

Can the presence of alien Ponto–Caspian gobies affect shelter use by the native European bullhead?

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Abstract Extensive invasion of Ponto–Caspian gobies raised the question how they affect recipient ecosystems. The round and racer goby pose a threat to their native counterparts, cottid species, but the influence of other gobiids is still not sufficiently demonstrated. We experimentally assessed how monkey and western tubenose goby, two of the most widespread species across Central and Western Europe, affected time spent by bullhead in the shelter in different seasons and light conditions. Direct and indirect aggression and guarding the shelter by the fish were also checked. We observed the behaviour of single-species and mixed-species pairs in the presence of a single shelter, with bullhead as a resident and one of three species as an intruder introduced to the tank

24 h later. Neither tubenose nor monkey goby was the stronger competitor, capable of outcompeting bullhead from the shelter. Their influence on the resident bullhead was the same as that of intruding bullhead: all intruders made resident bullhead increase shelter occupancy in spring at night. Moreover, compared to the monkey goby, the tubenose goby spent more time in the shelter, occupied it similarly in both seasons, guarded it more intensely and was more aggressive. The monkey goby displayed indirect aggression more often in spring. Despite these differences, bullhead responded to the presence of both goby species similarly, especially during reproductive season.

Keywords Competition · *Proterorhinus semilunaris* · *Neogobius fluviatilis* · *Cottus gobio*

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Introduction

Ponto–Caspian gobies are among the most successful fish invaders, which spread across Central and Western Europe (Copp et al. 2005; Roche et al. 2013) and in the Laurentian Great Lakes (Ricciardi and MacIsaac 2000; Ricciardi 2001). As introduced species, gobies can affect the recipient community through many ways, e.g. as predators, parasite vectors or competitors of native fish species (Gherardi 2006; Gozlan et al. 2010). Some examples of competitive interactions between the alien round goby *Neogobius*

melanostomus and native fish are documented in the Great Lakes (e.g. Dubs and Corkum 1996; Jude and DeBoe 1996; Janssen and Jude 2001; Balshine et al. 2005; Bergstrom and Mensinger 2009). In European waters, where six alien goby species have been recorded so far (Copp et al. 2005), their impact was tested in a few studies concerning mainly the racer goby *Babka gymnotrachelus* (Kakareko et al. 2013; Jermacz et al. 2015; Grabowska et al. 2016), except Van Kessel et al. (2011) who studied four goby species. These studies indicate that cottids are among the most vulnerable native fish species considering the possible impact of the Ponto–Caspian gobies.

In Europe, the co-occurrence of the European bullhead *Cottus gobio* and alien gobies was recorded in the Danube River (Austria) (Ahnelt et al. 1998; Wiesner 2005; Polačik et al. 2008), Rhine River (France) (Manné and Poulet 2008), Moselle River (Germany) (Von Landwüst 2006), Meuse River (Van Kessel et al. 2011, 2016) and Brda River (Poland) (Kakareko et al. 2016). In their non-native area, gobies are found on various substrates but, especially in the Danube River, they are particularly numerous in stony, rip-rap habitats (Ahnelt et al. 1998; Wiesner 2005; Polačik et al. 2008), also preferred by bullheads. Both invasive gobies and native cottids are small (up to several cm in total length), bottom-dwelling fish, which are most active at dusk and during the night, preying mainly on benthic macroinvertebrates (Mills and Mann 1983; Grabowska and Grabowski 2005; Kobler et al. 2012; Kornis et al. 2012). For both groups, the accessibility of shelter habitat is essential during the spawning period due to their similar reproductive strategies, including nest guarding and parental care of eggs (Goto 1982; Miller 2003; Grabowska 2005). Moreover, shelters play also a significant role as a protection against predators, visual isolation and hydraulic cache (Allouche 2002). That is why for some species, shelter is fundamental not only during reproduction, but throughout the year (Mills and Mann 1983; Allouche 2002).

Shelter places vary in their quality, e.g. they may differ in the level of protection against predators, suitability as a nest or prey availability in adjacent foraging grounds. Thus, optimal shelter could be potentially a limited resource especially for species displaying similar territorial and aggressive behaviour, such as bullheads and gobies (Mills and Mann 1983; Davey et al. 2009; Grabowska et al. 2016), and

when species density is high. Bullheads exhibit strong interference competition for a shelter and aggressive interactions between them outside shelters are common (Ladich 1989; Davey et al. 2005). This competition increases during the spawning season when a suitable shelter becomes also a nesting place. Larger males monopolize larger territories than smaller individuals, and even if a sufficient number of nesting places is provided, the dominants (larger males) prevent the smaller males approaching them through aggressive behaviour (Natsumeda 2001; Davey et al. 2005; Natsumeda et al. 2012). Gobies typically exhibit such territorial and aggressive behaviour (Miller 1984). All those similarities in biology and reproductive behaviour between gobies and bullheads should result in interspecific antagonistic interactions if they co-occur under the conditions of limited resources. Indeed, the invasive round goby exhibited more aggressive behaviour and successfully outcompeted an American cottid species, *Cottus bairdi*, from the shelter (Dubs and Corkum 1996). A similar adverse effect of the racer goby on the shelter occupancy of the European bullhead was shown both during the reproductive period (Grabowska et al. 2016) and outside the spawning season (Jermacz et al. 2015). Van Kessel et al. (2011) observed that only two out of four tested Ponto–Caspian goby species managed to force bullheads to shift from their preferred habitat; however, this study was conducted outside the reproductive period.

