

Research Article

Population and reproductive characteristics of a non-native western tubenose goby (*Proterorhinus semilunaris*) population unaffected by gobiid competitors

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Abstract

The western tubenose goby (*Proterorhinus semilunaris*) is one of several Ponto-Caspian gobiid species currently extending their distribution range over Europe. As part of an ongoing study, we examined the annual dynamics of an established tubenose goby population from the River Dyje (Czech Republic, Danube basin), 13 years after its first occurrence. Monthly monitoring (February 2011–January 2012) confirmed that the species dominates the local fish assemblage (more than two thirds of fish caught) and is the only species captured every month. The population is female-dominated from March–August and displays a 1:1 sex-ratio from September on. Tubenose gobies in the Dyje are characterised by rapid reproduction and early maturation, with all 0+ fish ready to spawn at the start of the next spawning season. Females display significantly higher GSI but lower body condition and a shorter life-span than males. Female life-span was almost strictly annual, while males were more likely to survive to a second spawning season. This study is unique in presenting results for a tubenose goby population with long-term absence of other potentially competing gobiid species. These results provide new baseline data for future tubenose goby studies in areas both with and without competitors.

Key words: Gobiidae, invasive species, non-indigenous, tubenose goby, population dynamics

Introduction

The western tubenose goby (hereafter tubenose goby) *Proterorhinus semilunaris* (Heckel, 1837) is a small, benthic, Ponto-Caspian fish species of the family Gobiidae (Pinchuk et al. 2003), native to the Black Sea basin. In recent decades, this and other gobiid species have invaded new regions, their expansion probably facilitated and accelerated by transport in ballast water and possible transport of eggs on ship's hulls, as well as accidental stocking with other fish species and release of bait fish (Lusk and Hanel 1996; Roche et al. 2013). They have been particularly successful in colonising rip-rap structures along inland waterways (Ahnelt et al. 1998). First evidence of spreading beyond its native range was observed in 1965, when it began moving upstream along the Austrian Danube (Ahnelt et al. 1988).

Following completion of the Rhine–Main–Danube canal in 1992, the species was able to disperse into the lower (1999) and middle (2000) Rhine and the River Main (Manné et al. 2013), once

again most likely by ship. In addition to its expansion in Europe (e.g. Borchering et al. 2011; Cammaerts et al. 2012; Manné et al. 2013; Roche et al. 2013) the tubenose goby has also been introduced into the Laurentian Great Lakes (Ricciardi and MacIsaac 2000; Kocovsky et al. 2011; Grant et al. 2012).

The tubenose goby was first recorded in the Czech Republic in 1994 in the upper reservoir of the Nové Mlýny reservoir system (r. km 58, River Dyje, Danube basin; Figure 1), where its distribution was limited to about 500 m of shoreline (Lusk and Halačka 1995). The most probable origin of these gobies appears to be from baitfish released by foreign anglers (Lusk and Hanel 1996). By 1998, the species was found throughout the reservoir system and the first individuals had been recorded in the River Dyje as far away as its confluence with the River Morava (Prášek and Jurajda 2005). Later the same year, the first specimens appeared in the River Morava, upstream of the Dyje confluence (r. km 74; Prášek and Jurajda 2005). In the 17 years since its first record in the

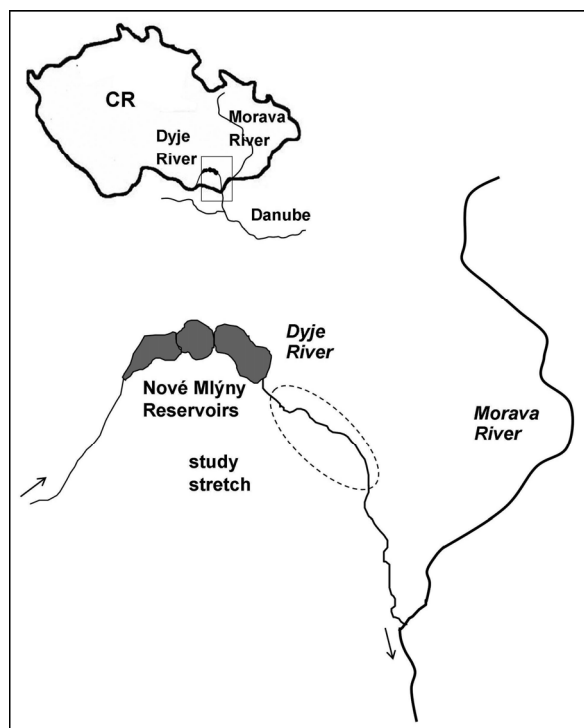


Figure 1. Map of the River Dyje system, with the study stretch and important features highlighted.

reservoir, the tubenose goby has colonised at least 117.7 km of the Dyje and 45.4 km of the Morava (Janáč et al. 2012; Kopeček 2013). As both the Dyje and Morava are non-navigable rivers (i.e. no ships/boats with ballast water use the rivers), the further spreading of tubenose goby has been through natural upstream expansion or by larval and juvenile drift (Janáč et al. 2012; Janáč et al. 2013a, b).

