

## Research Article

# Gut evacuation rate as a tool for revealing feeding patterns in the invasive round goby (*Neogobius melanostomus*) under different feeding modes, food types and temperatures

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## Abstract

The round goby (*Neogobius melanostomus*) is a well-known invasive fish. Knowledge of its feeding habits and means of food processing is key in understanding its impact on aquatic food webs. The present study assessed the gut evacuation rate of round gobies feeding on three different types of prey occurring naturally in the diet of this species (small native freshwater clams, an invasive amphipod and chironomid larvae), at two different temperatures (14 and 20 °C) and under different food availability scenarios (continuous and non-continuous feeding). Gut evacuation rates varied significantly between the prey availability scenarios and, specifically, round gobies processed prey significantly faster in the continuous feeding mode when food was regularly available than when fed only once. The highest evacuation rates were detected for individuals fed with clams, in which complete gut clearance was observed within 16 h, compared to within 24 h and 36 h for chironomid larvae and amphipods, respectively. Our study shows that round gobies evacuate chironomid and mollusc prey most rapidly, which suggests that potentially the highest predatory pressure will be exerted on these prey types, assuming that all three prey species are locally present. The slower processing and digestion of amphipods may be due to their bulkier shape, which makes them more difficult to swallow. The relatively high evacuation efficiency of the round goby observed in the continuous feeding mode suggests overall increased pressure on food resources, thereby potentially reducing availability for other consumers and accelerating resource depletion, mainly driven by the high local densities of the round goby populations.

**Key words:** intestine clearance, prey consumption, predatory impact, non-indigenous species, prey availability

## Introduction

Non-native invasive species are commonly regarded as one of the greatest contemporary threats to the functional, biological and genetic diversity of the world's biota (Dudgeon et al. 2006; Keller et al. 2011; Shuai et al. 2018). Both the magnitude of this problem and the number of newly established non-indigenous species continue to increase as a result of constant globalisation (Seebens et al. 2017, 2021), environmental homogenisation (McKinney and Lockwood 1999; Olden and Poff 2004; Olden and Rooney 2006) and progressive global warming (Leppäkoski et al. 2002; Rahel 2008; Penk et al. 2016). Non-native aquatic species are most often introduced into areas outside their native ranges through human-derived activities, such as ships' ballast water (Jude et al. 1992), aquaculture (Hill 2008), or indirectly through species' natural migration abilities (Šlapanský et al. 2017; Janáč et al. 2019) facilitated by man-made ecological corridors as initial expansion pathways (Bij de Vaate et al. 2002). Once established in new areas, they often have particularly pronounced impacts on native communities and overall ecosystem functioning (Strayer 2010; Simberloff et al. 2013; Pyšek et al. 2020; Ricciardi et al. 2021).

The round goby *Neogobius melanostomus* (Pallas, 1814), which originates from the Ponto-Caspian region and is one of the most notorious examples of an invasive aquatic species, has been the object of much laboratory and field research in recent decades (Cerwenka et al. 2023). Since it was first recorded outside its native range in 1990 in the Gulf of Gdansk (Skóra and Stolarski 1993), this fish has quickly established highly abundant populations (Corkum et al. 2004; Johnson et al. 2005) in areas stretching from the Great Lakes of North America (Jude et al. 1992, Ricciardi and MacIsaac 2000) to numerous Central and Western European rivers (Jurajda et al. 2005; Manné et al. 2013; Roche et al. 2015) and localities around the Baltic Sea (Azour et al. 2015; Quattrocchi et al. 2023). This species possesses many traits that allow it to successfully colonise novel ecosystems (Corkum et al. 2004; Kornis et al. 2012), including, above all, early sexual maturation coupled with short life cycles and small individual adult size (Simonović et al. 2001; Gutowsky and Fox 2012; Masson et al. 2018); a highly effective reproductive strategy (including portioned spawning several times a year) (Kulikova and Fandeeva 1975; Stammer and Corkum 2005); parental care (Sapota 2004; Vivó-Pons et al. 2023); high tolerance towards abiotic factors such as temperature and salinity (Behrens et al. 2017; Christensen et al. 2021); and aggressive behaviour (L'avrinčíková and Kováč 2007; Vivó-Pons et al. 2023). Adding to these, the successful establishment and spread of round goby depend on the ability to exploit food resources in the newly invaded ecosystem, as generally observed in the non-native species, which is usually achieved via opportunistic feeding strategies and the capacity to adapt rapidly (Diggins et al. 2002; Carman et al. 2006; Copp et al. 2008). The introduction of the invasive round goby into a new ecosystem can have a significant impact on food web dynamics, mainly through direct predation on macroinvertebrate communities (Kornis et al. 2012; Barton et al. 2005; Krakowiak and Pennuto 2008; Mikl et al. 2017; van Deurs et al. 2021). Generally, as an invasion progresses, the early stages are characterised by low population densities and abundant potential food sources, which allow invaders to extensively exploit a wide range of resources (Iacarella et al. 2015). This leads to high feeding rates and the rapid expansion of the invading population, as observed in studies by Phillips (2009), Raby et al. (2010) and Burton et al. (2010). By contrast, resource availability is more restricted in mature and established populations with higher conspecific densities where there is greater intraspecific competition (Brownscombe and Fox 2012; Azour et al. 2015).

Most of the numerous studies focussing on characterising the round goby's predatory activity (summarised by Marsden et al. 1996; Kornis et al. 2012; Števo ve and Kováč 2016; Dashinov and Uzunova 2020) have relied on descriptive approaches (via gut content analysis) and only provide limited information on, for example, niche and food preferences in specific localities (Amundsen et al. 1996). While some studies have emphasised the importance of certain taxa in the diet of round gobies (Polačik et al. 2009; Kirilenko and Shemonaev 2012) and the threat this species can pose to macroinvertebrates assemblages (Kuhns and Berg 1999; Barton et al. 2005; Mikl et al. 2017; van Deurs et al. 2021), such methods may overestimate prey significance by not accounting for food digestion rates and dynamics. This approach captures only a snapshot of prey present at a given time, which potentially distorts total consumption estimations since the relative abundance of prey items in the digestive tract may not reflect the proportion of actually consumed prey (Lagru e and Bollache 2006).

