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

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31

32 **Abstract**

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

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

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50 **Introduction**

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

60 The appearance of Ponto-Caspian gobies in North America happened via ballast water
61 transport of transoceanic vessels (Jude et al., 1992). However, the relative role of human
62 mediated transport vs natural dispersal processes in rivers flowing into the Black Sea is still
63 disputed among scientists (Harka and Bíró 2007; Kornis et al., 2012). The fast spread of
64 gobies in the Danube River, for example, has been connected to ballast water transport too,
65 which may explain why these species were found first in the vicinity of urbanised areas
66 sometimes even some hundreds of kilometres away from their original range limit (Roche et
67 al., 2013). Small crevices can provide an ideal “spawning substrate” for these speleophil
68 species, which could explain the vector role of ships in their dispersal. Other factors, such as
69 different hydro-technical constructions (i.e. rip-rap, groynes, revertment and dykes) or even
70 increasing mean water temperature of the river have been also related to their fast spread and
71 successful invasion (Harka and Bíró, 2007). However, due to the lack of a consistent
72 monitoring system, which could have exactly tracked the proliferation of gobies along their
73 invasion route, it is hard to answer questions related to their saltatoric vs continuous upstream

74 movement. Therefore, transboundary surveys on large international rivers with a standardized
75 methodology are necessary to understand spatial and temporal changes in fish assemblages
76 and to reveal the crucial parameters for the invasion of individual species over large spatial
77 extents.

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

87 The aim of this paper is to present the first standardized catch per unit effort (CPUE) offshore
88 data on the actual abundance of gobies in the main channel of the Danube River using the
89 results of the Joint Danube Survey 3 expedition. The Joint Danube Survey 3 was an
90 international river research expedition which was organized by the International Commission
91 for the Protection of the Danube River (ICPDR) between 13th of August to 26th of September
92 2013 (<http://www.icpdr.org/jds/>). The survey covered the sampling of several biotic and
93 abiotic components of the Danube from Regensburg (i.e. the first bigger town), South
94 Germany to the Danube Delta in Romania.

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98 **Material and methods**

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

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121 **Results and Discussion**

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

128 Gobies comprised 50.4 % of the catches and occurred with high frequency in the samples
129 (Table 1). Altogether 6 goby species were found: *Neogobius melanostomus* the round goby,
130 *N. fluviatilis* (Pallas, 1814) the monkey goby, *Ponticola kessleri* (Günther 1861) the bighead
131 goby, *Babka gymnotrachelus* (Kessler 1857) the racer goby, *Benthophilus stellatus* (Sauvage
132 1874) the stellate tadpole-goby and *Proterorhinus semilunaris* (Heckel, 1837) the tubenose
133 goby.

134 Mean total density (i.e. CPUE data) of gobies per site varied between 0.00 and 83.67 ind.
135 500 m⁻¹. No significant relationship was found between offshore density and upstream
136 downstream position along the river ($R_S = -0.318$; $n = 22$; $p = 0.148$). *N. melanostomus* was the
137 most abundant species in the overall catch of gobies (73.2%), and clearly the most dominant
138 species in the Middle- and Upper Danube region (Fig. 1). Its density did not show a
139 correlation with upstream-downstream position along the river ($R_S = -0.100$; $n = 22$; $p = 0.656$)
140 and showed high variations among sites (mean density per site = 11.03 ± 2.33 ind. 500 m⁻¹). *N.*
141 *fluviatilis* was the second most abundant species in the catch (21.7%; mean density per
142 site = 3.27 ± 1.27 ind. 500 m⁻¹). Although, it was rather rare in the Upper and Middle Danube,
143 its abundance and density increased significantly in the Lower Danube (i.e. below the Iron

