**This manuscript is textually identical with the published paper:**
Szalóky, Z.; Bammer, V.; György, Á I.; Pehlivanov, L.; Schabuss, M.; Zornig, H.; Weiperth, A.; Erős, T. (2015) Offshore distribution of invasive gobies (Pisces: Gobiidae) along the longitudinal profile of the Danube River. FUNDAMENTAL AND APPLIED LIMNOLOGY, 187: (2) pp. 127-133. DOI: http://dx.doi.org/10.1127/fal/2015/0768

# Offshore distribution of invasive gobies (Pisces: Gobiidae) along the longitudinal profile of the Danube River

Z. Szalóky[a], V. Bammer[b], Á.I. György[a], L. Pehlivanov[y], M. Schabuss[d], H. Zornig[d], A. Weiperth[a], T. Erős[c,*]

[a] *Danube Research Institute, MTA Centre for Ecological Research, Jávorka Sándor u. 14., H-2131 Göd, Hungary*

[b] *Federal Agency for Water Management, Institute for Water Ecology, Fisheries and Lake Research A-5310 Mondsee, Austria*

[c] *Balaton Limnological Institute, MTA Centre for Ecological Research, Klebelsberg Kuno u. 3., H-8237 Tihany, Hungary*

[d] *PRO FISCH OG Ecological Consultants, Hörlgasse 6/13,1090 Vienna, Austria*

[y] *Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences 2., Major Yurii Gagarin str., 1113 Sofia, Bulgaria*

*Corresponding author:
Tibor ERŐS
Balaton Limnological Institute, MTA Centre for Ecological Research
Klebelsberg K. u. 3., H-8237 Tihany, Hungary
Tel.: +36 87 448 244
Fax.: +36 87 448 006
E-mail address: eros.tibor@okologia.mta.hu

## Abstract

The invasion of fish and invertebrate species of Ponto-Caspian origin is in the forefront of freshwater research due to the extremely fast range expansion of many species and their radical effects on the structure and functioning of ecosystems in their non-native habitat. This study provides the first assessment of the offshore distribution of invasive Ponto-Caspian gobies along the longitudinal profile of the Danube River using the data of the Joint Danube Survey 3 research expedition. Six goby species were collected, the round goby *Neogobius melanostomus*, the monkey goby *N. fluviatilis*, the Kessler goby *Ponticola kessleri*, the racer goby *Babka gymnotrachelus*, the stellate tadpole-goby *Benthophilus stellatus*, and the tubenose goby *Proterorhinus semilunaris*, which showed large differences in their offshore distribution along the river. *N. fluviatilis* was found for the first time as a new species in Austria, which shows the slow spread of this species upstream in the Danube River or alternatively, its introduction by ships. Offshore trawling confirmed the use of deep channel habitats by gobies, and is suggested as a useful tool for monitoring spatial and temporal trends in the dynamics of invasive benthic species for riverine fish biological research.

Key words: invasive species, Gobiids, benthic trawling, large river

## Introduction

The invasion of fish and invertebrate species of Ponto-Caspian origin is in the forefront of freshwater research due to the extreme fast range expansion of many species and their radical effects on the structure and functioning of ecosystems in their non-native habitat (Ricciardi and MacIsaac, 2000; Borza et al, this issue). Gobiids (Pisces, Gobiidae) are one of the most characteristic examples of this process. For example, the round goby *Neogobius melanostomus* (Pallas 1814) was first discovered in North America in 1990 in the St Clair River (Jude 1992). During its invasion over less than 25 years, the species spread through the Laurentian Great Lakes at a faster rate than any previous fish invader. Its invasion led to significant changes of entire food webs (Kornis et al., 2007).

The appearance of Ponto-Caspian gobies in North America happened via ballast water transport of transoceanic vessels (Jude et al., 1992). However, the relative role of human mediated transport vs natural dispersal processes in rivers flowing into the Black Sea is still disputed among scientists (Harka and Bíró 2007; Kornis et al., 2012). The fast spread of gobies in the Danube River, for example, has been connected to ballast water transport too, which may explain why these species were found first in the vicinity of urbanised areas sometimes even some hundreds of kilometres away from their original range limit (Roche et al., 2013). Small crevices can provide an ideal "spawning substrate" for these speleophil species, which could explain the vector role of ships in their dispersal. Other factors, such as different hydro-technical constructions (i.e. rip-rap, groynes, revertment and dykes) or even increasing mean water temperature of the river have been also related to their fast spread and successful invasion (Harka and Bíró, 2007). However, due to the lack of a consistent monitoring system, which could have exactly tracked the proliferation of gobies along their invasion route, it is hard to answer questions related to their saltatoric vs continuous upstream movement. Therefore, transboundary surveys on large international rivers with a standardized methodology are necessary to understand spatial and temporal changes in fish assemblages and to reveal the crucial parameters for the invasion of individual species over large spatial extents.

