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Dietary habits of invasive Ponto-Caspian gobies in the Croatian part of the Danube River basin and their potential impact on benthic fish communities

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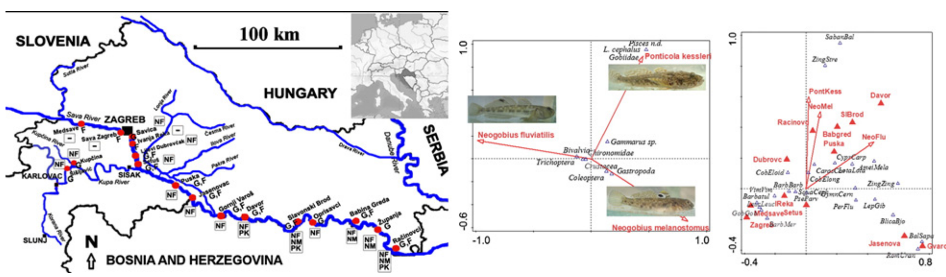
HIGHLIGHTS

- Dietary habits and impacts of invasive P-C gobies on other fish were studied
- Monkey and round goby preferred Trichoptera, Megaloptera and Coleoptera
- Bighead goby preferred Trichoptera, Gammarus and Pisces
- No negative impacts of the most abundant, monkey goby, on native fish populations
- Round goby negatively impacts native zingel, and bighead goby - chub populations

GRAPHICAL ABSTRACT



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ABSTRACT

Invasive Ponto-Caspian (P-C[1]) gobies have recently caused dramatic changes in fish assemblage structures throughout the Danube basin. While their presence in the Croatian part of the basin has been noted and distribution studied, their dietary habits and impacts on native fish communities have, until now, been unknown. In 2011, 17 locations in the Sava River Basin were sampled for fish and 15 for benthic invertebrates. Fish population monitoring data, available for nine seasons (2003–2006 and 2010–2014) and 12 locations, were used to analyse the impacts of P-C gobies on benthic fish abundance. Gut content analysis indicates that the monkey goby *Neogobius fluviatilis* diet is very diverse, but dominated by Trichoptera, Chironomidae, Bivalvia and Odonata. The diet overlaps considerably with the round goby *Neogobius melanostomus* diet, although Gastropoda are dominant in the latter's diet. Small fish and *Gammarus* sp. dominate the bighead goby *Ponticola kessleri* diet. Comparison of gut content with the prey available in the environment indicates that monkey and round gobies exhibit preference for Trichoptera, Megaloptera and Coleoptera, and bighead goby for Trichoptera, *Gammarus* sp. and Pisces. P-C gobies in the Sava River are spreading upstream, towards the reaches with lower fish diversity. Analyses indicate potentially positive impacts of P-C gobies' presence on some fish populations: round and bighead goby on Balkan golden loach *Sabanejewia balcanica* and monkey goby on common carp *Cyprinus carpio*, crucian carp *Carassius carassius*, burbot *Lota lota* and Balkan loach *Cobitis elongata*. However, there are also indications that bighead and round goby could adversely impact the native chub *Squalius cephalus* and zingel *Zingel zingel* populations, respectively. As P-C gobies are still in the expansionary period of invasion and the

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[1] P-C = Ponto-Caspian; NF = *Neogobius fluviatilis*; NM = *Neogobius melanostomus*; PK = *Ponticola kessleri*; CCA = canonical correspondence analysis; GLM = generalized linear model; n.d. = not determined.

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ecosystem still adapting to new circumstances, continued monitoring of fish population dynamics in the Sava basin is needed to determine the outcome and impacts of this invasion.

1. Introduction

The ongoing spread and proliferation of Ponto-Caspian (P-C) gobies, and particularly the dramatic changes they have caused in fish assemblage structures throughout Europe, have attracted a considerable amount of attention from conservationists and scientists alike (Jazdzewski and Konopacka, 2002; Copp et al., 2005a; Jurajda et al., 2005; Borza et al., 2009; Leuven et al., 2009; Manné et al., 2013). Expansion of four species of P-C gobies has been noted and studied in Croatia as well: monkey goby *Neogobius fluviatilis* (Pallas, 1814), bighead goby *Ponticola kessleri* (Günther, 1861), round goby *Neogobius melanostomus* (Pallas, 1814) and racer goby *Babka gymnotrachelus* (Kessler, 1857) (Polačik et al., 2008a; Piria et al., 2011a, 2011b; Šanda et al., 2013; Jakovlić et al., 2015). All four species are considered invasive (Copp et al., 2005a), as confirmed by the risk assessment analysis of non-native species (Copp et al., 2005b) in Croatia and Slovenia, which indicated a "high risk" for round goby and "medium to high risk" for the other three species (M. Piria, unpublished data).

