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RESEARCH PAPER — OPEN ACCESS

Round goby *versus* marbled crayfish: alien invasive predators and competitors

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Abstract – Aquatic biodiversity is threatened by spread of invasive alien species. Round goby *Neogobius melanostomus* is an invasive fish in large European rivers as well as in coastal waters near their mouths and marbled crayfish *Procambarus virginalis* is a highly invasive crustacean. Both are small, bottom-dwelling species occupying similar habitat and shelters and utilizing similar food sources. We hypothesized that goby presents a threat to both native and non-native astacofauna in invaded ecosystems. We tested this through laboratory experiments designed to determine aggressiveness and competitiveness of goby against marbled crayfish as a model for other North American cambarid crayfish, assessing goby prey size selection and competition with marbled crayfish for space and shelter. Gobies showed high aggressiveness and dominance over the crayfish. Goby predation on juvenile crayfish was limited by mouth gape size. In goby/crayfish pairs of similar weight, gobies were more aggressive, although each affected the behavior of the other.

Keywords: Biological invasion / freshwater / predation / shelter competition / species interaction

Résumé – **Le gobie à tache noire versus l'écrevisse marbrée: des espèces exotiques envahissantes prédatrices et concurrentes.** La biodiversité aquatique est menacée par la propagation d'espèces exotiques envahissantes. Le gobie à taches noires *Neogobius melanostomus* est un poisson envahissant dans les grands fleuves européens ainsi que dans les eaux côtières près de leur embouchure et l'écrevisse marbrée *Procambarus virginalis* est un crustacé très envahissant. Toutes deux sont de petites espèces vivant sur le fond, occupant des habitats et des abris similaires et utilisant des sources de nourriture similaires. Nous avons émis l'hypothèse que le gobie constitue une menace pour l'astacofaune indigène et non indigène dans les écosystèmes envahis. Nous avons testé cette hypothèse par le biais d'expériences en laboratoire conçues pour déterminer l'agressivité et la compétitivité du gobie contre l'écrevisse marbrée comme modèle pour les autres écrevisses cambarides d'Amérique du Nord, en évaluant la sélection de la taille des proies du gobie et la compétition avec l'écrevisse marbrée pour l'espace et les abris. Les gobies ont montré une grande agressivité et une forte dominance sur les écrevisses. La prédation des gobies sur les écrevisses juvéniles était limitée par la taille de l'ouverture de la bouche. Dans les paires gobie/écrevisse de poids similaire, les gobies étaient plus agressifs, bien que chacun ait affecté le comportement de l'autre.

Mots clés : Invasion biologique / eau douce / prédation / compétition pour l'abri / interaction des espèces

1 Introduction

Fish and crayfish have multiple relationships and portraying them solely as prey or predator can be misleading. In aquatic habitats, both groups often represent keystone species, and their competition for resources can have high impact (Bond, 1994; Crandall and Buhay, 2008). Although the ranges of some non-native fish overlap with those of non-native crayfish, with the exception of information with respect to aggressive encounters, little is known about interactions between invasive benthic fish and native and invasive crayfish, although they co-exist and use similar niches and substrates as shelter (Church *et al.*, 2017). Filling the knowledge gaps is worthwhile, because both fish and decapods may regulate community biodiversity through their longevity and trophic

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Table 1. Biometric data of marbled crayfish size groups used in experiment on round goby predation on crayfish (Experiments 1–3). Data are presented as mean ± standard deviation (SD) and range.

Exp.	Size class	Weight (mg)	Total length (mm)	Carapace length (mm)	Carapace height (mm)
1,2	Small	15.2 ± 4.0 (10–25)	10 ± 1.4 (8–12)	4.7 ± 0.9 (3–6)	2.3 ± 0.5 (2–3)
	Medium	58.6 ± 12.7 (40–80)	15.3 ± 0.5 (15–16)	7.3 ± 0.6 (6–8)	3.7 ± 0.8 (3–5)
	Large	210.6 ± 60.3 (100–300)	22.8 ± 1.7 (21–25)	11 ± 0.6 (10–12)	5.7 ± 0.8 (5–7)
3	Small juveniles	5.7 ± 0.6 (5.1–7)	7.1 ± 0.4 (6.5–8)	3.9 ± 0.4 (3–4.5)	–
	Medium juveniles	13.8 ± 4.8 (9–22)	10 ± 1.4 (8–12)	4.7 ± 0.9 (3–6)	2.3 ± 0.5 (2–3)

specialization (Reynolds, 2011). Alien predatory fishes can negatively impact native crayfishes, while fish populations may be affected by predation and competition from multiple organisms, including exotic crayfish (Degerman *et al.*, 2007).