Fish assemblage surveys are usually methodologically restricted to sampling shoreline habitats in very large rivers. Although offshore main channel habitats have a much larger extent than shoreline areas and have been shown to be intensively utilized by fish (Dettmers et al., 2001; Szalóky et al., 2014), detailed knowledge about the composition of offshore fish assemblages is limited. Several studies reported on the shoreline distribution and habitat use of invasive gobies in large European rivers (see e.g. Erős et al., 2005; Jurajda et al., 2005; Kakareko et al., 2009), but how and to what extent gobies utilize offshore areas is still unknown. Knowledge about the offshore distribution and abundance of gobies could help to better evaluate invasion success and ecological importance of these species.

The aim of this paper is to present the first standardized catch per unit effort (CPUE) offshore data on the actual abundance of gobies in the main channel of the Danube River using the results of the Joint Danube Survey 3 expedition. The Joint Danube Survey 3 was an international river research expedition which was organized by the International Commission for the Protection of the Danube River (ICPDR) between 13$^{th}$ of August to 26$^{th}$ of September 2013 (http://www.icpdr.org/jds/). The survey covered the sampling of several biotic and abiotic components of the Danube from Regensburg (i.e. the first bigger town), South Germany to the Danube Delta in Romania.

## Material and methods

Offshore distribution of gobies was examined at 22 sampling sites along a 2214 km long river section. Sampling in offshore areas was done by drift net (mesh size 5 and 8 mm for the inner and outer mesh bag, respectively) attached to stainless steel frame (2 m wide × 1 m high) (for details see Szalóky et al., 2014). The frame was electrified with a Hans-Grassl EL65 IIGI electrofishing device operated with a VANGUARD HP21 14.9 KW generator. A 6 m long copper cathode cable was connected freely and pulled approx. 2 m before the electrified frame. The fishing team consisted of two people handling the framed net, one handling the electrofishing device and one operating the boat. Trawling was conducted during daytime with a 6.3 m long boat powered by a 50 horsepower outboard Mercury four stroke engine. Before starting trawling, the operators lowered the frame to the bottom while the boat was slowly moving downstream with the flow. Measurement of the trawling route using a GARMIN 60CSx GPS only began after the net reached the bottom, which could be easily felt while holding the central rope, and right after electroshocking started. The direct current (approx. 350 V, 33 A) was applied for 5-8 sec. with 3-5 sec. breaks between the operations to minimize fright bias and injury of fish. The applied trawling speed was slightly higher than the current velocity of the river (approx. 0.6 m $sec.^{-1}$). At each site 6 hauls were conducted on average (min. 3 max. 9) along predefined transects, excluding the littoral, less than 2 m deep, shoreline zone. Each haul had a length of 500 m. Based on these hauls mean CPUE was calculated for each site. Spearman rank correlation ($R_S$) analysis was used to test whether the mean abundance of gobies show a correlation with the upstream-downstream gradient.

## Results and Discussion

Altogether 37 fish species and 4213 specimens were collected during the survey (Table 1). Many benthic species, which were considered very rare in former littoral surveys, were relatively abundant in the offshore catches, such as for example the Danube streber *Zingel streber* (Linnaeus, 1766) and the golden loach *Sabanejewia bulgarica* (Drensky, 1928). A similar situation was recorded in previous surveys along much shorter sections of the Danube (Szalóky et al., 2012, 2014).

Gobies comprised 50.4 % of the catches and occurred with high frequency in the samples (Table 1). Altogether 6 goby species were found: *Neogobius melanostomus* the round goby, *N. fluviatilis* (Pallas, 1814) the monkey goby, *Ponticola kessleri* (Günther 1861) the bighead goby, *Babka gymnotrachelus* (Kessler 1857) the racer goby, *Benthophilus stellatus* (Sauvage 1874) the stellate tadpole-goby and *Proterorhinus semilunaris* (Heckel, 1837) the tubenose goby.

