Diet and feeding niche of five invasive species in the Bodrog River watershed

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Abstract. Spreading of invasive fish species in Europe still continues. Studies of their feeding behavior and diet habits have shown that these species could negatively affect native ichthyofauna by competition and predation; they could also cause changes in feeding behaviour and feeding niche of Central European native species. The aim of this study is to refer similarities and differences in feeding behavior / feeding niche of altogether 83 specimens of five different species, namely *Ameiurus melas*, *Lepomis gibbosus*, *Neogobius fluviatilis*, *Perccottus glenii* and *Proterorhinus semilunaris* from the Bodrog River. Non-metric (NMDS and Bray-Curtis index) and non-parametric (PERMANOVA and ANOSIM) tests were used for determination of differences in food. Piankas and Levins statistical models were used for feeding niche overlap and niche width. Statistically significant differences were found in the diet composition of analysed species by ANOSIM. Chironomidae larvae (Diptera), class Ostracoda (Crustacea), family Baetidae (Ephemeroptera) larvae, Corophiidae and Copepoda explained about 60% of the dissimilarity in the diet composition by SIMPER test. At the site Borša, large feeding niche overlap was found between *N. fluviatilis* and *P. semilunaris* and intermediate between *P. glenii* and *P. semilunaris*. Broad niche width was observed in *P. glenii* and *P. semilunaris* differently of *N. fluviatilis* at this site. *N. fluviatilis* and *P. semilunaris* occurred at the site Ladmovce. Diet of these species was very similar in this biotope and we did not confirm significant difference in the food composition. Our findings suggest that in this case, the food offer in specific site was the most important factor in dietary habits and behavior of invasive fishes.
Key Words: feeding behavior, differences, non-native fish, niche overlap.

Introduction. Nowadays, invasive species of plants and animals are one of the main problems of declining biodiversity. This is a real worldwide problem, which is not only in areas like Australia or Madagascar, where is the highest amount of endemic species, but also in both, all aquatic and terrestrial habitats on the Earth. Occurrence of these invasive species has great consequences for ecosystems (Feroz Khan & Panikkar 2009; Cucherousset & Olden 2011; Kati et al 2015). It can cause homogenisation of flora and fauna species, leading to a global biomonotony (Brandner et al 2013). The important question is, how the invasive species may affect indigenous species through trophic relationships? The predation and competition of invasive species on native species can lead to changes in abundance of native species and ultimately can cause a local extinction of them (Kati et al 2015). The high plasticity in reproductive strategy, feeding behaviour and dispersion strategy is typical for invasive species. But it is impossible to identify a single model of life-history that would guarantee invasive success. It depends on multiple factors and interaction in specific situation (Grabowska & Przybylski 2015).

The knowledge on the diet, feeding niche and feeding strategies is still insufficient in invaded areas. However, the diet of invasive species of fish have been recently studied in many countries in Europe and North America (Brandner et al 2013; Almeida et al 2014; Vašek et al 2014; Všetičková et al 2014; Kati et al 2015; Piria et al 2016; Błońska et al 2017; Didenko et al 2017).

In this study, we compare diet of 5 invasive fish species: Western tubenose goby (*Proterorhinus semilunaris*), monkey goby (*Neogobius fluviatilis*), pumpkinseed (*Lepomis gibbosus*), amur sleeper (*Perccottus glenii*) and black bullhead (*Ameiurus melas*).

In the last few years, Ponto-Caspian (P-C) gobiids have invaded many European and North American habitats and their spreading continues (Vašek et al 2014). Diet of the monkey goby (*N. fluviatilis*) is very diverse. Trichoptera, Chironomidae, Bivalvia and Odonata are dominant part of food and the diet overlaps with round goby (*N. melanostomus*). Gut content comparison indicates that monkey and round gobies prefer Trichoptera, Megaloptera and Coleoptera in comparison with another P-C gobies. Even the presence of P-C gobies suggests potentially negative impact on native fish populations - round and bighead gobies on *Sabanejewia balcanica* and monkey goby on *Cyprinus carpio* or *Cobitis elongata* (Piria et al 2016). The spectrum of their food is wide, but Chironomidae larvae prevail (Grabowska et al 2009). There are many studies based on feeding activity of round goby *N. melanostomus* for example (Didenko et al 2017), which was considered to be one of the most important invasive species, due to its high overlap in diet with native species of fish, spawning behaviour and high spreading behavior into new invaded areas (Hempel et al 2018). The western tubenose goby *P. semilunaris* is also one of the P-C gobiids with native distribution in South-East European waters. Nowadays, spreading of this species is in north-west area along the river Danube and Rhine (Všetičková et al 2014). Food composition of this species consists of Trichoptera, Chironomidae and zooplankton. There are some differences between seasons, where Trichoptera dominate earlier and zooplankton later in the same year. It is feeding opportunist that can successfully create new populations also in habitats with poor food availability. No predation pressure on native fishes is known, but it may be feeding competitor, mainly for small and juvenile fish species (Všetičková et al 2014).