Most of the continental Croatia (and a significant part of the Southeast Europe) belongs to the Sava River Basin, which represents 12% of the total Danube Basin area (Komatina and Grošelj, 2015). While there are presently relatively few significant hydromorphological alterations, mostly limited to Sava tributaries (Ogrinc et al., 2015), and the water quality has improved since the beginning of the 1990s, mostly as a result of the decline of heavy industry and mining (Treer et al., 2007), the presence of invasive species is becoming an increasingly important stressor in the basin (Copp et al., 2005a; Simonović et al., 2015). In the Sava basin, monkey goby is the most abundant and widely distributed among the P-C gobies, which has been brought in correlation (Jakovlić et al., 2015) to the abundance of its preferred habitat, sandy substrate (Simonović et al., 1998; Čápová et al., 2008; Kessel van et al., 2011). However, all four species can successfully subsist on mixed gravel/sand and rocky substrates (Kottelat and Freyhof, 2007). They feed on invertebrates and fish (Kakareko et al., 2005; Adámek et al., 2007; Borza et al., 2009; Grabowska et al., 2009; Brandner et al., 2013), depending on the habitat type, time of day and year, as well as the size of goby (Kornis et al., 2012). Their presence can have adverse impacts on benthic fauna diversity and ecosystem functioning (Kipp and Ricciardi, 2012). It has also been reported that their proliferation may have caused a progressive decline in some native benthic fish populations, such as *Cottus gobio* (Linnaeus, 1758), *Romanogobio albipinnatus* (Lukasch, 1933) and *Barbatula barbatula* (Linnaeus, 1758) (Borcherding et al., 2011; Copp et al., 2008; Jurajda et al., 2005; Corkum et al., 2004; Charlebois et al., 2001). Thus, it is possible that they might be adversely affecting the native populations of fish species occupying similar ecological niches in the largest (by discharge) Danube tributary, the Sava River, and its sub-tributaries. Accordingly, the main objectives of this study were to examine the: 1) dietary habits of P-C gobies and their preferences for prey available in the environment; and 2) effect of P-C gobies on other benthic-feeding fish using the multivariate approach.

2. Materials and methods

2.1. Study area and fish sampling

17 locations in the Sava basin were sampled in 2011 (Fig. 1, Appendix I) using electrofishing method as previously described (Jakovlić et al., 2015). Fish specimens were measured for standard length (SL) and total length (TL) to the nearest 0.1 cm. Scales for the age determination were taken from above the lateral line, below the anterior part of the dorsal fin. Sampled P-C goby specimens were frozen immediately after capture in order to preserve the gut contents. P-C goby specimens that were undamaged after thawing were dissected and the entire content of the anterior third of the gut was weighed and fixed in 96% ethanol. Recognisable organisms were subsequently identified to the family or, when possible, genus level. The age of specimens was estimated by counting annuli on scales using a microscope (×100) under transmitted light. All ages were determined twice by the same reader.

2.2. Benthic fauna sampling

In addition to fish fauna, sampling of the benthic fauna was carried out at the same locations, only at the Sava River (15 locations), from a diverse range of substrate types: stones (>6 cm), pebbles (2–64 mm), sand (0.06–2 mm) and mud (<0.06 mm). Sampling was carried out in triplicate, using a Surber sampler (30 × 30 cm, 250 mm mesh size). All sampled benthic fauna was preserved in 96% ethanol in the field. Identification of invertebrates from digestive tracts and benthic fauna samples was carried out in a laboratory according to Nilsson (1996, 1997) and the density of all benthos classes per square metre was calculated.

2.3. P-C goby gut content analysis, diet overlap and prey importance

Assessment of the fish diet was based on the frequency of occurrence *F%*, numerical frequency *N%* and mass frequency *W%* of the different diet components, using the following formulas:

$$F\% = \frac{f_i}{\sum f} \cdot 100$$

where f_i is a number of guts containing each prey item and $\sum f$ is the total number of guts with food;

$$N\% = \frac{n_i}{\sum n} \cdot 100$$

where n_i is the total number of a particular prey item and $\sum n$ is the total number of prey items consumed by the gobies;

$$W\% = \frac{w_i}{\sum w} \cdot 100$$

where w_i is the total mass of a single prey item and $\sum w$ is the total mass of prey items consumed by the gobies (Holden and Raitt, 1974).

The analysis of changes in feeding habits was performed using the following indexes (Hyslop, 1980):

$$Fulness\,index(FI\%) = \frac{Total\,stomach\,contents\,weight}{Fish\,weight} \cdot 100$$

$$Vacuity\,coefficient(VI\%) = \frac{Number\,of\,empty\,stomachs}{Total\,number\,of\,guts\,analysed} \cdot 100.$$

The mean percentage of each prey category was calculated for all three P-C gobies. Diet overlap was calculated using the index proposed by Schoener (1970):