Mean total density (i.e. CPUE data) of gobies per site varied between 0.00 and 83.67 ind. 500 $m^{-1}$. No significant relationship was found between offshore density and upstream downstream position along the river ($R_S$=-0.318; n=22; p=0.148). *N. melanostomus* was the most abundant species in the overall catch of gobies (73.2%), and clearly the most dominant species in the Middle- and Upper Danube region (Fig. 1). Its density did not show a correlation with upstream-downstream position along the river ($R_S$=-0.100; n=22; p=0.656) and showed high variations among sites (mean density per site=11.03±2.33 ind. 500 $m^{-1}$). *N. fluviatilis* was the second most abundant species in the catch (21.7%; mean density per site=3.27±1.27 ind. 500 $m^{-1}$). Although, it was rather rare in the Upper and Middle Danube, its abundance and density increased significantly in the Lower Danube (i.e. below the Iron

Gate) ($R_S$=-0.785; n=22; p<0.001). An interesting faunistic finding of the survey was the occurrence of *N. fluviatilis* in the Middle Danube in Austria, since the uppermost reported distribution of the species in the Danube was at river km 1791 at Gönyü, Hungary (Erős et al., 2008). Note, however that anglers report the occurrence of the species above Gönyü, even from the Szigetköz area in Hungary. In Austria, the species was found in a non-typical habitat of the main channel, where part of the channel was closed by a rip-rap embankment. Two specimens, 43 and 33 mm long (SL) were collected on silty-sandy substrate at a mean depth of 2.9 m on 19.08.2013 close to the settlement Oberloiben at river km 2008 (site position N48.38.507, E15.52.298). This new occurrence is thus 217 river km upstream from the last documented (i.e. published) occurrence of the species, and importantly the natural spread of *N. fluviatilis* upstream of the Gabcikovo dam (river km 1816), to our knowledge, has never been proved. Interestingly, *P. kessleri* was rare offshore (1.8%; mean density per site=0.27±0.08 ind. 500 $m^{-1}$), albeit the species was formerly commonly found in inshore catches along the whole river (Erős et al., 2005; Borza et al., 2009; Polačik et al., 2009) (Fig. 1). Its density did not correlate significantly with upstream-downstream gradient ($R_S$=-0.390; n=22; p=0.073). *B. gymnotrachelus* was relatively rare (2.2%; mean density per site=0.34±0.09 ind. 500 $m^{-1}$), although it was found along the longitudinal profile of the whole river (Fig. 1). Its density did not correlate significantly with upstream-downstream position ($R_S$=-0.403; n=22; p=0.063). *B. stellatus* was a rare species in the river (0.8%; mean density per site=0.13±0.05 ind. 500 $m^{-1}$), and was found only in the Lower Danube at five offshore sites. Finally, *P. semilunaris* was also very rare offshore (0.3%; mean density per site=0.04±0.03 ind. 500 $m^{-1}$) and was found only in the Lower Danube region at 3 sites.

While Ponto-Caspian gobies were always relatively abundant in the Lower Danube, they became more abundant in the Middle and afterwards in the Upper Danube region during the last decade of the 20$^{th}$ century (Erős et al., 2005; Jurajda et al., 2005; Wiesner, 2005). The only exception is the western tubenose goby *P. semilunaris*, which had already been discovered in the Middle Danube at the end of the 19th century (Ahnelt et al., 1998). The present offshore survey confirmed the occurrence of the species in the middle of the river, but only from the Lower Danube region. Although *P. semilunaris* occurs along the Danube in very low numbers (Erős et al., 2008; Roche et al., 2013), the species is more abundant in lowland tributary streams and rivers. It seems that the species avoids offshore areas in the Middle and Upper Danube region. *P. semilunaris* is probably the rarest species in the main channel of the Danube River at present, at least with the exception of *B. stellatus*, which clearly remains a species of the Lower Danube (Otel, 2007). It is likely that the partial exclusion of *P. semilunaris* from the river is due to competition for space with the more aggressive and larger gobies, like the *P. kessleri*, which also prey upon *P. semilunaris* (Borza et al., 2008).

At present *N. melanostomus* is the most successful invader of the Danube above the Iron Gate dam. It occurs along the whole river section with relatively high abundance in both inshore (Borza et al., 2009; Polačik et al., 2009) and offshore habitats (this study). Interestingly, it is even more successful than the bighead goby in its spread, although the bighead goby appeared in the Middle and subsequently in the Upper Danube approx. 5 years earlier than *N. melanostomus*. The round goby seems to outcompete the larger bodied bighead goby, probably due to its more aggressive territorial behaviour and its more favourable life-history strategy for colonization (Kováč et al., 2009). Although the fish survey of the JDS3 core team did not involve the uppermost section of the Danube, recent studies show that *N. melanostomus* is the most dominant goby in this most upstream Danubian section as well, and forms highly abundant populations at its invasion front in Germany (Brandner et al., 2013a, b).