Several methods can be employed to quantify prey consumption and thus the predatory impact of a species (Elliot and Persson 1978; Durbin et al. 1983), including indirect methods based on bioenergetic models (Karjalainen et al. 1997; Hartman and Hayward 2007) and a direct method that estimates the predator's daily food intake *in situ* (Sun et al. 2016). The *in situ* approach is based on measuring stomach or gut fullness, which indicates the quantity of prey consumed. It is corrected by the evacuation rate ( $R$ ) that quantifies the mass of food expelled from the digestive tract per unit of time (Talbot 1985; Bromley 1994). This correction is necessary because  $R$  primarily regulates feeding rates (Hedden et al. 2020). While both methods have limitations, *in situ* estimations appear more accurate (Bajkov 1935; Elliott and Persson 1978; Kawaguchi et al. 2007; Richter et al. 2018). This approach can be used under field conditions (Worischka and Mehner 1998; Rindorf 2004) and is based on fewer assumptions (Bartell et al. 1986), which improves its reliability. Hence, knowledge about the evacuation rate can be essential for the correct evaluation of the importance of particular food items found in the fish by preventing the underestimation of easily digestible foods and the overestimation of foods that are more difficult to digest (MacNeil et al. 2001; Gerking 1994). This enables us to potentially estimate the direct impact of non-native fish species on food webs and their indirect influence on native fish through competition for food.

The evacuation rate can be affected by various factors including which part of the digestive tract is considered, i.e. the stomach, foregut or the entire tract (Bromley 1994; Grove and Crawford 1980). However, temperature and predator size are considered to be the most important factors (Noble 1973; Garber 1983; Mills et al. 1984; Temming and Andersen 1992; Temming et al. 2002). Multiple studies have confirmed that there are significant variations in evacuation rates difficult to digest between prey species (Jones 1974; Macdonald et al. 1982), primarily due to their body structures (Singh-Renton 1990; dos Santos and Jobling 1992; Andersen 1999) since, in general, hard-bodied prey tends to be expelled much more slowly than soft-bodied items (Jones 1974). Given that an inverse dependency has also been observed (Hölker and Temming 1996), an analysis of the integument structure of the prey (Macdonald et al. 1982; Couturier et al. 2013), as well as its nutritional value and content (Bromley 1994; Zhu et al. 2015; Bonvini et al. 2018), should also be undertaken.

In terms of feeding strategies, previous experimental studies have primarily focused on quantifying the evacuation rate after single-food treatments (Pääkkönen and Marjomäki 1997; Bromley 1987). However, Tekinay et al. (2003) highlight certain limitations associated with this approach, largely because it does not replicate the natural feeding rhythms and evacuation patterns usually observed in nature, especially in omnivorous species (Hofer et al. 1982; Bromley 1994). Notably, the application of multiple meals (continuous feeding) has been reported

to induce a significantly higher evacuation rate (García-Ortega et al. 2010) in comparison to fish fed only once (Bromley 1988). Considering the pivotal role of both food resource availability and species-specific traits in determining the success of biological invasion processes (Phillips 2009; Brownscombe and Fox 2012; Vivó-Pons et al. 2023), we opted to compare these two feeding regimes in this study.

Comprehensive data on the consumption rates of individual prey species (including the gut evacuation rate) providing accurate evaluations of the predation pressure exercised by the highly invasive round goby are still lacking. Therefore, while considering how the round goby's diet changes in terms of ontogeny, season, daytime and environmental conditions, which can lead to shifts in food resource dynamics and availability (Walsh et al. 2007; Pennuto et al. 2010; Borcherding et al. 2013; Bhagat et al. 2015; Števo ve and Kováč 2016; Olson and Janssen 2017), we aimed to estimate and test gut evacuation rates in the round goby in terms of different prey types and under variable food availability scenarios (continuous feeding vs. non-continuous feeding), and diverse thermal conditions.

Based on the previous findings outlined above, we hypothesized that gut evacuation rates would differ depending on the type of prey (hard-bodied vs. soft-bodied prey), temperature conditions, and prey availability. We predicted that the round goby would exhibit faster food evacuation rates when fed continuously, evacuate soft-bodied prey more rapidly than hard-bodied ones, and have faster evacuation rates at higher temperatures.

## Materials and methods

### Collection and maintenance of study species

The round gobies were collected in late May and at the end of August 2019 using electrofishing (a backpack-pulsed-DC electrofishing unit ELT60II GI HONDA GXV 50; Hans Grassl GmbH, Germany) in the river Elbe near Dolní Žleb (North Bohemia, Czech Republic GPS: 50°50'39.9"N, 14°13'01.5"E) from a recently established population (Buřič et al. 2015). Specimens representing the most common size cohort at the invaded locality (total length, TL = 55–75 mm) were transported to the experimental facilities of the Institute of Aquaculture and Protection of Waters FFPW USB in České Budějovice and acclimated in a recirculating aquaculture system (RAS, total volume of 1600 l) for at least one week prior to the experiments. Physical-chemical water parameters such as, temperature ( $20.22 \pm 0.07$  and  $14.19 \pm 0.04$  °C), oxygen saturation ( $94.05 \pm 0.25$  and  $92.05 \pm 0.33\%$ ) and pH ( $7.67 \pm 0.02$  and  $7.68 \pm 0.02$ ), were controlled on a daily basis using an HQ40d digital multimeter (Hach Lange GmbH, Düsseldorf, Germany). The experimental temperature was gradually adjusted by approximately 1 °C every four days during the initial acclimatization period.