At least within a European context, the tubenose goby can be considered a pioneer species as its range expansion took place earlier than other Ponto-Caspian gobiids and, therefore, it has usually been first to colonise new areas (Roche et al. 2013). Moreover, it appears to be more successful at colonising smaller rivers (Kopeček 2013). As such, there is concern about the species' potential impact on native species and ecosystem function, particularly as regards making invaded areas more vulnerable to expansion of other gobiid species (e.g. through outcompeting native competitors or other ecosystem disturbances; see the “invasional meltdown hypothesis” of Simberloff and Von Holle 1999). Despite this concern, relatively little is known about tubenose goby life-history traits, the attention of fish biologists tending to be directed more toward larger, “more attractive”

gobiid species such as the round goby *Neogobius melanostomus* (Pallas, 1814) (Kornis et al. 2012). The lack of information on their impact on local biota has been exacerbated by the species' low density and patchy distribution over much of its newly colonised area (Freyhof 2003; Naseka et al. 2005; Dopazo et al. 2008; Manné et al. 2013).

In recent years, a number of studies have helped remedy this gap in knowledge, including those of Adámek et al. (2010) and Vašek et al. (2014) on diet, Van Kessel et al. (2011) and Janáč et al. (2012) on habitat preference, Dopazo et al. (2008) and Gaygusuz et al. (2010) on diel activity and Janáč et al. (2013a,b) on propagule dispersal patterns. Detailed and comparative information on reproduction mode, sex ratio, size structure and condition are still lacking, however, with just fragmentary data available from earlier studies (e.g. Georghiev 1966; Ragimov 1986; Harka and Farkas 2006).

To address this, we evaluated a number of fundamental population and reproductive characteristics for tubenose goby, focusing on seasonal variation in population abundance, size structure, sex-ratio, condition and reproductive characteristics.

Materials and methods

Fish sampling was conducted monthly from February 2011 to January 2012 along the river Dyje (Danube basin, Czech Republic) between Břeclav (48°44'23.859"N, 16°53'27.509"E; r. km 23.5) and the Nové Mlýny Reservoir (48°51'27.200"N, 16°43'30.701"E; r. km 45.9; Figure 1). The river banks throughout this stretch have been modified and are stabilised with 10–100 cm stony rip-rap. Aquatic vegetation is absent and submerged vegetation only present during times of higher discharge.

Fish were caught during the day along the river bank (depth not exceeding 80 cm) using single pass continual electrofishing (SEN battery-powered backpack electrofishing gear; Bednář, Czech Republic), with 300 m of shoreline generally being sampled. Sampling was spread over the whole study stretch, with at least 50 m sampled in the downstream, middle and upstream sections. All tubenose gobies sampled were immediately killed with an overdose of clove oil and preserved in 4% formaldehyde for further analysis in the laboratory. Other fish species were identified, measured and released back to the water. Fish abundance was expressed as catch-per-unit-effort (CPUE), i.e. the number of fish captured per 10 m of shoreline surveyed.

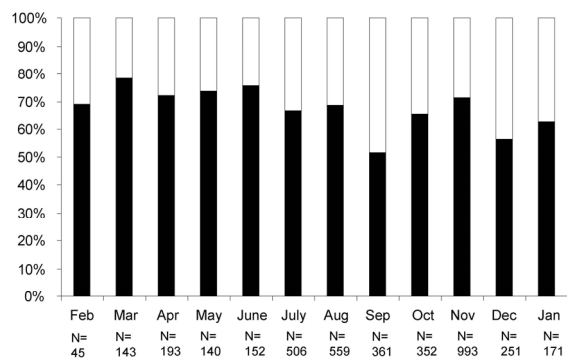


Figure 2. The relative proportion of tubenose goby (black bars) and other species combined (white bars) in the fish assemblage at the River Dyje study stretch between 2011 and 2012.

In the laboratory, the tubenose gobies were measured to the nearest mm using digital callipers (total length [TL]; standard length [SL]) and weighed to the nearest 0.01 g (total weight [W_T]). The fish were then eviscerated and the carcass and gonads reweighed (eviscerated fish weight [W_{ev}]; gonad weight [W_G]). Sex was determined during fish dissection based on the presence and type of gonads. Fish with absent or indistinguishable gonads were considered as juveniles and those with clearly distinguishable gonads as adults. All adults were considered as “mature” as they were all ready to spawn in the following spawning season (see Results). The proportion of each sex (juveniles excluded) was used to calculate the sex-ratio, and length-frequency distribution was used to calculate population age structure (see Konečná and Jurajda 2012). The relationships between fish SL and (i) proportion of juveniles and adults in the population (assessing the maturity size) and (ii) proportion of females in the adult population (assessing changes in sex ratio with size) were determined using generalised linear models (GLM; binomial distribution).

In order to assess fecundity, we undertook a detailed study of female gonads from the April sample (start of the spawning season). An image of each ovary was taken using a digital camera (uEye-1540C) coupled with a binocular microscope (20 \times , 25 \times and 32 \times zoom; Olympus SZX7) in order to enable precise measurement of oocyte size and assessment of individual fecundity. Individual oocyte developmental stage (primary growth/previtellogenic, cortical alveoli, vitellogenic or mature oocyte) was characterised according to Cinquetti and Rinaldi (1987) and Konečná and Jurajda (2012). All oocytes were measured

(maximum length of ovoid egg shape) and counted using LUCIA 5 image analysis software (Laboratory Imaging Ltd.). For each female with ovaries containing oocytes of at least the cortical alveoli stage, absolute fecundity (F_A) was counted as the total number of oocytes in the ovaries minus previtellogenic (primary growth) oocytes and the relative fecundity (number of oocytes per 1 gram) was assessed using the formula: $F_R = F_A / W_T$. The relationship between F_A and fish length was described using linear regression. For further comparison with fecundity in the literature sources, females were separated into three size categories (28–40 mm, 41–50 mm and > 50 mm) based on their length-frequency distributions.