$$\alpha = 1{-}0.5\left(\sum_{i=1}^{n} |PV_{xi}{-}PV_{yi}\right)$$

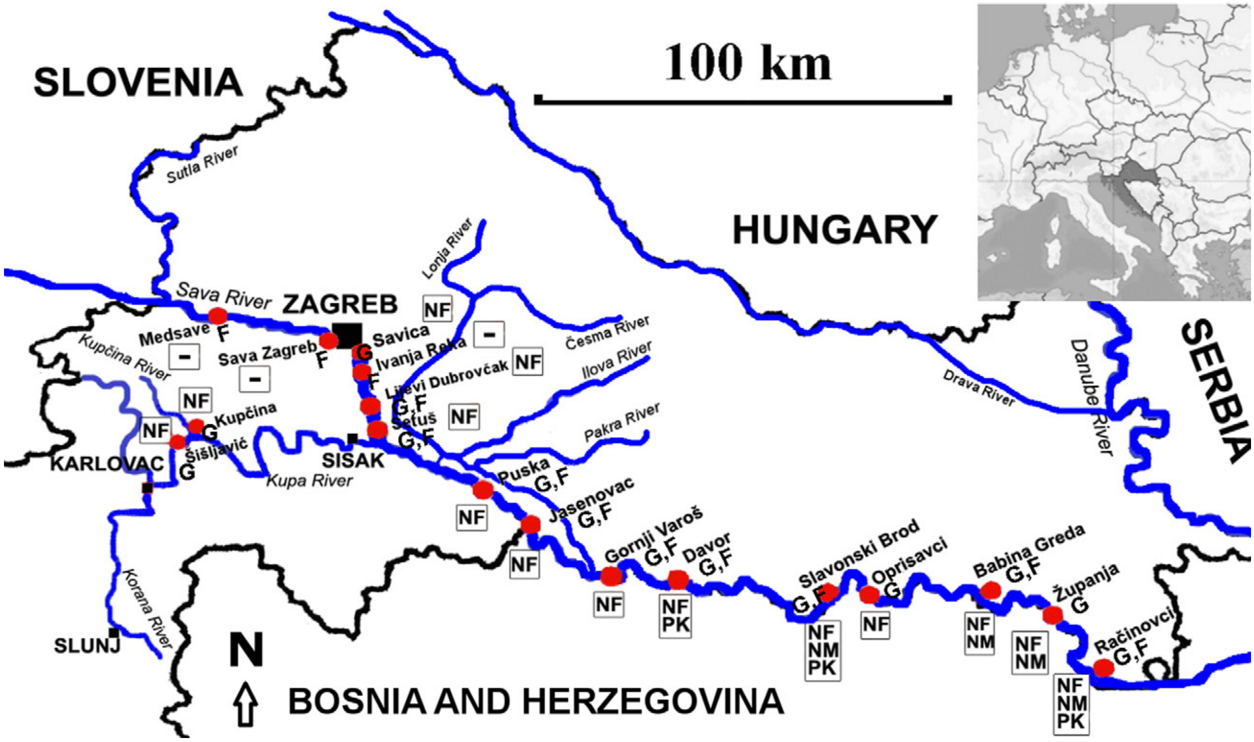

Fig. 1. Locations sampled 2003–2014 on the Sava River and in 2011 on the Kupa River in Croatia, with the note of presence of Ponto-Caspian goby specimens: NF = *Neogobius fluviatilis*, NM = *N. melanostomus*, PK = *Ponticola kessleri*, – = none; G indicates locations where the gut content analysis was applied and F where the impacts of P-C gobies on benthic fish were analysed; black lines represent the country borders and blue lines represent rivers, where the thickness corresponds to the approximate size of the river.

where n = number of prey items PV_{xi} = percentage of prey item i in species x and PV_{yi} = percentage of prey item i in species y. Values range from 0 (no feeding overlap) to 1 (total feeding overlap).

To test the importance of each individual prey item for all three P-C gobies separately, the gut content data were analysed using the generalized linear model (GLM) module implemented in CANOCO software package. Poisson generalized linear models with log link were used, where gobies were the response variables for each prey item found in gut content (Ter Braak and Šmilauer, 2012).

2.4. P-C gobies' preferences for available prey in the environment

Ivlev's index (Ivlev, 1961) was used as a measure of selectivity (E) for various prey items in the fish rations: $E = (r_i - p_i) / (r_i + p_i)$, where r_i is the relative abundance of prey category i in the digestive tract (as a proportion or percentage of all digestive tract contents) and p_i is the relative abundance of this prey in the environment. Values range from −1 to +1, with negative values indicating rejection or inaccessibility of the prey, zero – random feeding, and positive values – active selection.

2.5. Statistical analysis of the effects on benthic fish communities

Monitoring data available for nine seasons, 2003 to 2006 and 2010 to 2014 (Aničić et al., 2014 and references within), and additional sampling data (2004) for several locations along the Sava (Mustafić, 2005), were used to analyse the effects of P-C gobies on fish abundance in the Sava. Benthic fish species were chosen as response variables to analyse the relationships between fish abundance (the number of fish sampled per 100 m shoreline) and invasive fish as environmental (explanatory) variables, with locations as nominal explanatory variables, using constrained canonical correspondence analysis (CCA) with response data log transformations, all constrained axis test and unrestricted permutations, implemented in CANOCO 5 software package. The same programme was used to analyse the additional effects of invasive gobies, as response variables, on fish abundance using Poisson generalized linear models with log link (Ter Braak and Šmilauer, 2012).

3. Results

3.1. Gut content analysis, prey composition, prey importance and diet overlap

The total of 278 P-C goby specimens belonging to three species, as racer goby was not found, were collected in 2011: 173 monkey, 84 round and 21 bighead gobies. Gut content analyses were performed on 242 P-C goby specimens (Tables 1 and 2) from 14 locations on the Sava and one on the Kupa River (Fig. 1). Monkey and round gobies consumed a larger variety of prey items than bighead goby. Even though monkey goby was the most numerous species, no specimens were found in the 4+ age-class.

Table 1
Number of analysed specimens (n), total (TL) and standard length (SL), mean value ± standard deviation ($\bar{x} \pm$ SD), standard error (STD ERR) and coefficient of variation (CV).