In accordance with our observations of the invaded localities along the river Elbe, as well as findings from other river systems (Ghedotti et al. 1995; Ray and Corkum 1997; French and Jude 2001; Diggins et al. 2002; Barton et al. 2005), three different types of prey (molluscs, amphipods and insect larvae) were chosen to represent the predominant macrozoobenthic groups in the round goby's diet (e.g. Phillips et al. 2003; Borza et al. 2009; Števo ve and Kováč 2016; Všeticková et al. 2018). Specifically, the killer shrimp *Dikerogammarus villosus* (Sowinsky, 1894), pill clam (*Pisidium* sp.) and chironomid larvae (*Chironomus* sp.) were used. Specimens of *D. villosus* were collected at the same localities as the round gobies were captured. *Pisidium* sp. were sampled in a tributary pond (Točník, South Bohemia, Czech Republic GPS: 48°56'57.8"N, 14°55'39.3"E), while live chironomid larvae were purchased from a pet store.

## Experimental design

Based on the modified methodology proposed by Richter et al. (2018), we performed a series of six experimental trials to test gut evacuation rates in round gobies under different prey availabilities (two different feeding modes), prey types (three different prey items) and seasons. The latter were represented by two temperatures corresponding to the common/actual thermal conditions of the water at invaded localities on the river Elbe (taken from long-term data gathered during field surveys) in summer ( $T = 20 \pm 0.035$  °C; mean  $\pm$  SE; season A; series of experiments 1–3 performed in July 2019) and spring/autumn ( $T = 14 \pm 0.027$  °C; mean  $\pm$  SE; season B; series of experiments 4–6 performed in October 2019). Prey availability was varied by providing (i) food only at the beginning of the experiment (non-continual feeding) or (ii) continuously throughout the experiment (continual feeding).

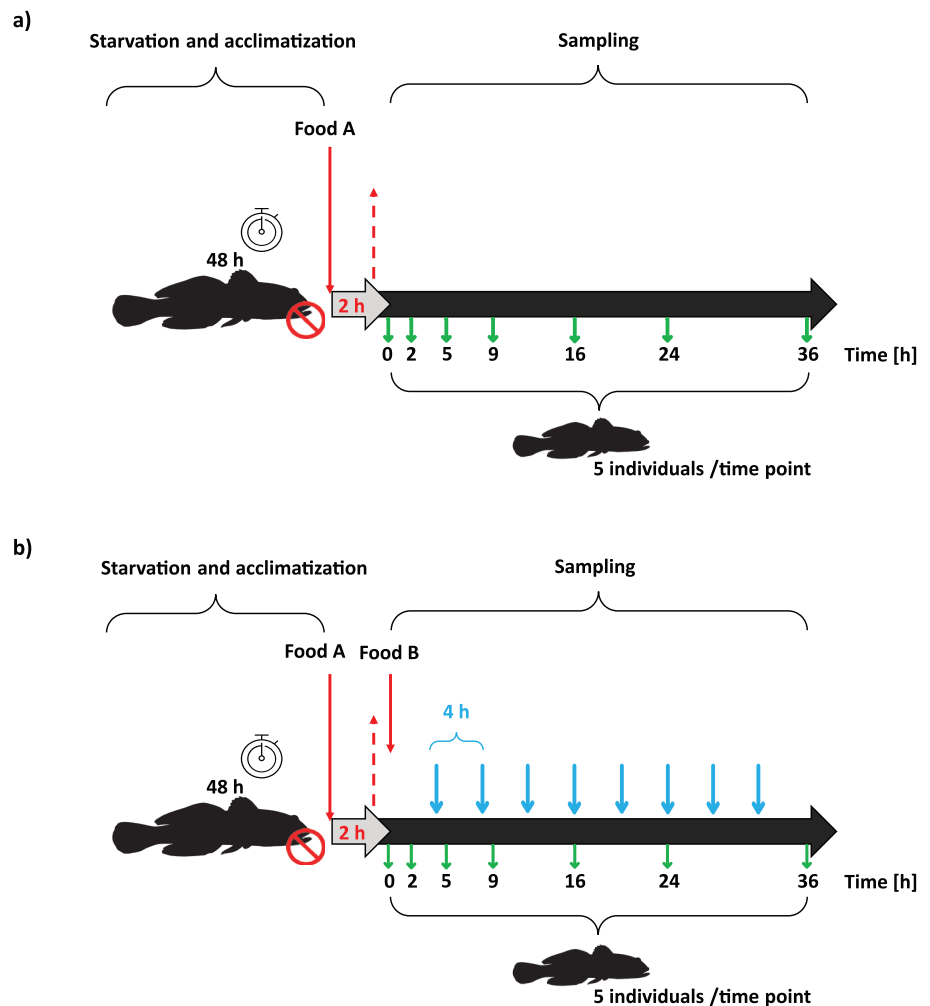
Fish were starved for 48 h prior to each experimental trial to ensure the complete emptying of their alimentary tracts. To avoid possible cannibalism provoked by starvation and to ensure that a particular individual consumed a given amount of food, all fish were placed separately in experimental arenas (plastic boxes, each with a total volume of 2.4 l and water volume of 2.2 l) equipped with aeration and shelter. All experimental arenas had opaque walls to prevent visual contact between tested fish or any other visual disturbance that might represent an additional stressor. The light regime was set to 14 h light (intensity of 1000 lux.m<sup>2</sup>): 10 h dark, corresponding to the light regime during the vegetation season in the field, with simulations of dusk and dawn. The water parameters (T, O<sub>2</sub> and conductivity) in each experimental arena were monitored daily.

After the 48-hour starvation period, the round goby were fed with the tested food (food A) consisting of one of three different types of prey depending on the trial number (Table 1a, Fig. 1). Each type of prey was provided in quantities corresponding to similar weights to standardise the initial mass between treatments, regardless of the prey species (Table 1b). Subsequently, two hours later, any remains of tested prey (food A) were removed from all experimental arenas, which signalled the start of the main experiment phase and sampling. Fish whose tested prey (food A) intake was not observed were excluded from the experiment. Then, experimental arenas were randomly marked with colour clips to distinguish between non-continuous and continuous feeding modes. Following this, half of the individuals were not fed with any additional prey (the non-continuous feeding mode) (Fig. 1a), while the other half of the fish were continuously fed with a different type of prey (food B) (Table 1a, Fig. 1b), which was easily distinguishable to ensure the correct identification of the tested type of prey (food A) during subsequent fish dissection. To maintain constant food availability and experimental arenas were checked every four hours (Fig. 1b).

