Knowledge and Management of Aquatic Ecosystems (2014) 414, 10 http://www.kmae-journal.org
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DOI: 10.1051/kmae/2014022

Non-native western tubenose gobies *Proterorhinus semilunaris* show distinct site, sex and age-related differences in diet

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Received February 17, 2014
Revised June 16, 2014
Accepted June 16, 2014

ABSTRACT

Key-words: *invasive species, Gobiidae, Proterorhinus semilunaris, diet, site-effect, sex-differences*

The tubenose goby, *Proterorhinus semilunaris*, has expanded its range throughout Europe. Comprehensive studies to identify its impact on native riverine ecosystems, however, are relatively scarce. Here, we evaluate dietary preferences of *P. semilunaris* on a non-native river to reveal any such impacts. Fish were sampled monthly over 2011–2012 from three sites along the River Dyje (Czech Republic; Danube basin) with differing population levels and food availability. Both the amount of food consumed and dietary composition varied seasonally, with site having a strong effect. Food consumed differed between males and females, but not dietary composition; while diet differed between age classes, but not food consumed. In general, Trichoptera, Chironomidae and zooplankton dominated the diet at all sites, with Trichoptera taken more often earlier in the year and zooplankton later. Mussels were always avoided, despite high abundance, while less preferred prey were occasionally taken in high numbers in response to increased availability or energy demand (*e.g.* spawning). Fish eggs and fry (all *P. semilunaris*) were only taken in high numbers at one site, being related to high population level and fry drift from a reservoir. Male feeding declined over the breeding season, presumably due to nest guarding, while female feeding declined over winter, possibly resulting in high mortality. *Proterorhinus semilunaris* is a feeding opportunist, which may facilitate colonisation in habitats with poor prey availability. No predation pressure on native species was observed through consumption of eggs or fry, though small/young indigenous fish may be affected through diet competition.

RÉSUMÉ

Les gobies demi-lune, non autochtones, *Proterorhinus semilunaris,* montrent des différences dans le régime alimentaire liées au site, au sexe et à l'âge.

Mots-clés : *espèces envahissantes, Gobiidae, Proterorhinus semilunaris*

Le gobie demi-lune, *Proterorhinus semilunaris*, a élargi son aire de répartition à travers l'Europe. Cependant, les études approfondies afin d'identifier son impact sur les écosystèmes lotiques indigènes, sont relativement rares. Ici, nous évaluons les préférences alimentaires de *P. semilunaris* dans une rivière où il n'est pas autochtone pour révéler ces impacts. Les poissons ont été échantillonnés mensuellement sur 2011–2012 à partir de trois sites le long de la rivière Dyje (République tchèque ; bassin du Danube) avec différents niveaux de population et de disponibilité de la nourriture. À la fois la quantité de nourriture consommée et la composition

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régime alimentaire, effet site, différences sexuelles

alimentaire variaient saisonnièrement, le site ayant un effet important. La quantité d'aliments consommés différait entre les sexes, mais pas la composition alimentaire ; le régime alimentaire différait entre les classes d'âge. En général, les trichoptères, les larves de chironomes et le zooplancton ont dominé le régime à tous les sites, avec les trichoptères consommés plus souvent plus tôt dans l'année et le zooplancton plus tard. Les moules ont été toujours évitées, malgré leur forte abondance, bien que cette proie moins préférée ait été parfois prise en grand nombre en réponse à sa disponibilité accrue ou à la demande d'énergie (par exemple, pour l'ovogénèse). Les œufs de poissons et les alevins (tous de *P. semilunaris*) n'ont été pris en grand nombre qu'à un seul site, étant lié à un niveau élevé de la population et à la dérive des alevins à partir d'un réservoir. L'alimentation des mâles a diminué au cours de la saison de reproduction, probablement en raison de la garde des nids, tandis que l'alimentation des femelles a baissé pendant l'hiver, ce qui peut entraîner une mortalité élevée. *Proterorhinus semilunaris* est un opportuniste dans son alimentation, ce qui peut faciliter la colonisation dans les habitats avec une faible disponibilité en proies. Aucune pression de prédation sur les espèces indigènes n'a été observée par la consommation d'œufs ou d'alevins, mais les petits/jeunes poissons indigènes peuvent être affectés par la concurrence alimentaire.

INTRODUCTION

The tubenose goby *Proterorhinus semilunaris* (Pallas, 1811) is one of five Ponto-Caspian Gobiid species that have expanded their ranges throughout Europe in recent decades (for a review of expansion along the Rivers Danube and Rhine, see Roche *et al*., 2013). The species was first introduced into the Czech Republic in 1994 as angler's live-bait in the shallow Nové Mlýny reservoir system on the River Dyje (Figure 1; Lusk and Halačka, 1995). Soon after, it was found in the River Dyje itself and it subsequently spread rapidly downstream to the Dyje's confluence with the River Morava (a main tributary of the Danube). It has since continued to spread more slowly up the Morava (Figure 1; Janáč *et al*., 2012; Prášek and Jurajda, 2005).

In order to evaluate the impact of this non-native species on the native ecosystem, *e.g.* through competition, predation and/or as prey, a long-term study has been put in place to obtain detailed information on the species' biological requirements, especially as regards dietary preference. Information on *P. semilunaris* diet in its non-native area is relatively scarce compared with the related round goby *Neogobius melanostomus* (Pallas, 1814) (but see Borcherding *et al*., 2013; Borza *et al*., 2009; Brandner *et al*., 2013). Further, studies have tended to focus mainly on marine and estuarine habitats (*e.g.* Andriyashev and Arnoldi, 1945; Burnashev *et al*., 1955; Grinbart, 1960); hence, there is limited information available for freshwaters, despite this being the habitat within which *P. semilunaris* populations are expanding (for exceptions see Adámek *et al*., 2007; French and Jude, 2001). Moreover, dietary studies on *P. semilunaris* have usually been limited to a single summer sample; just one study to date dealing with annual diet composition, *i.e.* Adámek *et al*. (2010), who examined the original introductory population in the Nové Mlýny reservoir. In general, the results of these previous studies have been highly variable, with Crustacea, various aquatic insect larvae or zooplankton being presented as the dominant dietary item (Adámek *et al*., 2010; French and Jude, 2001; Vašek *et al*., 2011).

Here, we aim to address the general lack of studies describing *P. semilunaris* dietary preference in freshwater systems by describing annual dietary patterns along the River Dyje. The river itself is naturally split into three 'zones' by weirs, with the downstream stretch connected with the larger River Morava (and not far below that the River Danube) and the upstream stretch situated immediately below the reservoir outlet. The upstream stretch is known to offer greater prey availability (Adámek *et al*., 2010) and harbour higher numbers of *P. semilunaris* (Prášek and Jurajda, 2005), partly due to larval drift over the dam from the reservoir (Janáč *et al*., 2013). Differences in fish density, prey availability and habitat quality in the three zones could all potentially result in differences in *P*. semilunaris diet. Furthermore, several

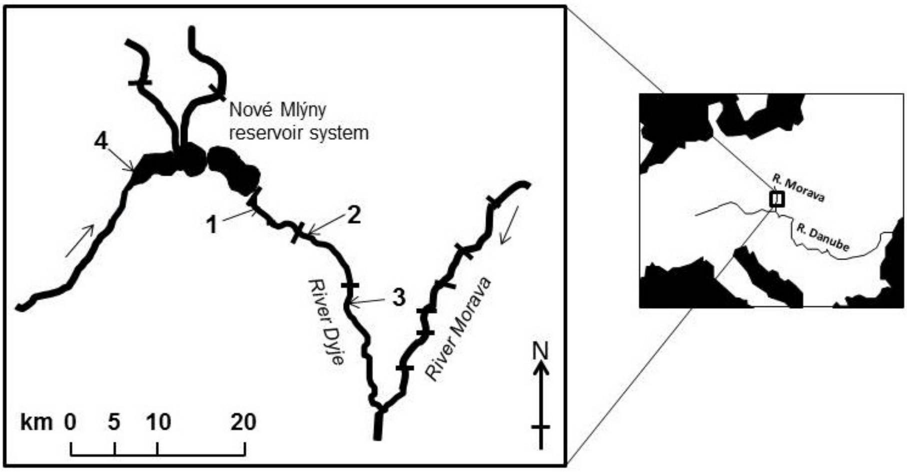


Figure 1
Map of the study site. 1 = Nové Mlýny study site, situated below the outlet of the Nové Mlýny reservoir system, 2 = Bulhary study site, 3 = Břeclav study site, 4 = site of first P. semilunaris introduction.

previous studies have suggested that distinct dietary preferences may be displayed by particular sizes/ages (*e.g.* Adámek *et al.*, 2010; Vašek *et al.*, 2011) and by males and females, particularly during the mating season when dominant males are assumed to be guarding the nest (*e.g.* see Adámek *et al*., 2010). In this study, therefore, we not only examine potential differences in diet between different sites but also between different sexes and age groups. As such, this paper presents the first comprehensive overview of *P*.semilunaris diet in its non-native riverine habitat and builds upon the lentic study of Adámek *et al*. (2010).