Parameter	Monkey goby n = 156		Round goby n = 73		Bighead goby n = 15	
	TL	SL	TL	SL	TL	SL
$\bar{x} \pm$ SD	7.52 ± 2.02	6.35 ± 1.76	5.97 ± 1.81	4.93 ± 1.48	7.76 ± 2.59	6.53 ± 2.21
Min	3.9	3.2	4.0	3.2	4.6	3.8
Max	12.6	10.8	13.6	11.3	11.9	10.0
STD ERR	0.16	0.14	0.21	0.17	0.67	0.57
CV (%)	26.86	27.72	30.32	30.02	33.38	33.84

Table 2
Variation with age (0+ to 4+) of frequency of occurrence (F%), numerical frequency (N%), mass frequency (W%), vacuity coefficient (VI%) and fullness index (FI%) of the food items consumed by monkey goby (NF), round goby (NM) and bighead goby (PK) (n is the number of analyzed specimens; n.d. — not determined).

Food item	0+									1+									2+								
	NF FI = 2.06 VI = 0 n = 34			NM FI = 1.37 VI = 0 n = 5			PK FI = 5.26 VI = 0 n = 1			NF FI = 2.22 VI = 0 n = 46			NM FI = 1.15 VI = 7.62 n = 26			PK FI = 5.39 VI = 0 n = 4			NF FI = 1.85 VI = 0 n = 54			NM FI = 2.17 VI = 0 n = 27			PK FI = 3.44 VI = 0 n = 4		
	N	F	W	N	F	W	N	F	W	N	F	W	N	F	W	N	F	W	N	F	W	N	F	W	N	F	W
Trichoptera	30.3	12.5	11.4							32.3	12.9	18.3	28.8	17.1	71.6	20.0	25.0	10.7	22.1	9.0	9.9	4.0	2.3	1.1			
Chironomidae	16.7	7.1	2.0	7.1	10.0	3.7				34.1	12.9	24.3	37.9	14.6	5.7				37.2	9.8	9.7						
Tipulidae																20.0	25.0	5.3	0.4	0.8	1.0						
Megaloptera										0.6	1.1	0.2	1.5	2.4	0.8				0.4	0.8	0.2						
Gastropoda				42.9	30.0	50.1				7.9	2.2	3.2	9.1	7.3	2.1							37.3	14.0	33.6			
Bivalvia										4.9	6.5	2.6							9.7	6.8	11.1						
Crustacea n.d.										1.2	2.2	2.1	3.0	4.9	1.6				3.5	4.5	5.8						
Gammarus sp.							100	100	100	1.8	1.1	2.9	1.5	2.4	1.6	40.0	25.0	46.6	3.1	3.0	4.2	5.3	7.0	12.8			
Odonata										0.6	1.1	1.2							1.3	2.3	6.8						
Coleoptera	7.6	5.4	4.5										1.5	2.4	7.4				1.8	0.8	0.6	30.7	7.0	3.7			
Oligochaeta										0.6	1.1	0.4							1.3	0.8	0.7						
Insecta n.d.	45.5	30.4	37.5	50.0	30.0	33.7				15.9	21.5	13.6	16.7	19.5	5.6				18.6	17.3	24.1	22.7	27.9	25.7	80.0	40.0	16.2
Detritus		44.6	44.6		30.0	12.5					37.6	31.2		29.3	3.6					28.6	23.5		41.9	23.1		40.0	76.5
Pisces n.d.																									20.0	20.0	7.3
Cyprinidae																			0.4	0.8	2.6						
Gobiidae																											
S. cephalus																20.0	25.0	37.4									

[a] No NF specimens in the category 4+.

Table 2 (*continued*)

Food item	3+									4+[a]					
	NF FI = 2.52 VI = 0 n = 22			NM FI = 2.20 VI = 7.69 n = 13			PK FI = 3.80 VI = 0 n = 4			NM FI = 2.52 VI = 0 n = 2			PK FI = 4.21 VI = 0 n = 2		
	N	F	W	N	F	W	N	F	W	N	F	W	N	F	W
Trichoptera	22.2	15.1	12.4	5.6	4.2	1.1									
Chironomidae	14.8	3.8	2.1												
Tipulidae	2.5	3.8	1.2												
Megaloptera															
Gastropoda	7.4	5.7	5.5	38.9	16.7	7.4									
Bivalvia	14.8	9.4	6.8												
Crustacea n.d.	8.6	5.7	8.9												
Gammarus sp.	8.6	7.5	11.5	33.3	16.7	24.5	50.0	28.6	53.9						
Odonata	3.7	3.8	15.5												
Coleoptera				5.6	4.2	0.9									
Oligochaeta	1.2	1.9	0.3	5.6	4.2	1.5									
Insecta n.d.	16.0	17.0	10.4	11.1	8.3	14.5	25.0	14.3	5.9	100	50.0	65.8	33.3	33.3	23.1
Detritus		26.4	25.4		45.8	50.1		42.9	10.7		50.0	34.2			
Pisces n.d.							25.0	14.3	29.5				33.3	33.3	34.6
Cyprinidae															
Gobiidae													33.4	33.4	42.3
S. cephalus															

Approximately 7.6% of round goby specimens in 1+ (VI = 7.62) and 3+ (VI = 7.69) age-classes had empty guts, while the highest fullness indexes in all age-classes (FI from 3.44 to 5.39) were found in bighead goby specimens (Table 2). GLM analysis of gut contents indicates that Trichoptera, Chironomidae, Bivalvia and Odonata are highly important prey items for monkey goby, small fish and *Gammarus* sp. for bighead goby, and Gastropoda for round goby. The analysis also indicates particularly low importance of various insect larvae (Trichoptera and Chironomidae) for round goby (Table 3; Appendix IIIA). Diet analysis among the three P-C gobies reveals a significant feeding overlap between monkey and round gobies ($\alpha = 0.87$, $p < 0.05$), while bighead goby diet does not overlap significantly with others' ($\alpha = 0.21$ with monkey and $\alpha = 0.33$ with bighead gobies).