Round goby *Neogobius melanostomus* (Pallas, 1814) is an alien invasive species from the Eurasian Ponto-Caspian region that presents high potential for competition with native species (Jude, 1997; Borcherding *et al.*, 2011; Brandner *et al.*, 2013). The species has invaded, or expanded its range in, large European rivers including the Danube (Vanderploeg *et al.*, 2002), Rhine (Van Kessel *et al.*, 2009), Vistula (Grabowska *et al.*, 2008), and Volga (Copp *et al.*, 2005) and has established invasive populations in North America (Kornis *et al.*, 2013). A primary characteristic is its formation of vital and dense populations with rapid spread both upstream and downstream (Roche *et al.*, 2015; Verliin *et al.*, 2017). Round goby is an adaptable generalist benthic feeder with a broad diet spectrum including zooplankton, benthic invertebrates, and fish eggs and larvae (Kornis *et al.*, 2012). Small benthic fish like round goby can feed on small juvenile crayfish as well as exploit the same food sources and be subject to the same predators as larger crayfish (Dorn and Mittelbach, 1999).

The marbled crayfish *Procambarus virginalis* (Lyko, 2017) is a unique invasive crayfish that reproduces parthenogenetically and has been included in the list of European Union invasive species of concern since August 2016 (EU regulation No. 1143/2014 and Commission Implementing Regulation No. 2016/1141). The marbled crayfish has been reported established in many European countries and on other continents (Chucholl and Pfeiffer, 2010; Lipták *et al.*, 2016; Hossain *et al.*, 2018; Andriantsoa *et al.*, 2019). It is omnivorous, feeding on algae, detritus, zoobenthos, and macrophytes. It can become abundant and form high-density populations in a short time (Lipták *et al.*, 2019). As *Procambarus fallax* (Hagen, 1870) is a crayfish endemic to Florida and closest relative to the parthenogenetic *P. virginalis*, we used marbled crayfish as a representative of other successful invasive members of the Cambaridae originally from North America (Kouba *et al.*, 2014; Patoka *et al.*, 2016).

Direct interactions between fish and crayfish include predation and competition for shelter. When co-existing fish and decapods are omnivores, there will be competition and mutual predation, depending on relative size and vulnerability (Reynolds, 2011). Bottom-dwelling fish such as round goby can potentially exert negative effects on crayfish in addition to predation, as they use similar food sources and compete for shelter (Gebauer *et al.*, 2019). Limited shelter availability can increase the vulnerability to predation of the weaker opponent (Church *et al.*, 2017).

We hypothesized that (a) round goby represents a predatory threat to smaller crayfish (tested in experiments 1, 2 and 3 in multiple scenarios), (b) this predatory impact and food selection is depending on marbled crayfish sizes available, and (c) round goby is more aggressive and dominates over larger crayfish in competition for shelter (experiments 4 and 5). The goal of the present study was to determine the effect of round goby predation, aggressiveness, and shelter dominance on crayfish under laboratory conditions, using the marbled crayfish as a model for other invasive species.

2 Materials and methods

Round goby (TL 63.54 ± 7.6 mm) were collected from the River Elbe in September 2018 (Ústí and Labem, north of Czech Republic) using a battery powered backpack electrofishing unit (FEG 1500, EFKO, Leutkirch, Germany) while experiments were carried out during November and December 2018. Fish were transferred to the experimental facility of the Research Institute of Fish Culture and Hydrobiology in Vodňany, University of South Bohemia in České Budějovice and held in troughs embedded in a small recirculating system for acclimatization to laboratory conditions. Troughs were filled with aged tap water and cleaned every second day. Fish were fed *ad libitum* with frozen chironomid larvae daily.

Marbled crayfish were obtained from our own culture and fed *ad libitum* on chironomid larvae and carrot daily. Continuous culture enabled the use of all developmental stages in this research.

Animals were weighed using a digital precision balance (Kern 572-35, Kern and Sohn, Germany) to the nearest 0.5 mg. Crayfish total length (TL, from tip of the rostrum to the posterior median edge of telson), carapace length (CL, from tip of the rostrum to the posterior median edge of the cephalothorax) and carapace height (CH) were measured with Vernier calipers, and fish TL (from the tip of the snout to the tip of the tail) was measured with a ruler to the nearest 1 mm. All crayfish individuals were measured (TL, CL, CH) before performing the following experiments (1, 2, and 3) thus separated according to their weight (small, medium, large, small juveniles, medium juveniles) in five different aquariums that later could be used for the experiments (Tab. 1), same as for following experiments (4 and 5) weight-matched pairs of

Table 2. Biometric data of marbled crayfish and round goby used in experiments on competition for shelter (Experiments 4–5). Data are presented as mean ± standard deviation (SD) and range.

Exp.	Animal	Weight (mg)	Total length (mm)
4	Marbled crayfish	116.9 ± 27.5 (60–180)	32.52 ± 14.8 (31–44)
	Round goby	123.5 ± 32.1 (60–190)	44.7 ± 11.29 (38–53)
5	Marbled crayfish	255 ± 10.4 (200–500)	20.61 ± 2.72 (16–25)
	Round goby	272 ± 95.8 (200–500)	57.27 ± 4.02 (52–65)

round goby and crayfish for each experiment were selected and separated before (Tab. 2).

Animals appeared healthy and active and were used only once for each experiment to avoid any learning effect during the experiments. Crayfish with missing or regenerating chelae or showing signs of approaching molt or not fully hardened following molting were omitted from experiments. No specific permissions were required for the location in this study. All facilities used for housing of experimental animals and for experimental procedures were located indoors in separate units with no direct connection to surface waters and were protected against the escape of any organisms used.