STUDY AREA

This study was conducted at three sites along the River Dyje (Danube Basin; Czech Republic) stretching from the site of first introduction (Nové Mlýny reservoir outlet) to the Dyje's confluence with the River Morava (Figure 1). The first locality is situated 100-300 m below the reservoir's outlet (Nové Mlýny; 48°51′27.200″N, 16°43′30.701″E; river km 45.9); the second is situated below a large weir near the village of Bulhary (Bulhary; 48°49′38.468″N, 16°46′11.489″E; river km 39.5); and the third is situated near the town of Břeclav, not far from the Dyje's confluence with the River Morava (Břeclav; 48°44′23.859″N, 16°53′27.509″E; river km 23.5). All sites have similar habitat with modified river banks stabilised by rip-rap, average depth of around 1 m and only rare presence of vegetation (mainly during times of higher discharge). Microhabitat variation was slightly higher at Břeclav, the channel including a number of deeper, faster-flowing sections.

MATERIALS AND METHODS

Fish were collected each month from February 2011 to January 2012 (sampling was impossible in January 2011 due to ice cover on the Dyje) from a 50–150 m section of rip-rap river bank at each site using electrofishing gear (SEN, Fa. Bednář, Olomouc, 220–240 V, 1.5–2 A, 80–90 Hz). All *P. semilunaris* were overdosed with clove oil and preserved in 4% formaldehyde for further analysis in the laboratory. Water temperature was measured at the same time as sampling in order to assess any impact on fish presence or food abundance.

All adult fish captured (*i.e.* those whose sex could be determined; all >22 mm·SL^{-1}) were measured to the nearest 0.001 mm (standard length [SL] and total length [TL]) and weighed to the nearest 0.001 g. Two distinct size groups are regularly observed in *P. semilunaris* samples, with 40 mm SL representing an approximate threshold between fish of <1 year and those ⩾1 year (Jurajda, unpublished data). Hence, in our samples, we use this 40 mm threshold to separate *P. semilunaris* into two age groups, *i.e.* fish of <1 year and those ⩾1 year. The fish were again weighed following evisceration, as was the digestive tract. The contents of the digestive tract were removed, weighed and determined to the lowest possible taxonomic group under a binocular microscope. The contents were sorted into categories to simplify analysis, *i.e.* Annelida (Oligochaeta, Hirudinida), zooplankton, benthic Crustacea (*Asellus*), Ephemeroptera, 'other' items (*e.g.* Odonata, Heteroptera, Megaloptera, Coleoptera), Trichoptera, Chironomidae larvae, Chironomidae pupae, Diptera (except Chironomidae), terrestrial insects, fish (eggs and fish fry), and detritus (detritus, sand, macrovegetation residues; not included in dietary analysis).

In order to document availability of prey, macrozoobenthos samples were taken seasonally (spring-April, summer-July, autumn-September, winter-January) over 2011 from the same sites where fish sampling took place. Previous macrozoobenthos studies have documented low intra-season variability at these sites; hence it was not felt necessary to sample macrozoobenthos each month (Všetičková, unpublished data). Macrozoobenthos samples were collected semi-quantitatively via a standard three minute kick-sample using a sweep net with a mesh size of 500 μm (Kokeš and Němejcová, 2006). The final sample also included zootecton scraped manually from three large boulders. The samples, along with organic and inorganic debris, were sorted using a 500 μm mesh sieve and preserved in a 4% formaldehyde solution. Macroinvertebrates were sorted in the laboratory and determined to the lowest possible taxonomic level (mostly species).

DATA ANALYSIS

Food bulk in each digestive tract was assessed using an index of digestive tract fullness IF (‰), calculated as the ratio between diet weight (w) and eviscerated fish weight (W_{evi}):

$$IF = 10^4 \times (w/W_{\text{evi}})$$

The percentage representation of each dietary item in each digestive tract was expressed visually as the percentage contribution ($\%V_i$; volumetric method, Hyslop, 1980):

$$\%V_i = 100 \times (V_i/\Sigma V_i)$$

where V_i is the volume of dietary item i in a digestive tract. Analysis of seasonal variance in diet composition was based on these $\%V_i$ values (see below).

The percentage representation of each dietary item (pooled for all digestive tracts) was expressed as relative percentage weight ($\%W_i$), calculated according to Hyslop (1980):

$$\%W_i = 100 \times (W_i/\Sigma W_i)$$

where W_i is the weight of diet item i (summed for all digestive tracts) and ΣW_i represents the total weight of all dietary items (summed for all digestive tracts).

Frequency of occurrence ($\%F_i$) was specified as the percentage of digestive tracts including dietary item i:

$$\%F_i = 100 \times (n_i/n)$$

where n_i is the number of fish with dietary item i in the digestive tract and n is the total number of fish whose digestive tracts contained food items (non-empty).

The index of preponderance (I_{Pi}) was used to assess the "importance" of dietary item i, according to Natarajan and Jhingran (1961):

$$I_{\text{Pi}} = (\%W_i \times \%F_i)/(\Sigma\%W_i \times \%F_i) \times 100$$

where $\%W_i$ is the percentage weight of dietary item i and $\%F_i$ is the frequency of occurrence of dietary item i (see above).

Similarities between sites in composition of available prey were assessed in each season using the Bray-Curtis similarity index. Ivlev's electivity index was calculated for each site in each season using the formula:

$$E = (r_i - p_i)/(r_i + p_i)$$

where E is the electivity index, r_i is the proportion of prey item i in the gut contents (calculated as mean $\%V_i$) and p_i is the proportion of prey item i in the corresponding macrozoobenthos sample (volumetric %). Only Mollusca, Annelida, Crustacea, Ephemeroptera, Trichoptera, Chironomidae (larvae) and Diptera were considered in the electivity index as zooplankton and 'fish' were largely underestimated by macrozoobenthos samples and other prey items were of minor importance.

Three feeding characteristics were considered as response variables: (1) proportion of fish with non-empty digestive tracts, (2) *IF* and (3) diet composition. The effect of predictors on the proportion of fish with non-empty digestive tracts was tested using a generalised linear model (GLM; binomial distribution). Each GLM was checked for possible over- or under-dispersion (absence of both confirmed). The effect of predictors on *IF* was tested using a linear model (LM; data $\log(IF + 1)$ transformed to follow normal distribution). The validity of each LM was confirmed through residual analysis (no shape in residuals vs. fitted values; normality of residuals). The effect of predictors on diet composition was tested using non-parametric permutational multiple analysis of variance (PERMANOVA; Anderson, 2001). The response variable (distance matrix of samples (*i.e.* digestive tracts)) for each PERMANOVA was calculated from an original "sample *x* diet-item" matrix of *%Vi*, using Bray-Curtis dissimilarity as a distance measure. PERMANOVA uses a multivariate analogue of Fisher's *F* ratio to compare variability within groups versus variability between different groups, *P*-values being obtained using permutations (Anderson, 2001). In this study, 999 permutations were conducted for each PERMANOVA. Diet composition data were visualised in two-dimensional space through non-metric multidimensional scaling (NMDS), using Bray-Curtis dissimilarity as a distance measure and the metaMDS function (Oksanen *et al.*, 2012), which runs NMDS with several random starts and returns the best solution, rotated such that the largest variance of samples is on the first axis.

For each feeding characteristic, intra-annual difference was tested first (using the analyses described above) and differences between months described based on a visual comparison of the data. Subsequently, the effect of sex, size and site was tested for each month using a full model containing all three predictors. The full model for each month was simplified by removing non-significant predictors, removal of which had no significant effect on the models (likelihood ratio tests significant at α level of 0.05 for GLM and LM, and an analogous procedure in PERMANOVA that detects significance of a predictor for all possible model combinations). In each final model containing two predictors (none contained all three predictors), both were significant. When the effect of site was significant in the final model, three multiple tests were conducted to reveal differences between separate sites (α level of each multiple test was reduced following Bonferroni correction of α in multiple testing to $0.05/3 = 0.017$).

All analyses were conducted using R 2.14.2 (R Development Core Team, 2012) using the *stats* (R Development Core Team, 2012) and *vegan* packages (Oksanen *et al*., 2012).

RESULTS

Overall, 1291 adult fish were examined (*i.e.* SL $>$22 mm), of which 1053 had digestive tracts containing food (Table I). More females were caught than males throughout the year, except for July. Note, however, that low numbers of *P. semilunaris* were caught at some sites during February, July and January (no *P. semilunaris* were caught at Bulhary and Břeclav in February), and this may have affected analysis outputs. In general, more fish were caught later in the year following the spawning period (between august and December), and more $\geqslant$1 year fish were caught than $<$1 year.