Another invasive fish species is Amur sleeper *P. glenii*. It comes from far East Asia, from Russia and Northern China. It was spreading very fast into the Eastern Europe and now, Balaton is considered to be one of the most western situated invaded area of Europe. Between the most common food belong chironomid (Chironomidae) larvae, damselfly (Odonata, Zygoptera) larvae, crustaceans (Crustacea), and mayfly (Ephemeroptera) larvae. Resulting from the study, body size has a stronger effect on diet composition, than habitat or season. This species is predator of invertebrates, fishes are consumed rarely, only by the biggest individuals, but it may influence several levels of aquatic food web (Kati et al 2015). Another study, which has made by Reshetnikov (2003) claims that the species also decreases populations of some native amphibians in small waterbodies by consuming their larvae.

The North America species pumpkinseed (*L. gibbosus*) has been also widely established in Europe. It is another very destructive species that causes changes in behaviour and feeding of native species and benefits by human interventions (Almeida et al 2009; Bhagat et al 2011; Almeida et al 2014).

The last mentioned invasive fish species, which also come from North and Central America is black bullhead *A. melas*. Resulting from studies, this very opportunistic species could negatively affect native ichthyofauna by direct predation and competition, but the most frequented food in all size categories and all researching localities is family Chironomidae. From the feeding strategy point of view, black bullhead is generalist, consuming the most common food (García-Berthou & Morena-Amich 2000; Leunda et al 2008).

The aim of this study is to refer similarity and difference in the diet and feeding niche of five invasive species of fish. The research took place on three sites on the Bodrog river basin (Eastern Slovakia, Tisza river catchment).

Material and Method

Sampling. Fish sampling was performed during October 20th, 2017, using battery electrical aggregate (IUP-12) at three sites in the Bodrog river watershed: Bodrog-Ladmovce (48°24’45”N; 21°46’57”E), Bodrog-Borša (48°22’50”N; 21°42’07”E), and Somotorský kanál, near Somotor village (48°23’43”N; 21°49’09”E) (Figure 1). The length of the sampled sections ranged between 50 and 100 m. Acquired individuals of invasive fish were killed by increasing the anesthetical concentrate (clove oil), and then fish were fixed in 6% solution of formaldehyde after identification, at the sampling areas. Other analyses were performed in laboratory conditions. For our study, altogether 83 specimens of 5 species were used (Table 1).

