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Non-native Ponto-Caspian Gobies in the mouths of the Vistula River tributaries

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Original article

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At present, four non-native gobiid species (racer goby *Babka gymnotrachelus*, monkey goby *Neogobius fluviatilis*, round goby *Neogobius melanostomus* and western tubenose goby *Proterorhinus semilunaris*) are listed as occurring in the Vistula River of Poland. In this work, the distribution and densities of gobiids in the river-mouth stretches of the Vistula tributaries flowing downstream from the Włocławek Reservoir in the Baltic Sea direction are presented. The fish were collected by electrofishing from a boat or while wading. Non-native gobiids were noted in 15 of the 18 tributaries studied. Among the 1,075 gobies caught, the western tubenose goby was the most common and occurred in 15 of the sites examined. The racer goby was registered in seven of the tributaries, while the monkey goby was recorded in only three. No round goby was caught. The highest densities of the western tubenose goby and racer goby were noted in the small stream located directly downstream from the Włocławek Reservoir. Among the species identified, the western tubenose goby seems to be more eurytopic because it was the last of these species to invade the Vistula River, but it is now the most widespread. In addition to a morphological identification of the fish, samples of each species were confirmed by an analysis of subunit I of the cytochrome oxidase (COI) barcode sequences. The genetic analysis of the COI subunit revealed that this sequence is effective for the taxonomic differentiation of the Ponto-Caspian gobies occurring in Poland. The results showed that gobiids have become a permanent element of the ichthyofauna of the Vistula and its tributaries that are available for migration

Key words: invasive species, Gobiidae, *Neogobius fluviatilis*, *Babka gymnotrachelus*, *Proterorhinus semilunaris*.

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By the end of the twentieth century, Ponto-Caspian gobies had increased their native range and colonised large European river systems, including those of the Danube, Rhine and Main, through artificial waterways and discharged ballast water (Copp *et al*. 2005; Roche *et al*. 2013). Similarly, the ichthyofauna of the Vistula Basin has included four non-native Ponto-Caspian gobiid fish since the 1990s: round goby *Neogobius melanostomus* (Pallas, 1814); racer goby *Babka gymnotrachelus* (Kessler, 1857); monkey goby *Neogobius fluviatilis* (Pallas, 1814); and western tubenose goby *Proterorhinus semilunaris* (Heckel, 1837).

The Vistula River is the longest river flowing into the Baltic Sea, and its runoff is second only to that of the Neva River (BACC II 2015). The round goby is known to have ascended the Vistula from the Gulf of Gdańsk, into which it was probably introduced along with discharged ballast water (Sapota & Skóra 2005). The species was first observed in 1990 (Skóra & Stolarski 1992), following which it was reported in the Vistula up to 130 km from the river mouth

(Kostrzewa & Grabowski 2002). The other three species migrated to Poland from the Black Sea basin via the Dnieper River and the Pripyat-Bug Canal to the Vistula, through the so-called central corridor (Semenchenko *et al*. 2011). This upstream expansion of fish in Poland was also observed in the case of the racer goby in Polish tributaries of the Dniester River (Kukuła & Bylak 2013).

The racer goby was caught for the first time in the Bug River, a Vistula tributary, in 1995 (Danilkiewicz 1996); while the monkey goby was also observed there in 1997 (Danilkiewicz 1998). After another decade, the western tubenose goby was first registered in the Włocławek Reservoir (Grabowska *et al*. 2008). All three species are distributed in the lower Vistula as far downstream as its mouth, although only the monkey goby has been reported in close proximity to the Gulf of Gdańsk and in the Vistula Lagoon (Lejk *et al*. 2013). However, the racer goby and monkey goby were also noted in the estuarine Mikoszewskie Lake close to the largest mouth branch of the Vistula (Kuczyński & Pieckiel 2018).