In total, we tested 420 fish, of which 70 were sampled during each experimental trial (which included one tested prey type, one temperature and two feeding modes) over 36 h at seven time points: 0, 2, 5, 9, 16, 24 and 36 h after the start of the main part of the experiment trial. At each time point, five randomly chosen fish from each feeding mode (non-continuous and continuous) were sampled. The collected fish were killed by overdosing with an MS-222 anaesthetic solution before being immediately frozen in dry ice to inhibit all metabolic processes in the digestive system. Specimens were then stored in a deep freezer (-80 °C) until processing, which took place within maximum of two weeks.

**Table 1.** Description of tested food and mean wet weights of experimental prey species. a) Combinations of tested food (Food A) and easily distinguishable additional food (Food B) used in the experiment. b) The mean wet weights of the experimental food prey species used in estimating the interaction between prey type and the gastric evacuation rate of round goby and their corresponding numbers of provided prey individuals *n*.

a)		
Experiment no.	Food A (tested food)	Food B (additional food)
1,4	<i>Chironomus</i> sp. larvae	<i>Dikerogammarus villosus</i>
2,5	<i>Pisidium</i> sp.	<i>Chironomus</i> sp. larvae
3,6	<i>Dikerogammarus villosus</i>	<i>Pisidium</i> sp.
b)		
Prey type	Wet weight [g (mean ± S.D.)]	<i>n</i>
<i>Chironomus</i> sp. larvae	0.0620 ± 0.0085	12
<i>Pisidium</i> sp.	0.0746 ± 0.0154	6
<i>Dikerogammarus villosus</i>	0.0696 ± 0.0153	4



**Figure 1.** Gut evacuation rate experimental design. (a) Non-continual feeding mode; (b) Continual feeding mode. Solid red arrows represent the application of tested food (Food A) and additional food (Food B); dashed lines indicate the removal of tested food (food A) remains. Blue arrows represent the checking times (every 4 hours) when additional food (Food B) was provided if necessary to ensure continuous feeding. Green arrows represent the sampling time points.

## Sample processing

Before the gut determination of the round gobies was performed, measurements of total length (TL; mm), standard length (SL; mm) and body weight (W; g) were taken, and their sex determined (Kornis et al. 2012). Subsequently, fish were individually dissected. The gut extraction was conducted following Manko (2016). The removed complete gut (together with the food content) was weighed (EX224M analytical balance, Ohaus Corporation, USA), its length measured, and the position of each food type (food A and B) observed. Then, the gut content (foods A and B separately) was carefully placed on previously heated (30 minutes at 500 °C in a muffle furnace) and weighed glass-microfibre discs (grade MGF, diameter 50 mm, Sartorius, Goettingen, Germany) to determine its wet weight (WW) with an analytical microbalance (M3P, Sartorius, Goettingen, Germany). Finally, samples of the gut content (food A and B), the fish carcasses and the empty dissected gut were dried at 105 °C overnight and stored in a desiccator to determine their dry weight (DW) with a microbalance.

## Statistical analyses

The evacuation rate ( $R$ ), represented by the regression coefficient, was determined through statistical modelling of the relative gut content weight over time. The relative gut content ( $GI$ ) was defined as the proportion of the dry weight (DW) of the tested food (food A) remaining in the gut at a given time vs. the fish's dry body weight. The evacuation rate, expressed as the proportion lost from the gut per hour, can be described by several models, the most frequently used of which are linear and exponential functions (Elliott and Persson 1978; Bromley 1994). We found that the exponential function was the most suitable for our data as it had the fewest residual variations. Specifically, we applied the model represented by

$$GI_t = GI_0 e^{-Rt}$$

where  $GI_t$  is the relative gut content at time  $t$ ,  $GI_0$  is initial intestine content at time  $t_0$  after feeding (h), and  $R$  is the evacuation rate (Elliott and Persson 1978).

We fitted the equation using generalised linear models (GLMs) with a Gaussian distribution (the log link function applied) and tested the relationship between the relative gut content  $GI$  used as a response variable and explanatory variables: (I) 'Time' (continuous numerical variable), (II) 'Prey availability' (two-level factor), and (III) 'Prey type' (three-level factor) separately for each season (see below). The improvement of the fitted relationships by the addition of interaction terms – one- and two-order interactions between all given explanatory variables – was tested using likelihood ratio tests. In order to avoid errors with log-transforming observations of empty guts at specific times, all zero values of relative gut content were replaced by 0.871 mg to represent half of the overall minimum gut content mass. The use of a Gaussian distribution was appropriate given that the histogram of model residuals was found to be close to the Gaussian; the assumption of homoscedasticity was also fulfilled. The significances of all relationships were tested by partial  $F$ -tests.

The independence of the initial consumption of prey and size of fish – used as explanatory variables – during the experimental treatments was tested given that this issue may potentially affect the obtained conclusions. Using GLMs with the binomial distribution (logistic link function applied), the consumption probability was detected as significantly greater when round gobies were fed with chironomids

than when fed with either *Dikerogammarus villosus* or *Pisidium* sp. (Suppl. material 1;  $p < 0.05$ ). Therefore, individual values of  $GI$  were weighted by the proportions between prey consumed by the round goby and the total amount of offered prey, i.e. a weighted GLM was used to fit a shift in  $GI$ . In addition, significantly different consumption probabilities and significant differences in  $SL$  (tested using GLM with gamma distribution with the log link function applied) were also detected between the two seasons (Suppl. material 3;  $p < 0.05$ ). Thus, two separate models with  $GI$  as a response were fitted for each 'season' to yield the regression coefficients representing the evacuation rate. Nevertheless, the fitted round goby  $SL$  in season B was only 2.06 mm higher than in season A, whilst  $SL$  variance was very low and similar in both seasons (Suppl. material 2). Based on these findings and given that it was the focus of our study, the factor 'season' (which represents different temperatures and temporarily distinct round goby stocks) was also left as the explanatory variable in a single  $GI$  model to test the significance of its additive effect. No other significant differences in the consumption probability or  $SL$  range for any explanatory variables of the interactions were detected ( $p > 0.05$ ; sporadically marginal interactions detected). All data analyses and graphical procedures were performed using R software (CRAN, version 4.1.1).