2.1 Goby predation on crayfish

2.1.1 Experiment 1, single size predation

Thirty round gobies (TL 74.2 ± 7.25 mm) were placed in separate 27L × 19W × 7.5H cm plastic boxes containing 2 l aged tap water and 150 cm^3 of fine sand for acclimatization and starvation. Water temperature was set at 21 °C and the light regime to 12:12 h light: dark. After 24 h, water was exchanged, and one crayfish was randomly added to each of 30 boxes containing one round goby.

We selected three size classes of crayfish: small (10–25 mg), medium (40–80 mg) and large (100–300 mg) (Tab. 1) with 10 replicates of each size class to assess round goby ability to swallow crayfish of various sizes.

After 24 h we evaluated crayfish type of response with paired goby and we noted if crayfish was consumed, killed and partially consumed or still alive. Following the experiment, we measured round goby total length (TL) and the smallest internal dimension of the fish mouth (here referred as mouth gape) was measured using a set of conical plastic tips consisting of a plastic body with different measurement head attachments. The plastic tip was inserted into the fish mouth until a marked resistance was reached (Supplement 2). In this position, mouth gape size could be determined from fine gradients of the plastic tip to the nearest 0.01 mm.

2.1.2 Experiment 2, choice-size predation

Following exp. 1., we increased crayfish number, so we stocked one round goby (TL 78.0 ± 7.85 mm) and three juvenile crayfish from each size class in 65L × 50W cm elliptical arenas with 650 cm^3 of sand and 10 l water (water level 5 cm). One half of each arena was shaded by an opaque cover to reduce stress during the light period (12:12 h light: dark). The experiment was conducted at 17 °C and at 21 °C (the higher temperature according to prevailing summer water temperatures of River Elbe and the lower one to its slightly colder tributaries). At each temperature, we carried out 18 replicates plus 7 control replicates in which crayfish were stocked without round goby to assess possible cannibalism. At 24 hours post-stocking, we counted the number of crayfish of each size class consumed by round goby and analyzed the association of round goby mouth gape and water temperature on consumption rate and size selection.

2.1.3 Experiment 3, biomass consumption

In the third experiment, single round goby (TL 76.3 ± 3.06 mm) were placed in the experimental elliptical arenas under the same light regime as in the size-choice experiment along with 50 small juveniles or 50 medium juvenile crayfish (Tab. 1). Water temperature was 17 °C. Four replicates were conducted with each class size. After 24 hours, we counted remaining crayfish and measured the weight of surviving and unconsumed crayfish to calculate the biomass consumed per 24 hours.

2.2 Competition for shelter

2.2.1 Experiment 4, daily observations of competition for shelter over 8 days

The experiment was conducted in aquaria (40L × 20W × 25H cm) with a layer of sand (1500 cm^3) and 7 l aged tap water aerated by a single air stone placed in a corner and equipped with a single shelter situated in the middle of the shorter side of the aquarium (half of a ceramic flowerpot, entry diameter: 4.7 cm, height: 4 cm, length 4.5 cm). Round goby and marbled crayfish were weighed to form weight-matched pairs with wet weight difference $<5\%$ (Tab. 2). Prior to the experiment, goby and marbled crayfish were placed separately in 27L × 19W × 7.5H cm plastic boxes with 150 cm^3 sand and 2 l water for 24 hours to standardize the starvation level. The light regime was 12:12 h light: dark and water temperature ~20 °C. The weight-matched pairs were placed simultaneously in each aquarium (20 replications) and observed for the following 8 days. Visual observations of all twenty weight-matched pairs were made only during daylight hours at 08.00, 11.00, 14.00, and 17.00 o'clock for total of 8 days. Animals did not receive supplemental food during the course of the experiment. The position of all individuals was described to record whether an animal was in the shelter, in the proximity of the shelter (near to entry or beside the shelter), hidden in the sand, in a corner, in the corner with the air stone, or active in other areas of the bottom. Mortality and molting events in crayfish were recorded.

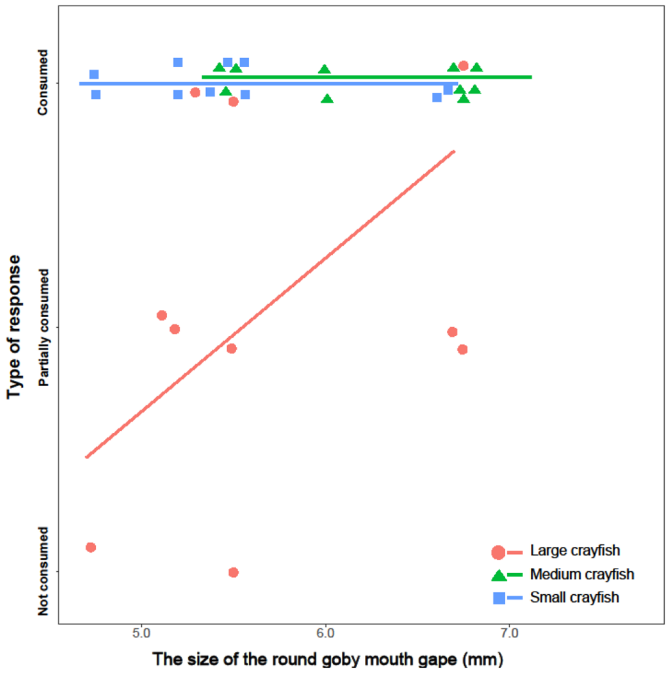

Fig. 1. Relationship of round goby *Neogobius melanostomus* mouth gape and marbled crayfish *Procambarus virginalis* size classes consumed over 24 h.