The Ponto-Caspian gobies have become essential food items of native predatory fishes such as the Northern pike *Esox lucius* Linnaeus, 1758, European perch *Perca fluviatilis* Linnaeus, 1758 and the pikeperch *Sander lucioperca* (Linnaeus, 1758) (Płąchocki *et al*. 2012). Additionally, these non-native species brought with them new fish parasites, such as *Gyrodactylus proterorhini* (Ergens, 1967)

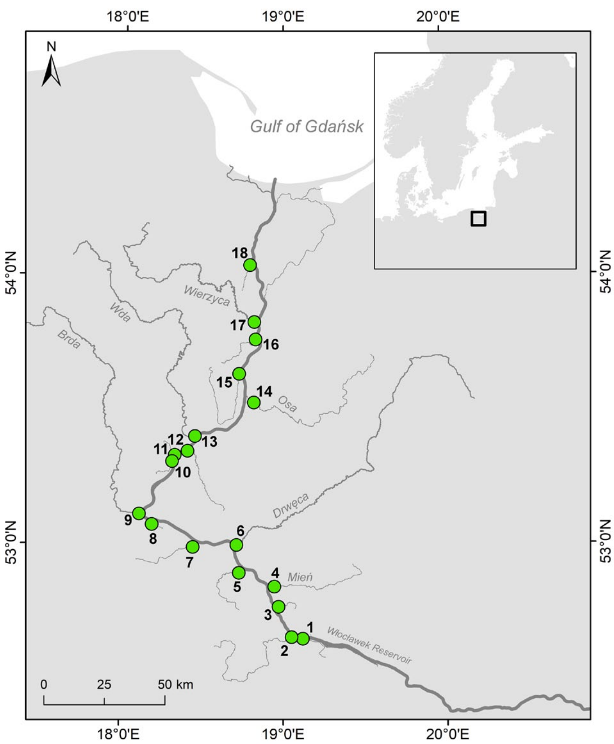

Fig. 1. Sampling sites in the tributaries of the lower Vistula River where the electrofishing was carried out in 2012.

(Mierzejewska *et al*. 2011). Observations from about 10 years ago have also shown there is competition for space with native stream fish such as the bullhead *Cottus gobio* (Linnaeus, 1758) (Kakareko *et al*. 2013).

Compared with other teleost fishes, Gobiidae is a hyperdiverse family with approximately 2,000 species occurring in varied aquatic habitats (Nelson 2006; Zander 2011). Mitochondrial cytochrome oxidase subunit I (COI) could serve as a rapid and reliable barcoding marker for identifying gobiid species (Jeon *et al*. 2012); therefore, this method could be a good complement to the classification of gobies based on the external morphology (Nelson 2006).

The aim of this study was to identify the distribution and density of invasive gobiid species in the river mouth stretches of the lower Vistula River tributaries located between the Gulf of Gdańsk and the Włocławek Reservoir using electrofishing, identification by morphological features and a mitochondrial DNA analysis. In addition, the results of the work are discussed in relation to the species composition of alien gobies in other large European rivers.

Material and methods

Study area and sampling

The surveys were carried out in 18 river mouth stretches of the Vistula tributaries located between the Gulf of Gdańsk and the Włocławek Reservoir between August and October 2012 (Fig. 1, Table 1). The fish samples were collected using the electric fishing method (Backiel & Penczak 1989). Electrofishing was chosen as the most representative approach for the sampling of all size classes of gobies (Brandner *et al*. 2013). A stationary generator (200 V, 5 A, smoothed AC) was used in the larger tributaries, and the catches were made by either wading upstream or, in the deepest stretches, by placing the device on a boat drifting downstream. A backpack generator (115-250 V, 1-2 A, DC) was used in the smallest streams (width <2.0 m).

The study sites were located close to the Vistula, and during high flows, they were often inundated by water from the main river. The geographic positions, which are presented in Table 1, show the beginning or end of the stretches of river during the fishing by wading and from a boat, respectively. The lengths of the river stretches examined ranged from 100 to 150 m when fishing by wading and from 400 to 700 m during the boat catches (Table 1). The fishing area covered the whole width of the smaller streams and approx. 2.5 m when sampling from a boat along one side of the river. Most of the studied stream sections were similar in terms of their habitats, but they dif-

Table 1

Details of the study sites. Site number, name of the Vistula tributary, sampling date, GPS location, site length (m), mean width (m) and depth (m), fishing method and the dominant bottom substrate (s – sand, st – stones, g – gravel)