## Results

A total of 420 round gobies (TL: 53–80 mm; mean  $\pm$  SD = 67.7  $\pm$  5.53 mm) were tested in a series of six experiments.

Seasonal variation represented by two different thermal conditions (14 and 20 °C) had a significant additive effect on changes in the relative gut content ( $p < 0.001$ , Table 2, Suppl. material 2). Specifically, in Season A (20 °C), a 12.4% decrease in  $GI$  was observed compared to Season B (14 °C), based on the respective regression coefficients.

### Season A: 20 °C

The second-order interaction of 'Time  $\times$  Prey availability  $\times$  Prey type' in modelling effects on the relative gut content ( $GI$ ) was significant ( $p < 0.01$ , Table 3) and both 'Prey type' and 'Time' had significant effects on the relative gut content itself (Table 3).

**Table 2.** Relationships between relative gut content ( $GI$ ) and explanatory variables, i.e. prey availability, prey type, season and time. Weighted generalised linear models (GLM) with Gaussian distribution (with the log link function applied) were used to fit and test the relationships. The significances of all relationships were tested using partial  $F$ -tests. Asterisks following  $p$ -values denote significance. Model  $R^2 = 0.77$ .  $df$  = degrees of freedom.

<i>GI</i> ~ Prey availability $\times$ Prey $\times$ Time + Season					
Variable	<i>df</i>	Deviance	<i>F</i> value	<i>p</i> -value	
Prey availability	1	0.0074	0.03	0.865	
Prey	2	0.0078	13.47	<0.001	***
Time	1	0.0090	90.06	<0.001	***
Season	1	0.0077	18.90	<0.001	***
Prey availability $\times$ Prey	2	0.0075	3.36	0.036	*
Prey availability $\times$ Time	1	0.0101	153.42	<0.001	***
Prey $\times$ Time	2	0.0074	1.07	0.344	
Prey availability $\times$ Prey $\times$ Time	2	0.0079	15.19	<0.001	***
Residual deviance: 0.0074 on 407 residual <i>df</i>					



**Table 3.** Relationship between relative gut content ( $GI$ ) and prey availability, prey type and time evaluated for both seasons (seasons A and B) separately. Generalised linear models (GLMs) with Gaussian distribution (with the log link function applied) were used to fit and test the relationships. Season A:  $R^2 = 0.75$ ; Season B:  $R^2 = 0.82$ ,  $df$  = degrees of freedom. The significances of all relationships were tested using partial  $F$ -tests. Asterisks following approximal  $p$ -values represent different significance levels: \* 0.05, \*\* 0.01, \*\*\* 0.001.

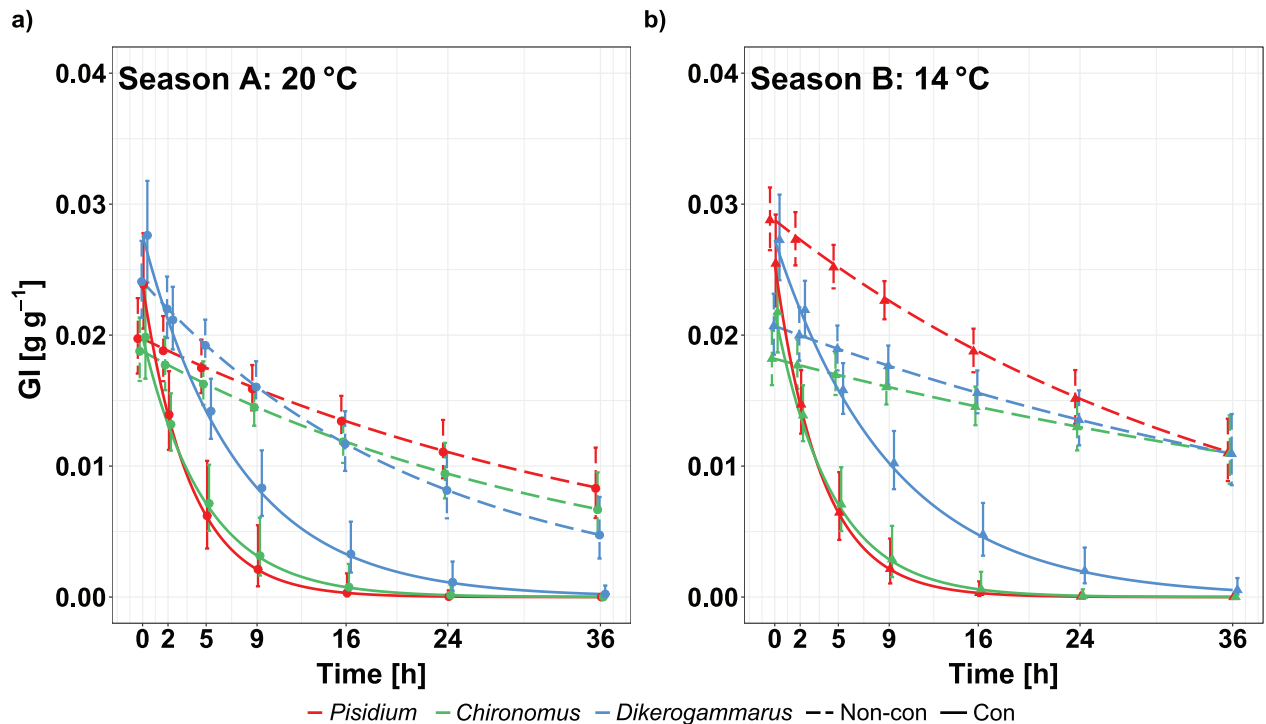
<b>Model: <math>GI \sim</math> Prey availability <math>\times</math> Prey <math>\times</math> Time</b>					
<b>Season A</b>					
<b>Variable</b>	<b><math>df</math></b>	<b>Deviance</b>	<b><math>F</math> value</b>	<b><math>p</math>-value</b>	
Prey availability	1	0.0036	3.00	0.085	.
Prey	2	0.0037	3.92	0.022	*
Time	1	0.0040	24.13	<0.001	**
Prey availability $\times$ Prey	2	0.0036	0.39	0.665	
Prey availability $\times$ Time	1	0.0046	58.39	<0.001	***
Prey $\times$ Time	2	0.0037	2.58	0.078	.
Prey availability $\times$ Prey $\times$ Time	2	0.0038	5.33	0.006	**
Residual deviance: 0.0036 on 198 residual $df$					
<b>Season B</b>					
Prey availability	1	0.0032	2.46	0.118	
Prey	2	0.0039	22.50	<0.001	***
Time	1	0.0043	70.84	<0.001	***
Prey availability $\times$ Prey	2	0.0034	6.71	0.002	**
Prey availability $\times$ Time	1	0.0049	103.82	<0.001	***
Prey $\times$ Time	2	0.0033	2.76	0.066	.
Prey availability $\times$ Prey $\times$ Time	2	0.0036	11.35	<0.001	***
Residual deviance: 0.0032 on 198 residual $df$					