3.2. Preferences of P-C gobies for the available benthic invertebrates

Gastropoda (54.49%), Chironomidae (20.08%) and *Gammarus* sp. (18.02%) were widely available in the environment, while Megaloptera and Coleoptera were not found (Fig. 2). Ivlev's selectivity index, calculated only for the 15 Sava locations, indicates active selection of Trichoptera for all three P-C goby species. Monkey and round gobies actively selected Megaloptera, Crustacea and Coleoptera, while bighead goby selected *Gammarus* sp. and Pisces. Monkey goby also actively selected Bivalvia, but the other two gobies avoided them. Although Gastropoda were abundant in the environment, monkey and bighead gobies avoided them, while random feeding is indicated for round goby (Figs. 2 and 3).

3.3. Influence of P-C gobies' abundance on benthic fish communities

12 locations along the Sava as nominal explanatory variables (where data from to 2004 to 2014 were available), 24 benthic fish species as response data and P-C gobies as explanatory variables were included in CCA and GLM analyses (Table 4). CCA analysis between fish abundance and P-C gobies explains 44.81% of variation on the first axis and 57.98% on the second (Appendix II, Fig. 4). Constrained test on all axes shows significant variation of all environmental variables ($F = 1.8$, $p = 0.002$) (Fig. 4). The ordination diagram indicates that P-C gobies are spreading from locations closest to the Danube confluence (Račinovci) upstream: Slavonski Brod, Babina Greda, Davor. A possibility of influence on Balkan golden loach *Sabanejewia balcanica*, zingel *Zingel streber*, Balkan loach *Cobitis elongata* and crucian carp *Carassius carassius* is indicated for round goby and bighead goby, while monkey goby could be impacting common carp *Cyprinus carpio*, crucian carp, burbot *Lota lota* and Balkan loach *C. elongata* populations (Fig. 4). GLM model was used to analyse the additional effects of the abundance of P-C gobies on each tested fish species. Abundance data for all three response variables had statistically significant relationships (Appendix IIIB). Due to its absence from many researched locations (Table 4), nine variables were excluded from the analysis for bighead goby. Negative intercept data for the bighead goby indicate a decline in the abundance, but during the period of invasion their presence may have had a negative effect on chub *Squalius cephalus* populations ($p < 0.05$) (Table 5). The abundance of monkey goby is still increasing ($p < 0.05$), which is not the case with round goby ($p > 0.05$). Zingel is the only species whose populations seem to have been adversely impacted by round goby with a significant statistical support ($p < 0.05$). In addition, an increasing trend for Balkan golden loach in response to round goby ($p < 0.001$), as well as for crucian carp, Balkan loach and burbot in response to monkey goby ($p < 0.05$) presence was noted. There was no significant effect on other fish species included in the analysis, so they are not presented in Table 5.

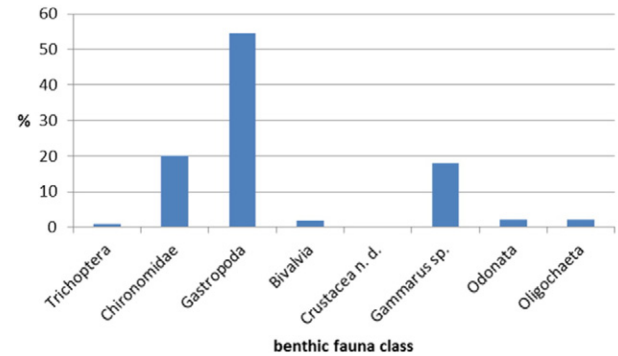

Fig. 2. The ratio of each class of benthic fauna, collected on all the sampled locations in the Sava River, expressed as the number of specimens (%) per square metre.

Table 3
The importance of prey categories for three P-C goby species analysed using the generalized linear model (GLM) method, where n.d. = not determined, b = regression coefficient, SE = standard error, T = corresponding T statistic (for the partial test of *H0: b(i) = 0*) and p(T) = corresponding significance value. Only the significant GLM coefficients of importance of particular prey items in P-C gobies diet are shown (GLM analysis parameters in Appendix III A).