Table I
Mean (± SD) standard length (in mm) and weight (in g), total number of fish sampled, number of fish with empty guts (Empty), and number of fish in each of the categories (sex, size, site) considered in IF and diet composition analysis (i.e. fish with non-empty digestive tracts) for P. semilunaris sampled over 2011/2012 from the River Dyje.

					sex		size		site		
Month	**Length**	**Weight**	**No. fish**	**Empty**	**Female**	**Male**	**<1 year**	**⩾ 1year**	**Břeclav**	**Bulhary**	**Nové Ml.**
February	42.8 ± 8.0	2.2 ± 1.1	25	10	9	6	7	8			15
March	45.2 ± 10.6	2.6 ± 2.2	112	43	51	18	21	48	25	18	26
April	46.0± 7.9	2.6± 1.5	82	8	55	19	16	58	18	11	45
May	45.5 ± 7.4	2.5 ± 1.5	94	1	73	20	23	70	30	16	47
June	45.4 ± 8.1	2.3 ± 1.7	54	10	35	9	15	29	10	8	26
July	35.8 ± 7.4	1.2 ± 1.0	36	2	9	25	26	8	5	4	25
August	40.2 ± 7.0	1.7 ± 1.1	227	44	143	40	100	83	23	94	66
September	44.9 ± 7.7	2.3 ± 1.2	116	27	44	45	18	71	9	55	25
October	44.1 ± 8.1	2.1 ± 1.3	195	6	98	91	66	123	30	66	93
November	46.0 ± 8.1	2.4 ± 1.4	161	27	72	62	30	104	21	65	48
December	46.9 ± 8.9	2.8 ± 1.8	107	26	42	39	17	64	14	38	29
January	43.1 ± 6.3	2.1 ± 1.0	82	34	21	27	16	32	4	36	8
Total	44.0 ± 8.3	2.2 ± 1.5	1291	238	652	401	355	698	189	411	453

Table II
Prey availability (% of total sample volume) assessed using a standard three-minute kick sample and rock scraping once per season and Bray-Curtis (B-C) similarity of the obtained assemblages within a respective season. Nm – Nové Mlýny, Bu – Bulhary, Bř – Břeclav, L. – larvae. Figures in bold show dominant items (i.e. >10%).

	April			July			September			January		
	Nm	Bu	Bř	Nm	Bu	Bř	Nm	Bu	Bř	Nm	Bu	Bř
Mollusca	**38.5**	**20.9**	**13.4**	**85.0**	**28.5**	**29.6**	**44.5**	**34.5**	**10.0**	**45.5**	5.3	**13.3**
Chironomidae L.	**20.9**	**12.7**	**15.2**	4.9	**35.8**	**38.4**	**34.9**	**19.1**	**36.7**	**38.8**	**36.3**	**25.3**
Annelida	**31.9**	9.1	**18.8**	0.2	5.3	**10.4**	**13.0**	**19.6**	3.3	**13.1**	**31.1**	1.2
Ephemeroptera	2.2	**34.6**	**46.4**		**17.2**	**12.0**	1.4	0.6	**30.0**	0.4	1.6	**14.5**
Trichoptera		**18.2**	3.6	0.6	**12.6**	7.2	4.1	**21.1**	**16.7**	2.2	**16.3**	
Crustacea	5.5			8.8							5.3	
Diptera L.		3.6	1.8		0.7						1.6	1.2
Other	1.1	0.9	0.9	0.4		2.4	2.1	5.1	3.3		2.6	**44.6**
B-C similarity:												
Nm-Bu	0.49			0.24			0.61			0.53		
Nm-Br	0.55			0.25			0.26			0.19		
Bu-Br	0.75			0.85			0.12			0.29		

FOOD RESOURCES

Although each site hosted the same prey taxa, prey availability differed between sites throughout the year (Bray-Curtis similarities often below 0.5; Table II). The Nové Mlýny site hosted a larger proportion of molluscs and a lower proportion of EPT (Ephemeroptera, Plecoptera and Trichoptera) taxa compared to the other two sites. EPT fluctuated throughout the year, the main changes presumably being related to timing of mass hatching of different species. There was, however, a clear trend for Trichoptera larvae being more abundant in Bulhary and Ephemeroptera larvae being more abundant in Břeclav. Mollusca (mostly *D. polymorpha*) were always more abundant at Nové Mlýny, and were virtually the only prey item found in July (Table II). Chironomids were abundant at all three sites, with proportions fluctuating throughout the year (mostly at Nové Mlýny). Permanent macrozoobenthos fauna (*e.g.* Annelida) fluctuated through the year, with lowest numbers found in July at Nové Mlýny and Bulhary, and in September and January at Břeclav. A large proportion of 'other' prey items occurred in January at Břeclav, though this comprised almost entirely Heteroptera. The Bray-Curtis index indicated that Bulhary and Břeclav hosted similar prey assemblages in April and July (*i.e.* Nové Mlýny differed), and that Nové Mlýny and Bulhary hosted similar prey assemblages in September and January (*i.e.* Břeclav differed).

NUMBER OF FISH WITH DIGESTIVE TRACTS CONTAINING FOOD

The proportion of fish with digestive tracts containing food differed throughout the year (GLM; $df = 11$ and 1279; $P < 0.001$; the model explained 11.5% of variability). In general, two peaks were identifiable, *i.e.* around May and October (Figure 2). Proportions clearly decreased from October onwards and increased from March onwards (Figure 2).

Few significant inter-sex, inter-size or inter-site differences were observed (GLM; $P < 0.05$). Differences between male and female proportions occurred in June, with a sudden decrease in males with full digestive tracts, and January, when females showed a pronounced drop (Figure 2). Size associated differences occurred in September with a decrease in the proportion of full digestive tracts in small fish (<1 year; Figure 2). Site differences occurred in March, April and September, with a drop in proportion at Nové Mlýny in March and April, and at Bulhary in September (Figure 2). While the differences between sexes and sizes were variable (*i.e.* neither sex nor size showed consistently lower values in one representative), this did not

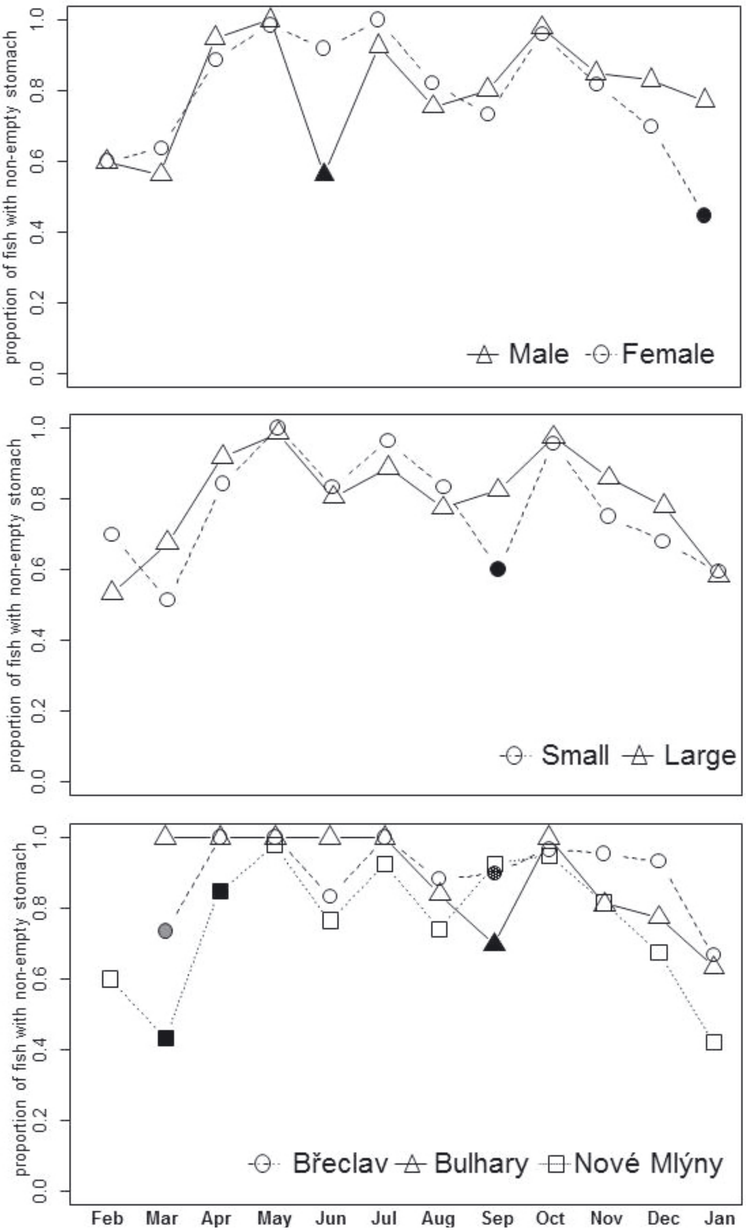


Figure 2

Proportion of P. semilunaris (categorised for sex, site and size) from the River Dyje with non-empty digestive tracts over 2011/2012. Symbols with different colours (white, black) indicate a significant difference between levels in each month (GLM; $P < 0.05$ for sex and size, $P < 0.017$ for site). Hatched symbols represent values that do not differ from either black or white.

hold true for site, with *P. semilunaris* from Nové Mlýny having the lowest proportion of full digestive tracts in most months (though the difference was not usually significant; Figure 2).

INDEX OF FULLNESS

IF differed throughout the year (LM; $df = 11$ and 1041; $P < 0.001$; the model explained 30.1% of variability) in a similar manner to the proportion of fish with full digestive tracts. In general, *IF* showed an increase from February to May with two major drops from July to September and from December to February (Figure 3).