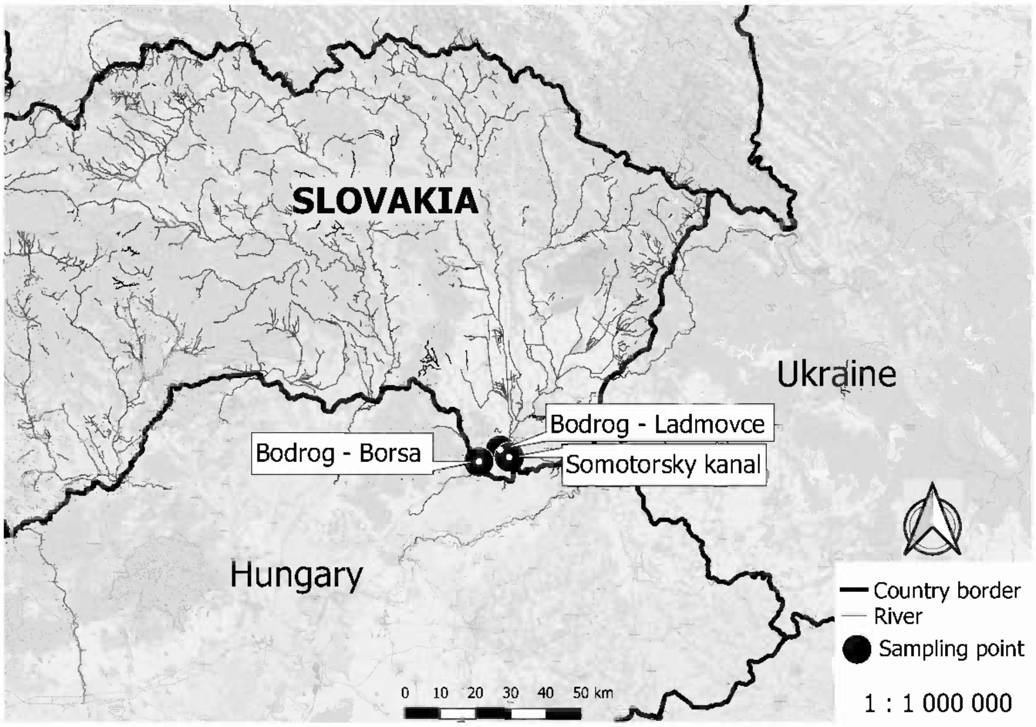

Figure 1. Map with sampling points (made using OpenStreetMaps and QGIS - A Free and Open Source Geographic Information System; author: Peter Manko).

Laboratory analyses. Total weight (WT; weight before dissection), weight after dissection (W-after; weight without gut, liver, and gonads in females), standard length (SL; distance, which is measured from the upper jaw to the base of the caudal fin) were measured before the food analysis. After dissection, the gut was removed from each individual, placed to the marked Petri dish and weighed. Subsequently, we opened the stomach or gut segment with fine scissors and washed out the contents with a small amount of water from a squirt bottle or pipette.

Food items identification. Stereomicroscope Motic SMZ 168 (0.75 - 50x), with Canon 1200D and software Canon Eos Utility 2 were used for microscopic food identification. Food items were identified to the lowest possible taxonomic level and recorded in the laboratory protocol (Manko 2016).

Statistical analyses. For our analysis we used descriptive statistics (Table 1), which determined median of total weight (*Wt*), weight after dissection (*W*), standard length (*SL*) values and total number of individuals.

For quantitative analysis, determination of feeding niche overlap and width, we used different specific indexes, namely Piankas index for determination of diet similarity and Levins index for niche width determination. In Piankas index are values from 0 to 1, where value of 0 suggests that two species don't share the same food resources, value 1 suggests complete overlap and other intermediate values show partial overlap.

ANOSIM is a non-parametric statistical analysis, which tests if there are any significant differences between two or more sampling units. The test is based on comparing the distance of the values in one group with values between two or more groups. Another analysis we used was SIMPER test, which showed the main contribution of food components in every species of fish. Obtained results were analysed using non-metric multidimensional scaling (NMDS), with Bray-Curtis index, which showed us differences in the food composition of species population from sampling sites in the Bodrog River basin.

The results of all analyses were carried out in PAST 3 (Hammer et al 2019) and in the R environment for statistical computing (R Core Team 2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.) with package spaa (Zhang J (2013). spaa: Species Association Analysis. R package version 0.2.1, URL (https://CRAN.R-project.org/package=spaa.).

Results. In total, 83 fish individuals belonging to five invasive species were analysed in this study. The descriptive statistics presented in Table 1 show high variability in species sample sizes, sex ratios, ontogenetic stage, and basic morphological parameters of fish analysed.

Table 1

Total number of individuals (*N*), median of total weight (*Wt*), weight after dissection (*W*), and standard length (*SL*) values

	A. melas	*L. gibbosus*	*N. fluviatilis*	*P. glenii*	*P. semilunaris*
N	10	5	14	4	50
Males	3	0	6	3	13
Females	7	3	6	1	37
Juveniles	0	2	2	0	0
Wt (g)	28.32	2.15	1.25	2.02	1.64
W (g)	25.22	1.8	1.1	1.56	1.44
SL (mm)	101.5	39	44	42	43

The diet of analysed fish species in sampled habitats consists mainly of aquatic insects (Ephemeroptera, Diptera, Odonata) and crustaceans (Ostracoda, Amphipoda) (Figure 2). However, statistically significant differences were found in the diet composition of analysed species by ANOSIM (9999 perm., mean rank within: 1456; mean rank between: 1869; R: 0.243; p: 0.0002) although the separation of the species diet seems low. The pairwise test confirmed significant differences between six pairs of species and they are presented in Table 2. The SIMPER test showed the main contribution of Chironomidae larvae (Diptera), class Ostracoda (Crustacea) and family Baetidae (Ephemeroptera) larvae to the differences in the food composition and this is presented in Table 3. These species along with Corophiidae and Copepoda explained about 60% of the dissimilarity in the diet composition.