Food items	b	SE	T	p(T)
Monkey goby				
Intercept	−0.621	0.081	−7.68	<0.001
Trichoptera	0.068	0.022	3.16	0.002
Chironomidae	0.046	0.017	2.79	0.006
Bivalvia	0.137	0.047	2.93	0.004
Odonata	0.419	0.202	2.08	0.039
Round goby				
Intercept	−0.710	0.127	−5.58	<0.001
Trichoptera	−0.233	0.084	−2.77	0.006
Chironomidae	−0.167	0.066	−2.53	0.012
Gastropoda	0.070	0.034	2.05	0.041
Bighead goby				
Intercept	−1.869	0.309	−6.04	<0.001
Gammarus sp.	0.546	0.212	2.58	0.010
Pisces n.d.	0.803	0.294	2.73	0.007

4. Discussion

Based on the gut content analyses, prey consumption of invasive P-C gobies in the Sava and Kupa Rivers is generally similar to some previous reports: monkey goby in the Vistula River system (Baltic basin, Poland) and the Slovak part of the Danube also consumes a broad range of prey items, including insect larvae and pupae, amphipods, crustaceans, annelids, gastropods and fish. Insects (Chironomidae, Trichoptera) are the dominant diet component in all size-groups, followed by amphipods at some localities (Tables 2 and 3; Kakareko et al., 2005; Grabowska et al., 2009). Even though monkey goby and round goby diets appear to overlap considerably, beside Trichoptera, Chironomidae and other insects, gastropods are highly important prey item only for round goby. This is in accordance with the findings from the Serbian part of the Danube, where molluscs are the prevalent prey item (Simonović et al., 1998, 2001). However, Chironomidae are the most important prey item for round goby in the Slovakian part of the Danube (Števove and Kováč, 2013) and, along with other aquatic insects, in Lake Erie, Pennsylvania (Phillips et al., 2003). Bighead goby diet in the Sava is based on *Gammarus* sp. and fish, which is in accordance with studies conducted in the Danube in Austria (Polačik et al., 2009), Slovakia (Števove and Kováč, 2013) and Hungary (Borza et al., 2009). Contrary to Števove and Kováč (2013) findings, bighead goby and round goby diets do not overlap in our study. Another research, conducted in Hungary, found that their diets do not overlap in spring, but significantly overlap in summer and autumn (Borza et al., 2009). Beside this, overlap in individual diet items varies by season (Števove and Kováč, 2013). A low total number of bighead goby specimens analysed for this study, along with a very low number of prey items found in the guts of some of the specimens, could have affected the results.

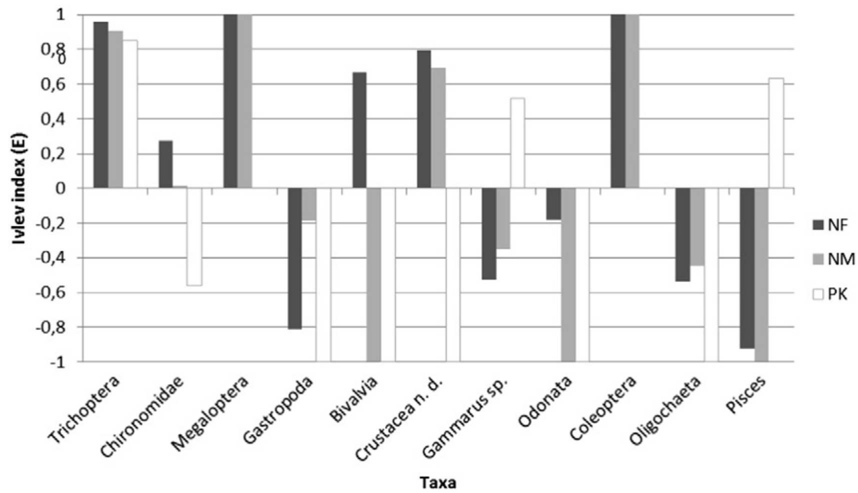

Fig. 3. Ivlev's selectivity index (E) for monkey goby (NF), round goby (NM) and bighead goby (PK) prey preferences in the Sava River. Positive values indicate active selection of the benthos item, negative values indicate the avoidance of the item and zero indicates neutrality.

Based on the comparison of the gut content and the prey available in the environment, monkey goby seems to exhibit a strong dietary preference for Trichoptera, Megaloptera and Coleoptera, as the Ivlev's selectivity index (E) values for all three prey categories are close to 1 (E = 0.96, 1.00, 1.00, respectively; Fig. 3). Almost random feeding (E = 0.24) is indicated for monkey goby with regard to its main prey category, Chironomidae, even though this group is freely available in the environment. In the Vistula River system, the same result was found at one studied site, with more pronounced preferences for Chironomidae at the other two sampled locations (Grabowska et al., 2009). Round goby has similar dietary preferences: even though Gastropoda is its main, or most important, prey category and the most abundant item in the environment (Fig. 2), analysis indicates avoidance of this item (E = −0.19). Similar phenomenon was previously reported by Polačik et al. (2009), who hypothesized that Mollusca are an unavoidable alternative, rather than the most preferred prey. Bighead goby dietary preferences reveal Trichoptera, *Gammarus* sp. and Pisces (E = 0.85, 0.52, 0.63, respectively, Fig. 3) as preferred prey items. Trichoptera are not abundant in the environment (Fig. 2) and are not significantly important prey item in the gut content (Tables 2 and 3), but this item can't be excluded as a secondary food resource for bighead goby (Adámek et al., 2007; Števove and Kováč, 2013).