Site	Tributary	Date	GPS	Length	Width	Depth	Method	Substrate
1	Zuzanka	30.08.2012	19.133, 52.656	150	7	0.2	wading	s
2	Zgłowiączka	04.09.2012	19.062, 52.656	150	12	0.3	wading	s
3	Dopływ z Gnojna	04.09.2012	18.976, 52.767	150	2	0.35	wading	s
4	Mień	29.08.2012	18.951, 52.843	150	5	0.25	wading	s
5	Tążyna	30.08.2012	18.743, 52.895	100	3.5	0.2	wading	s>>g
6	Drwęca	23.10.2012	18.711, 52.996	500	27	0.7	boat	st>s
7	Zielona Struga	05.09.2012	18.447, 52.992	150	4	0.2	wading	s
8	Dopływ z Solca	10.10.2012	18.186, 53.077	150	2.5	0.25	wading	s
9	Brda	23.10.2012	18.099, 53.113	700	35	1	boat	s>>st
10	Struga Niewieścińska	11.10.2012	18.310, 53.314	150	0.9	0.15	wading	s
11	Dopływ z Gawrońca	11.10.2012	18.325, 53.332	150	1.5	0.3	wading	s
12	Fryba	11.10.2012	18.412, 53.345	150	5	0.2	wading	s
13	Wda	24.10.2012	18.437, 53.400	500	25	1	boat	s
14	Osa	25.10.2012	18.821, 53.524	500	9	0.7	boat	s
15	Mątawa	25.10.2012	18.723, 53.632	400	6.5	0.8	boat	s
16	Młyńska Struga	18.10.2012	18.820, 53.764	150	1.8	0.2	wading	s
17	Wierzyca	24.10.2012	18.815, 53.831	500	20	1.2	boat	s
18	Drybok	29.10.2012	18.777, 54.034	150	1.7	0.15	wading	s>st

fered significantly in width (Table 1). The catch per unit effort (CPUE) was estimated as the number of fish caught per unit of time, similarly to the approach applied by Penczak and Kruk (2000), where the CPUE varied according to the river characteristics. We applied the stream size as the main parameter and: (a) for small streams with a width <2.5 m where we electrofished on a section of 150 m by wading upstream the time equivalent was 0.5 h; (b) for sites wider than 2.5 m where we electrofished on a section of 150 m by wading the time equivalent was 0.75 h; (c) for the sites where we electrofished from a boat downstream on a section of 500 m it was 0.5 h. For sites with different lengths, the numbers of fish were recalculated to 150 or 500 metres, depending on the fishing method.

The fish we caught were lethal anaesthetised with etomidate (Kazuń & Siwicki 2012), and were then counted and preserved in an ethanol solution (70%). Later, in the laboratory, the gobies were identified to the species based on morphologic features (Kottelat & Freyhof 2007). The smallest individuals were identified under a magnifier. Finally, every specimen was placed on a plastic plate and the total length was measured to the nearest 1 mm.

Molecular analysis of the COI gene

An analysis of subunit I of the cytochrome oxidase (COI) barcode sequences of three randomly selected specimens of monkey goby, and two specimens each of racer goby and western tubenose goby from the Mień, Fryba and Brda Rivers, respectively, were used to confirm the earlier species identification. DNA extraction was performed with Genomic Mini Kits (A&A Biotechnology, Poland). The purity and concentration of the DNA eluates were assessed on 1.5% agarose gel with a NanoDrop spectrophotometer (Thermo Fisher Scientific). Sets of PCR primers targeting COI [COI-Fish-F 5'-TTCTCAACTAACCAYAAAGAYATYGG-3', COI-Fish-R 5'-TAGACTTCTGGGTGGCCRAARAAYCA-3'] were applied (Kochzius *et al*. 2010). The COI polymerase chain reaction (PCR) with a total volume of 20 µl contained 10x reaction buffer, 1 mM dNTPs, 2.5 mM $MgCl_2$, 0.33 µM of each primer, 1.25 U Tag Polymerase (Run A&A Biotechnology, Poland) and 3 µl of DNA-extract. The thermal profiles from Kochzius *et al*. (2010) were used. The results of each PCR were verified by separating the analysed samples in 1.5% agarose gel, and each PCR product was then sequenced bidirectionally according to Sanger's method (Genomed, Poland). The sequences obtained were then analysed with BioEdit 7.0.5 software (Hall 1999), and the consensus versions were deposited in GenBank (29 January 2018) under Numbers MG865725 to MG865731. The sequences are also available in the corresponding BOLDSYSTEM (Ratnasingham & Hebert 2007). Species matching was performed using the BLAST tool assuming the highest value of taxonomic percent identity. The descriptive statistics were performed using Statistica 8 (StatSoft Inc., Tulsa, OK, USA).