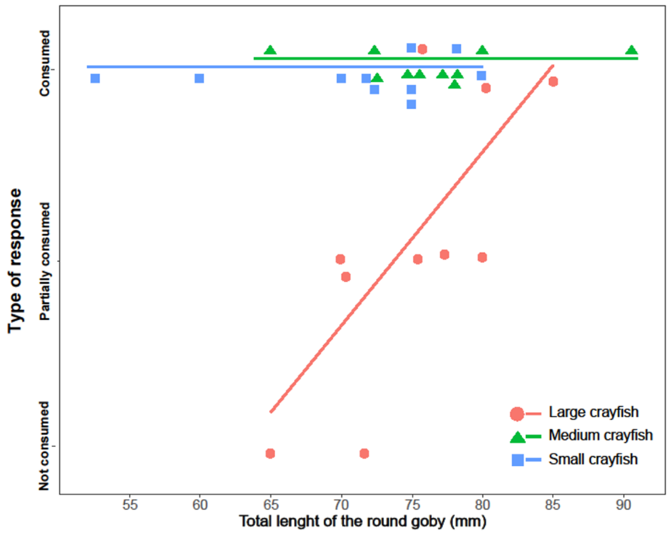

Fig. 2. Relationship of round goby *Neogobius melanostomus* total length and marbled crayfish *Procambarus virginalis* size classes consumed over 24 h.

2.2.2 Experiment 5, twenty-four-hour observation of competition for shelter

We used similar conditions as for the eight-day experiment but with the air stone removed to omit recording disturbance in the experiment of continuous observation for 24 hours. Another 16 pairs of round goby and marbled crayfish were weight-matched (Tab. 2) and acclimated as described in previous experiment. Inter-specific pairs were stocked in aquaria and video-recorded. We conducted 16 replicates. Animal activity was recorded as aggressive interaction (attack, biting, pursuit), time spent in shelter, and avoidance (retreat from opponent prior to attack) (Tabs. 4 and 5), detailed and carefully analyzed by eye. Duration of attack was recorded as the time from the first aggressive act to cessation of interaction (Tab. 5). At the conclusion of the experiment, we measured round goby/marbled crayfish length and weight and assessed injuries.

2.3 Data analysis

Since many data sets did not meet the assumptions for parametric tests, even after transformation, nonparametric tests were used. For experiment 1, we used Firth's bias-reduced penalized-likelihood logistic regression to analyze the type of response between marbled crayfish (which was taken as a factor and assessed as: 0 = not consumed, 0.5 = killed and partially consumed, 1 = consumed) and relationship with round goby mouth gape and total length. The simple linear regression between round goby mouth gape and round goby total length was evaluated. In experiment 2, a generalized linear model (hereafter, GLM) with an assumed quasi-binomial distribution, that accounts for data underdispersion, was used to analyze the quantity of three crayfish size categories consumed and their relationship with round goby mouth gape under two different temperatures. Also, linear relationship between round goby mouth gape and crayfish size class under two temperatures was calculated. For experiment 3, we performed a test using GLM with quasi-binomial distribution, that accounts for data overdispersion, to assess the difference in wet mass of two offered crayfish size groups consumed by round goby. For experiment 4, we used GLM with Poisson distribution. As post-hoc testing was not possible for Poisson distribution errors, results are based on predictions and estimations. We used detailed data from visual observations to better understand interactions of round goby and marbled crayfish over the course of 24 hours. For experiment 5 we performed GLM with Gaussian distribution to analyze the difference in time spent in shelters and to test the number of attacks per animal during the light and dark periods. Analysis was conducted with R software and package ggplot 2 was used for data visualization (R Development Core Team, v. 4.0.3., 2020). In the case of Figures 1-3 points are dispersed within 3 or more lines of the y-axis because of a jitter-like function enabling to visualize individual points without their coverage.