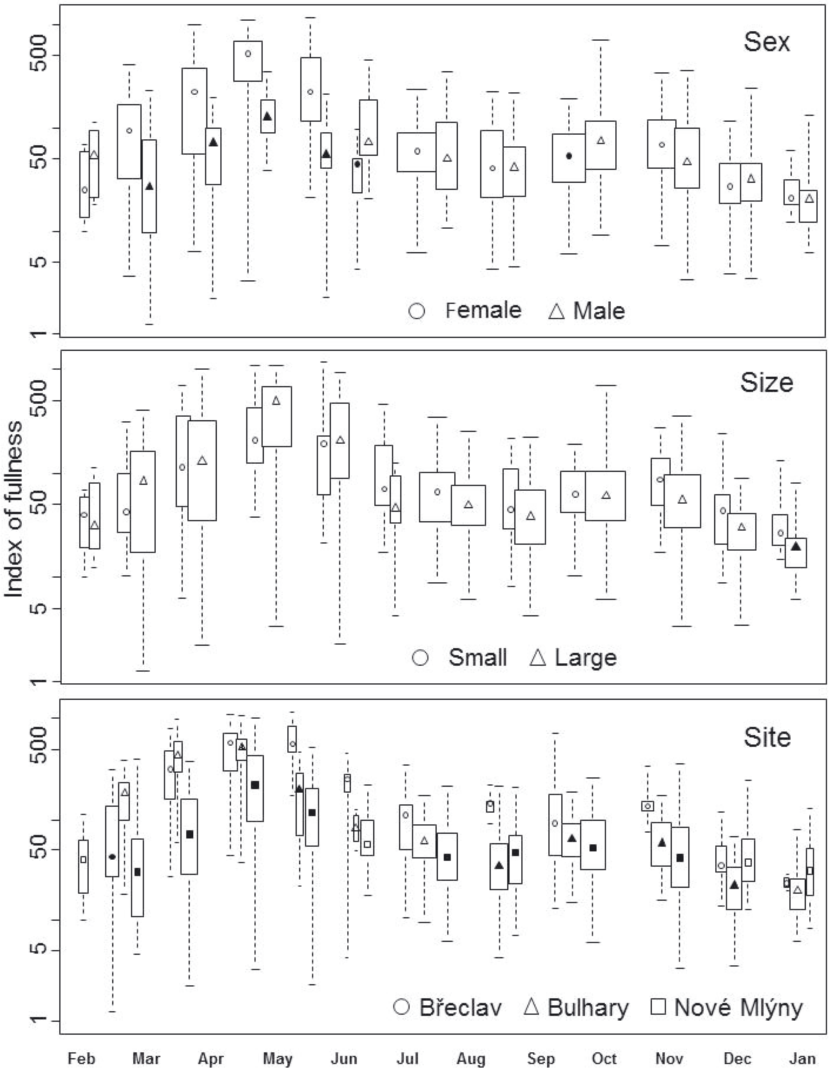


Figure 3

Gut fullness of P. semilunaris (categorised for sex, size and site) over 2011/2012 in the River Dyje. Symbols with different colours (white, grey, black) indicate a significant difference between levels in each month (LM; $P < 0.05$ for sex and size, $P < 0.017$ for site). Hatched symbols represent values that do not differ from either black or white. Centre point = median, box = inter-quartile range, whiskers = range, box widths = square root of sample size. All data presented left to right as February 2011 to January 2012.

Males consistently had significantly lower *IF* than females from March to June (LM; $P < 0.05$; Figure 3), but significantly higher *IF* over July and October (LM; $P < 0.05$; Figure 3). Size difference in *IF* was not significant throughout most of the year, with the exception of January when $\geqslant 1$ year fish had significantly lower *IF* than <1 year fish (LM; $P < 0.05$; Figure 3). Site differences were significant through most of the year (LM; $P < 0.05$), with the exception of July and January. Lowest *IF* values occurred most frequently at the Nové Mlýny site and highest at the Břeclav site (Figure 3).

DIET COMPOSITION

Trichoptera, zooplankton and chironomid larvae were the most important diet items of *P. semilunaris* in the River Dyje, both in terms of percentage weight, frequency of occurrence and *IP* (Table III; results shown for *IP* only). Trichoptera larvae were important from February to September (with Ephemeroptera larvae as an alternative from May to June [July]),

Table III
IP values of dietary items found in digestive tracts of tubenose gobies over 2011/2012 in the River Dyje. Items are shown in descending IP calculated for the whole year (Total). Figures in bold indicate dominant dietary items ($>$10%). Tric = Trichoptera, Chir = Chironomidae larvae, Zoo = zooplankton, Eph = Ephemeroptera, Crust = Crustacea, Dipt = Diptera larvae, Ann = Annelida, Chir = Chironomidae pupae, Ins = terrestrial insect, Oth = others.

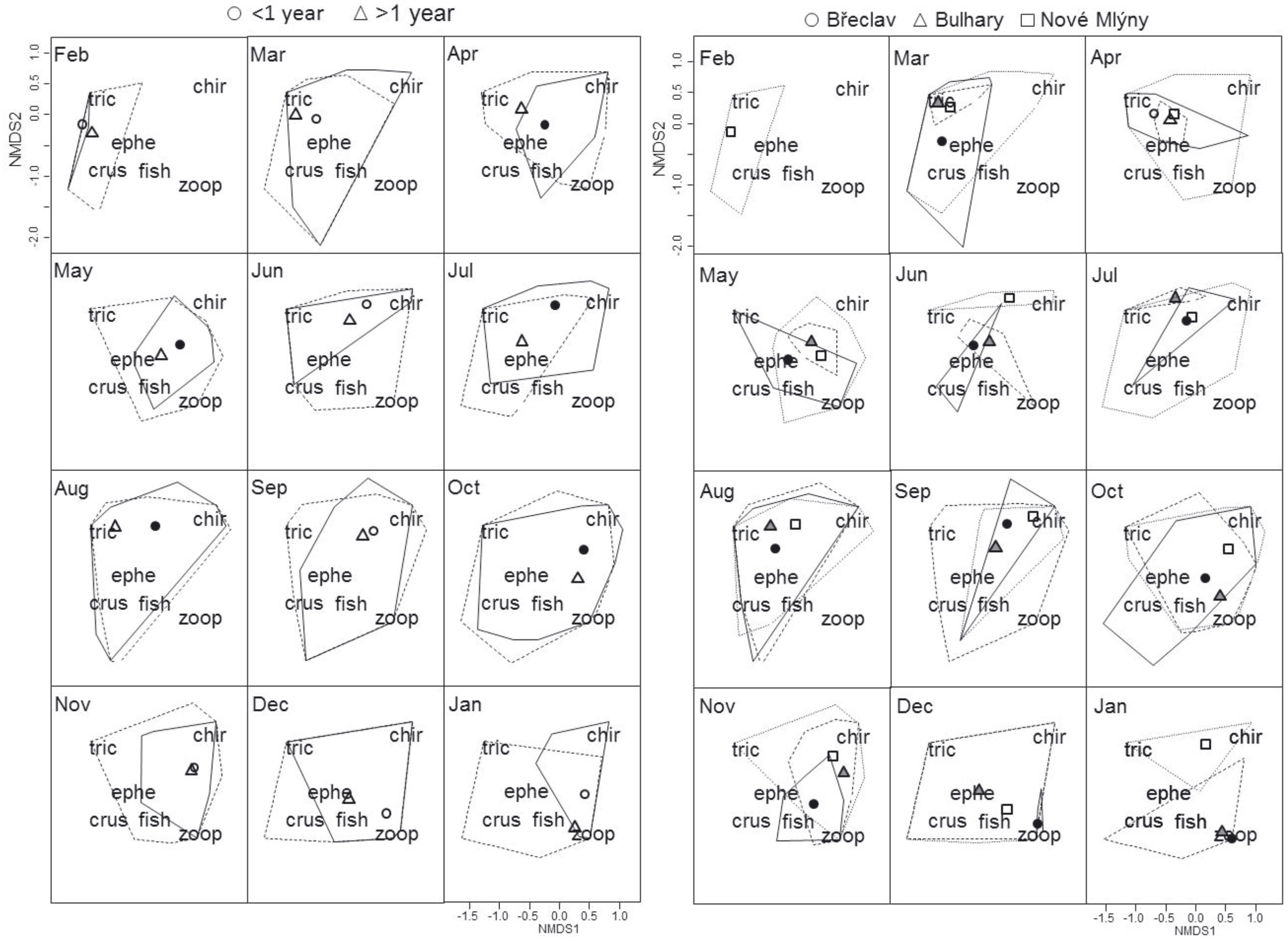
Month	Tric	Chir	Zoo	Eph	Crust	Fish	Dipt	Ann	Chip	Ins	Oth
February	**64.9**	0.6			**33.3**	0.8		0.3			
March	**93.8**	0.7	<0.1	0.3	5.2	<0.1		<0.1			<0.1
April	**89.9**	3.5	2.4	0.2	2.1	1.3	<0.1	0.1	0.5		0.1
May	**27.9**	**18.3**	**34.1**	**13.8**	0.5	2.5	0.8	0.1	2.0		<0.1
June	**17.1**	**37.2**	0.3	**44.5**	<0.1	0.1	<0.1	0.5	<0.1		0.2
July	**45.9**	**41.6**	0.1	9.4	0.6			0.7		1.6	<0.1
August	**75.5**	**20.2**		2.7	<0.1		<0.1	0.5	0.1	1.0	<0.1
September	**27.0**	**65.6**	1.4	0.9	<0.1		3.2	0.1	0.4	1.5	
October	2.9	**26.8**	**66.8**	0.4	0.2		1.0	1.6	<0.1	0.4	<0.1
November	3.5	**59.0**	**37.3**	<0.1	0.2		<0.1		<0.1	<0.1	<0.1
December	**19.7**	3.6	**74.6**		2.1				<0.1		
January	0.5	4.1	**94.1**		1.3						
All-year	**42.7**	**27.5**	**23.2**	4.3	0.9	0.3	0.3	0.3	0.3	0.1	<0.1

Chironomids from May to November and zooplankton from October to January. Overall, the dominant prey classes appear to switch from Trichoptera-chironomid to chironomid-zooplankton around autumn. Note also the sharp peak for zooplankton in May and the levels for Ephemeroptera just below dominance (10%) in July (Figure 4a; Table III). Notably, the only visible increase in fish (egg) consumption was documented in May and crustaceans were important only in February (Table III). All other items were taken at very low levels.