The analyses revealed that in continual feeding mode, the highest evacuation rate was for the *Pisidium* sp. ( $R = 0.2694 \pm 0.044$  g·g<sup>-1</sup> fish·h<sup>-1</sup>; mean i.e. grams of prey per gram of fish per hour  $\pm$  SE; further h<sup>-1</sup>; Table 4a) as complete food depletion took place 16 h after food intake (Fig. 2a), while chironomid larvae were completely evacuated within 24 hours, representing the second highest value in terms of evacuation rate ( $R = 0.2043 \pm 0.029$  h<sup>-1</sup>). *Dikerogammarus villosus* had the lowest evacuation rate of all prey types ( $R = 0.1330 \pm 0.013$  h<sup>-1</sup>), with total food depletion taking place only after 36 hours (Fig. 2a). Conversely, the lowest evacuation rate in non-continual feeding mode was observed for *Pisidium* sp. ( $R = 0.02407 \pm 0.002$  h<sup>-1</sup>; Table 4a) and the fastest for *Dikerogammarus villosus* ( $R = 0.04516 \pm 0.005$  h<sup>-1</sup>; Table 4a), the latter represented by the lowest relative gut content 36 h after food intake. None of the tested 'Prey types' were completely digested within 36 h in the non-continual feeding mode (Fig. 2a).

### Season B: 14 °C

The second-order interaction of 'Time  $\times$  Prey availability  $\times$  Prey type' in modelling effects on the relative gut content was significant ( $p < 0.001$ , Table 3) and the effect of 'Prey type' on the relative gut content was also significant ( $p < 0.001$ ).

The evacuation rates in the continual feeding mode varied between ( $R = 0.1091 \pm 0.009$  h<sup>-1</sup>) for *Dikerogammarus villosus* and the highest evacuation rate ( $R = 0.2746 \pm 0.032$  h<sup>-1</sup>) for *Pisidium* sp. (Table 4b), which significantly differed from the evacuation rate of chironomid larvae under the same feeding mode (Fig. 3b). The round gobies tested in continual feeding mode evacuated all the experimental food within 36 h (*D. villosus*), with complete gut evacuation after 24 h for chironomid larvae and after 16 h for *Pisidium* sp. (Fig. 2b). The lowest evacuation rate in the non-continual feeding mode was observed for chironomid larvae ( $R = 0.01408 \pm 0.002$  h<sup>-1</sup>) and the highest evacuation



**Figure 2.** Change in the relative gut content ( $GI$ ) over time in the round goby (*Neogobius melanostomus*) in two different seasons (season A represented by  $T = 20\text{ }^{\circ}\text{C}$  and season B represented by  $T = 14\text{ }^{\circ}\text{C}$ ) fed with three different prey species: 1. *Pisidium* sp. (red); 2. *Chironomus* sp. larvae (green); 3. *Dikerogammarus villosus* (blue), and in two different feeding modes: 1. Non-con = non-continual feeding mode (shown by dashed lines) and 2. Con = continual feeding mode (shown by solid lines). Relative gut content expressed as a ratio of the dry weight (DW) of the tested food (food A) for each treatment at the time of sampling vs. fish dry body weight [ $\text{g}\cdot\text{g}^{-1}$ ]. Whiskers represent 95% confidence intervals around mean estimates.

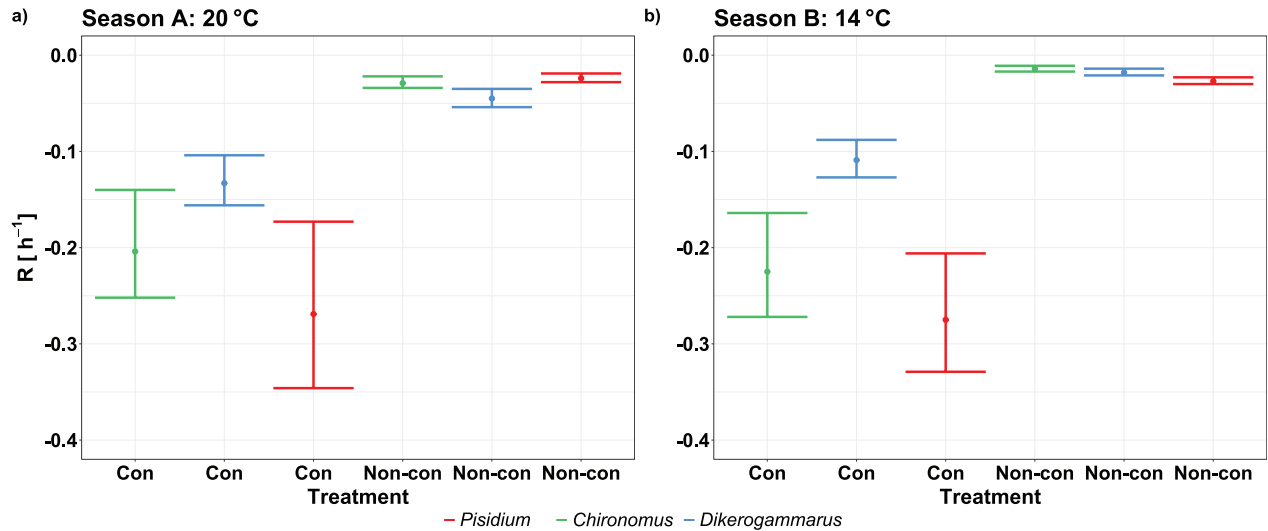
rate for *Pisidium* sp. ( $R = 0.02676 \pm 0.002\text{ h}^{-1}$ ) (Table 4b). None of the tested 'Prey types' were completely digested within 36 h in non-continual feeding mode (Fig. 2b).