3 Results

3.1 Round goby predation on crayfish

3.1.1 Experiment 1, single size predation

Mean round goby mouth gape (diameter) was 5.7 ± 0.74 (4.7–7.7) mm. The type of response of crayfish due to round goby predation (which was taken as a factor and assessed as: 0 = not consumed, 0.5 = killed and partially consumed, 1 = consumed) did differ significantly between size groups (likelihood ratio test = 16.49; df = 5; $p = 0.005$). The type of response of all size classes of marbled crayfish was correlated with mouth gape and total length of round goby individuals ($p = 0.005$), indicating that round goby was not able to

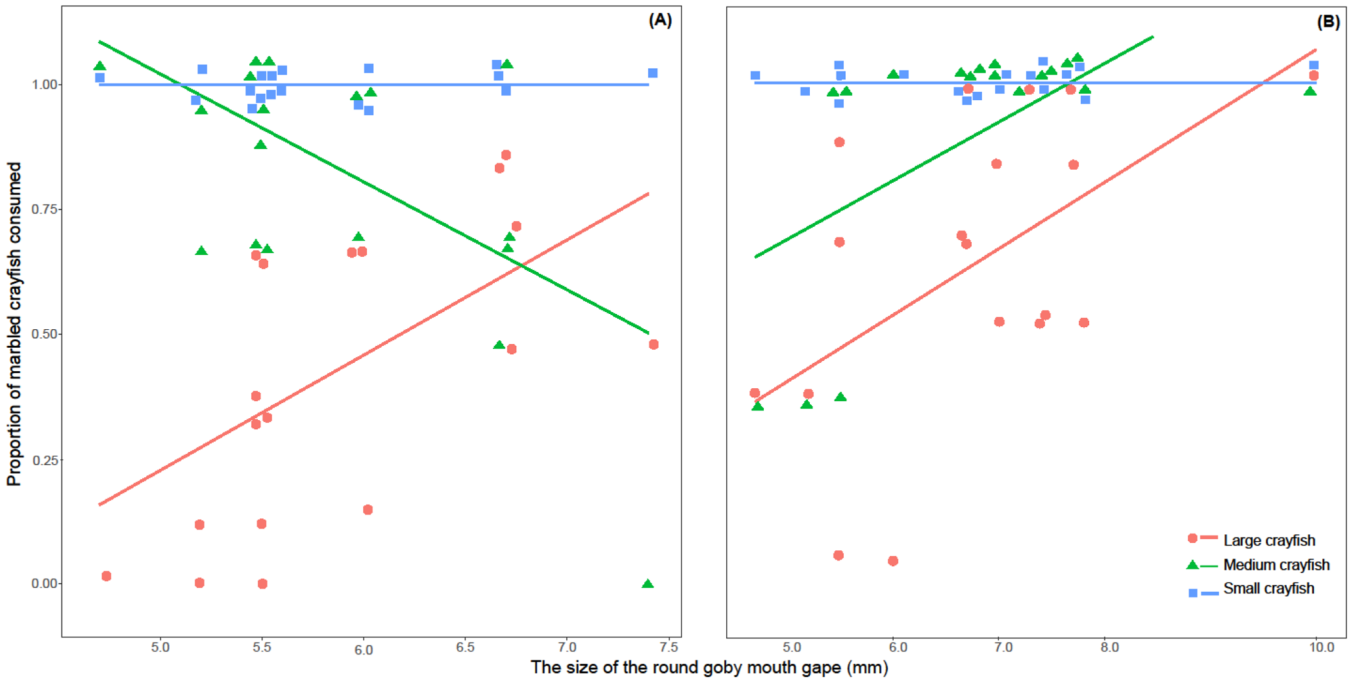

Fig. 3. Relationship of mouth gape of round goby *Neogobius melanostomus* and the proportion of consumed marbled crayfish *Procambarus virginalis* of 3 size classes at 17°C (A) and 21°C (B).

Table 3. Linear relationships between round goby mouth gape and crayfish class size under two temperatures. Negative effect (−), positive effect (+), no effect (0), n = 18.

Crayfish size class	Temperature 17 °C		Temperature 21 °C	
	p-value	R^2	*p*-value	R^2
Small	0.764 (0)	0.480	0.621 (0)	0.472
Medium	0.003 (−)	0.308	0.008 (+)	0.322
Large	0.005 (+)	0.281	0.018 (+)	0.257

consume all provided prey size classes. Due to the significant linear relationship between round goby mouth gape and total length (Supplement 1) we used mouth gape size in further analysis as confounding factor as it is more directly causing the limitation of predation than total length.

Generally, all small and all medium crayfish were consumed (Figs. 1 and 2), while 70% of large crayfish were killed and partially consumed with parts of crayfish found in the experimental arena.

3.1.2 Experiment 2, choice-size predation

Mean mouth gape (diameter) of round goby was 6.2 ± 1.1 (4.7–10) mm. We found a significant relationship among size of the round goby mouth gape and crayfish size class consumption under two different water temperatures ($F_{[2,\ 111]}$ = 17.54, $p < 0.0001$). At 17 °C all small crayfish were eaten in 24 h, with a slightly less number of medium crayfish consumed and few large-sized eaten (Fig. 3). With increasing gape size, round gobies consumed more crayfish of the larger size class, while the number of medium-sized crayfish eaten was negatively correlated with mouth gape (Tab. 3). At 21 °C, all small crayfish were eaten, and the number of consumed crayfish increased compared to 17 °C (Fig. 3). At 21 °C, a positive correlation of mouth gape with the quantity of crayfish of all size classes eaten was observed (Fig. 3, Tab. 3). In general, with an increase of temperature and gape size, predation on crayfish increased. As all small crayfish were consumed at both temperatures, total biomass consumed was 45.6 mg. Average biomass of medium eaten crayfish at 17 °C was 89.29 mg, and at 21 °C was much higher at 156.2 mg. Average biomass of large crayfish eaten was 258.6 mg at 17 °C and 391.95 mg at 21 °C.