While no significant differences were observed in diet composition between male and female *P. semilunaris* (PERMANOVA, $P > 0.05$; Table IVa), dietary differences between $<$1 year and $\geqslant$1 year fish were more pronounced, being significantly different in April, May, July, August and October (PERMANOVA, $P < 0.05$; see Table IVb and Figure 4a). In general, the diet of $<$1 year *P. semilunaris* was shifted more to chironomids and less to Trichoptera than $\geqslant$1 year fish, and the switch to a chironomid-dominated diet occurred sooner than in larger gobies (May in $<$1 year fish, September in $\geqslant$1year fish; Table IVb, Figure 4b). Significant differences were observed in diet composition between the three sites for all months (PERMANOVA, $P < 0.05$; Table IVc), with the exception of April (and February, when no fish were captured at Bulhary and Břeclav). In general, fish from Nové Mlýny tended to consume more chironomid larvae than those from the other sites (Figure 4b, Table IVc). Interestingly, the May increase in fish egg/larvae consumption was caused by fish from Nové Mlýny only (*IP* of fish raised to 15.8 in May). Nové Mlýny was also the only site where Crustacea (Asellus) was of any importance, though only in February and, to a lesser extent, January (Table IVc). Between February and September, when aquatic insect larvae are most abundant, fish from Břeclav consumed larger numbers of Ephemeroptera (mostly between May and August), while those from Bulhary ate more Trichoptera (Figure 4b; Table IVc). Following the autumn shift to a chironomid-zooplankton-based diet (Tables III and IVc), Břeclav fish were distinguished from those at the other sites by consuming a larger proportion of non-chironomid Diptera (October, November) or zooplankton (December, January; Table IVc, Figure 4b); while Bulhary fish fed almost entirely on zooplankton (October, January), zooplankton-Trichoptera (November) or chironomids (December).

ELECTIVITY

Mollusca and Annelida were regularly avoided in *P. semilunaris* diet at all three sites, while Crustacea (Asellus) were often preferred (Figure 5). These results should be treated with



(a) (b)

Figure 4

Non-metric multidimensional scaling ordination representing the diet of P. semilunaris categorised for (a) size and (b) site in the River Dyje over 2011/2012. Points represent group centroids and lines border group hulls. Text indicates the position of dietary items in the ordination. Chir = Chironomidae larvae, tric = Trichoptera, ephe = Ephemeroptera, crus = Crustacea, zoop= zooplankton. Less important dietary items are not shown (positions of Diptera larvae, terrestrial insect, Annelida and 'others' are all close to that of 'fish', and that of Chironomidae pupae close to Chironomidae larvae). Different coloured symbols (white, grey, black) indicate a significant difference between levels in each month (PERMANOVA; $P < 0.05$ for size, $P < 0.017$ for site).

Table IVa
IP values of dietary items found in digestive tracts of female and male P. semilunaris over 2011/2012 in the River Dyje. Items are shown in descending IP calculated for the whole year (Total). Figures in bold indicate dominant dietary items (>10%). Tric = Trichoptera, Chir = Chironomidae larvae, Zoo = zooplankton, Eph = Ephemeroptera, Crus = Crustacea, Dipt = Diptera larvae, Ann = Annelida, Chip = Chironomidae pupae, Ins = terrestrial insect, oth = others.

Females	Tric	Chir	Zoo	Eph	Crus	Fish	Dipt	Ann	Chip	Ins	Oth
February	**81.3**				**18.8**						
March	**92.8**	0.9	<0.01	0.5	5.8	<0.01		<0.01			<0.01
April	**89.6**	3.5	1.9	0.3	2.5	1.5	<0.01	0.1	0.5		0.1
May	**30.8**	**18.3**	**30.2**	**15.7**	0.4	1.8	0.8	0.1	1.8		<0.01
June	**17.5**	**32.0**	0.3	**49.5**	<0.01		<0.01	0.4	<0.01		0.3
July	**28.6**	**52.8**		0.5	3.6			1.3		**13.2**	
August	**75.9**	**21.5**		1.1	<0.01		<0.01	0.3	0.1	1.1	<0.01
September	7.7	**67.7**	1.2	1.1			**20.6**	0.6	0.1	1.0	
October	1.8	**41.1**	**56.0**	0.1	0.1		0.2	0.7	<0.01	0.1	
November	5.1	**53.9**	**40.3**	<0.01	0.6					<0.01	
December	9.8	1.1	**86.8**		2.4				<0.01		
January	0.3	**11.7**	**87.6**		0.5						
All-year	**51.1**	**24.7**	**15.9**	6.0	1.0	0.4	0.3	0.2	0.4	0.1	<0.01

Males	Tric	Chir	Zoo	Eph	Crus	Fish	Dipt	Ann	Chip	Ins	Oth
February	**44.1**	2.3			**49.5**	3.0		1.2			
March	**95.8**	0.3	<0.01	<0.01	3.8	0.1					
April	**90.4**	3.4	4.8		0.4	0.5	0.1		0.3		<0.01
May	**11.9**	**12.4**	**60.2**	3.1	0.9	8.2	0.2		3.0		<0.01
June	8.8	**79.5**	0.8	1.3		8.3		1.3			
July	**46.9**	**40.0**	0.1	**11.6**	0.3			0.5		0.5	<0.01
August	**70.3**	**14.4**		**13.4**			<0.01	1.1	0.1	0.7	
September	**39.6**	**56.2**	1.3	0.6	0.1				0.7	1.6	
October	3.7	**20.1**	**70.5**	0.6	0.2		1.9	2.2	<0.01	0.7	<0.01
November	2.5	**62.5**	**34.8**	<0.01	<0.01		0.1		<0.01		0.1
December	**27.3**	6.1	**64.7**		1.9				<0.01		
January	0.7	1.1	**96.1**		2.1						
All-year	**27.4**	**31.5**	**37.8**	1.2	0.7	0.2	0.4	0.4	0.1	0.3	<0.01

caution, however, as highest index values ($E = 1$) were often caused by a lack of Crustaceans in the macrozoobenthos sample, and very few examples occurring in fish digestive tracts. Trichoptera larvae were generally strongly preferred (with the exception of Břeclav, where Ephemeroptera were taken preferentially in summer and Diptera in autumn; Figure 5). Chironomidae were taken preferentially at Nové Mlýny and, to a lesser extent, at Bulhary, but were avoided at Břeclav. Index values for Ephemeroptera and Diptera larvae varied greatly and often reached extreme values (–1, +1) due to their low proportion/absence in the diet and/or macrozoobenthos samples.

DISCUSSION

Proterorhinus semilunaris on the River Dyje displayed distinct differences in dietary composition and food consumption based on sex, site and size. In addition, there was a strong seasonal aspect in both amount of food consumed and dietary composition.

Food consumption was low over winter and increased from March to around May, reflecting both rapid replacement of body mass following winter and the need to increase energy

Table IVb
IP values of dietary items found in digestive tracts of <1 year and ⩾1 year old P. semilunaris over 2011/2012 in the River Dyje. Items are shown in descending IP calculated for the whole year (Total). Tric= Trichoptera, Chir = Chironomidae larvae, Zoo = zooplankton, Eph = Ephemeroptera, Crus = Crustacea, Dipt = Diptera larvae, Ann = Annelida, Chip = Chironomidae pupae, Ins = terrestrial insect, oth = others.

<1 year	Tric	Chir	Zoo	Eph	Crus	Fish	Dipt	Ann	Chip	Ins	Oth
February	**65.6**				**34.4**						
March	**62.5**	**12.0**		1.6	**23.7**			0.1			0.1
April	**63.6**	**14.3**	**17.9**	0.7	1.8	0.4	0.4		0.5		0.6
May	5.9	**56.8**	**26.4**	1.7	0.7	3.9	0.1		4.4		<0.01
June	6.8	**77.1**	0.3	**15.0**	<0.01			0.7	<0.01		
July	**26.6**	**55.9**	0.1	**16.9**	0.4			0.1			<0.01
August	**32.9**	**56.9**		8.1	<0.01		<0.01	0.3	0.1	1.7	<0.01
September	2.1	**77.5**	0.1	1.4			**14.6**	1.4		2.7	
October	2.4	**69.5**	**26.1**	0.3	0.2		1.1	0.2	0.1	<0.01	<0.01
November	3.4	**50.7**	**45.9**								
December	0.2	**21.9**	**77.1**		0.8				<0.01		
January	0.5	**18.0**	**75.0**		6.5						
All-year	**18.5**	**63.1**	**12.6**	3.8	0.9	0.1	0.3	0.2	0.3	0.2	<0.01