Both *N. fluviatilis* and *B. gymnotrahelus* show a more restricted distribution and much lower colonization rate, and we believe this is due to the differences in their habitat preferences from *N. melanostomus and P. kessleri* (see Erős et al., 2005), since both species, especially *N. fluviatilis*, prefer sandy habitats, which are relatively rare in the Upper and Middle Danube. Our offshore trawling data support this argument. While sandy or silty-sandy mesohabitat patches can be relatively easily found along the shoreline, and especially in the side arms, providing possible habitat patches for colonization, offshore sandy substrate becomes dominant only downstream from ~1530 rkm. Correspondingly, *N. fluviatilis* was only be collected offshore  from this section with relatively high abundance (Fig. 1). Nevertheless, the first occurrence data of the species in Austria proves its slow upstream spread in the river, or alternatively its introduction by ships in the section above the Gabcikovo dam. We believe, however that the species could reach Austria by “natural” dispersion since the Gabcikovo dam cannot be an insurmountable obstacle for the gobies and the species was already relatively abundant in the litoral zone of the upper Hungarian Danube about ten years ago (Erős et al., 2008). Interestingly, *B. gymnotrachelus* and *N. fluviatilis* were the first and most abundant invaders in the Vistula river system in Poland (Kostrzewa & Grabowksi 2003; Kakareko et al. 2009), a very different pattern than  that observed in the Danube. It may well be that the settlement and invasion dynamics of a goby species can determine the settlement and invasion speed of later arriving goby species in rivers, although further detailed investigations are needed to support this hypothesis.

This is the first study which provides data about the offshore distribution of invasive gobies in a large European river.  No data exist on the offshore abundance and habitat use of gobies in other river systems, but studies on *N. melanostomus* from the Laurentian Great Lakes and on other goby species from other areas show that some gobies can be quite abundant in deep benthic areas in lacustrine environments (Johnson et al., 2005a; Guo et al., 2012). It seems that similar to large lakes, bottom trawling can be a useful method for providing standardized (i.e. CPUE) data on the abundance and composition of benthic species from offshore areas in large rivers (Szalóky et al., 2014), including small bodied gobies. Results on the Danube indicate that, in addition to their presence in littoral areas, *N. melanostomus* and *N. fluviatilis* are especially abundant offshore, while the distribution of *P. kessleri* is confined to the shoreline zone. In summary this study presents the first standardized reference data for further investigations of the invasion patterns and changes in the abundance of gobies along the Danube River offshore. Studying the spatial and temporal distribution of gobies is not only important for understanding their dispersion in the river. Since these small, benthic fishes become keystone species in the food web of many invaded habitats both as predators and prey (Johnson et al., 2005b; Kornis et al., 2012), they have the potential to transform the structure and function of the Danubian ecosystem.

**Acknowledgements**

We would like to thank the national team members for their help in the field work. This study was performed by the kind permission and contribution of the ICPDR. The work of TE was supported by the Bolyai János Scholarship of the Hungarian Academy of Sciences and the OTKA 104279 grant. This ms is part of the PhD thesis of ZSz under the supervision of TE. The study was designed by TE, ZSz and GYÁI. All authors contributed to the field work which was led by ZSz. The data were prepared by ZSz and TE. ZSz and TE wrote the ms with the feedback of all co-authors.