Changes in body condition were expressed using Clark’s condition coefficient (K_C), expressed as $K_C = (W_{ev}/T_L^3) \times 100,000$ (Clark 1928). Gonad development and maturity was determined using the gonadosomatic index (GSI), expressed as $GSI = W_G/W_T \times 100$ (West 1990). The delimitation of the spawning season was assessed using both GSI values and number of females ready to spawn (containing mature oocytes).

Any difference in sex ratio from 1:1 each month was tested using the Chi-square test (χ^2). Differences in male or female K_C or GSI between months was determined using the Kruskal-Wallis test, while differences between male and female K_C within each month were determined using the Mann-Whitney test. All statistical analyses were conducted using R 2.14.2 (R Foundation for Statistical Computing, Vienna, Austria; R Development Core Team 2012).

Results

1. Abundance and proportion of tubenose gobies in the fish assemblage

A total of 3866 fish, comprising 28 species, was captured along the study stretch over the sampling campaign. Tubenose gobies occurred in all monthly samplings and were the only species with 100% frequency of occurrence. Tubenose gobies were also the dominant species caught each month, comprising 67.1% of the total fish assemblage overall (Table 1) and ranging between 51.3 and 78.3% over the year (Figure 2). Other abundant species included round goby (*Neogobius melanostomus*, 7.8%), zander (*Sander lucioperca*, 6.3%), common bream (*Abramis brama*, 5.9%) and burbot (*Lota lota*, 2.6%). No other fish species exceeded 2% dominance (Table 1). Tubenose goby abundance varied over the year, ranging from 1.6 to 16.9

Table 1. Abundance (CPUE = ind./10 m), dominance (%) and frequency of occurrence (%) of fish species at the River Dyje study site between 2011 and 2012.

Common name	Scientific name	CPUE	Dominance	Frequency
pike	<i>Esox lucius</i> (Linnaeus, 1758)	0.00	0.03	8.3
roach	<i>Rutilus rutilus</i> (Linnaeus, 1758)	0.10	0.90	58.3
dace	<i>Leuciscus leuciscus</i> (Linnaeus, 1758)	0.01	0.08	16.7
chub	<i>Squalius cephalus</i> (Linnaeus, 1758)	0.18	1.65	66.7
ide	<i>Leuciscus idus</i> (Linnaeus, 1758)	0.16	1.47	75.0
rudd	<i>Scardinius erythrophthalmus</i> (Linnaeus, 1758)	0.00	0.03	8.3
asp	<i>Leuciscus aspius</i> (Linnaeus, 1758)	0.06	0.59	33.3
gudgeon	<i>Gobio gobio</i> (Linnaeus, 1758)	0.01	0.13	25.0
whitefin gudgeon	<i>Romanogobio albipinnatus</i> (Lukasch, 1933)	0.01	0.10	16.7
topmouth gudgeon	<i>Pseudorasbora parva</i> (Temminck&Schlegel, 1846)	0.02	0.16	33.3
barbel	<i>Barbus barbus</i> (Linnaeus, 1758)	0.07	0.65	41.7
bleak	<i>Alburnus alburnus</i> (Linnaeus, 1758)	0.03	0.28	33.3
white bream	<i>Blicca bjoerkna</i> (Linnaeus, 1758)	0.07	0.67	16.7
common bream	<i>Abramis brama</i> (Linnaeus, 1758)	0.63	5.87	66.7
bitterling	<i>Rhodeus amarus</i> (Bloch, 1782)	0.04	0.34	41.7
Prussian carp	<i>Carassius gibelio</i> (Bloch, 1782)	0.03	0.26	58.3
carp	<i>Cyprinus carpio</i> (Linnaeus, 1758)	0.02	0.21	16.7
spined loach	<i>Cobitis</i> sp.	0.01	0.05	16.7
wells	<i>Silurus glanis</i> (Linnaeus, 1758)	0.04	0.34	58.3
eel	<i>Anguilla anguilla</i> (Linnaeus, 1758)	0.01	0.08	16.7
burbot	<i>Lota lota</i> (Linnaeus, 1758)	0.28	2.61	91.7
perch	<i>Perca fluviatilis</i> (Linnaeus, 1758)	0.20	1.86	91.7
zander	<i>Sander lucioperca</i> (Linnaeus, 1758)	0.68	6.33	50.0
ruffe	<i>Gymnocephalus cernuus</i> (Linnaeus, 1758)	0.04	0.36	50.0
Danube ruffe	<i>Gymnocephalus baloni</i> (Holcik&Hensel, 1974)	0.01	0.08	8.3
zingel	<i>Zingel zingel</i> (Linnaeus, 1766)	0.00	0.03	8.3
tubenose goby	<i>Proterorhinus semilunaris</i> (Heckel, 1837)	7.18	67.06	100.0
round goby	<i>Neogobius melanostomus</i> (Pallas, 1814)	0.83	7.78	83.3

Table 2. Number of tubenose gobies from the River Dyje study site used for analysis of GSI and KC.