⩾1 year	Tric	Chir	Zoo	Eph	Crus	Fish	Dipt	Ann	Chip	Ins	Oth
February	**61.5**	1.6			**34.0**	2.1		0.8			
March	**95.5**	0.2	<0.01	0.2	4.0	0.1					
April	**92.4**	2.3	1.1	0.1	2.1	1.4	<0.01	0.1	0.4		<0.01
May	**31.1**	**13.2**	**33.4**	**17.2**	0.4	1.9	0.8	0.1	1.7		<0.01
June	**19.4**	**24.6**	0.3	**54.8**	<0.01	0.2	<0.01	0.2			0.4
July	**67.4**	8.7			1.3			3.9		**18.7**	
August	**93.8**	4.6		0.6	<0.01			0.5	0.1	0.5	<0.01
September	**32.7**	**61.1**	1.6	0.7	<0.01		2.0	<0.01	0.5	1.2	
October	2.9	**18.1**	**74.9**	0.4	0.1		0.8	2.2	<0.01	0.5	
November	3.5	**60.2**	**35.9**	<0.01	0.2		<0.01		<0.01	<0.01	<0.01
December	**27.1**	1.2	**69.2**		2.4				<0.01		
January	0.5	1.2	**98.0**		0.3						
All-year	**48.1**	**19.5**	**25.9**	4.1	0.9	0.4	0.3	0.3	0.3	0.1	<0.01

reserves prior to spawning. Throughout the following period (roughly May to October) variation in both *IF* and the proportion of fish with full digestive tracts varied, mainly as a result of temporal changes in available prey. Indeed, the majority of available prey over this period consisted of temporal benthic fauna (*e.g.* Ephemeroptera larvae), known for their intermittent occurrence (due to collective hatching) and high variability, even on a daily basis (Kosnicki and Sites, 2011; Kukula, 1997). There was a distinct drop in food consumption over winter at all sites; attributable to a lack of available prey. The decrease in food consumption over winter may also be associated with changes in *P. semilunaris* metabolism as the mean water temperature dropped to 1.0–3.5 °C at this time. In *N. melanostomus*, Skazkina (1972) observed greatly reduced metabolic rates at temperatures of 0.5–3.5 °C, resulting in lower thermostability and disintegration of digestive enzymes (Kanduk, 1967). Indeed, some fish species actually cease activity and feeding at such low temperatures (Banarescu and Paepke, 2001).

Diet also changed temporally, with *P. semilunaris* responding to seasonal changes in availability of particular prey classes. EPT taxa were important in the diet from February to August, while chironomid larvae (mostly *Chironomus*, *Tanytarsus* and *Orthocladius* in autumn) and zooplankton (dominant over winter and in October) were consumed most from September onwards, when EPT availability were limited. The order of preference in prey taxa

Table IVc
IP values of dietary items found in digestive tracts of P. semilunaris over 2011/2012 at the three study sites on the River Dyje. Items are shown in descending IP calculated for the whole year (Total). Figures in bold indicate dominant dietary items (>10%). Tric= Trichoptera, Chir = Chironomidae larvae, Zoo = zooplankton, Eph = Ephemeroptera, Crus = Crustacea, Dipt = Diptera larvae, Ann = Annelida, Chip = Chironomidae pupae, Ins = terrestrial insect, oth = others.

Břeclav	Tric	Chir	Zoo	Eph	Crus	Fish	Dipt	Ann	Chip	Ins	Oth
February											
March	**80.3**	0.9		3.9	**15.0**	0.1					
April	**93.0**	0.6	0.6	1.5	1.3	1.6		0.3	0.5		0.6
May	**37.3**	1.2	**21.8**	**35.5**	0.3		3.4	0.4	0.1		<0.01
June	<0.01	1.8		**97.0**				0.5			0.7
July	1.1	**11.4**		**86.4**	1.1					0.1	
August	**25.2**	**10.9**		**53.4**				**10.3**		0.3	
September		**25.8**					**74.2**				
October	<0.01	**22.1**	**29.8**	2.4	1.7		**33.5**	**10.2**	0.1	0.1	<0.01
November	9.3	6.3	**82.8**		1.5						
December		1.6	**98.2**						0.2		
January			**100.0**								
All-year	**37.8**	5.5	**19.5**	**30.8**	1.1	0.1	3.7	1.2	0.1	<0.01	0.2
Bulhary	**Tric**	**Chir**	**Zoo**	**Eph**	**Crus**	**Fish**	**Dipt**	**Ann**	**Chip**	**Ins**	**Oth**
February											
March	**94.8**	0.4			4.7	<0.01					
April	**73.8**	5.9	1.7		**14.7**	2.8			1.1		
May	**26.5**	**36.1**	**23.5**	8.1	1.0				4.8		
June	**73.0**	**15.9**	7.4	0.4	0.3	2.8	0.1		<0.01		
July	**81.7**	**16.6**		0.3	0.6			0.8			
August	**89.1**	8.5		0.6	<0.01		<0.01	0.2	<0.01	1.6	<0.01
September	**55.1**	**34.0**	4.3	2.3	0.1			0.3	0.5	3.4	
October	3.3	3.8	**90.9**	<0.01	<0.01			1.3		0.6	
November	0.7	**62.4**	**36.9**	<0.01	<0.01					<0.01	
December	**56.3**	0.7	**40.9**		2.2						
January		0.8	**98.9**		0.2						
All-year	**48.1**	**20.8**	**28.8**	0.6	0.9	0.1	<0.01	0.2	0.2	0.4	<0.01
Nové Mlýny	**Tric**	**Chir**	**Zoo**	**Eph**	**Crus**	**Fish**	**Dipt**	**Ann**	**Chip**	**Ins**	**Oth**
February	**64.9**	0.6			**33.3**	0.8		0.3			
March	**96.1**	1.1	0.1	0.2	2.3	0.1		<0.01			<0.01
April	**88.8**	5.4	5.1	<0.01	0.1	0.3	0.1	<0.01	0.2		<0.01
May	7.7	**33.1**	**39.3**	0.1	0.3	**15.8**			3.5		<0.01
June	**18.8**	**80.8**		0.2	<0.01			0.2			
July	**45.1**	**51.1**	0.2	0.1	0.3			0.8		2.5	<0.01
August	**49.7**	**49.1**		0.5	0.1		<0.01		0.2	0.3	0.1
September	5.1	**94.2**		<0.01			0.1		0.3	0.2	
October	3.3	**63.4**	**32.7**	0.2	<0.01			0.2	<0.01	0.2	
November	6.9	**76.9**	**15.5**	<0.01	0.1		0.3		<0.01		0.2
December	6.9	8.6	**80.1**		4.3						
January	**18.1**	**58.7**	5.2		**18.1**						
All-year	**29.1**	**52.7**	**15.9**	0.2	0.5	1.1	<0.01	<0.01	0.4	0.1	<0.01

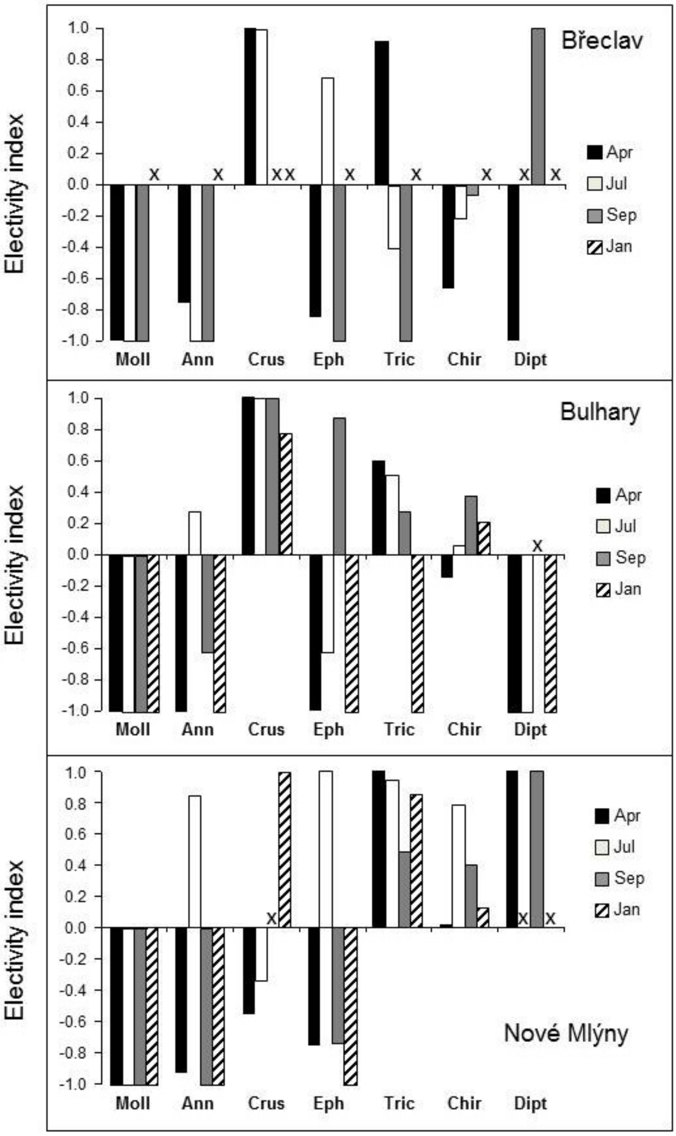


Figure 5
Ivlev's electivity index (E) for P. semilunaris dietary items during winter, spring, summer and autumn of 2011/2012 at the three study sites on the River Dyje. Positive values show increasing preference, negative values indicate increasing avoidance of the diet item. Crosses identify samples/items for which E could not be calculated (null proportion both in diet and benthos sample). Moll = Mollusca, annel = Annelida, crus = Crustacea, ephe = Ephemeroptera, chir = Chironomidae larvae, dipt = Diptera larvae (without Chironomidae).