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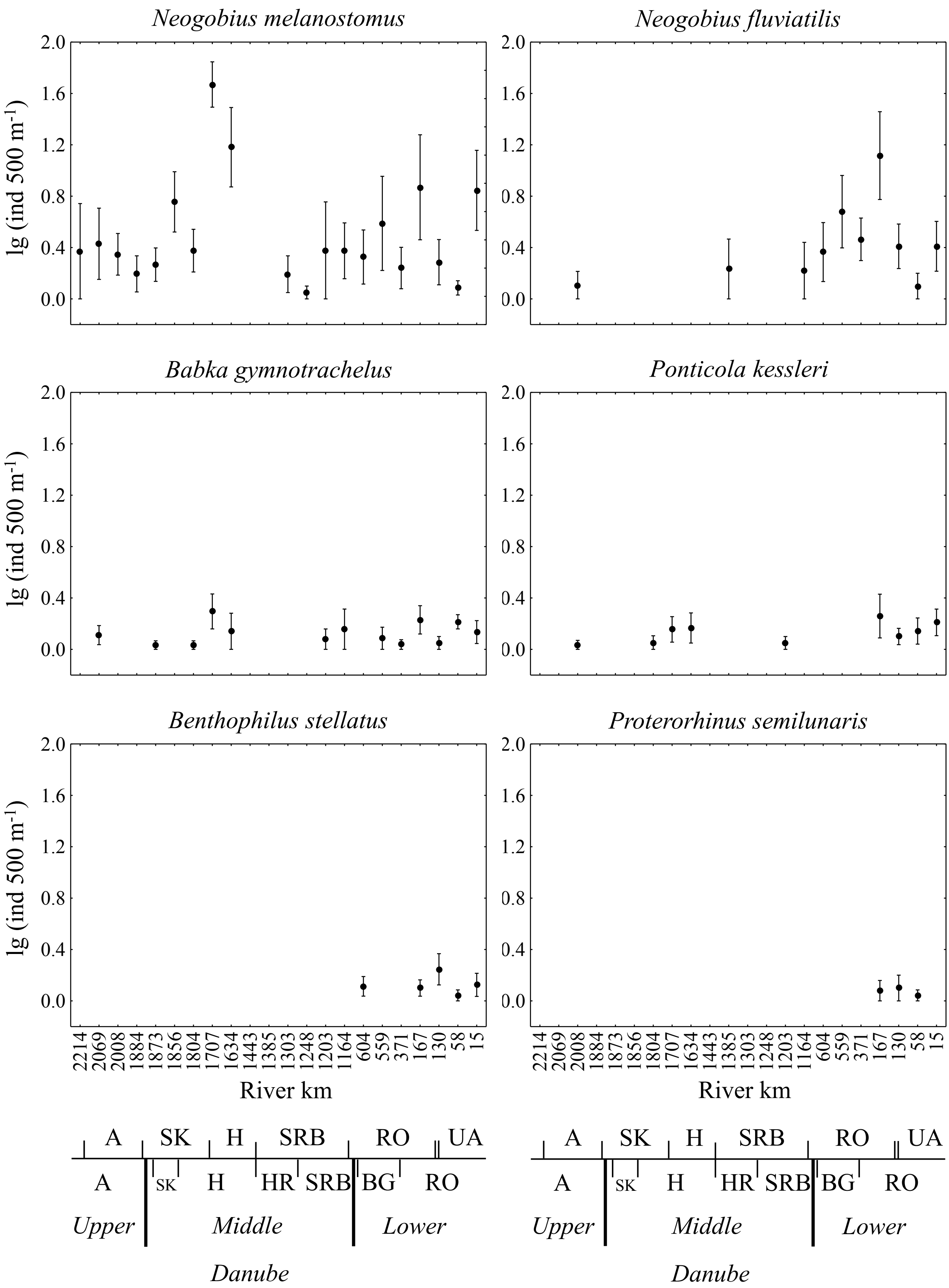
Table 1 The relative abundance (RA %), frequency of occurrence (FRO %), mean CPUE (ind 500 m $^{-1}$ ±SD) data of fishes in the Danube River based on offshore trawling samples. Species are ordered according to their relative abundance in the overall catch.