Although Ponto–Caspian gobies share a similar mode of arrival, vectors and dispersal pathways (Mombaerts et al. 2014), they differ in their activity and competitive behaviour (Borcherding et al. 2013a, b) and habitat demands, which was confirmed experimentally (Kakareko 2011; Van Kessel et al. 2011). Two species of contrasting preferences are the western tubenose goby *Proterorhinus semilunaris* and monkey goby *Neogobius fluviatilis*. In its native range, the tubenose goby is associated with aquatic vegetation in rivers, but in lakes, it may be found on the hard substrate (Miller 2003). Outside their indigenous area (e.g. Czech Republic, Germany, Austria, Poland), tubenose goby was often found in rip-rap and stones (Jude and DeBoe 1996; Ahnelt et al. 1998; Wiesner 2005; Grabowska et al. 2008; Adamek et al. 2010; Janáč et al. 2013). On the contrary, the monkey goby occurs natively on sandy shoals with moderate current (Miller 2003). This species reveals a morphological

specialization for sandy substrata (Čápková et al. 2008), and in newly invaded ecosystems, it is often found on sandy bottom, e.g. in the Vistula River system (Danilukiewicz 1998; Kostrzewa and Grabowski 2002), Danube (Holčík et al. 2003; Erös et al. 2005; Polačik et al. 2008) and Rhine (Borcherding et al. 2011). Throughout the year, monkey gobies were caught usually on sand except for the spawning season when they occupied artificial shelters (Kakareko 2011). Although habitat preferences of gobies differ somewhat from those of bullheads (Van Kessel et al. 2011; Kakareko et al. 2016), they tend to use crevice shelters, such as spaces between or under stones (Kakareko et al. 2016), especially during the most important period, the spawning season.

The purpose of the present study was to examine whether the monkey or western tubenose goby can influence the shelter use by the European bullhead. We conducted a laboratory experiment to investigate interactions between the above-mentioned species and check whether this interference depends on season (spring/autumn), light conditions (day/night) and goby species identity. We hypothesized that the tubenose goby would be determined to compete for a shelter independently of season due to its habitat preferences, while the monkey goby would try to take over the shelter in the spawning season (spring) only. As the tested species display nocturnal activity, we expected that most competitive interactions would occur during the daylight, when they seek for a refuge.

Materials and methods

Study sites and sampling

Gobies and bullheads were collected on two dates, in autumn (September) 2014 and spring (May) 2015. Monkey and western tubenose gobies were sampled from the lower section of the Vistula River (in the backwater of the Włocławski Reservoir), near the city of Płock (19°31'E; 52°36'N), central Poland. European bullheads are legally protected in Poland, so they were collected under permit (no. WPN-II.6401.1.162.2014.IW.3) from the Pilica River (a tributary of the Vistula River, 20°04'E; 51°53'N). Both gobies and bullheads were collected in localities with similar environmental conditions (shallow, near-shore areas with moderate water flow, stony riverbank with

sandy bottom) in which they do not coexist; therefore, they did not experience any encounters with each other before the experimental trials.

Fish were caught using electrofishing (IUP-12, Poznań, Poland and type EFGI 650, BSE Bretschneider Spezial elektrotechnik, Germany), transported to the laboratory in aerated tanks and after 24 h of acclimation placed in 72-L (50 × 40 × 36 cm) single-species aquaria filled with filtered, aerated water at room temperature (7–10 specimens per aquarium). Before the experimental trials, the fish were kept in the aquaria for a month in the presence of shelters (in the number exceeding the number of fish) made of PVC half-pipes (about 10 cm long and wide), without any substrate. Additionally, the aquaria were connected with one another in a system of circulating water (the same water volume was exchanged constantly) to provide the fish with proper living conditions. Fish were fed with living chironomid larvae every second day. To reflect natural conditions, photoperiod in the laboratory was set to 14 h L:10 h D (day 06:00–20:00) in spring and 11 h L:13 h D (day 05:00–16:00) in autumn. The stock and experimental aquaria were located in the same laboratory room, under similar light and temperature (17–19 °C) conditions. For all experimental procedures, we obtained permission from the Local Ethic Committee and Regional Directorate for Environmental Protection (no. 41/ŁB720/2014; WPN-II.6401.1.162.2014.IW.3, respectively).