144 Gate) ($R_S=-0.785$; $n=22$; $p<0.001$). An interesting faunistic finding of the survey was the
145 occurrence of *N. fluviatilis* in the Middle Danube in Austria, since the uppermost reported
146 distribution of the species in the Danube was at river km 1791 at Gönyü, Hungary (Erős et al.,
147 2008). Note, however that anglers report the occurrence of the species above Gönyü, even
148 from the Szigetköz area in Hungary. In Austria, the species was found in a non-typical habitat
149 of the main channel, where part of the channel was closed by a rip-rap embankment. Two
150 specimens, 43 and 33 mm long (SL) were collected on silty-sandy substrate at a mean depth
151 of 2.9 m on 19.08.2013 close to the settlement Oberloiben at river km 2008 (site position
152 N48.38.507, E15.52.298). This new occurrence is thus 217 river km upstream from the last
153 documented (i.e. published) occurrence of the species, and importantly the natural spread of
154 *N. fluviatilis* upstream of the Gabčíkovo dam (river km 1816), to our knowledge, has never
155 been proved. Interestingly, *P. kessleri* was rare offshore (1.8%; mean density per
156 site= 0.27 ± 0.08 ind. 500 m^{-1}), albeit the species was formerly commonly found in inshore
157 catches along the whole river (Erős et al., 2005; Borza et al., 2009; Polačik et al., 2009) (Fig.
158 1). Its density did not correlate significantly with upstream-downstream gradient ($R_S=-0.390$;
159 $n=22$; $p=0.073$). *B. gymnotrachelus* was relatively rare (2.2%; mean density per
160 site= 0.34 ± 0.09 ind. 500 m^{-1}), although it was found along the longitudinal profile of the
161 whole river (Fig. 1). Its density did not correlate significantly with upstream-downstream
162 position ($R_S=-0.403$; $n=22$; $p=0.063$). *B. stellatus* was a rare species in the river (0.8%; mean
163 density per site= 0.13 ± 0.05 ind. 500 m^{-1}), and was found only in the Lower Danube at five
164 offshore sites. Finally, *P. semilunaris* was also very rare offshore (0.3%; mean density per
165 site= 0.04 ± 0.03 ind. 500 m^{-1}) and was found only in the Lower Danube region at 3 sites.

166 While Ponto-Caspian gobies were always relatively abundant in the Lower Danube, they
167 became more abundant in the Middle and afterwards in the Upper Danube region during the
168 last decade of the 20th century (Erős et al., 2005; Jurajda et al., 2005; Wiesner, 2005). The

169 only exception is the western tubenose goby *P. semilunaris*, which had already been
170 discovered in the Middle Danube at the end of the 19th century (Ahnelt et al., 1998). The
171 present offshore survey confirmed the occurrence of the species in the middle of the river, but
172 only from the Lower Danube region. Although *P. semilunaris* occurs along the Danube in
173 very low numbers (Erős et al., 2008; Roche et al., 2013), the species is more abundant in
174 lowland tributary streams and rivers. It seems that the species avoids offshore areas in the
175 Middle and Upper Danube region. *P. semilunaris* is probably the rarest species in the main
176 channel of the Danube River at present, at least with the exception of *B. stellatus*, which
177 clearly remains a species of the Lower Danube (Otel, 2007). It is likely that the partial
178 exclusion of *P. semilunaris* from the river is due to competition for space with the more
179 aggressive and larger gobies, like the *P. kessleri*, which also prey upon *P. semilunaris* (Borza
180 et al., 2008).

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

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

213 This is the first study which provides data about the offshore distribution of invasive
214 gobies in a large European river. No data exist on the offshore abundance and habitat use of
215 gobies in other river systems, but studies on *N. melanostomus* from the Laurentian Great
216 Lakes and on other goby species from other areas show that some gobies can be quite
217 abundant in deep benthic areas in lacustrine environments (Johnson et al., 2005a; Guo et al.,

218 2012). It seems that similar to large lakes, bottom trawling can be a useful method for
219 providing standardized (i.e. CPUE) data on the abundance and composition of benthic species
220 from offshore areas in large rivers (Szalóky et al., 2014), including small bodied gobies.
221 Results on the Danube indicate that, in addition to their presence in littoral areas, *N.*
222 *melanostomus* and *N. fluviatilis* are especially abundant offshore, while the distribution of *P.*
223 *kessleri* is confined to the shoreline zone. In summary this study presents the first
224 standardized reference data for further investigations of the invasion patterns and changes in
225 the abundance of gobies along the Danube River offshore. Studying the spatial and temporal
226 distribution of gobies is not only important for understanding their dispersion in the river.
227 Since these small, benthic fishes become keystone species in the food web of many invaded
228 habitats both as predators and prey (Johnson et al., 2005b; Kornis et al., 2012), they have the
229 potential to transform the structure and function of the Danubian ecosystem.