### Prey type-specific differences between seasons

We observed prey-type-specific differences in evacuation rates between seasons. For *Dikerogammarus villosus*, the highest evacuation rate was observed in season A ( $20\text{ }^{\circ}\text{C}$ ) for both continual and non-continual prey availability (Fig. 2, Table 4). Conversely, all individuals fed with *Pisidium* sp. had faster evacuation rates in season B ( $14\text{ }^{\circ}\text{C}$ ) in both feeding modes (Table 4). Overall, the fastest evacuation rate for the *Chironomus* sp. larvae was observed in the continual prey availability at the lower temperature, i.e. in season B ( $14\text{ }^{\circ}\text{C}$ ) (Table 4). For the non-continual feeding mode, the evacuation rate of chironomid larvae observed in season A ( $20\text{ }^{\circ}\text{C}$ ) ( $R = 0.02873 \pm 0.003\text{ h}^{-1}$ ) was faster than for the lower temperature conditions (Table 4).

### Gut evacuation as a function of the food availability

According to the 95% confidence intervals, there was no observed overlap between the two feeding modes in the mean values of the evacuation rates in each season (Fig. 3), which indicates that there were significant differences between them. All prey types were digested and evacuated significantly faster in round gobies fed continuously (Table 4). By contrast, in the non-continual feeding mode, no round goby had completely evacuated its gut content 36 hours after feeding, unlike the continuously fed fish (Fig. 2).



**Figure 3.** Overlap between the 95% confidence intervals of the gastric evacuation rates ( $R$ ;  $n = 420$ ) for all experimental treatments (three different prey types and two feeding modes) in both experimental seasons ( $T = 14\text{ }^{\circ}\text{C}$  and  $T = 20\text{ }^{\circ}\text{C}$ ) in the round goby (*Neogobius melanostomus*).

**Table 4.** Gastric evacuation rates  $R$  [ $\text{h}^{-1}$ ] in the round goby (*Neogobius melanostomus*) under different treatments. Each experimental treatment combined different season (water temperature), prey type and feeding mode. A, Season A (temperature  $20\text{ }^{\circ}\text{C}$ ). B, Season B (temperature  $14\text{ }^{\circ}\text{C}$ ). Evacuation rates (mean  $\pm$  SE) are given as absolute values, while values in parentheses represent real  $R$  values. Prey types: pill clam (*Pisidium* sp.), chironomid larvae (*Chironomus* sp.) and killer shrimp (*Dikerogammarus villosus*). For the gastric evacuation rate the 95% confidence interval (95% CI) is also presented.

Treatment			$R$ [ $\text{h}^{-1}$ ]		95% CI	
Season	Prey availability	Prey type	(mean $\pm$ SE)		lower	upper
A	Non-continual	<i>Pisidium</i> sp.	<b>0.024</b> (-0.024)	$\pm 0.002$	-0.028	-0.019
A	Non-continual	<i>Chironomus</i> sp. larvae	<b>0.029</b> (-0.029)	$\pm 0.003$	-0.034	-0.022
A	Non-continual	<i>Dikerogammarus villosus</i>	<b>0.045</b> (-0.045)	$\pm 0.005$	-0.054	-0.035
A	Continual	<i>Pisidium</i> sp.	<b>0.269</b> (-0.269)	$\pm 0.044$	-0.346	-0.173
A	Continual	<i>Chironomus</i> sp. larvae	<b>0.204</b> (-0.204)	$\pm 0.029$	-0.252	-0.140
A	Continual	<i>Dikerogammarus villosus</i>	<b>0.133</b> (-0.133)	$\pm 0.013$	-0.156	-0.104
<b>b)</b>						
Treatment			$R$ [ $\text{h}^{-1}$ ]		95% CI	
Season	Prey availability	Prey type	(mean $\pm$ SE)		lower	upper
B	Non-continual	<i>Pisidium</i> sp.	<b>0.027</b> (-0.027)	$\pm 0.002$	-0.030	-0.023
B	Non-continual	<i>Chironomus</i> sp. larvae	<b>0.014</b> (-0.014)	$\pm 0.002$	-0.017	-0.011
B	Non-continual	<i>Dikerogammarus villosus</i>	<b>0.018</b> (-0.018)	$\pm 0.002$	-0.021	-0.014
B	Continual	<i>Pisidium</i> sp.	<b>0.275</b> (-0.275)	$\pm 0.032$	-0.329	-0.206
B	Continual	<i>Chironomus</i> sp. larvae	<b>0.225</b> (-0.225)	$\pm 0.028$	-0.272	-0.164
B	Continual	<i>Dikerogammarus villosus</i>	<b>0.109</b> (-0.109)	$\pm 0.009$	-0.127	-0.088