The spread and proliferation of invasive P-C gobies in Europe have coincided with a decline in several native benthic fish species: bullhead *C. gobio*, stone loach *B. barbatula* and white-finned gudgeon *R. albipinnatus* (Charlebois et al., 2001; Corkum et al., 2004; Copp et al., 2005a; Jurajda et al., 2005; Kornis et al., 2012). Among these, presently only stone loach shares the same habitat in the Sava (Simonović et al., 2015), however there is no evidence for adverse impacts on its populations. Similarly, even though a possibility of competition for food between P-C gobies and Eurasian perch *Perca fluviatilis*, as well as Balon's ruffe *Gymnocephalus baloni*, has been proposed (Copp et al., 2008), our results did not show any significant influence on perch or ruffe (*Gymnocephalus cernua*, a species closely related to *G. baloni*, but more abundant in the Sava) populations. The data about feeding habits of these species in the studied part of the Sava Basin is rather scarce, however, it has been proposed that the main food item for Balkan golden loach and Balkan loach is Chironomidae (Mičetić et al., 2008), which suggests an overlap with the main diet spectrum of monkey and round gobies (Table 3). Even though the possibility of negative impacts of bighead goby on chub (*S. cephalus*) populations has been indicated (Table 5), it is not highly likely to be a consequence of diet overlap, as chub is an omnivorous fish (Piria et al., 2005), very adaptable to various environmental stressors (Piria, 2007). The negative impact on its populations is probably much better explained by a rather large number of chub specimens found in bighead goby guts (Table 2). Data about the diets of zingel, burbot and crucian carp are still not available, so it is hard to assess the extent of the diet overlap and possible adverse impacts of direct food competition.

In a previous research (Jakovlić et al., 2015), temporal population dynamics at Lijevi Dubrovčak location suggested a decline in the ratio of gudgeon *Gobio gobio* in the total catch in response to the increasing ratio of monkey goby from 2003 to 2013, but in this research the GLM test did not indicate any significant impact of the presence of monkey goby on gudgeon abundance for any of the analysed sites over time, including Lijevi Dubrovčak (Tables 4 and 5). Along the longitudinal profile of the River Sava, P-C gobies are the most abundant in mid and downstream reaches, with the monkey goby distribution spreading the farthest upstream. However, round and monkey gobies are both still spreading upstream (Fig. 4) (Jakovlić et al., 2015), towards the locations with lower fish diversity and predominantly upper rhithron fish communities (Aničić et al., 2014; Simonović et al., 2015), where the impacts could also be different. Positive response of three vulnerable (Mrakovčić et al., 2006) fish species (crucian carp, Balkan loach and burbot) to the presence of monkey goby (Table 5) is rather intriguing. A possible explanation could be that, since monkey goby has been present in the Sava for many years before it was scientifically reported (Piria et al., 2011b), possibly even before the year 2000 (M. Piria, pers. obs.), this could have been caused by the recovery of these native species after the period of adjustment to the invasion. Bighead goby was not present in the monitoring data after 2011, which was reflected in the GLM test as a significantly negative intercept value (Table 5). Due to the short presence of round goby in the Sava, there is no conclusive evidence for significant impacts on other fish, however the possibility of adverse impacts on zingel should not be excluded. This calls for closer attention, as the conservation status of this species in Croatia has been categorized as "vulnerable" (Mrakovčić et al., 2006). It should be noted that the significance of these analyses could be somewhat limited due to the sampling method bias (Polačik et al., 2008b) and a relatively small number of obtained samples at some locations. In conclusion, somewhat inconclusive results of the impact analyses could also be seen as a minor contribution to the ongoing scientific debate about the possibility that in some cases, instead of causing significant disturbances in the ecosystems, the introduction of non-native species can result merely in an increase of biodiversity (Gozlan, 2008), or even have positive impacts on native species (Rodriguez, 2006; Schlaepfer et al., 2011). Even though P-C gobies have caused significant disturbances elsewhere in the Danube Basin, their population densities and impacts still seem to be relatively limited in the Sava. Whether it is the

Table 4
List of the 27 sampled benthic fish species, including the P-C gobies, their scientific and common names, abbreviations, locations and the total number caught at each location; *species not included in the GLM analysis for PK; **data available for 2003, 2004, 2005, 2006, 2010, 2011, 2012, 2013 and 2014 (Aničić et al., 2014); *** data available for 2011, 2012, 2013 and 2014 (Aničić et al., 2014); underline data available only for 2004 (Mustafić, 2005).