3.1.3 Experiment 3, biomass consumption

Mean mouth gape (diameter) of round goby was 5.1 ± 0.34 (4.5–5.5) mm. We found a significant relationship in the number of the two juvenile crayfish classes preyed by round goby ($F_{[1,6]}$ = 11.73, p = 0.01). There was a significant relationship in the amount of consumption of small

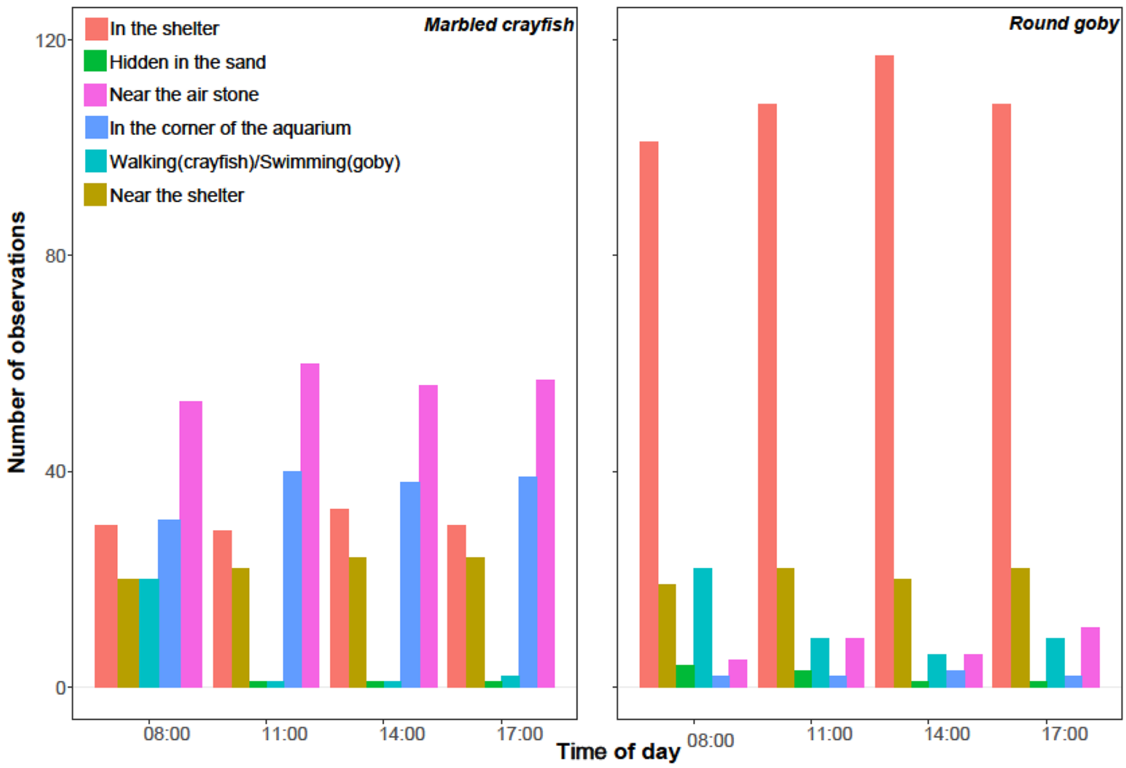

Fig. 4. Number of observations of round goby *Neogobius melanostomus* and marbled crayfish *Procambarus virginalis* at specific locations in the experimental arena with respect to time (08:00, 11:00, 14:00, and 17:00).

(5.7 ± 0.63 mg, n = 10) and medium (13.8 ± 4.82 mg, $n = 10$) crayfish biomass by round goby. Round goby consumed a mean of 285 mg small crayfish juveniles (all 50) and ~593.4 mg of medium juvenile crayfish in 24 h. They consumed fewer of the larger group, in average 43 ± 6.48 medium juveniles. The mean biomass of juvenile crayfish consumed was 45.7% of round goby wet weight.

3.2 Competition for shelter

3.2.1 Experiment 4, daily observations of competition for shelter over 8 days

We made 1225 observations. Round goby spent the majority of time in or near the shelter, while marbled crayfish took alternative shelter near the air stone or in the corner of the aquarium (Fig. 4). By day six, 40% of the crayfish had molted and were subsequently killed and partially eaten by round gobies. A single crayfish (weight: 130 mg/TL: 38 mm) was observed (day two) to capture, kill, and consume a round goby (weight: 120 mg/TL: 50 mm).

A GLM analysis with Poisson distribution (dispersion parameter set at 1) showed the number of observations at a given position to differ significantly ($\chi^2 < 806.89$, df = 5, $p < 0.001$). Individuals were observed leaving positions between recorded time points. The number of observations at specific locations at 08:00, 11:00, 14:00, 17:00 h differed significantly ($\chi^2 = 17.36$, df = 18, $p < 0.01$) as well as the number of observations at the locations at 08:00, 11:00, 14:00, 17:00 h between species ($\chi^2 < 526.03$, df = 6, $p < 0.001$) (see Fig. 4).