(*i.e.* EPT > Chironomidae > zooplankton) is most likely based on food quality (*i.e.* energy return), while shifts in dominance are likely to be related to changes in availability (*e.g.* abundance) of the more preferred item(s). In May, there appears to be a general broadening of the diet spectrum, in opposition to the general trend of increased specialisation over spring-summer, suggesting that the diet becomes more opportunistic at the start of the spawning season, when energy costs are high and 'mobility' reduced.

In general, there was no significant difference in the prey types consumed by males and females, the main differences in *IP* being attributable to consumption of minor items and slight differences in when major items were consumed (see Table IVa). Female *IF*, however, was significantly higher than that for males throughout March–May, which probably reflects the greater need for females to increase energy reserves prior to spawning. Males also consumed significantly less prey than females from May to June. Indeed, a significant number of males ($>50\%$) had no food at all in their digestive tracts in June. In Central Europe, May and June are considered the breeding season for *P. semilunaris* (see Ladich and Kratochvil, 1989) and, while there is no information available on sex-specific behaviour for *P. semilunaris* during the breeding season (or, indeed, other aspects of their biology), some clues may be deduced from studies on the related *N. melanostomus*, which have described male gobies as guarding the nest after egg laying (Japoshvili *et al*., 2012; Wickett and Corkum, 1998). Early studies suggested that nest guarding resulted in males ceasing to feed altogether (Kostyuchenko, 1961, 1970); however, it is more likely that food intake is greatly reduced at this time (depending on local conditions) rather than stopped as some nest-guarding males have been observed consuming prey (Charlebois *et al*., 1997; Všetičková, unpublished data). We assume, therefore, that *P. semilunaris* males also guard nests during the May-June breeding season, as previously suggested by Adámek *et al*. (2010), and that this also explains the significant reduction in food consumption by males over this period in this study.

The significant and steep decrease in female feeding over winter (compared to males) is harder to explain but may be related to poor female condition following the spawning period. This steep decline in feeding corresponds to a decline in the number of 2 year females caught in spring at these sites (Jurajda, unpublished data), suggesting high female mortality over winter.

In general, Trichoptera, chironomid larvae and zooplankton were the major dietary items in the diet of both <1 year and $\geqslant 1$ year fish. The lack of major dietary shift in *P. semilunaris* ontogenesis contradicts the significant ontogenetic dietary shifts observed in related Ponto-Caspian gobiid species (*e.g.* Brandner *et al*., 2013; French and Jude, 2001). All of these studies, however, examined much larger species (*e.g. N. melanostomus*) and mostly documented shifts to large and relatively unpalatable prey items (*i.e.* a large proportion of indigestible matter) such as molluscs (mainly *Dreissena polymorpha*) in larger and older fish. The short life-span of *P. semilunaris* (maximum of two years; Jurajda, unpublished data) and its small size (the vast majority of $\geqslant 1$ year fish were smaller than 6 $\mathrm{cm \cdot SL^{-1}}$) preclude such major dietary shifts. Indeed, unlike *N. melanostomus*, for whom molluscs are frequently reported as a dietary item (Ghedotti *et al*., 1995; Taraborelli *et al*., 2010), molluscs (mostly *D. polymorpha*, *Bithynia tentaculata*, *Lymnaea* sp. and *Pisidium* sp.) were actively avoided in this study, despite being abundant at all times at each site. Adámek *et al*. (2010) suggested that this was in part due to their hard, indigestible shell and their strong attachment to the rocks; however, it is just as likely that most molluscs are simply too big for the average *P. semilunaris* to consume with its small gape size. In this respect, therefore, *P. semilunaris* show lower diet plasticity in winter than *N. melanostomus*, putting them at a competitive disadvantage.

Overall, the most obvious difference between the two age groups was in increased consumption of smaller items by <1 year fish, reflected particularly in their increased intake of chironomids. Not only did <1 year fish concentrate on chironomids over most of the year, they also switched to a chironomid-dominated diet earlier than $\geqslant 1$ year fish (*i.e.* <1 in May and $\geqslant 1$ year in September). Even within the chironomid taxon, <1 year fish displayed a preference for smaller prey, taking the smaller *Tribus tanytarsini* more often. Likewise, smaller Trichoptera larvae were taken in preference by <1 year fish as documented by greatly reduced Trichoptera consumption from May onward when larger (older) Trichoptera dominate (Trichoptera remained dominant in $\geqslant 1$ year fish until August). Studies dealing with <1 year *P. semilunaris* are rare; however, Vašek *et al*. (2011) also found small-sized items (mostly zooplankton) prevailing in the digestive tracts of <1 year fish. Based on the observed differences, we suggest that difference in gape size is responsible for the fine dietary shifts observed during *P. semilunaris* ontogeny.

Inter-site differences in diet composition were largely related to differing prey availability at the three sites. In general, Břeclav provided the most diverse range of prey, resulting in higher consumption of Ephemeroptera in spring and summer and more (non-chironomid) Diptera in the autumn. The sharp peak in Diptera consumption at Břeclav was probably connected with a short period of high larval abundance, suggesting that conditions were more suitable for Diptera at this site.

The larger numbers of Ephemeroptera larvae (*Caenis* sp., *Baetis* sp., *Potamanthus luteus*) consumed (and present) at Břeclav are likely to be connected with site morphology as this site had a greater microhabitat variety, including the deeper, faster-flowing habitats preferred by oxygen-demanding Ephemeroptera. Gobies from Nové Mlýny, on the other hand, consumed more chironomid larvae than those at the other two sites and over a longer period, reflecting a relative lack of EPT taxa at this site and the high abundance of mussels. The relatively low availability of preferred food types at Nové Mlýny (availability dominated by Molluscs and annelids, both of which were actively avoided as prey) was also reflected in significantly lower *IF* values at Nové Mlýny for much of the year.

Adámek *et al*. (2010) also found that chironomids dominated the diet in the Nové Mlýny reservoir, reflecting their dominance in the macrozoobenthos assemblage. As the outlet from the reservoir is underwater, it is possible that macrozoobenthos at the Nové Mlýny sample site more closely resembles that of the reservoir than downstream sites. The proximity of the reservoir also helps explain both the higher abundance of *P. semilunaris* found at Nové Mlýny (approximately 2x that downstream) and differences in fish prey taken. The increased occurrence of fish larvae in the diet, for example, results from feeding on the large numbers of *P. semilunaris* fry drifting out of the reservoir outlet (Janáč *et al*., 2013) – the large numbers of fry also contributing to higher overall *P. semilunaris* abundance. High fish abundance will also lead to increased intra-specific food competition, possibly reflected in increased egg predation. As all fish eggs and fry consumed were of *P. semilunaris*, and no predation on native fish eggs and/or fry was observed, there appears to be no impact on native fish fauna through direct predation. Such opportunistic egg cannibalism (see also Vašek *et al*., 2014 and Adámek *et al*., 2010) has also been observed in *N. melanostomus* under laboratory conditions (Meunier *et al*., 2009), where it was connected with males abandoning nests. The fact that egg and fry consumption at Nové Mlýny occurs primarily in May tends to confirm that the behaviour is connected primarily with *P. semilunaris* breeding behaviour and is not a general behaviour pattern.

This study confirms that *P. semilunaris* are feeding opportunists, consuming prey (especially EPT, chironomids and zooplankton) in relation to their relative availability (abundance) and that some taxa, such as Mollusca, are avoided, despite high availability, due to inappropriate size and indigestibility. Preference for individual food types changed based on local and seasonal changes in relative abundance. Such diet plasticity has allowed *P. semilunaris* to successfully colonise even those habitats with poor diet availability, which has probably contributed to its successful range expansion (see Roche *et al*., 2013). In addition, we were able to demonstrate differences in diet related to both sex and age, with inter-sex differences most likely related to breeding behaviour and energy expenditure over the breeding season, and age-related differences most likely related to gape-size.

Whereas any direct effect of *P. semilunaris* on the native ecosystem cannot be assessed from our relatively short-term study, we assume that its generalist feeding style results in equal pressure on all prey types, resulting in little threat to native invertebrate diversity. Further, predation pressure on native fish species through consumption of eggs and fry appears to be negligible. The preference shown by *P. semilunaris* for small prey items, however, suggests the potential for dietary competition with small and juvenile native species that specialise on small crustaceans, oligochaetes, chironomids and other such insect larvae (Garner, 1996; Nunn *et al*., 2008).

ACKNOWLEDGEMENTS

This study was supported by the Grant Agency of the Czech Republic, Grant No. P505/11/1768. We thank M. Konečná and Z. Valová for help with fieldwork. We are much indebted to representatives of the Moravian Angling Union and their Local Clubs (Rakvice, Lednice and Břeclav) for allowing this research in their waters.

REFERENCES

Adámek Z., Andreji J. and Gallardo J., 2007. Food habits of four bottom-dwelling gobiid species at the confluence of the Danube and Hron Rivers (South Slovakia). *Int. Rev. Hydrobiol.*, *92*, 554–563.