Month	Male	Female
February	10	15
March	35	77
April	20	63
May	20	72
June	16	38
July	8	5
August	11	48
September	59	56
October	93	102
November	69	69
December	48	59
January	35	47

specimens per 10 m of shoreline (Figure 3). CPUE was lowest in winter, with an increase in April and highest value in July, when high numbers of 0+ juveniles were noted. CPUE decreased slowly from July to November, whereupon values decreased more rapidly (Figure 3).

2. Size structure

The month-to-month distribution of length-frequency peaks (Figure 4) suggests an annual or annual-like life-cycle for tubenose goby. Growth of fish from the 1+ year cohort (hatched in 2010) was measured from February to May 2011, with mean SL increasing from 44.6 to 49.2 mm over this period. The first juveniles of 2011, with a mean SL of 14.8 ± 0.39 mm, were observed in June and these increased in number and size in subsequent collections. Juvenile tubenose gobies

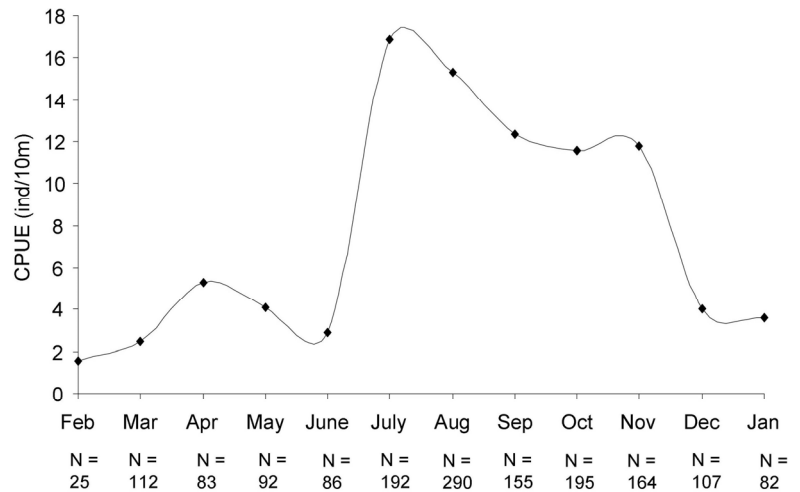


Figure 3. Tubenose goby abundance (catch-per-unit-effort; ind./10m) at the River Dyje study stretch between 2011 and 2012.

displayed rapid growth over summer, reaching a mean size of 34.6 ± 0.43 mm (in August). Individuals from the 1+ cohort were no longer observed in length-frequency distribution graphs from July. Note, however, that some larger male specimens (SL = 75.4 to 84.5 mm) were caught in March, April, May and June (Figure 4), and these probably represent 2+ individuals from the 2009 cohort. While this would indicate a possible exception, the majority of the population do appear to be following an annual life-cycle.

The proportion of adults in the population increased significantly with fish size (GLM; $P < 0.001$), with the model predicting a 50% proportion of the population at SL = 35.1 mm and proportions of 10% and 90% at 27.0 mm and 43.2 mm, respectively (Figure 5).

The proportion of females in the adult population decreased significantly with fish size (GLM; $P < 0.001$), with the model predicting a 50% proportion at SL = 48.9 mm and 90% and 10% proportions at 33.9 mm and 63.7 mm, respectively (Figure 5).

3. Sex ratio

The pooled data indicate a sex-ratio differing significantly from 1:1 (χ^2 test, $df=1$, $P < 0.001$), with females representing 60.2% of the adult population. Overall, the sample was female-dominated from March to August (χ^2 test, all $df=1$, $P < 0.05$), with the exception of July (Figure 6). In February, July and September to December, the sex-ratio did not differ significantly from 1:1 (χ^2 tests, all $df=1$, $P > 0.05$; Figure 6).

4. Condition (K_C)

Both male and female K_C differed significantly over the year (Kruskal-Wallis, both $P < 0.001$). While there was no significant difference between male and female K_C in February and July (Mann-Whitney tests, both $P > 0.05$), females displayed significantly lower K_C than males in all other months (Mann-Whitney tests, all $P < 0.05$; Figure 7). Note, however, that low sample size may have been responsible for the lack of significance in July (Table 2). Overall, males and females show a similar yearly trend in K_C , with peaks in February and August i.e. before and after the spawning season. Whereas male K_C stayed relatively stable at around 1.2 either side of these two peaks, female K_C was lower during spawning (March-June) than during the latter part of the year (September-January).

5. GSI and number of females ready to spawn

In total, 424 males and 651 females were available for analysis of male and female GSI (Table 2). Both male and female GSI differed significantly over the year (Kruskal-Wallis, both $P < 0.001$). Changes in female GSI over the year indicate that the spawning season started in April, when highest GSI values were recorded, and lasted until July, when lowest values were recorded following a rapid decrease. In both males and females, GSI values gradually rose again from August (Figure 8).