Table 2

Differences in the food composition of five invasive species in the Bodrog River basin based on the pairwise ANOSIM (Bray-Curtis; *p* values above and *R* values below the diagonal)

R\p	*A. melas*	*L. gibbosus*	*N. fluviatilis*	*P. glenii*	*P. semilunaris*
A. melas		**0.0498**	**0.0036**	0.0583	**0.0012**
L. gibbosus	0.26		**0.0478**	0.1484	**0.0052**
N. fluviatilis	0.2433	0.2238		0.348	**0.0151**
P. glenii	0.3088	0.1812	0.03074		0.1014
P. semilunaris	0.2969	0.3579	0.1809	0.1939	

Note: Bold text indicates a statistically significant difference with a p-value less than 0.05.

Table 3

The contribution of prey to the differences in the food composition of five invasive species in the Bodrog River basin based on the SIMPER analysis (only prey contributing to the cumulative contribution of 60% are presented; for abbrev. explanations see Figure 1)

Taxon	*Av. dissim*	*Contrib. %*	*Cumulat . %*	*Mean AmeMel*	*Mean LepGib*	*Mean NeoFlu*	*Mean PerGle*	*Mean ProSem*
Chiro	16.64	19.46	19.46	9.2	0.6	2.57	0.75	4.34
Ostr	12.16	14.22	33.68	0.4	0.6	0.571	0.75	4.46
Baet	11.96	13.98	47.66	8.4	1.6	0.286	1.25	0.78
Coro	5.128	5.996	53.65	0	9.2	0	0	0.22
Cope	4.736	5.537	59.19	1.9	1.4	0.214	0	0.52