230

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239

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331

332 Table 1 The relative abundance (RA %), frequency of occurrence (FRO %), mean CPUE (ind
 333 500 m⁻¹ ±SD) data of fishes in the Danube River based on offshore trawling samples. Species
 334 are ordered according to their relative abundance in the overall catch.
 335

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

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339 Captions to figures

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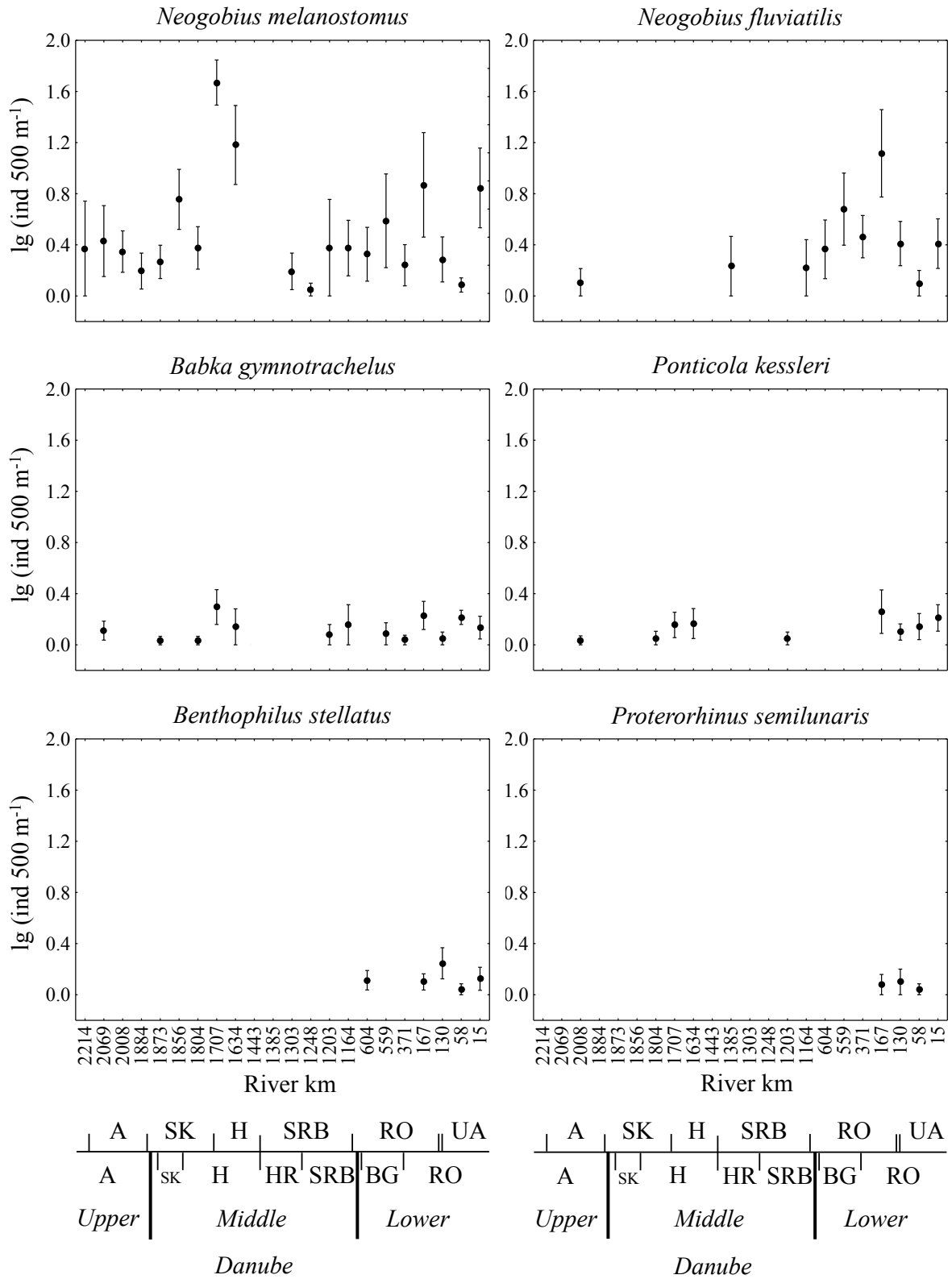
341 Fig. 1. Mean abundance (CPUE, log transformed data) of gobies at each sampling site (n=22)

342 along the longitudinal profile of the Danube River based on offshore samples. Ranges show

343 the standard error of the mean. A, Austria; SK, Slovakia; H, Hungary; SRB, Serbia; HR,

344 Croatia; RO, Romania; BG, Bulgaria; UA, Ukraine.

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