Table 4. Mean time spent in shelter by round goby *Neogobius melanostomus* and marbled crayfish *Procambarus virginalis* during light and dark periods. The data analyzed by GLM with Gaussian distribution. Different superscripts indicate significant differences ($p < 0.05$) between species. Data are presented as mean ± standard deviation.

Animal	Photoperiod	Average time spent in shelter (s)
Round goby	Light	15851 ± 11420^a
	Dark	12378 ± 13640^a
Marbled crayfish	Light	22365 ± 18435^b
	Dark	15723 ± 11114^a

3.2.2 Experiment 5, twenty-four-hour observation of competition for shelter

Individuals of both species were always observed to leave the occupied shelter when a specimen of the other species entered. Crayfish pursued goby with open chelae, one capture was observed during the experiment.

We found a significant difference between species in the use of shelter during light and dark periods ($t = 0.51$, $p < 0.05$), as the species alternated in shelter occupancy (Tab. 4). Marbled crayfish spent more time in shelters during light hours. Using GLM analysis, we found a significantly greater number of attacks during the light period than during the dark period by both species ($t = 1.78$, $p < 0.05$) (Tab. 5), with round goby

Table 5. The total number and percentage of attacks observed by round goby *Neogobius melanostomus* and marbled crayfish *Procambarus virginalis* during light and dark periods and the total time spent (s) in aggressive behavior. The data analyzed by GLM with Gaussian distribution. Different superscripts indicate significant differences ($p < 0.05$).

Animal	Photoperiod	Number of attacks (%)	Duration of attacks (s)
Round goby	Light	237 (60.61)	2140[b]
	Dark	127 (54.04)	324[a]
Marbled crayfish	Light	154 (39.39)	926[b]
	Dark	108 (45.96)	274[a]

showing higher aggression. Round goby attacked crayfish more during day, while it was less active during night but still performing attacks more than crayfish (Tab. 5).

4 Discussion

Round goby has colonized major European river systems and coastal waters as well as North American freshwater ecosystems and has the potential to cause ecological regime shifts (Borcherding *et al.*, 2011; Hempel and Thiel, 2013; Roche *et al.*, 2015). Many European lake and stream communities also harbor non-native crayfish species that have considerable impact on native species, such as the signal crayfish *Pacifastacus leniusculus* (Dana, 1852), the spiny cheek crayfish *Faxonius limosus* (Rafinesque, 1817), and, relatively recently, the marbled crayfish *P. virginalis* (Kouba *et al.*, 2014; Kubec *et al.*, 2019). We focused on interaction of round goby, recently spread to upper stretches of the River Elbe (Roche *et al.*, 2015; Buřič *et al.*, 2015), with marbled crayfish as a model species representing the North American crayfish family Cambaridae (Hossain *et al.*, 2019).

Round goby usually preys upon small bottom-dwelling vertebrates and invertebrates (Mikl *et al.*, 2017), but is adaptable to feeding on a wide array of available prey types, including scavenging of carcasses, and particle sizes (Polačik *et al.*, 2009, 2015; Perello *et al.*, 2015). Crustaceans and mollusks represent the most important food items (Brandner *et al.*, 2013). Crayfish can serve as an important food source for predatory fish, as demonstrated by a study of marbled crayfish (Lipták *et al.*, 2019). Due to the results of experiment 1, it is hard to interpret if round goby was size-selective or not, but mouth gape and total length were significant limiting factors for consumption of larger crayfish class. Gobies were able to swallow all small and medium crayfish, but also to kill and partially consume the larger prey as larger crayfish were rather too big for smaller goby individuals. Non-selectivity of round goby was confirmed with mussels as prey (Perello *et al.*, 2015). Ray and Corkum (1997) observed that round gobies spit out the entire mussel shell as well as pieces, similar to our observations of crayfish cuticula in aquaria. Higher temperature induced higher consumption of marbled crayfish (especially medium size class) by round goby. The positive temperature effect on round goby food consumption confirmed results of Lee and Johnson (2005) who showed increased consumption to 23–26 °C. But, results of experiment 2 suggest an interaction in proportion of marbled crayfish consumed between medium and large crayfish size with increasing mouth gape (Fig. 3) at 17 °C but not at 21 °C. This indicates that larger gobies might prefer larger prey at lower temperatures while showing no preference at higher temperatures. As the consumption of larger prey items is more energy-efficient (Sih, 1980), this feeding behavior might be advantageous especially at low temperatures because energy loss is more important for a predator (Rall *et al.*, 2010). In contrast, in exp. 1. bigger crayfish were less consumed but here no free prey size selection was possible as we used pairs of single goby and single crayfish and crayfish were randomly put in aquarium, thus it could be that smaller gobies were combined with bigger crayfish and could not predate on them. Round goby was able to consume a large number of crayfish at higher temperatures without showing signs of size-selectivity. This is contrary to crayfish size selection of smallmouth bass *Micropterus dolomieu* (Lacépède, 1802), which chose the smallest crayfish *Faxonius propinquus* (Girard, 1852) first and then consumed animals in ascending order of size (Stein, 1977), but also noted by other predatory fishes. All in all, round goby in our study consumed from 200 to 500 mg of crayfish biomass per day, which was almost half of their own weight.