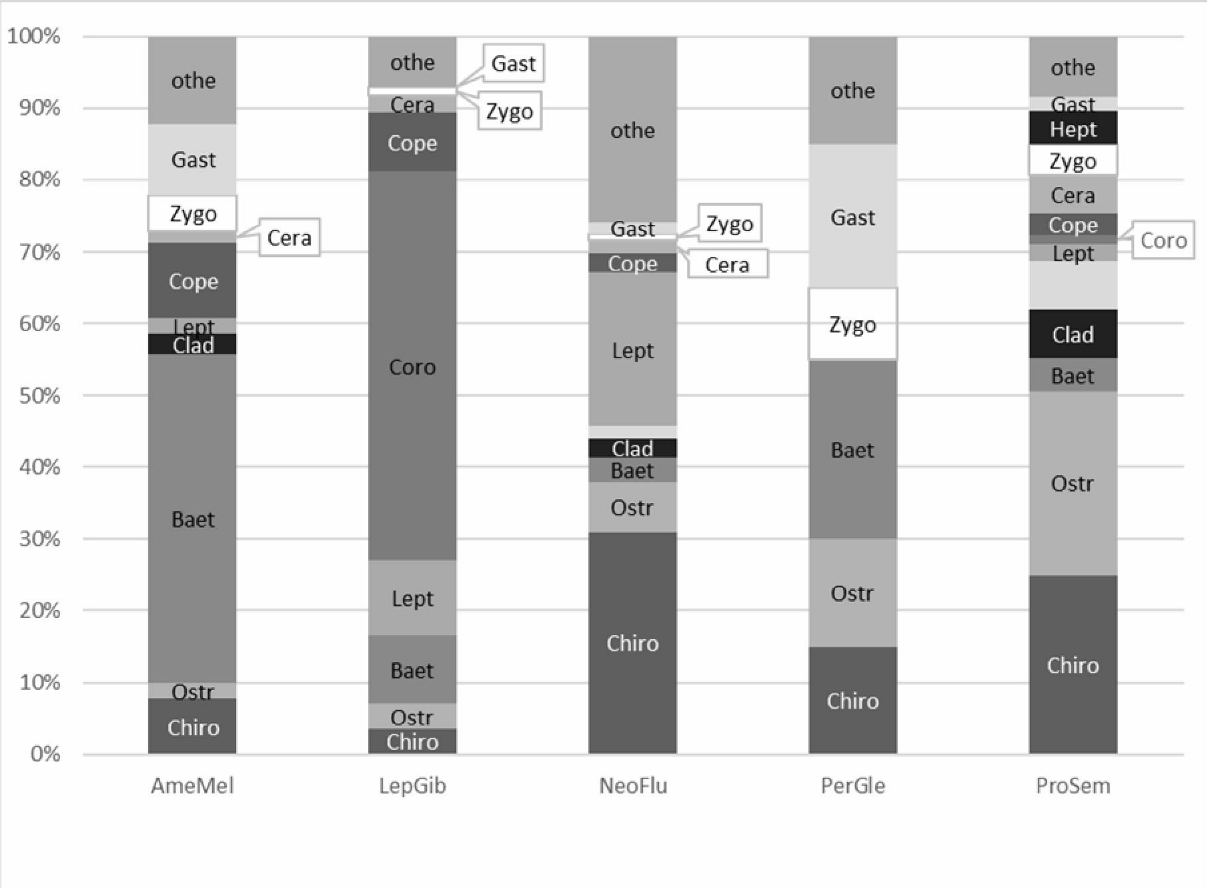

Figure 2. Food composition of five invasive species in the Bodrog river basin (Abbreviations explanation: AmeMel – *A. melas*; LepGib – *L. gibbosus*; NeoFlu – *N. fluviatilis*; PerGle – *P. glenii*; ProSem – *P. semilunaris*; Chiro – Chironomidae (Diptera) larvae; Ostr – Ostracoda (Crustacea); Baet – Baetidae (Ephemeroptera) larvae; Lept – Leptoceridae (Trichoptera) larvae; Coro – Corophiidae (Crustacea); Cope – Copepoda (Crustacea); Cera – Ceratopogonidae (Diptera) larvae; Zygo – Zygoptera (Odonata) larvae; Hept – Heptageniidae (Ephemeroptera) larvae; Gast – Gastrotricha (Mollusca); othe – other taxa).

Differences were also found between populations of species at different sites. The ANOSIM (9999 perm., mean rank within: 514.4; mean rank between: 715.7; R: 0.3287; p: 0.0001) showed that the diet of *P. semilunaris* significantly differed between sites although the NMDS results only slightly point out the differences (Figure 2).
Differences between populations of *N. fluviatilis* were found by ANOSIM (9999 perm., mean rank within: 33.11; mean rank between: 45.58; R: 0.3287; p: 0.0229) and they are also visible in the results of NMDS (Figure 3).

Analyses of the diet composition and feeding niche of coexisting invasive fish species show that there is no niche overlap (*O* = 0.074) and there are also differences in the food composition of *A. melas* and adult *L. gibbosus* while juveniles of *L. gibbosus* seems to have similar diet to *A. melas* at this sampling site (Figure 4). Corophidae consumed only by adult *L. gibbosus*, Baetidae larvae consumed much more by *A. melas* and juveniles *of L. gibbosus*, and Chironomidae larvae contributed most to the dissimilarity of the diet (Table 4). Niche width was broader in *A. melas* (*O* = 4.1) than in *L. gibbosus* (*O* = 2.3).

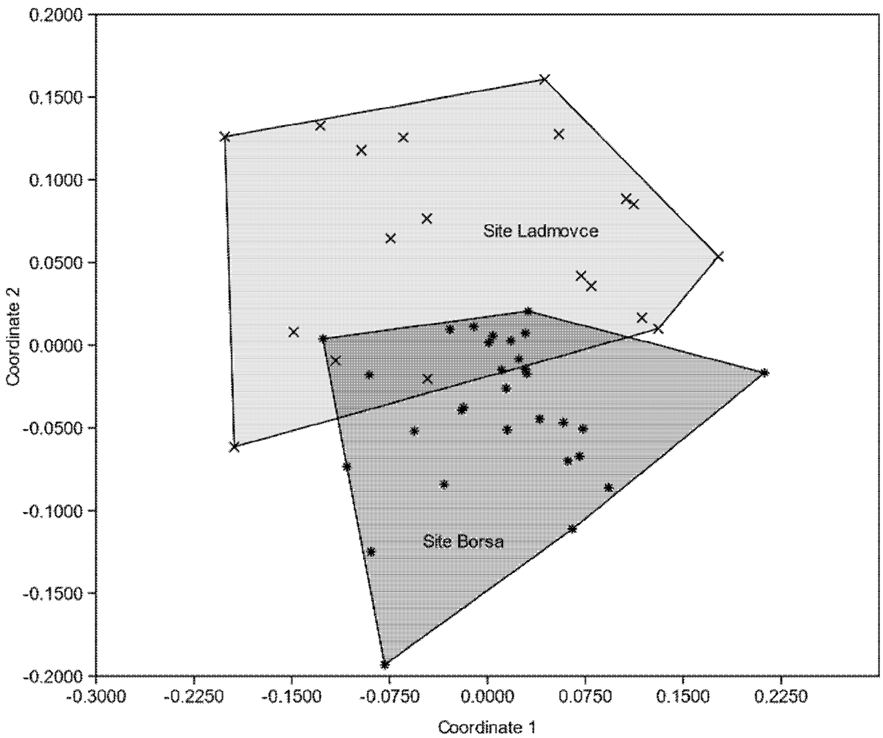

Figure 2. Differences in the food composition of *P. semilunaris* populations from two sampling sites in the Bodrog River basin based on the NMDS graphical output (3D, Bray-Curtis; Stress factor: 0.2158).