Ethical approval

All of the research methods were carried out in accordance with the relevant ethical guidelines and regulations. The study complies with the current laws of the Republic of Poland. Field protocols for the capture, handling and release of the fish were approved by the Department of Environmental Protection, Marshal's Office of the Pomeranian and Kuyavian-Pomeranian Voivodeships. The research did not require special approval from the Local Ethics Committee.

Results

General data and fish measurements

Ponto-Caspian gobies were found in 15 of the river tributaries (88%). No gobies were observed in the Struga Niewieścińska or in the Wierzyca and Drybok tributaries located closest to the mouth of the Vistula River. In total, 1,075 specimens of Ponto-Caspian gobies were caught. The western tubenose goby was the most common (n=533, 49.6% of the gobies) and occurred in 15 of the sites that were sampled, while the racer goby (n=522, 48.5%) was registered in seven tributaries and the monkey goby (n=20, 1.9%) was recorded in only three. No round goby was caught.

The highest values of CPUE for the western tubenose goby and the racer goby were noted in the Zuzanka River (330.4 and 490.0 CPUE, respectively), which is located close to the Włocławek Reservoir. The monkey goby was the most abundant in the Mień River (80% of the gobies collected; 21.0 CPUE) (Fig. 2).

During the survey, 38 species of fish and lampreys were caught (Radtke *et al*. 2016). Among all the fish that were caught, the most abundant were eurytopic species such as perch (Linnaeus, 1758), roach *Rutilus rutilus* (Linnaeus, 1758) and white bream *Blicca bjoerkna* (Linnaeus, 1758). The most widespread species were the three-spined stickleback *Gasterosteus aculeatus* Linnaeus, 1758, tubenose goby and spined loach *Cobitis taenia* Linnaeus, 1758.

The western tubenose goby measured from 23 to 93 mm in total length, while the racer goby had a wider length range and measured from 24 to 109 mm. The twenty monkey gobies had lengths ranging from 35 to 91 mm (Table 2).

Taxonomic identification by the molecular analysis

The genetic analysis fully confirmed the accuracy of the taxonomic identification. In all cases, the sequences obtained showed a 100% probability of placement (Table 3). The highest second species similarity for the monkey goby was found for the Caspian goby *Neogobius caspius* (Eichwald, 1831), while for the racer goby it was the Iranian goby *Ponticola iranicus* (Vasil'eva, Mousavi-Sabet & Vasil'ev, 2015) and for the western tubenose goby it was *Proterorhinus semipellucidus* (Kessler, 1877) (Table 3).