Sex was determined by examining the shape of a urogenital papilla in gobies (Charlebois et al. 1997) in both seasons and based on the presence of dark body colouration with bright cream edge of the first dorsal fin in male bullheads in spring only (Tomlinson and Perrow 2003). Our objection was to investigate the influence of male gobies, which usually display aggressive behaviour and due to prolonged spawning can be distinguished from females even in late summer (Grabowska 2005), on bullhead specimens. We assumed that in spring male bullheads would be more subjected to goby competition, as they select and guard spawning sites. Therefore, we used only male bullhead in the spring treatments. However, it was not possible to determine the sex of the specimens in autumn when sexual dimorphism of the bullhead disappeared (Kobler et al. 2012), and we were not allowed to dissect the used fish due to their protected status in Poland. Nevertheless, in autumn, which is the post-spawning period for bullheads (Mills and Mann

1983; Davey et al. 2005) as well as for gobies (Pinchuk et al. 2003), both sexes will use shelters similarly, for protection against unsuitable environmental conditions and display similar levels of aggression as it was demonstrated by Kobler et al. (2011). Therefore, we conducted the autumn treatments without determining sex in bullhead.

Experimental setup

Fish were tested in 72-L tanks filled with settled, aerated tap water. Tanks were mildly aerated, deprived of substrate and contained one shelter (PVC half-pipe; similar to those used in the stock tanks) placed in the centre, imitating a limited rock cavity habitat. Each tank was isolated from external laboratory conditions with opaque Styrofoam screens on the bottom and sides. Each tank was equipped with a video camera (CCTV day and night video camera, SDC425P, Samsung, South Korea) suspended 65 cm above the water level and infrared (850 nm) illuminator (MFL-I/LED5-12, Eneo, Germany), which allowed observations of fish in darkness. Before introducing into the experimental tank, each fish was measured (total length) with a ruler to the nearest 1 mm. To standardize the hunger level, the tested fish were deprived of food during the experimental procedure. Each fish was used only once in the experiment.

Experimental protocol

We examined single bullheads in the presence of monkey or tubenose goby of the same size (Table 1). Paired Student's *t* tests performed on each pair of

species showed no differences in size between specimens. We ran 10 replicates in each season (spring and autumn) for each pair of species. The experiment was conducted for 48 h. At the beginning of each trial (always at 9.00 a.m.), a single bullhead was placed in the aquarium equipped with a single shelter. Hereafter, we refer to the bullhead at this stage of the trial as 'solitary' as it was a single fish in the aquarium. After 24 h, a single goby or another bullhead was added to the tank and since then the bullhead became a 'resident', as opposed to the newly added 'intruder' fish. Both fish were kept together for the next 24 h. We used this design, as our main intention was to test the occurrence of the negative effect of an invader on a native species. Therefore, we placed the native species in a privileged position, as a shelter 'owner', to check whether the alien fish would be able to displace their native competitor in such situation. The single-species treatment (with bullheads both as residents and intruders) was used as a control to check whether the fish responses to gobies differ from those exhibited in the presence of conspecifics. Such a difference could be regarded as a sign of the impact of the alien species on bullhead behaviour.

The fish behaviour was observed during four selected hours from each 24-h period (with a solitary fish and a pair of fish): 2 h during the light period (11.00–12.00 and 14.00–15.00) and 2 h at night (23.00–24.00 and 04.00–05.00). We measured time spent by fish in the shelter. Additionally, we distinguished three types of behaviour: (1) 'direct aggression' (DA) when one fish bites, darts towards or chases another; (2) 'indirect aggression' (IA), when one fish seizes the shelter, attempts to overtake or moves

Table 1 Mean and range of total length TL (mm) of fish used in the each treatment of experiment

	Pair (resident vs. intruder)	Resident mean length (range min–max) (mm)	Intruder mean length (range min–max) (mm)
Fish: <i>MG</i> monkey goby, <i>TG</i> western tubenose goby, <i>BH</i> bullhead	Spring		
	BH vs. MG	79.0 (57–100)	79.2 (61–98)
	BH vs. TG	78.3 (72–89)	78.2 (70–85)
	BH vs. BH	81.5 (75–108)	81.4 (70–105)
	Autumn		
	BH vs. MG	91.0 (64–105)	90.0 (66–107)
	BH vs. TG	66.0 (57–75)	65.9 (57–80)
	BH vs. BH	92.9 (78–102)	88.4 (76–101)

slowly towards the shelter; and (3) ‘guarding the shelter’ (GS), when a fish is inside the shelter and leans out to watch. Fish behaviour of a given type was scored if it appeared at least once in a single 5-min episode during the recording (thus the maximum number of recorded attacks per an hour was 12: at least one per each episode).

Data analysis

Time spent by bullhead in the shelter was expressed as a proportion of 4 h of observation. The data were arcsine transformed to achieve normality. A four-way mixed model ANOVA was used to compare differences in time spent in the shelter by the tested fish with ‘bullhead status’ (solitary, resident) and ‘light condition’ (day, night) as within-subject factors and ‘season’ (spring, autumn) and ‘intruder species’ (tubenose goby, monkey goby and bullhead) as between-subject factors. A three-way mixed model ANOVA was used to test the behaviour of different intruder fish, with ‘light conditions’ (day, night) as a within-subject factor and ‘season’ (spring, autumn) and intruder species (tubenose goby, monkey goby and bullhead) as between-subject factors.