Adámek Z., Jurajda P., Prášek V. and Sukop I., 2010. Seasonal diet pattern of non-native tubenose goby (*Proterorhinus semilunaris*) in a lowland reservoir (Mušov, Czech Republic). *Knowl. Manag. Aquat. Ecosyst.*, *397*, 1–12.

Anderson M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecol*., *26*, 32–46.

Andriyashev A.P. and Arnoldi L.V., 1945. Concerning the feeding of several bottom fishes from the Black Sea. *Zh. Obtshei Biol*., *6*, 53–61 [in Russian].

Banarescu P.M. and Paepke H.J., 2001. The Freshwater Fishes of Europe, Cyprinidae 2, Part III: *Carassius* to *Cyprinus*, Gasterosteidae. AULA-Verlag GmbH Wiebelsheim, Verlag für Wissenschaft und Forschung.

Borcherding J., Dolina M., Heermann L., Knutzen P., Krüger S., Matern S., van Treeck R. and Gertzen S., 2013. Feeding and niche differentiation in three invasive gobies in the Lower Rhine, Germany. *Limnologica*, *43*, 49–58.

Borza P., Erös T. and Oertel N., 2009. Food resource partitioning between two invasive gobiid species (Pisces, Gobiidae) in the littoral zone of the River Danube, Hungary. *Int. Rev. Hydrobiol*., *94*, 609−621.

Brandner J., Auerswald K., Cerwenka A.F., Schlieven U.K. and Geist J., 2013. Comparative feeding ecology of invasive Ponto-Caspian gobies. *Hydrobiologia*, *703*, 113–131.

Burnashev M.S., Chepurnov V.S. and Rakitina N.P., 1955. Fishes of the Dubossarskoye Reservoir and questions of fishing development. In it. *Uch. Zap. Kishinevsk. Gos. Univ*., *20*, 3–31 [in Russian].

Charlebois P.M., Marsden J.E., Goettel R.G., Wolfe R.K., Jude D.J. and Rudnika S., 1997. The Round Goby, *Neogobius melanostomus* (Pallas), a review of European and North American literature. Illinois Natural History Survey Special Publication No. 20, 76 p.

French J.R.P. and Jude D.J., 2001. Diets and diet overlap of nonindigenous gobies and small benthic native fishes co-inhabiting the St. Clair River, Michigan. *J. Great Lakes Res*., *27*, 300–311.

Garner P., 1996. Microhabitat use and diet of 0+ cyprinid fishes in a lentic, regulated reach of the River Great Ouse, England. *J. Fish Biol*., *48*, 367–382.

Ghedotti M.J., Smihula J.C. and Smith G.R., 1995. Zebra mussel predation by round gobies in the laboratory. *J. Great Lakes Res*., *21*, 665–669.

Grinbart S.B., 1960. Towards the study of the feeding of fishes in Grigoryevsky lagoon. Odessk. *Gos. Univ. Biol. Fak*., *2*, 167–172 [in Russian].

Hyslop E.J., 1980. Stomach content analysis-a review of methods and their application. *J. Fish Biol*., *17*, 411–429.

Janáč M., Valová Z. and Jurajda P., 2012. Range expansion and habitat preferences of nonnative 0+ tubenose goby (*Proterorhinus semilunaris*) in two lowland rivers in the Danube basin. *Fund. Appl. Limnol*. / *Arch. Hydrobiol*., *181*, 73–85.

Janáč M., Jurajda P., Kružíková L., Roche K. and Prášek V., 2013. Reservoir to river passage of age – 0+ year fishes, indication of a dispersion pathway for a non-native species. *J. Fish Biol*., *82*, 994−1010.

Japoshvili B., Lehtonen T.K., Wong B.B.M. and Lindstrom K., 2012. Repeatability of nest size choice and nest building in sand gobies. *Anim. Behav*., *84*, 913–917.

Kanduk R.P., 1967. Comparative evaluation of the activity and thermostability of digestive enzymes of some fish of the northwestern part of the Black Sea. *In*: Kovalevskii A.O. (ed.), Biochemistry of Marine Organisms: 122–133. Inst. Biol. Southern Seas, Akad. Nauk Ukrain. SSR, Kiev [in Russian].

K'neva-Abadzhiyeva B. and Marinov T., 1963. Feeding of several species of the fam. Gobiidae (Pisces) from the Bulgarian Black Sea coast. *Izv. Centr. Nauchn. Issled. Inst. Rybov., Rybol.*, *3*, 149–172 [in Bulgarian].

Kokeš J. and Němejcová D., 2006. Methodology of running waters macrozoobenthos sampling and sample processing using the Perla method. VÚV TGM, Prague.

Kosnicki E. and Sites R.W., 2011. Seasonal predictability of benthic macroinvertebrate metrics and community structure with maturity-weighted abundances in a Missouri Ozark stream, USA. *Ecol. Indic*., *11*, 704–714.

Kostyuchenko V.A., 1961. Age and growth of the round goby *N. melanostomus* (Pallas) in the Azov Sea. *Tr. Azov.-Chernom. Nauchn.-Issled. Inst. Rybn. Khoz. Okeanogr.*, *19*, 45–60 [in Russian].

Kostyuchenko V.A., 1970. On the regulation of the Gobiidae fishery in the Azov Sea. *Tr. Azov.-Chernom. Nauchn.-Issled. Inst. Rybn. Khoz. Okeanogr.*, *71*, 51–67 [in Russian].

Kukula K., 1997. The life cycles of three species of Ephemeroptera in two streams in Poland. *Hydrobiologia*, *353*, 193–198.

Ladich F. and Kratochvil H., 1989. Sound production by the marmoreal goby *Proterorhinus marmoratus* (Pallas) (Gobiidae, Teleostei). *Zoologische Jahrbucher – Abteilung Fur Allgemeine Zoologie Physiologie der Tiere 93*, 501–504.

Lusk S. and Halačka K., 1995. The first finding of the tubenose goby, *Proterorhinus marmoratus* in the Czech Republic. *Folia Zool*., *44*, 90–92.

Meunier B., Yavno S., Ahmed S. and Corkum L.D., 2009. First documentation of spawning and nest guarding in the laboratory by the invasive fish, the round goby (*Neogobius melanostomus*). *J. Great Lakes Res*., *35*, 608–612.

Natarajan A.V. and Jhingran A.G., 1961. Index of preponderance – a method of grading the food elements in the stomach analysis of fishes. *Indian J. Fish*., *8*, 54–59.

Nunn A.D., Harvey J.P. and Cowx I.G., 2008. Winter diet and condition of two 0+ year cyprinid fish species in the lower River Trent, England. *J. Fish Biol*., *72*, 1529–1533.

Oksanen J., Blanchet F.G., Kindt R., Legendre P., Minchin P.R., O'Hara R.B., Simpson G.L., Solymos P., Stevens M.H.H. and Wagner H., 2012. vegan: Community Ecology Package. R package version 2.0-3. Available online at: http://CRAN.R-project.org/package=vegan (accessed June 25th 2013).

Prášek V. and Jurajda P., 2005. Expansion of *Proterorhinus marmoratus* in the Morava River basin (Czech Republic, Danube R. watershed). *Folia Zool*., *54*, 189–192.

R Development Core Team., 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, http://www.R-project.org/.

Roche K.F., Janač M. and Jurajda P., 2013. A review of Gobiid expansion along the Danube-Rhine corridor – Geopolitical change as a driver for invasion. *Knowl. Manag. Aquat. Ecosyst*., *411*, 1–23.

Shorygin A.A., 1939. Food and food preference of some Gobiidae of the Caspian Sea. *Zool. Zh*., *18*, 27–53.

Simberloff D. and Von Holle B., 1999. Positive interactions of nonindigenous species: invasional meltdown? *Biol. Invasions*, *1*, 21–32.

Skazkina E.P., 1972. On active metabolism in Azov goby. *Tr. Azov.-Chernom. Nauchn.-Issled. Inst. Rybn. Khoz. Okeanogr.*, *85*, 138–144 [in Russian].

Taraborelli A.C., Fox M.G., Johnson T.B. and Schaner T., 2010. Round goby (*Neogobius melanostomus*) population structure, biomass, prey consumption and mortality from predation in the Bay of Quentine, Lake Ontario. *J. Great Lakes Res*., *36*, 625–632.

Vašek M. and Kubečka J., 2004. In situ diel patterns of zooplankton consumption by subadult/adult roach *Rutilus rutilus*, bream *Abramis brama*, and bleak *Alburnus alburnus*. *Folia Zool*., *53*, 203−214.

Vašek M., Jůza T., Čech M., Kratochvíl M., Prchalová M., Frouzová J., Říha M., Tušer M., Seía J. and Kubečka J., 2011. The occurrence of non-native tubenose goby *Proterorhinus semilunaris* in the pelagic 0+ year fish assemblage of a central European reservoir. *J. Fish Biol*., *78*, 953–961.

Vašek M., Všetičková L., Roche K. and Jurajda P., 2014. Diet of two invading gobiid species (*Proterorhinus semilunaris* and *Neogobius melanostomus*) during the breeding and hatching season: no evidence of extensive predation on fish eggs and fry. *Limnologica*, *46*, 31–36.

Wickett R.G. and Corkum L.D., 1998. Nest defence by the non-indigenous fish, the round goby, *Neogobius melanostomus* (Gobiidae), on a shipwreck in western Lake Erie. *Can. Field-Naturalist*, *112*, 653–656.