| Species name | RA % | FRO % | mean CPUE (ind 500 m $^{-1}$) ±SD |
|---|---|---|---|
| *Neogobius melanostomus* (Pallas, 1814) | 36.91 | 41.13 | 11.03±27.68 |
| *Romanogobio vladykovi* (Fang, 1943) | 13.65 | 54.61 | 4.08±10.23 |
| *Neogobius fluviatilis* (Pallas, 1814) | 10.94 | 19.15 | 3.27±15.1 |
| *Blicca bjoerkna* (Linnaeus, 1758) | 9.26 | 24.11 | 2.77±11.51 |
| *Gymnocephalus schraetser* (Linnaeus, 1758) | 5.98 | 24.82 | 1.79±5.03 |
| *Zingel streber* (Siebold, 1863) | 2.89 | 21.99 | 0.86±2.72 |
| *Sabanejewia bulgarica* (Drensky, 1928) | 2.60 | 9.93 | 0.78±4.93 |
| *Sander lucioperca* (Linnaeus, 1758) | 2.57 | 16.31 | 0.77±2.19 |
| *Gymnocephalus baloni* (Holcík & Hensel, 1974) | 2.44 | 8.51 | 0.73±4.12 |
| *Abramis brama* (Linnaeus, 1758) | 2.41 | 20.57 | 0.72±2.42 |
| *Ballerus sapa* (Pallas, 1814) | 2.30 | 13.48 | 0.69±2.94 |
| *Babka gymnotrachelus* (Kessler, 1857) | 1.13 | 16.31 | 0.34±1.03 |
| *Ponticola kessleri* (Günther, 1861) | 0.89 | 11.35 | 0.27±0.97 |
| *Zingel zingel* (Linnaeus, 1766) | 0.84 | 14.18 | 0.25±0.79 |
| *Syngnathus abaster* (Risso, 1827) | 0.75 | 2.84 | 0.22±1.44 |
| *Barbus barbus* (Linnaeus, 1758) | 0.57 | 12.77 | 0.17±0.47 |
| *Ballerus ballerus* (Linnaeus, 1758) | 0.50 | 4.96 | 0.15±1.28 |
| *Benthophilus stellatus* (Sauvage, 1874) | 0.43 | 7.09 | 0.13±0.56 |
| *Perca fluviatilis* (Linnaeus, 1758) | 0.36 | 4.96 | 0.11±0.78 |
| *Vimba vimba* (Linnaeus, 1758) | 0.34 | 7.09 | 0.1±0.47 |
| *Carassius gibelio* (Bloch, 1782) | 0.33 | 2.84 | 0.1±0.86 |
| *Rutilus rutilus* (Linnaeus, 1758) | 0.28 | 4.96 | 0.09±0.44 |
| *Alburnus alburnus* (Linnaeus, 1758) | 0.27 | 4.26 | 0.08±0.55 |
| *Chondrostoma nasus* (Linnaeus, 1758) | 0.26 | 5.67 | 0.08±0.4 |
| *Acipenser ruthenus* (Linnaeus, 1758) | 0.19 | 3.55 | 0.06±0.31 |
| *Silurus glanis* (Linnaeus, 1758) | 0.15 | 4.26 | 0.05±0.24 |
| *Leuciscus aspius* (Linnaeus, 1758) | 0.14 | 2.84 | 0.04±0.26 |
| *Proterorhinus semilunaris* (Heckel, 1837) | 0.14 | 2.13 | 0.04±0.31 |
| *Pelecus cultratus* (Linnaeus, 1758) | 0.09 | 1.42 | 0.03±0.27 |
| *Cottus gobio* (Linnaeus, 1758) | 0.07 | 1.42 | 0.02±0.19 |
| *Esox lucius* (Linnaeus, 1758) | 0.07 | 1.42 | 0.02±0.19 |
| *Cyprinus carpio* (Linnaeus, 1758) | 0.05 | 1.42 | 0.01±0.12 |
| *Leuciscus idus* (Linnaeus, 1758) | 0.05 | 1.42 | 0.01±0.12 |
| *Sander volgensis* (Gmelin, 1789) | 0.05 | 1.42 | 0.01±0.12 |
| *Anguilla anguilla* (Linnaeus, 1758) | 0.02 | 0.71 | 0.01±0.08 |
| *Alburnus mento* (Heckel, 1837) | 0.02 | 0.71 | 0.01±0.08 |
| *Gymnocephalus cernua* (Linnaeus, 1758) | 0.02 | 0.71 | 0.01±0.08 |
| Number of species | | | 37 |
| Number of individuals | | | 4213 |
| Number of samples | | | 141 |

Captions to figures

Fig. 1. Mean abundance (CPUE, log transformed data) of gobies at each sampling site (n=22) along the longitudinal profile of the Danube River based on offshore samples. Ranges show the standard error of the mean. A, Austria; SK, Slovakia; H, Hungary; SRB, Serbia; HR, Croatia; RO, Romania; BG, Bulgaria; UA, Ukraine.


Neogobius melanostomus
Neogobius fluviatilis
Babka gymnotrachelus
Ponticola kessleri
Benthophilus stellatus
Proterorhinus semilunaris
lg (ind 500 m-1)
2.0
1.6
1.2
0.8
0.4
0.0
2214 2069 2008 1884 1873 1856 1804 1707 1634 1443 1385 1303 1248 1203 1164 604 559 371 167 130 58 15
River km
A SK H SRB RO UA
A SK H HR SRB BG RO
Upper Middle Lower
Danube