To compare recorded aggressive behaviours (direct and indirect aggression), we used square-root transformed counts of aggression events and performed a four-way mixed model ANOVA with ‘fish status’ (resident, intruder) and ‘light conditions’ as within-subject factors and ‘season’ and ‘intruder species’ as between-subject factors. Shelter guarding was expressed per time spent by fish in the shelter ($\log(x + 1)$ transformed) as it could be exhibited only by fish staying in the shelter. Only fish which spent at least 2 min in the shelter, were included in this analysis to avoid overestimation of casual behaviour of specimens that visited shelters occasionally. Therefore, we could not include intruder monkey gobies and bullheads into the analysis due to the insufficient number of appropriate cases. Thus, we applied a three-way mixed ANOVA with fish status and light conditions as within-subject factors and season as a between-subject factor using the data from the treatment with the tubenose goby. Moreover, we performed a three-way mixed model ANOVA using only the data for resident bullhead, with ‘light conditions’ as a within-subject factor and ‘season’ and ‘intruder species’ as between-subject factors.

When necessary, significant ANOVA effects were further analysed using paired or unpaired *t* tests.

Results

Time spent in the shelter

In both seasons, solitary bullheads occupied the shelter during the day for most of the observation time: 88 and 71 % in spring and autumn, respectively. At night, solitary bullheads were more active and stayed in the shelter only 26 and 35 % time, respectively (Fig. 1). For resident bullheads, a similar circadian activity was observed as they spent in the shelter more time during the daylight in both seasons (79 and 82 % in spring and autumn, respectively) than at night (50 and 43 %, respectively).

Season and light conditions affected the effect of intruders on the time spent in the shelter by bullhead, as indicated by a significant bullhead status \times light condition \times season interaction (Table 2). In spring, at night, the resident bullhead spent in the shelter twice as much time as during its solitary period (Fig. 1). On the other hand, in autumn, bullheads did not respond significantly to the presence of an intruder. However, the identity of an intruding fish was not important as shown by a non-significant main effect and interactions of the intruder species (Table 2).

Time spent in the shelter by intruding species depended on season and light conditions, as shown by a significant light condition \times intruder species \times season interaction (Table 3). Tubenose goby spent more time in the shelter in autumn than in spring and more time during the light period than at night in both seasons. No significant differences in shelter occupancy between the light conditions or seasons were found for the other species. In spring, all the intruder species occupied shelters similarly to one another. In autumn, the tubenose goby occupied the refuge longer than the monkey goby and bullhead (only during the day). Also, bullhead stayed in the shelter longer than monkey goby at night (Fig. 1).

Aggressive behaviours

Acts of direct aggression were affected by intruding fish species, light conditions and season as shown by a significant fish status \times light condition \times season \times

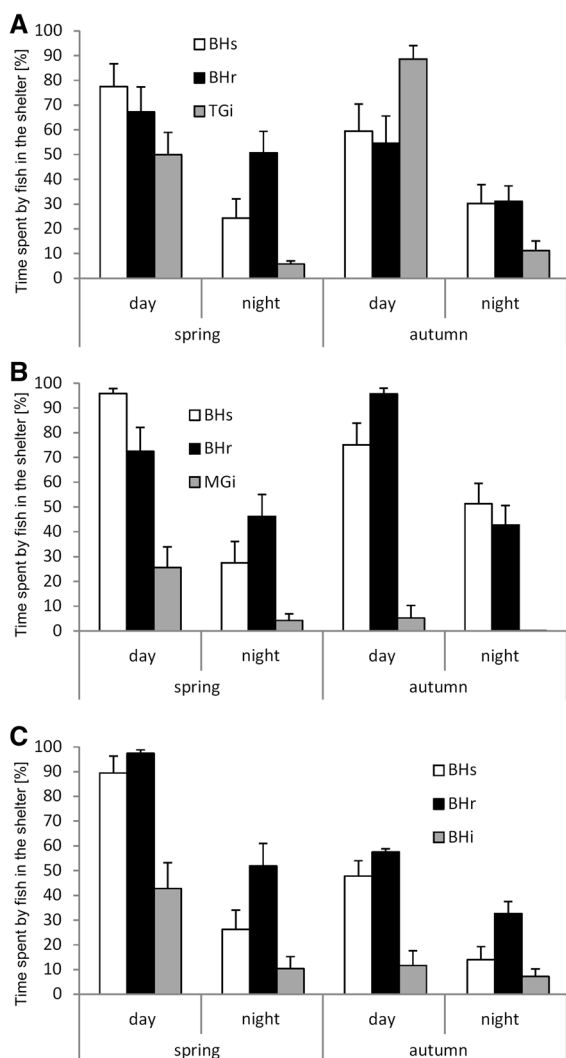


Fig. 1 Comparison of seasonal and light condition difference in time (\pm SE) spent in the shelter (%) by solitary, resident and intruder fish in the combinations: **a** western tubenose goby intruder (TG_i) versus bullhead (BHs—solitary bullhead, BHr—resident bullhead); **b** monkey goby intruder (MG_i) versus bullhead (BHs—solitary bullhead, BHr—resident bullhead); and **c** bullhead intruder (BH_i) versus bullhead (BHs—solitary bullhead, BHr—resident bullhead)

intruder species interaction (Table 4). When a conspecific intruder was present, resident bullhead displayed more DA than in the presence of monkey goby independently of light conditions in both seasons (Fig. 2). Moreover, in spring, the resident bullhead was more aggressive towards another bullhead than

towards the tubenose goby. Tubenose goby was a more aggressive intruder than bullhead intruder (in spring during the day and in autumn at night) and monkey goby (in autumn at night).