Generally, crayfish species share habitats with small bottom-dwelling fish species, such as darters (Mayden *et al.*, 1992) and gobies (Church *et al.*, 2017). Benthic fish can affect crayfish abundance through predation, and crayfish may compete with them for food resources (Thomas and Taylor, 2013). Benthic fish and crayfish naturally overlap in habitat use, with both primarily utilizing natural shelter beneath stones (Kubec *et al.*, 2019), and exhibit a strong temporal overlap in their requirement for shelter (Cooper *et al.*, 2009). In addition, both tested species are mainly nocturnal (Savino *et al.*, 2007; Kornis *et al.*, 2012; Kubec *et al.*, 2019), and competition for shelter is likely to be strongest during the daylight hours (Hill and Lodge, 1994). Despite the fact that round gobies feed more during daylight hours, nocturnal feeding is facilitated by well-developed sensory systems enabling rapid and precise localization and capture of prey (Jude, 1997). With the adaptability to utilize food sources and space upon which crayfish depend (Kornis *et al.*, 2012, 2013), it can be a strong competitor.

The previously reported decreased shelter occupancy by round goby during the night (Dubs and Corkum, 1996; Savino *et al.*, 2007) was confirmed in our study. Crayfish, which show highest activity at dusk and during the night, were also reported to seek shelter during daylight (Bubb *et al.*, 2009). An experiment confronting non-native signal crayfish with bottom-dwelling European bullhead *Cottus gobio* (Linnaeus, 1758), resulted in reduced shelter use by the fish (Bubb *et al.*, 2009). However, our results from the experiment 4 with daily observations showed that round goby spent more time in shelters than marbled crayfish, which used alternative hiding places. Round goby and marbled crayfish spent roughly equal amounts of time in the shelter as well as equal time in avoidance. This could be the result of avoidance after dominance establishment subsequent to aggressive interactions, as well as the dominant status of round goby, as crayfish remained outside shelter more than expected. Bilateral avoidance confirms reciprocal alteration of behavior of the tested species.

Due to the results of experiment 5, observations of aggressive encounters showed that crayfish were usually able to resist round goby attacks, except after molting. Their size itself was not an obstacle to consumption by round goby as long as the carapax was intact. Following molt, even a large crayfish can be extremely vulnerable to predation (Stein, 1977), and it is reported that small crayfish in particular may be vulnerable to predation by round goby when they are soft-shelled immediately following a molt (Ray and Corkum, 1997). Our results showed that even crayfish of similar weight to the fish could be preyed upon by round goby after molting, which is remarkable when the mouth gape size of round goby is taken into account.

Both species showed intense aggressive behavior in experiment 5. Round goby displayed more aggressive behavior than crayfish in our study, as reported by Church *et al.* (2017), but we observed a single instance of a crayfish attacking, killing, and feeding on a round goby. Round goby can hide in the sand and attack crayfish from behind, but were more likely to occupy shelters also used by crayfish. Round goby presence altered the shelter use by crayfish, but round goby behavior is also affected by crayfish presence, demonstrating a mutual interaction of two unrelated invasive species that occupy similar habitats.

Further investigation is necessary to elucidate interactions of round goby and larger crayfish in more complex conditions, as well as to estimate an effect on crayfish population structure. Altered behavior in both species can lead to higher vulnerability to predators (Blake and Hart, 1993; Kubec *et al.*, 2019). Competition for resources can induce increased pressure on resources and accelerate migration to habitats with lower pressure as well as to shifts in ecological niche (Gherardi, 2007). Findings of this study can be useful in the management and conservation of the native North American Cambarid species, but also round goby could exert a control on other species juvenile crayfishes, so eradication of round goby would be considered.

As many other factors are responsible for invader success in natural conditions (Gebauer *et al.*, 2018), the present study provides a baseline for ongoing study of particular scenarios and factors that influence the success and spread of alien aquatic organisms. Our results are in accordance with the known predatory capability of round goby and broaden it from the point of interactions with crayfish representative. Early juvenile crayfish are most vulnerable due to round goby predation. Round goby can possibly cause considerable declines in invasive crayfish species of genus Cambaridae in Europe. Naturally, they can have similar effects on other cambarid species in their native range in North American freshwaters. Hence our results can be easily transferred also to localities in North America inhabited by round goby.

Supplementary Material

Figure S1. The simple linear relationship between round goby mouth gape and round goby total length were undertaken using the default lm function in the R statistical program (R Core Team, 2020). Correlations which had an adjusted $R^2 > 0.5$ and $p < 0.05$ were considered to represent significant relationship. Significant relationships between mouth gape and total length would represent a potential confounding factor in further analysis.

Figure S2. Tools used for mouth gape measurement.

The Supplementary Material is available at https://www.kmae-journal.org/10.1051/kmae/2021019/olm.

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