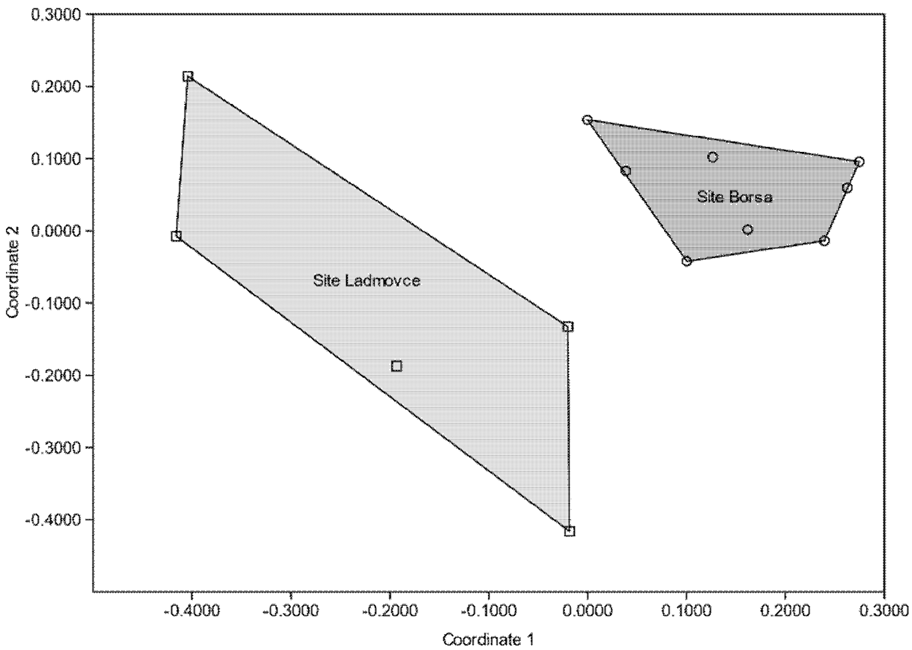

Figure 3. Differences in the food composition of *N. fluviatilis* populations from two sampling sites in the Bodrog River basin based on the NMDS graphical output (2D, Bray-Curtis; Stress factor: 0.247).

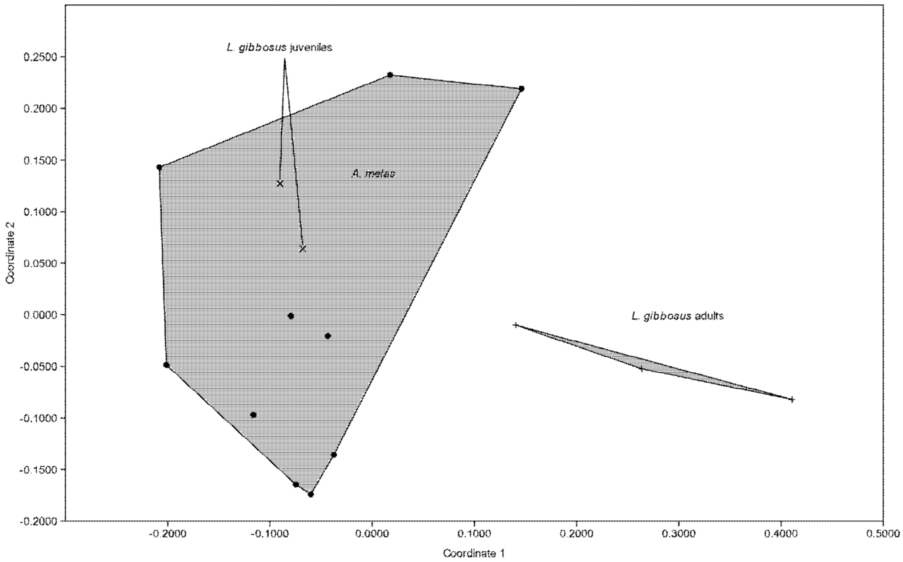

Figure 4. Differences in the food composition of two invasive fish (*A. melas*, *L. gibbosus* – adults and juveniles separratelly) in the Somotorsky kanal (channel) based on the NMDS graphical output (2D, Bray-Curtis; Stress factor: 0.08806).