Indirect acts of aggression depended on fish species and season (significant fish status \times season \times intruder species interaction, Table 4). There was no difference in IA of the resident bullhead in the presence of various intruding fish. Monkey goby was less aggressive than tubenose goby in spring and bullhead intruder in autumn (Fig. 3). Intruding fish always exhibited a greater IA than resident bullheads except monkey goby in autumn.

Guarding the shelter

Guarding the shelter was exhibited only by individuals staying in the shelter. In the treatment with tubenose goby, GS was displayed more often by the invader than resident species (significant fish status effect, Table 5; Fig. 4) as well as in spring (significant season effect, Table 5) and at night (significant light condition effect, Table 5). Resident bullheads guarded the shelter more often in spring (significant season effect, Table 6) and at night (significant light condition effect, Table 6), regardless of the identity of the intruding species.

Discussion

The influence of the tubenose goby and monkey goby on the shelter use of the European bullhead was not as strong as we expected from the studies on other goby species (Dubs and Corkum 1996; Van Kessel et al. 2011; Kakareko et al. 2013; Jermacz et al. 2015; Grabowska et al. 2016). Both tubenose and monkey goby did not outcompete bullhead from the shelter, but their influence on the shelter occupancy by the resident bullhead was the same as that of intruding bullhead. Independently of the intruding species, resident bullhead increased shelter occupancy at night in spring compared to the situation when it was alone, but this 'intruder effect' was not observed in autumn. The season was also an important factor affecting the fish behaviour and intensity of interaction between them.

Table 2 Four-way mixed model ANOVA table to test the effects of intruder species (western tubenose goby, monkey goby and bullhead) and season (spring, autumn) as a between-subject factors, as well as bullhead status (solitary, resident) and light condition (day, night) as within-subject factors on time spent in the shelter by resident bullhead. Significant differences are marked in bold

Effect	<i>df</i>	<i>F</i>	<i>P</i>
Bullhead status	1	6.199	0.016
Bullhead status × intruder species	2	2.742	0.073
Bullhead status × season	1	0.244	0.623
Bullhead status × intruder species × season	2	0.722	0.491
Error	54		
Light condition	1	100.244	<0.001
Light condition × intruder species	2	1.982	0.148
Light condition × season	1	0.866	0.356
Light condition × intruder species × season	2	0.198	0.821
Error	54		
Bullhead status × light condition	1	6.148	0.016
Bullhead status × light condition × intruder species	2	0.305	0.738
Bullhead status × light condition × season	1	6.269	0.015
Bullhead status × light condition × intruder species × season	2	1.399	0.256
Error	54		
Intruder species	2	3.003	0.058
Season	1	0.405	0.527
Intruder species × season	2	0.646	0.528
Error	54		

Table 3 Three-way mixed model ANOVA table to test the difference in time spent by intruder fish in the shelter with intruder species (tubenose goby, monkey goby and bullhead)

and season (spring, autumn) as between-subject factors and light condition (day, night) as a within-subject factor. Significant differences are marked in bold

Effect	<i>df</i>	<i>F</i>	<i>P</i>
Light condition	1	45.085	<0.001
Light condition × intruder species	2	8.478	0.001
Light condition × season	1	0.826	0.367
Light condition × intruder species × season	2	5.921	0.005
Error	54		
Intruder species	2	17.120	<0.001
Season	1	0.003	0.959
Season × intruder species	2	5.526	0.007
Error	54		

For both goby species, shelter is a more important resource in spring than outside the reproductive season, as it is not only a refuge but also a potential nest. This applies both to the monkey goby, associated with shelters only for spawning (Kakareko 2011), and to the western tubenose goby that use them throughout the year. Possibly that is why in spring the resident bullhead in our studies spend more time in the shelter than in the absence of an intruder, irrespective of the intruder species. It refers also to guarding the shelter behaviour as the resident bullhead exhibited it

significantly more often in spring and at night. It could be explained as a response of the resident to the indirect aggression, i.e. attempts to overtake the shelter or moving slowly towards the shelter, expressed more often by all intruding fish. Thus, the resident bullhead responded similarly to conspecific intruders and unknown gobies. The only exception was acts of direct aggression, such as biting, chasing or darting towards another fish, that were directed by the resident bullhead more often towards conspecifics than towards gobies. Intraspecific aggression is