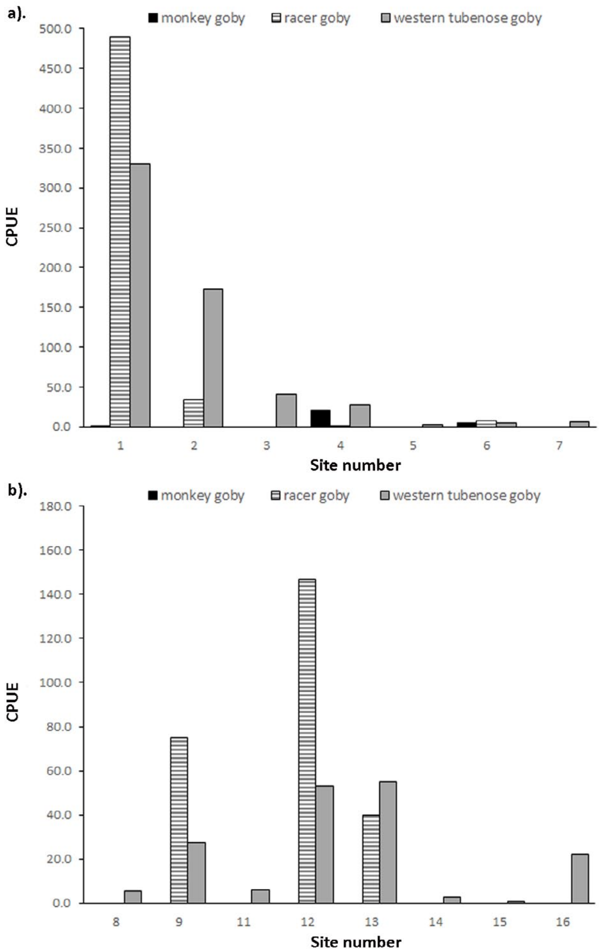

Fig. 2. Density of three species of gobies in the 15 sites studied where gobies were found. Graph: a – Sites 1-7, b – Sites 8-9 and 11-16.

Table 2

Length ranges of gobies caught in the Lower Vistula tributaries in 2012

Species	n	Mean TL (mm)	±SD	TL min-max
western tubenose goby	431	42.6	14.4	23-93
racer goby	481	44.3	13.5	24-109
monkey goby	20	47.5	12.6	35-91

Table 3

Details of the cytochrome oxidase subunit 1 (COI) sequences obtained: goby species, sequence length, efficiency of species identification, and GeneBank accession numbers, best next species similarity

Species	bp	Taxon identity	Accession numbers	Species similarity
monkey goby	590–619	100%	MG865725-MG865727	Caspian goby (93.9%)
racer goby	524–539	100%	MG865728-MG865729	Iranian goby (92.3%)
western tubenose goby	565–566	100%	MG865730-MG865731	*Proterorhinus semipellucidus* (96.7%)

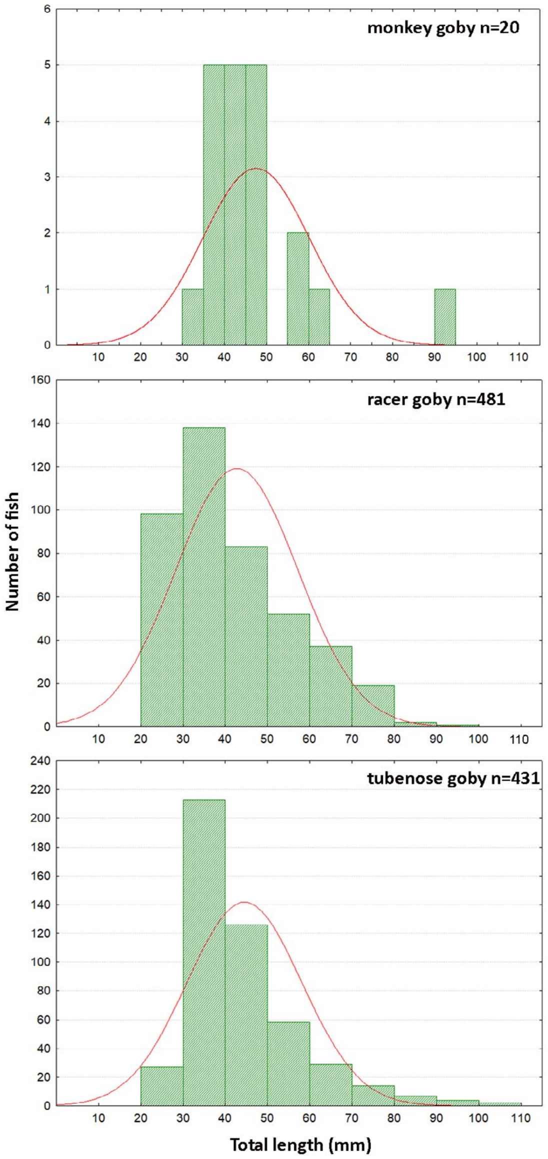

Fig. 3. Length-frequency distributions of the gobiids species caught by electrofishing in the lower Vistula tributaries in 2012. Red lines – the theoretical normal distribution density curve.