Table 4

The contribution of prey to the differences in the food composition of two invasive species in the Bodrog River basin based on the SIMPER analysis (only prey contributing with more than 10% are presented; LepGibJ – *L. gibbosus* juveniles; LepGibA – *L. gibbosus* adults; for other abbrev. explanations see Figure 1)

Taxon	*Av. dissim*	*Contrib. %*	*Cumulat. %*	*Mean AmeMel*	*Mean LepGibJ*	*Mean LepGibA*
Coro	21.62	26.78	26.78	0	0	15.3
Baet	17.14	21.23	48.02	8.4	3	0.667
Chiro	10.99	13.62	61.63	9.2	0.5	0.667

At the site Borša, although large feeding niche overlap was found between *N. fluviatilis* and *P. semilunaris* (*O* = 0.72) and intermediate between *P. glenii* and *P. semilunaris* (*O* = 0.53), the diet of all three coexisting species differs significantly according ANOSIM (9999 perm., mean rank within: 354.8; mean rank between: 522.1; R: 0.3886; p: 0.0001). The pairwise test confirmed significant differences between all three pairs of species, and they are presented in Table 5. Three prey taxa (Chironomidae larvae, Ostracoda, and Zygoptera) explain about 60% of the overall dissimilarity in the diet composition of these three fish species (Table 6). Different food composition is indicated between *N. fluviatilis* and other cooccurring species but practically indistinct between *P. semilunaris* and *P. glenii* in the graphical output of the NMDS (Figure 5). Much broader niche width was observed in *P. glenii* (*B* = 5.6) and *P. semilunaris* (*O* = 4.9) than in *N. fluviatilis* (*O* = 1.2) at this site.

Table 5

Differences in the food composition of three coexisting invasive species at the site Borša in the Bodrog River basin based on the pairwise ANOSIM (Bray-Curts; *p* values above and *R* values below the diagonal)

R\p	*N. fluviatilis*	*P. semilunaris*	*P. glenii*
N. fluviatilis		**0.0158**	**0.0024**
P. semilunaris	0.5129		**0.002**
P. glenii	0.3283	0.5	

Note: Bold text indicates a statistically significant difference with a p-value less than 0.05.

Table 6
The contribution of prey to the differences in the food composition of three coexisting invasive species at the site Borša in the Bodrog River basin based on the SIMPER analysis (only prey contributing to the cumulative contribution of 60% are presented; for abbrev. explanations see Figure 1)

Taxon	*Av. dissim*	*Contrib. %*	*Cumulat. %*	*Mean NeoFlu*	*Mean ProSem*	*Mean PerGle*
Chiro	20.66	26.12	26.12	3.25	0.75	4.27
Ostr	18.86	23.83	49.95	0.125	0.75	4.07
Zygo	7.362	9.305	59.25	0	0.5	1.2

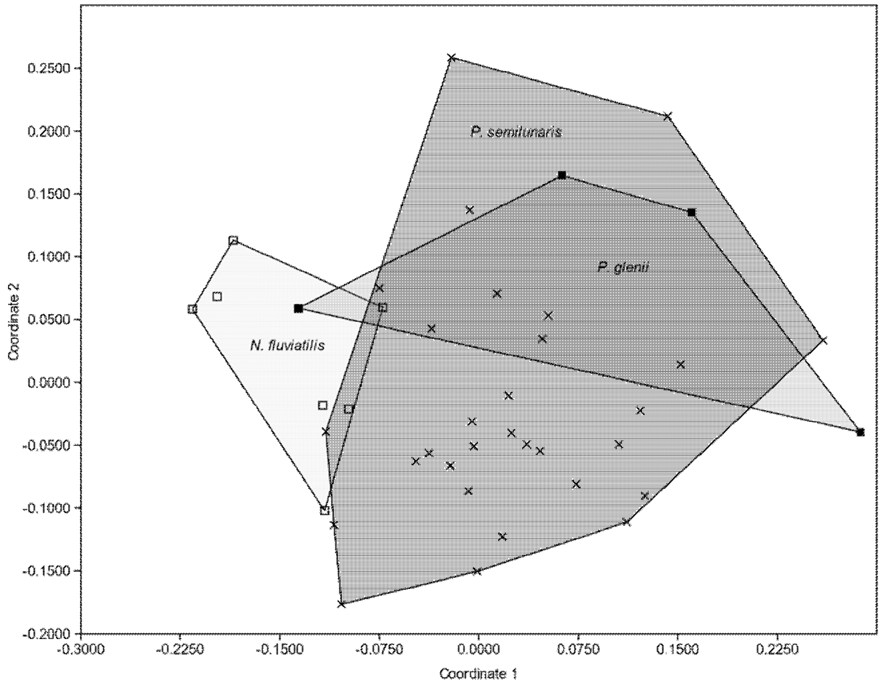

Figure 5. Differences in the food composition of three invasive fish at the site Borša based on the graphical output (NMDS; 2D, Bray-Curtis; Stress factor: 0.2514).