Table 4 Four-way mixed ANOVA table to test the effects of intruder species (western tubenose goby, monkey goby and bullhead) and season (spring, autumn) as a between-subject factors, as well as fish status (resident, intruder) and light

condition (day, night) as within-subject factors on different behaviours displayed by fish. Significant differences are marked in bold

Type of aggressive behaviour	Effect	df	F	P
Direct aggression	Fish status	1	0.286	0.595
	Fish status × intruder species	2	12.186	<0.001
	Fish status × season	1	0.029	0.865
	Fish status × intruder species × season	2	0.373	0.691
	Error	54		
	Light condition	1	0.434	0.513
	Light condition × intruder species	2	0.497	0.611
	Light condition × season	1	2.974	0.090
	Light condition × intruder species × season	2	3.088	0.054
	Error	54		
	Fish status × light condition	1	7.788	0.007
	Fish status × light condition × intruder species	2	2.511	0.091
	Fish status × light condition × season	1	0.001	0.976
	Fish status × light condition × intruder species × season	2	3.367	0.042
	Error	54		
	Intruder species	2	6.703	0.003
	Season	1	0.051	0.822
	Intruder species × season	2	0.580	0.563
	Error	54		
Indirect aggression	Fish status	1	84.845	<0.001
	Fish status × intruder species	2	3.479	0.038
	Fish status × season	1	12.358	0.001
	Fish status × intruder species × season	2	3.927	0.026
	Error	54		
	Light condition	1	0.459	0.501
	Light condition × intruder species	2	0.242	0.786
	Light condition × season	1	0.027	0.869
	Light condition × intruder species × season	2	0.853	0.432
	Error	54		
	Fish status × light condition	1	0.020	0.887
	Fish status × light condition × intruder species	2	0.185	0.832
	Fish status × light condition × season	1	0.178	0.675
	Fish status × light condition × intruder species × season	2	2.820	0.068
	Error	54		
	Intruder species	2	2.862	0.066
	Season	1	28.558	<0.001
	Intruder species × season	2	3.128	0.052
	Error	54		

usually stronger and more common than interspecific aggressive interactions (Connell 1983), as conspecifics compete for exactly the same resources.

Similar situation was observed by Jermacz et al. (2015), who found greater aggression of the racer goby towards conspecifics than towards bullheads during

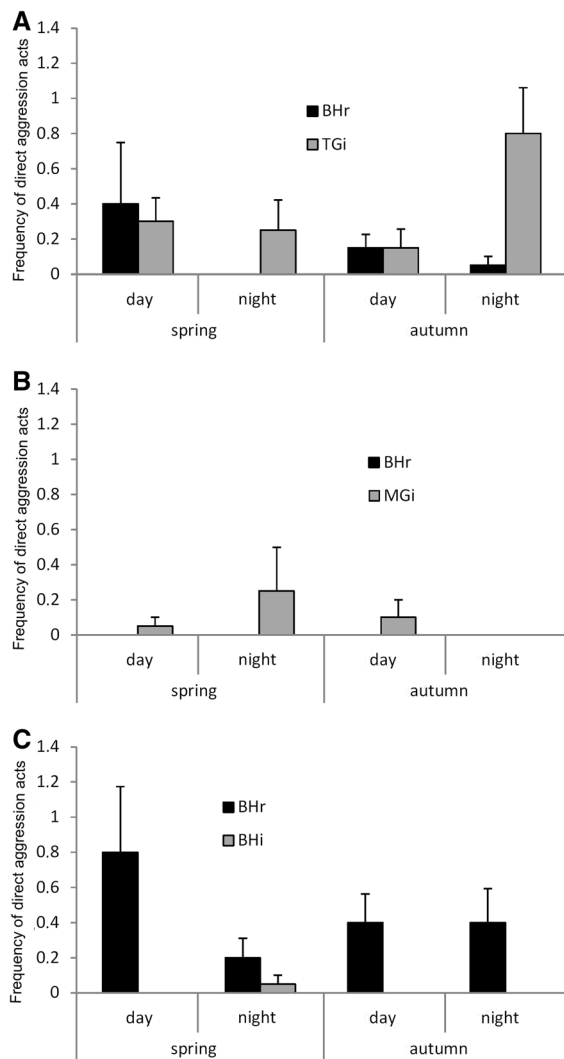


Fig. 2 Direct aggression displayed by resident and intruder fish expressed as the mean (\pm SE) frequency of 5-min episodes with an occurrence of a direct aggression act per 48 episodes analysed (total observation time: 4 h) in the combinations: **a** western tubenose goby intruder (TGi) versus resident bullhead (BHR); **b** monkey goby intruder (MGi) versus resident bullhead (BHR); and **c** bullhead intruder (BHi) versus resident bullhead (BHR)

their competition for a shelter. Nevertheless, this did not prevent the racer goby from displacing the bullheads from their shelters; thus, the lower inter-specific aggression does not explain the lack of success of the other goby species in our study. Interestingly, when food rather than shelter was a limiting resource, the racer gobies were similarly aggressive towards conspecifics and heterospecifics

