates (French and Jude 2001, Janssen and Jude 2001, Bergstrom and Mensinger 2009, Van Kessel et al. 2016). The upper sections of the Bulgarian Danube tributaries are the only habitats were the critically endangered *Cottus gobio* L. and *Cottus haemusi* Marinov & Dikov are found in Bulgarian waters (Stefanov and Trichkova 2012b, Uzunova et al. 2017). Hence, a possible future coexistence with the invasive round goby will most likely lead to intense competition with unknown consequences for this native species.

The present findings confirm the hypothesis that the round goby is potentially harmful to several local fish species. Efforts should be made to stop the upstream spread of this invader in Bulgaria. Possible mitigation efforts against the round goby in areas that are already infested could include preventing additional threats to native ichthyofauna such as poaching, pollution, and habitat degradation.

Funding

This study was funded by the Sofia University Scientific Fund grants no. 80-10-41/19.04.2017 and no. 80-10-140/2018.

Acknowledgments. We would like to thank Assoc. Prof. Ivan Traykov, Assoc. Prof. Lyubomir Kenderov, PhD candidate Emil Kanev, and students Kostadin Ignatov, Yonko Sashov and Yana Petkova of the Sofia University "St. Kliment Ohridski" for their help with fieldwork and collecting fishes. We would like to thank the student committee of the Sofia University "St. Kliment Ohridski" for facilitating funding for our participation in the Goby Meeting of February 2020 in Starnberg, Bavaria, Germany where the preliminary data of this paper were presented.

Author contributions. E.U. overall supervision, field collection, writing and revising of the manuscript; D.D. field collection, laboratory analysis, data analysis, writing and revising the manuscript.

ORCID iD

Eliza Uzunova: https://orcid.org/0000-0003-4130-3211

Dimitriy Dashinov: https://orcid.org/0000-0001-5108-2614

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