Two invasive fish species (*N. fluviatilis* and *P. semilunaris*) occur at the sampling site Ladmovce. Diet of these species is very similar in this biotope and we did not confirm significant difference in the food composition (ANOSIM; p = 0.1491). On the other hand, the Piankas index indicate only intermediate feeding niche overlap (*O* = 0.37) and the Levins index much larger width of the *P. semilunaris* feeding niche (*B* = 7.7) than the *N. fluviatilis* (*B* = 4.6).

Discussion. In the research that was performed on three sampling sites, altogether five invasive fish species were recorded: black bullhead (*Ameiurus melas*), western tubenose goby (*Proterorhinus semilunaris*), monkey goby (*Neogobius* fluviatilis), amur sleeper (*Perccottus glenii*) and pumpkinseed (*Lepomis gibbosus*).

The diet of analyzed fish species in sampled habitats consists mainly of aquatic insects (Ephemeroptera, Diptera and Odonata) and crustaceans (Ostracoda, Amphipoda) (Figure 1). However, we have found significant differences in diet composition of analysed species by ANOSIM. It can be caused by differences and high variability in size, ontogenetic stage and sex ratio. According to published information, these species feed on aquatic macroinvertebrates in both native and newly invaded areas. Main diet of these species constituted larvae of midges (Chironomidae), mayfly nymphae (Ephemeroptera), and caddisfly larvae (Trichoptera) (Leunda et al 2008; Grabowska et al 2009). Our results agree with these findings, where the largest portion of food accounted individuals of Chironomidae larvae (Diptera), Baetidae nymphae (Ephemeroptera), Ostracoda (Crustacea), and Leptoceridae (Trichoptera) family.

According to Abdoli et al (2002), in *N. fluviatilis*, the main components of food consist of Chironomidae (Diptera) larvae, Ephemeroptera and Trichoptera (larvae) groups in native range that is similar to our findings. Resulting from this, *N. fluviatilis* fed similar food both in native and non-indigenous range, in this case.

According to Grabowska et al (2009) and Piria et al (2016), the diet of *N. fluviatilis* is very diverse, which could have negative impact on our native species like *Cyprinus carpio* or *Cobitis elongata*. Their studies show that Trichoptera group, family Chironomidae and Odonata group are dominant parts of monkey goby diet. Gut content comparison indicates that monkey gobies prefer Trichoptera, Megaloptera and Coleoptera, which is in conflict with our results, where the largest representation of food composition had family Chironomidae (Diptera) larvae and family Leptoceridae (Trichoptera).

Another study by Všetičková et al (2014) shows food composition of invasive fish *P. semilunaris*, which is also one of the P-C gobiids, with native distribution in South-East European waters.

In this study, composition of food consists of Chironomidae (Diptera) larvae and Trichoptera group, which is similar to our results, where dominant parts of food consist of Chironomidae (Diptera) larvae and Ostracoda (Crustacea). These invasive species of fish are feeding opportunists and feeding competitors, mainly for small and juvenile fish species. Mentioned two invasive species of fish (monkey goby and western tubenose goby) had no trophic overlap with native species of fish, moreover, trophic niche was not larger than another coexisting fishes. This case suggest that monkey goby and western tubenose goby present no threat for native species, even, they can integrate into new habitats and fish communities and take advantage of underexploited resources and niches, which are relatively empty (Tarkan et al 2018).

The results from this study of all five invasive species indicated that species from every site received different types of food. It can be caused by different food offers. All five species in our study consumed mainly of aquatic macroinvertebrates, with domination of insects larvae (Chironomidae, Baetidae).

Pettitt-Wade et al (2015) found out no significant overlap between 2 non-native gobies (round goby and western tubenose goby) in the Great Lakes (North America). It can be caused by wide feeding niche and opportunistic feeding behavior of round goby. We found out statistical significant niche overlap and intermediate niche overlap between western tubenose goby and monkey goby. It can be caused by smaller niche width of monkey goby against round goby. The possibility to share a wide range and to have a great plasticity in food utilization can be one of the most important abilities for spreading of successful fish invaders.

Conclusions. The aim of this study was to describe feeding behavior, differences and similarities in feeding niche of 5 invasive species of fish. Research was performed on three sampling sites. Our results show that all 5 invasive species from every site received different types of food components, which can be caused by different offers of food. The main contribution of food components in all species was larvae of midges (Chironomidae) and mayfly nymphae (Ephemeroptera). Obtained data provide that the food offer is in this case the most important factor in feeding behavior of invasive fish.

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