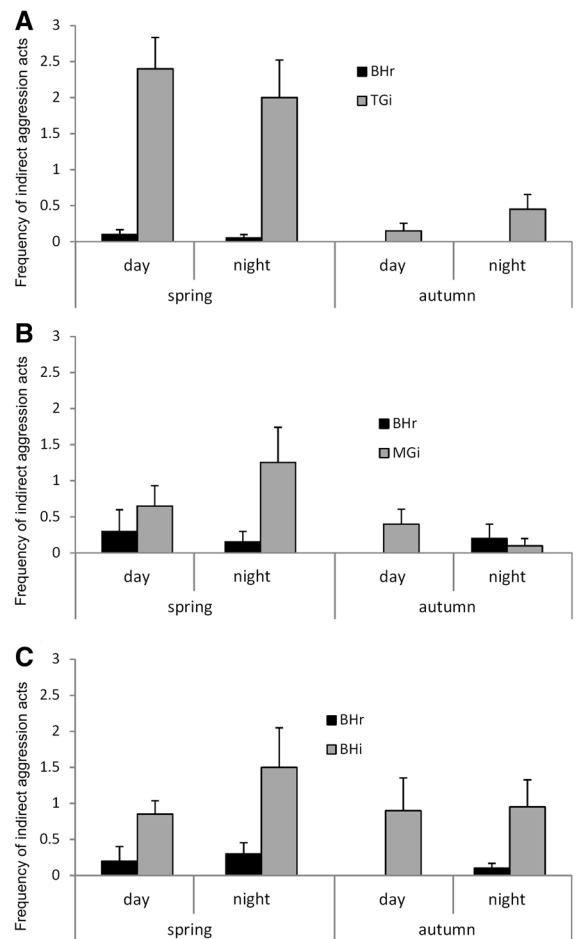


Fig. 3 Indirect aggression displayed by resident and intruder fish expressed as the mean (\pm SE) number of 5-min episodes with an occurrence of a direct aggression act per 48 episodes analysed (total observation time: 4 h) in the combinations: **a** western tubenose goby intruder (TGi) versus resident bullhead (BHR); **b** monkey goby intruder (MGi) versus resident bullhead (BHR); and **c** bullhead intruder (BHi) versus resident bullhead (BHR)

(Kakareko et al. 2013), showing that the resource type can also shape interactions among organisms.

Although, in contrast to our assumption, the influence of both goby species on resident bullhead was similar, their behaviour and shelter use differed from each other, as the tubenose goby spent more time in the shelter than the monkey goby. As we expected, the tubenose goby tended to occupy the shelter in both seasons. Besides, it guarded it more intensely and was also more aggressive than other intruders. Contrary to our assumptions, the monkey goby did not occupy the shelter significantly longer in spring than in autumn,

Table 5 Three-way mixed ANOVA table to test the effects of intruder species (western tubenose goby) on guarding the shelter behaviour displayed by fish (tubenose goby, bullhead) with fish status (resident, intruder) and light condition (day, night) as within-subject factors and season (spring, autumn) as a between-subject factor. Significant differences are marked in bold

Effect	<i>df</i>	<i>F</i>	<i>P</i>
Season	1	29.023	0.002
Error	6		
Fish status	1	103.816	<0.001
Season × fish status	1	0.024	0.881
Error	6		
Light condition	1	26.436	0.002
Light condition × season	1	0.525	0.496
Error	6		
Fish status × light condition	1	0.609	0.465
Fish status × light condition × season	1	1.627	0.249
Error	6		

but its acts of indirect aggression were observed more often in spring. Thus, its attempts to seize the shelter were probably sufficient to affect the shelter occupancy by the resident bullhead in spring equally to other intruders.

The tubenose goby and bullhead had a similar circadian activity. They stayed in the shelter more often during the day than at night. A similar pattern was observed for another Ponto–Caspian invasive goby, i.e. the racer goby (Grabowska et al. 2016). In contrast, the shelter occupancy of the monkey goby in our experiments did not differ depending on light conditions. Assuming that shelter occupancy differs between day and night, not only season but also time of the day should influence the magnitude of interference between the bullhead and both goby species. Bottom-dwelling fishes such as bullheads and some Ponto–Caspian gobies are usually nocturnal, seeking a hiding place during the day and being most active at dusk or at night (Tomlinson and Perrow 2003; Erös et al. 2005; Johnson et al. 2008; Gaygusuz et al. 2010; Grabowska et al. 2016). On the other hand, shelter occupancy by the monkey goby did not differ between night and day, which corroborates the lack of a clear diel pattern of its foraging activity (Grabowska et al. 2009). A combined effect of season and diel activity