Discussion

The racer goby was the most numerous goby species in the present study. It was the first noted in the Bug River, a Vistula tributary (Danilkiewicz 1996), while the first recorded catch in the Vistula was in the Włocławek Reservoir in 2000 (Kostrzewa & Grabowski 2001). The second goby species, the monkey goby, was also detected in the Vistula basin in the Bug River in 1997, and for first time in the Włocławek Reservoir in 2002. However, this species was the least abundant during the present study. Furthermore, in a net survey conducted in 2004 in the main course of the Vistula downstream from the Włocławek Dam, the monkey goby was the dominant species among all the fish caught (Kakareko *et al*. 2009). In more recent studies, the monkey goby has also been present throughout the lower Vistula and the Vistula Lagoon since at least 2006 (Lejk *et al*. 2013; Kuczyński & Pieckiel 2018), which proves that the species had already colonised a section of the lower Vistula and its estuary by this time. Therefore, there is no easy explanation as to why this species was rarely caught during the present survey. Among the gobies that we caught, the western tubenose goby seemed to be more eurytopic, because it was the last to invade the Vistula River (Grabowska *et al*. 2008) but now it is the most widespread.

The round goby, which is common in the Gulf of Gdańsk, seemed not to be invasive in the Vistula tributaries. This is puzzling, considering that it was previously observed more than 100 km upstream from the mouth of the Vistula (Kostrzewa & Grabowski 2002). It is likely that this species was then at the stage of spreading, but competition from other gobies or slightly different habitat requirements prevented its colonisation. High densities of round goby were also noted in the vicinity of the Motława River, a former Vistula estuary, and its tributary the Radunia River, but it does not coexist with other gobiid species there (Radtke *et al*. 2011). The observation that the round goby does not seem to be successful in the Vistula system is even more interesting because this species is already the dominant goby in many other large European river systems (e.g. Jurajda *et al*. 2005; Cerwenka *et al*. 2018; Gaye-Siessegger *et al*. 2022). One possible explanation for this is that the upper and middle sections of the Rhine and Danube Rivers are mostly dominated by gravel and stone substrate, whereas the lower Vistula is dominated by a sandy bottom (Tockner *et al.* 2009).

The genetic analysis of the COI subunit revealed that this sequence is effective for the taxonomic differentiation of the Ponto-Caspian gobies occurring in Poland and that it is a good complement to methods based on the morphological features (Knebelsberger & Thiel 2014). Currently, it is also a cost effective technique and in difficult cases it can be used, for example, for larval identification, analyses of remains and in diet studies.

The distribution of the gobies observed indicated that they inhabit almost all the tributaries of the lower Vistula. Their upstream penetration of the tributaries is only hampered by barriers to migration that limit their occurrence (Jones *et al*. 2021). As is generally known, tributary confluence zones usually provide inviting habitats of high ecological importance for aquatic biota (Rice *et al*. 2008). Moreover, the fact that we did not catch gobies in three of the tributaries in 2012 does not mean that they were not present at that time, as it is also known that tributary confluence zones are characterised by high species variability over time (Czeglédi *et al*. 2015). This has been confirmed by the results of several of our newer electrofishing sessions conducted at the sites during various occasions. For example, the racer goby was caught at Site 17 in the Wierzyca River in 2015, while we noted a monkey goby at Site 14 in the Osa River in 2017, and a racer goby was registered at Site 16 in the Młyńska Struga in 2019, despite the fact that these species were not previously noted in these locations. These findings indicate that the distribution of gobies and their share in fish communities is constantly changing and fluctuating. Nevertheless, these species have become a permanent element of the ichthyofauna of the Vistula and its tributaries that are available for fish migration.

Author contributions

Research concept and design: R.B., M.E.S., G.R.; Collection and/or assembly of data: R.B., M.E.S., G.R.; Data analysis and interpretation: R.B., M.E.S., G.R., A.W.-B.; Writing the article: R.B., M.E.S., G.R., A.W.-B.

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Conflict of Interest

The authors declare no conflict of interest.

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