Prior to the onset of the spawning season (April), ovaries of all females contained maturing (vitellogenic) eggs. Thus all females were

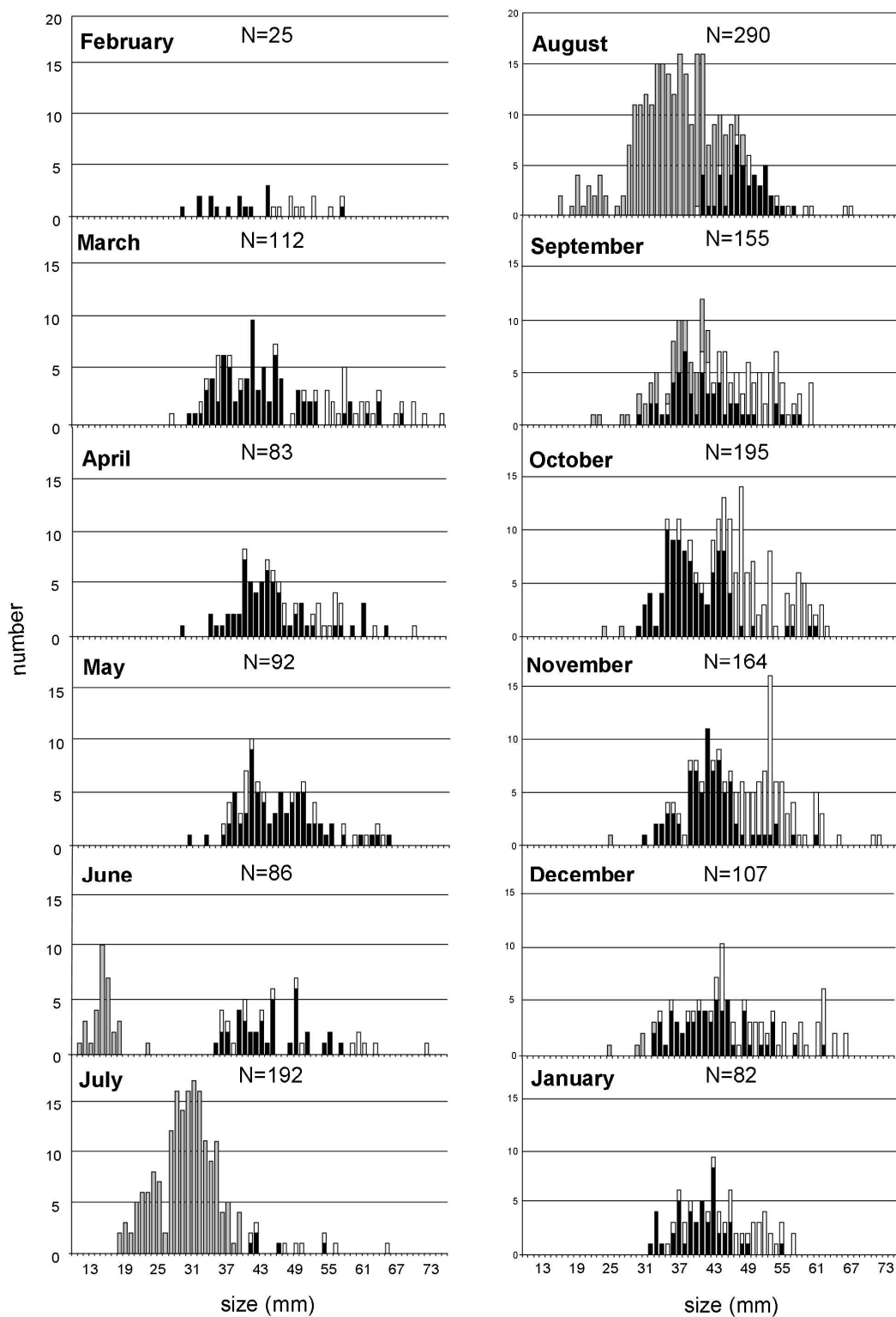


Figure 4. Tubenose goby length-frequency distribution at the River Dyje study stretch between 2011 and 2012 (white bars = male; black bars = female, grey bars = juvenile).

Figure 5. The relationship between fish length and (i) proportion of adults in the population (dashed line) and (ii) females in the adult population (solid line), as predicted by generalised linear models (95% confidence intervals shown). Model formula for adults: $y = 1/(1+e^{(9.501 - 0.271 * S_L)})$, and for females: $y = 1/(1+e^{(-7.199 + 0.147 * S_L)})$. Models are based on 483 juveniles, 663 females and 437 males.

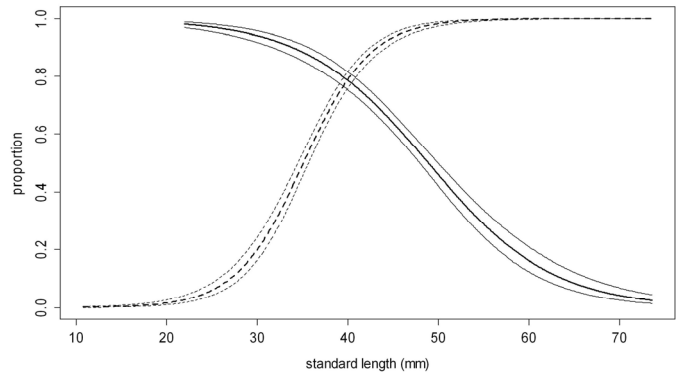


Figure 6. Proportion of females in the River Dyje tubenose goby population between 2011 and 2012. Empty symbols indicate a sex-ratio significantly different from 1:1 (χ^2 test, $p < 0.05$). N is the sum of males and females sampled.