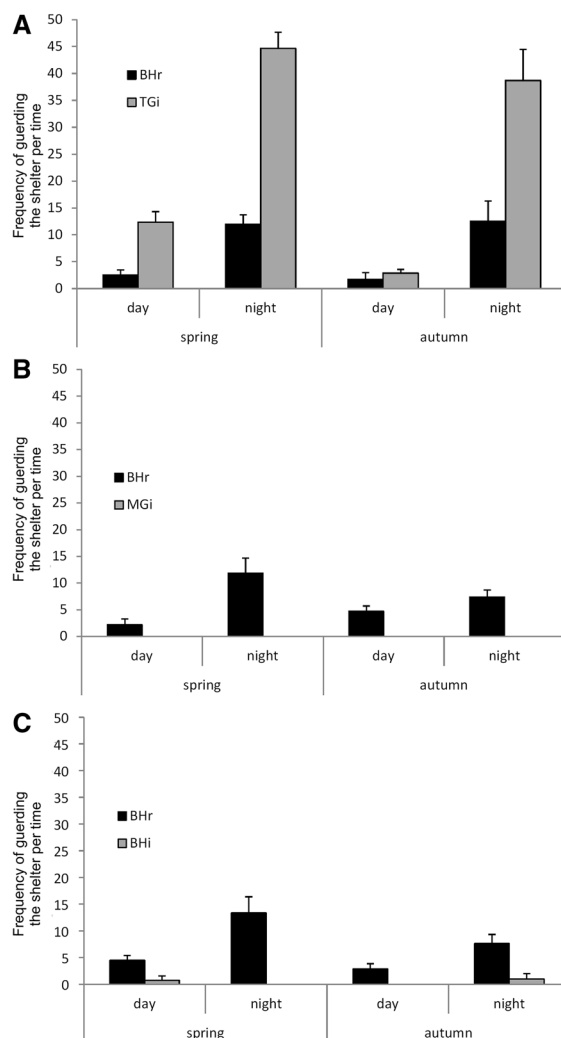


Fig. 4 Guarding the shelter exhibited by resident and intruder fish expressed as the mean (\pm SE) number of episodes per time spent in the shelter (total observation time: 4 h). Fish: *MGi* monkey goby intruder, *TGi* tubenose goby intruder, *BHr* resident bullhead and *BHi* intruder bullhead

pattern of fish was observed in the interaction between the European bullhead and racer goby (Grabowska et al. 2016). In these experiments, the goby competed with bullhead for the shelter predominantly in spring and mostly during the day. This interaction led to the modification of the circadian activity pattern of the resident bullhead in the presence of the intruder in comparison with solitary fish.

In our study, neither tubenose nor monkey goby was the stronger competitor and displaced the resident bullhead from the shelter nor considerably reduced its

Table 6 Three-way mixed ANOVA table to test the effects of intruder species (western tubenose goby, monkey goby and bullhead) on guarding the shelter behaviour displayed by resident bullhead with light condition (day, night) as within-

subject factor and season (spring, autumn) and intruder species (western tubenose goby, monkey goby and bullhead) as a between-subject factors. Significant differences are marked in bold

Effect	df	F	P
Intruder species	2	1.821	0.174
Season	1	6.154	0.017
Intruder species × season	2	1.387	0.261
Error	43		
Light condition	1	44.435	<0.001
Light condition × intruder species	2	0.978	0.384
Light condition × season	1	2.457	0.124
Light condition × intruder species × season	2	0.835	0.441
Error	43		

time of shelter occupancy. Moreover, even temporary success in seizing the shelter was not always equivalent with outcompeting a bullhead, as both fish often used to stay together for a while and then one of them left the shelter. Such results contradict previous studies conducted outside the reproductive period by Van Kessel et al. (2011), who observed that the tubenose goby was the species which, apart from the bighead goby *Ponticola kessleri*, outcompeted the bullhead and forced it to move to the less preferred habitat. A similar superior position in the competition for a shelter and/or food was observed for the round goby (Dubs and Corkum 1996) and the racer goby (Kakareko et al. 2013; Jermacz et al. 2015; Grabowska et al. 2016). Thus, the present and previous experimental studies considering the impact of invasive Ponto–Caspian gobies on bullhead show that the outcome of the interactions between these taxa is quite complex and depends on a number of factors, such as the goby species, season and environmental conditions.

To conclude, our studies do not show an impact of the tubenose goby and monkey goby on the European bullhead. However, it cannot be said that the bullhead was indifferent to their presence, as they had a similar influence on the shelter use as conspecific intruders. Apart from the already existing intraspecific competition among bullheads, the arrival of alien gobies can add the interspecific competition, though it would vary with season and, as other studies suggest, also with intruding goby species. In the wild, habitat partitioning between bullheads and gobies would solve this hypothetical problem of competition. Actually, such

space segregation with regard to differences in water velocity was observed between the invading racer goby and the native bullhead in the Brda River (Kakareko et al. 2016); especially, larger individuals displayed habitat partitioning, while smaller fish often co-occurred in intermediate habitats, not preferred by larger individuals. Thus, although the decrease in abundance of the European bullhead populations, observed in some rivers, e.g. the Slovak section of the Danube (Jurajda et al. 2005) or in Moselle River (Germany) (Von Landwüst 2006), coincides with the Ponto–Caspian goby expansion there, our results have shown that the impact of these invaders is not always an obvious reason of this decline. Instead, several additional factors may affect bullhead populations in natural conditions, as the species is vulnerable to a range of disturbances, such as increased siltation, channel modification and water pollution (Knaepkens et al. 2002).

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