Scientific name	Abbreviation	Common name	Location/the total fish number										
			Medsave**	Zagreb**	I Reka**	Lijevi Dubrovčak**	Setuš***	Puska***	Jasenovac***	G. Varoš***	Davor***	Sl. Brod***	Račinovci***
*Ameiurus melas**	AmeiMela	Black bullhead							1	1	3	3	1
*Ballerus sapa**	BalSapa	White-eye bream								2			
*Barbatula barbatula**	Barbatul	Stone loach		7		1							
Barbus barbus	BarbBarb	Barbel	1541	427	157	3	12	32	2	31	16	24	135
*Barbus meridionalis**	BarbMer	Mediterranean barbel	4	4			3						
Blicca bjoerkna	BlicaBjo	White bream							21	31	8	4	12
*Carassius carassius**	CarasCar	Crucian carp	4			1		6		1		4	
Carassius gibelio	CarasGib	Gibel carp	8	119	4			26	12	57	12	33	30
Cobitis elongatoides	CobEloide	Danubian spined loach	193	104	27	27	2	11	1	14	6	27	12
Cobitis elongata	CobElong	Balkan loach	64	535	2	53	8	14	19	23	2	43	17
*Cyprinus carpio**	CyprCarp	Common carp		8					1	2	1	3	
*Gobio gobio**	GobGob	Gudgeon	65	242	1	8	7	3					10
Gymnocephalus cernua	GymnCern	Ruffe				9	12	1	2	8	3	42	
Lepomis gibbosus	LepGib	Pumpkinseed	3				1	6	11	54	12	40	4
*Leuciscus leuciscus**	LeucLeuc	Dace	46	201	5	3				1		1	
Lota lota	LotaLota	Burbot				3	12	15	7	55	7	26	10
Neogobius fluviatilis	NeoFlu	Monkey goby	1	3		15	37	9	42	60	6	94	41
Neogobius melanostomus	NeoMel	Round goby				1				17		40	33
Perca fluviatilis	PerFlu	European perch	3	17	16	7	17	16	37	128	6	35	11
Pseudorasbora parva	PseParv	Topmouth gudgeon	28	18	29	1	1	5	14	5	9	6	19
Ponticola kessleri	PontKess	Bighead goby								1	2	11	4
Romanogobio uranoscopus	RomUran	Danubian longbarbel gudgeon							3	28			
Sabanejewia balcanica	SabanBal	Balkan golden loach								1	3	1	4
Squalius cephalus	SquaCeph	Chub	1855	1149	111	93	112	44	80	68	54	97	307
Vimba vimba	VimVim	Vimba bream	333	69	10	17	10	1	1	1	2	8	3
*Zingel zingel**	ZingZing	Zingel							1				
Zingel streber	ZingStre	Danube streber										1	1

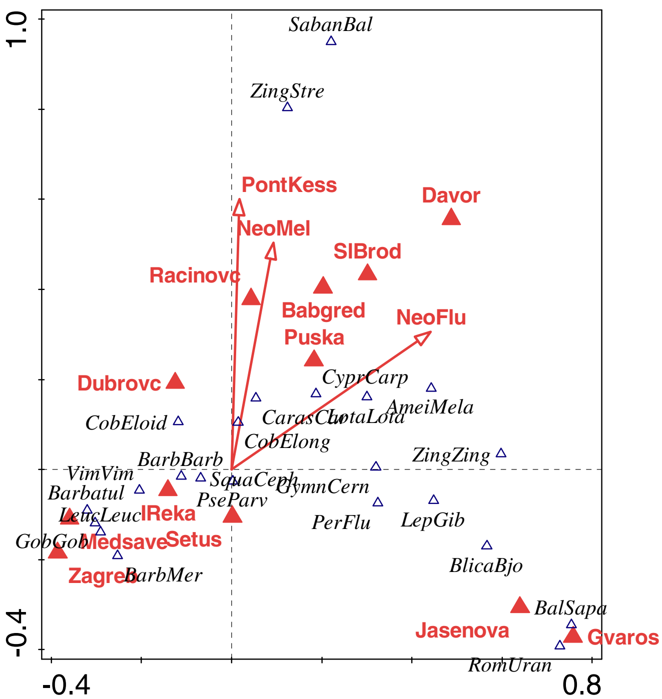

Fig. 4. Canonical correspondence analysis ordination diagram based on the abundance of 24 fish species, three environmental (response) and 12 nominal variables, where: ▲ represents nominal explanatory variables, Δ fish abundance and → explanatory/response variables. Total variation is 3.44376, explanatory variables account for 19.4% of variation, Monte Carlo permutation test results on all axes: pseudo-F = 1.8, P = 0.002. (See Table 4 for abbreviations and Appendix II for summary statistics).

consequence of the (un)availability of suitable habitat [while the Sava and its tributaries are abundant in suitable habitat for monkey goby, the situation seems to be reversed for bighead goby (Jakovlić et al., 2015)], or some other ecological factors, continued monitoring of fish population dynamics in the Sava basin will be necessary to determine the outcome and impacts of this invasion, as well as contribute to the understanding of complex ecological interactions that determine the successfulness of P-C gobies invasions in different aquatic environments.

Table 5
Impacts of monkey goby, round goby and bighead goby on bentic fish species abundance over time in the Sava River, calculated using the generalized linear models (GLM) analysis, where n.d. = not determined, b = regression coefficient, SE = standard error, T = corresponding T statistic (for the partial test of $H0$: $b(i) = 0$) and p(T) = corresponding significance value. (GLM analysis parameters in Appendix III B).

Species	b	SE	T	p(T)
Monkey goby				
Intercept	0.795	0.374	2.13	0.036
Carassius carassius	0.916	0.455	2.01	0.047
Cobitis elongata	0.402	0.185	2.17	0.032
Lota Lota	0.756	0.344	2.20	0.030
Round goby				
Intercept	0.068	0.869	0.08	0.937
Sabanejewia balcanica	4.552	1.182	3.85	0.0002
Zingel streber	−6.323	2.806	−2.25	0.027
Bighead goby				
Intercept	−2.533	0.831	−3.05	0.003
Carassius gibelio	1.445	0.531	2.72	0.008
Cobitis elongatoides	1.022	0.426	2.40	0.018
Sabanejewia balcanica	2.818	1.197	2.35	0.021
Squalius cephalus	−1.180	0.470	−2.51	0.014

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Appendix A. Supplementary data

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