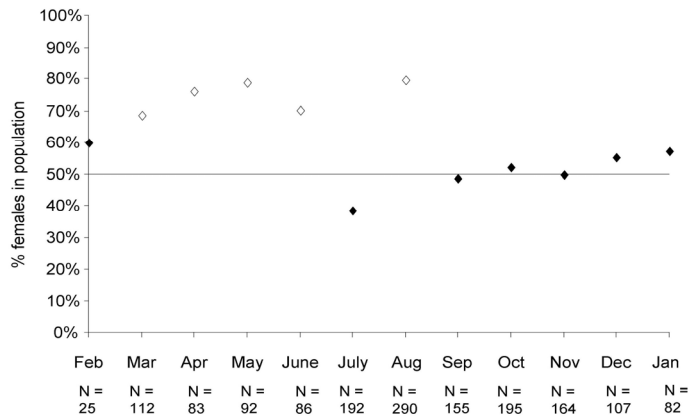


Figure 7. Male (empty circles, solid line) and female (crosses, dashed line) body condition for tubenose gobies at the River Dyje study stretch between 2011 and 2012 (medians (symbols) and interquartile ranges (whiskers) shown). Asterisks denote a significant difference (Mann-Whitney test, $p < 0.05$).

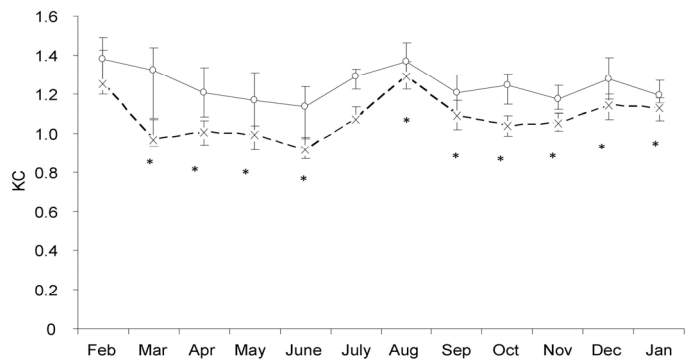


Figure 8. Male (empty circles, solid line) and female (crosses, dashed line) gonadosomatic index for tubenose gobies at the River Dyje study stretch between 2011 and 2012 (medians (symbols) and interquartile ranges (whiskers) shown; y axis = \log_{10} scale).

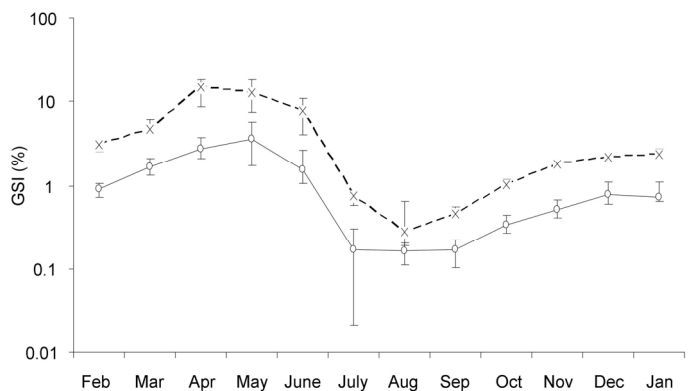


Table 3. Absolute (FA) and relative fecundity (FR) of tubenose gobies from the River Dyje in April 2011.

SL (mm)	Fecundity range	Mean FA	Mean FR
28–40 mm	140–658	379	309
41–50 mm	399–1018	628	296
51–65 mm	340–1349	1024	217

considered as reproducing during the upcoming spawning season. At the beginning of the spawning season, almost 80% of females sampled were ready to spawn immediately; i.e. each had a batch of ripe eggs clearly visible. This proportion decreased slowly (69% in May, 48% in June) until there were no ripe (or vitellogenic) oocytes observed in ovaries (July). Changes in the proportion of females ready to spawn confirm the duration of spawning season as being from April to July.

6. Fecundity

Tubenose goby F_A ranged from 140 to 1349 oocytes, with mean fecundity ranging from 379 to 1024 oocytes over the three size groups examined (Table 3). F_A increased significantly with female length ($df = 1$ and 60 , $P < 0.001$, $R^2 = 0.75$), the linear regression following the formula: $F_A = -796.89 + 32.07 \cdot SL$. Mean F_R ranged from 217 to 310 eggs/g (Table 2).

Discussion

Thirteen years after first being recorded, the tubenose goby has established an abundant population in the River Dyje and now represents an important component of the littoral fish assemblage. Our capture of a wide range of sizes (and hence age groups) indicates an abundant and self-sustaining population (see also Janáč et al. 2012). The tubenose goby is a benthic species that prefers shallow habitats, spawns on the underside of objects such as rocks and utilises small crevices in rocky areas, possibly to avoid predation (Kocovsky et al. 2011). The artificially reinforced stony rip-rap banks along our study stretch, therefore, represent an ideal habitat for the tubenose goby and provide an excellent opportunity for establishing an abundant population.

The dominant position of tubenose goby in the littoral fish assemblage of the Dyje is unique among previous reports on their occurrence, which always report low density, patchy distribution and a relatively low representation in the fish assemblage (Erös et al. 2005; Harka and Bíró 2007; Borcharding et al. 2011; Manné et al.

2013). We suggest that their dominance in the littoral-zone assemblage of the Dyje may be due to a very low abundance of potential competitors in the Dyje. The tubenose goby appears to be an inferior competitor to other Ponto-Caspian gobiid species, which usually dominate the fish assemblage soon after introduction (Erös et al. 2005; Harka and Bíró 2007; Borcharding et al. 2011; Manné et al. 2013). In our case, however, the main potential competitor of the tubenose goby, the round goby, has only recently entered the downstream stretch of the Dyje (Lusk et al. 2010) and, at most, has only a marginal impact on the tubenose goby population.

Due to its apparent inferiority as a competitor to other gobiid species (Jurajda et al. 2005), the tubenose goby tends to be considered the least threatening of potential invasive species. Manné et al. (2013), for example, assessed the tubenose goby as only of medium risk to virgin habitats, compared to the high risk posed by round and bighead gobies, due to its patchy distribution and low density. Tubenose goby populations, however, have only rarely been studied in the absence of other gobiids and our study demonstrates that the species can (numerically) dominate the fish assemblage when competing with native species only (see also Černý et al. 2003; Von Landwüst 2006). As a pioneering species, therefore, the tubenose goby may indeed be affecting local ecosystems (Kocovsky et al. 2011), which brings its relatively minor risk assessment status into question.

Seasonal variation in tubenose goby abundance in the littoral zone was influenced by two factors: high juvenile abundance following the peak in spawning and a decrease in abundance during the winter low temperature period. The population peak in July clearly reflected the recruitment of new young-of-the-year (note that the first influx of young-of-the-year appeared in June). Several mechanisms, however, could be responsible for the winter decrease in abundance, including increased predation (Hurst 2007), an increase in mortality related to low prey availability (Všetičková et al. 2014) or seasonal migration to deeper offshore areas. Moreover, electrofishing can be less effective

in winter due to the influence of low temperatures (Borkholder and Parsons 2001). We believe that the latter two reasons are the most probable explanation for the decrease in abundance observed in the littoral zone of the Dyje in this study. A similar winter decrease in abundance has also been noted in the Laurentian Great Lakes (Kocovsky et al. 2011) and the middle Danube (Erős et al. 2005); in both cases, the decrease being attributed to ‘seasonal migration in response to changing habitat conditions’, i.e. low temperatures in the littoral zone. Similar migrations have also been observed for round goby, which move into deeper water (≥ 60 m in the Laurentian Great Lakes) when the littoral zone becomes uninhabitable due to ice formation (Pennuto et al. 2010; Lynch and Mensinger 2011).

Over our study stretch, fish length-frequency distribution indicates an annual life-cycle. This type of life-cycle and a long breeding period, as observed for tubenose goby in this study, indicate early maturation and rapid reproduction. Such reproductive characteristics represent a distinct advantage when colonising new areas and are likely to contribute to the success of tubenose gobies as a “pioneer species”. The three age groups identified from the length-frequency data (0+, 1+ and 2+, with 2+ the likely maximum age) correspond with anecdotal information on tubenose goby size-structure from other non-native areas (e.g. Slynko 2008; Semenov 2011). A study describing tubenose goby in its native range, however, noted a mean life-span of two years, with a maximum age estimated at 4+ (Harka and Farkas 2006). This suggests a large variability in life-span between populations.

The overall sex-ratio observed in this study was clearly female dominated, with a female to male ratio of 1.5:1. There appears to be no general trend observable from the relatively sparse information available from other tubenose populations, however, with ratios ranging from 5:1 female dominance (middle Danube; Kux 1957), 1:1 with slight female dominance (Black Sea basin; Smirnov 1986) and 0.6:1 male dominance (Kuybyshev Reservoir; Semenov 2011). In this study, the sex ratio changed through the year, with females dominating during spring and summer (March-August) and equal sex distribution from September on. This pattern contradicts that of Prášek (2006), who reported equal distribution in the pre-spawning period and female-bias in the post-spawning period at the nearby Nové Mlýny Reservoir. This suggests that seasonal sex-ratio may vary greatly between (and perhaps even

within) populations, which further suggests that time of sampling could significantly affect the observed sex-ratio (Mazzoni and Caramaschi 1995). Note that, during the spawning season, males tend to defend territories in rock cavities, which may limit electrofishing sampling efficiency, as has been observed in other gobiid species (Brandner et al. 2013).

In general, however, we can state that the sex-ratio shifts post-spawning when older individuals are gradually lost from the population (in our case, mostly in July) and the new 0+ cohort shifts the ratio toward a female bias. This is supported by the observed significant shift in sex-ratio associated with fish size. The higher proportion of females identified in smaller size-classes could have been caused by either (i) misidentification of males as young females (or juveniles) as the gonads are less discernible in the youngest fish or, less probably, by (ii) males actually maturing later (i.e. at a larger size) than females, as observed for Belgian tubenose gobies (Verreycken 2013). Indeed, the higher proportion of larger, and presumably older, males in this study agrees with the results of Prášek (2006). We suggest that males and females exhibit different life-history strategies, with females having lower K_C over the course of the year, at least in our river site. This suggests that females invest more energy into reproduction than males and display an almost strictly annual life-span. Males maintain a higher K_C over the year and appear capable of diversifying their energy expenditure more, resulting in a notably higher proportion of males surviving into the second spawning season. Male gobies typically display high energy investment in nest defence, which could hypothetically lead to energy depletion and death where food, for example, is limiting (Kostyuchenko 1961). The existence of alternative male mating strategies such as ‘sneaking’, however, which appears to be common in other gobiid species (e.g. see Immler et al. 2004; Marentette et al. 2009), could help explain the higher survival rate of males into the next spawning season. Indeed, the results for K_C show a clear difference in male and female energy demand over the year. Overall, while K_C showed similar dynamics between the sexes, female K_C was significantly lower than that of males over most of the year. The greatest difference was observed at the beginning of the spawning season (February-March), when there was a steep drop in female K_C . At this time (see below), females display rapid growth and high investment in maturation of the gonads. In both males and

females, K_C continues to drop (or stay low) until the end of the spawning season (June), clearly reflecting the high energy investment in reproduction (gonad growth and mating in both sexes, nest guarding in males; Skolbekken and Utne-Palm 2001). The relatively stable K_C values post-spawning (and through the winter months) probably reflect (i) the rapid loss of the 2010 generation (presumably between June and July, following which K_C rapidly increases again) and (ii) the presence of sufficient food resources for the new, 2011 generation (Adámek et al. 2010).

In our population, gonad development began in the autumn (i.e. in early 0+ fish) and we were able to clearly distinguish male and female gonads in dissected fish from October onwards. Rapid growth and maturation of female gonads began in March and continued until April. Prior to the spawning season, female gonads contained two or three clearly distinguishable groups of oocytes (i.e. at various developmental stages), thereby confirming batch spawning in tubenose goby (at least two batches are visible from the fish length-frequency distribution in Figure 4). Our data indicate a relatively long spawning season lasting from April to the end of June, which corresponds with results from both the species' native (e.g. the Azov Molochnyi estuary (Yankovskiy 1966)) and non-native ranges (Ladich and Kratochvíl 1989). An even longer spawning season has been observed in Bulgaria (Georghiev 1966) and Hungary (Harka and Farkas 2006), however, with spawning taking place as late as August.

Mean fecundity ranged from 379 to 628 in fish of 28–50 mm (SL), which is well within the values for tubenose gobies from the Caspian Sea (i.e. 354–714; Ragimov 1986) and the Sea of Azov (i.e. 207–648; Smirnov 1986). Tubenose goby fecundity, therefore, is higher than that registered for other gobiid species of a similar size (Ľavrinčíková and Kováč 2007; Konečná unpublished data). Note, however, that the other invasive gobiids tend to be larger and the total number of eggs laid by an average female tubenose goby (or population) will be much lower. What is more, several generations of females are present and capable of spawning during one spawning season in the longer-lived gobiid species, compared to a single generation in tubenose goby.

A short life-span, therefore, represents a serious handicap for tubenose goby when facing competition from its larger relatives. The results of this study indicate that, while the tubenose goby may be a poor competitor when confronted with other gobiid species, it is a good pioneer

species. Fast reproduction, a prolonged spawning season, batch-spawning and a short (presumably annual) life-cycle all facilitate swift colonisation of novel areas. In the absence of competition from other gobiids, tubenose goby populations can reach high abundance and numerically dominate the fish assemblage, and as such may well have an influence on the native ecosystem.

We should emphasise that our study was not designed to quantify the possible effects of the tubenose goby on the native ecosystem. Despite this, we can suggest several possible ways in which the tubenose goby may be potentially interacting with native fish assemblages, including predation, competition for space and competition for food. The current state of knowledge assumes that tubenose goby has little or no influence on native fish species via predation, including predation on eggs and larvae (Vašek et al. 2014). Further, while small benthic fish species may suffer though interference competition for shelter and nesting sites (Balshine et al. 2005; Kocovsky et al. 2011; Van Kessel et al. 2011), this appears to play a minor role along our study stretch, where small benthic species have always represented a small proportion of the native assemblage, even prior to the introduction of tubenose gobies (Lusk et al. 2001). The tubenose goby may also compete with small native species for food (and especially with species with similar spawning seasons such as gudgeon *Gobio gobio* and whitefin gudgeon *Romanogobio albipinnatus*), as suggested both elsewhere (e.g. Dillon and Stepien 2001; Dopazo et al. 2008; Vašek et al. 2014) and by our own unpublished data. The effect of such competition on population parameters has yet to be studied.

Based on these results, therefore, it is possible that tubenose goby dominance in the fish assemblage does not result from outcompeting native species but that its dominance simply reflects their position in a realised niche. Prior to their arrival, the Dyje provided a relatively “vacant” fundamental niche following extensive river modification, i.e. channelisation of the river bed, disconnection of floodplains and backwaters and, especially, changing of the littoral-zone into a rip-rap habitat. Direct and conclusive evidence for the possible effects of tubenose goby on native fish and invertebrate assemblages can only be confirmed, however, via a comparison of fish and invertebrate assemblages before and after tubenose goby expansion that excludes the possible effect of other gobiid species. We aim to address this in future studies.

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