## Discussion

### Impact of food availability on round goby gut evacuation rates

Our study demonstrates differences in evacuation rates in the round goby, with the continual feeding mode resulting in the higher evacuation rates compared to the non-continual feeding mode. Multiple-food application during the continual feeding mode accelerated the process, causing fish to evacuate particular food types 5–10 times faster than those fed only once (Table 4). These observations are consistent

with previous studies (Noble 1973; Jones 1974; Elliott 1975; Persson 1981, 1982) demonstrating that the presence of additional food leads to higher evacuation rates (Tekinay et al. 2003). This can be seen by the steeper slope in Fig. 2, which indicates more rapid relative gut-content loss over time. This can be attributed to mechanical factors whereby the tested prey is pushed along the digestive tract by subsequently ingested food (Riche et al. 2004), possibly aided by the distribution of mucus-secreting goblet cells (Hur et al. 2016). However, the evacuation process is preceded by muscular and enzymatic activities (Nilsson and Brönmark 2000). Thus, one possible explanation for our findings could be intensified and accelerated metabolism involving greater enzymatic activity due to increased gut content (O'Connor et al. 2000), where the higher quantity of food, compared to fish fed only once, may affect digestive enzyme activity (Peres et al. 1998). Moreover, using two types of prey (Food A, Food B) can induce a potential synergistic metabolic response, where the digestion and metabolism of one prey type influence the processing of another, especially considering that different diet compositions (varied prey types) can selectively activate specific enzymes (German et al. 2004). Therefore, the biochemical composition appears to be a crucial factor (Caviedes-Vidal et al. 2000), affecting overall gut transition and digestion processes, which may be heightened in response to temporary increases in specific nutritional prey content (German et al. 2004). This effect is particularly significant for species known for their diverse diet, indicating modulation of overall digestive enzyme activities on a larger scale (Karasov 1992).

At the same time, differences in food digestion and evacuation rates according to food availability coincide with field observations since, in the early phases of invasions, individuals of a non-native species take advantage of low population densities and abundant food supply to exploit a wide range of accessible resources (Iacarella et al. 2015). This leads to higher feeding rates and rapid growth of the invading population (Phillips 2009; Burton et al. 2010; Raby et al. 2010). By contrast, in mature and established populations with higher conspecific densities resource availability is more restricted and leads to intensified intraspecific competition (Brownscombe and Fox 2012; Azour et al. 2015).

### Prey type-specific differences in gut evacuation rates

We expected to observe differences between fish fed with different prey species since prey body structure influences evacuation rates (Jones 1974; Singh-Renton 1990; dos Santos and Jobling 1992; Andersen et al. 1999). In our study, round gobies exhibited the fastest evacuation rates when fed on *Pisidium* sp. in both seasons (under different thermal conditions) and when food was continuously available. Those observations contradict the pattern observed in the majority of the studies whereby soft-bodied prey is digested and evacuated faster than hard-bodied prey (Jones 1974; Singh-Renton and Bromley 1999; Temming and Herrmann 2003). Typically, an external protective body structure such as a shell or exoskeleton acts as a barrier for digestive juices and slows down the digestion process (Jones 1974; dos Santos and Jobling 1992; Bromley 1994). One of the possible explanations behind our observations is that hard-shelled food resembles 'high-fibre' food or roughage and thus is transported faster through the digestive tract (Hilton et al. 1983). Moreover, it is important to consider species' digestive capabilities, which depend on differences between fish species in morphological features and levels of organisation in the digestive tract structure, in which the primary concern is to ensure optimum use of dietary nutrients (Ray and Ringø 2014). Therefore, depending on diet preferences and feeding behaviour, particular species adapt (Olsen and Ringø 1997; Ringø et al. 2003), which enhances the efficiency of their food processing and thus determines digestive tract

structure (Jiao et al. 2023). The omnivorous round goby is functionally classified as a stomachless fish due to its relatively simple, straight tubular-segmented digestive tract (Geevarghese 1983; Barton 2007), in which morphological adaptations are closely correlated to ontogenetic changes in the morphology of its robust molariform teeth (Ghedotti et al. 1995; Andraso et al. 2011a). Given that bivalves are the primary prey in round goby diets in both their native range (Demchenko and Tkachenko 2017) and in invaded localities (Diggins et al. 2002; Adámek et al. 2007; Kornis et al. 2012; van Deurs et al. 2021; Behrens et al. 2022), these adaptations enable it to exploit hard-shelled resources and feed on molluscs by crushing (Angradi 2018). This allows digestive enzymes to access soft tissues (Aarnio and Bonsdorff 1997) and ensure rapid gut transit through a short digestive tract that maximises food processing efficiency (Horn and Messer 1992), as observed in other stomachless species. This trait, which involves the mechanical breakdown of food before it reaches the intestines and is recognized as compensation for the lack of a stomach (Fänge and Grove 1979), likely explains the observed faster evacuation rates of *Pisidium* sp. in our study. Based on our post-experimental gut content analysis, it was evident that all *Pisidium* sp. individuals were thoroughly minced and crushed by the round gobies and, in almost all cases, after 16 hours it was impossible to discern the prey structure. In our opinion, this foraging adjustment almost certainly contributes to much faster evacuation rates.

In addition to the observed faster evacuation rates of *Pisidium* sp., it is crucial to consider the predator-prey size relationship. Although round gobies feed on a range of mussel sizes, experimental data reveal a preference for smaller individuals, most probably due to their ease of swallowing and thinner shells (Schwartzbach et al. 2020). Particularly interesting is the observation that some hard-bodied prey items such as Dreissenidae under 6 mm in size (Andraso et al. 2011b) remain intact after passing through the round goby intestine or like *Pisidium* sp. (Mack and Andraso 2015; Andraso et al. 2017) even survive gut passage. However, our observations differed as we failed to detect any persistent intact forms despite the individuals used in our experiments being smaller than 6 mm. Nonetheless, it is important to bear these findings in mind as they suggest that the actual predatory efficiency of round gobies on molluscs is unclear and could be overestimated in some cases. A similar pattern has been observed in *Ancylus fluviatilis* Müller, 1774 in the river Elbe, which appears to be a preferred food resource for the round goby (Richter et al. 2022). In this case, shell remnants persist in the rear sections of the digestive tract for an extended period and form stacked structures. This can lead to overestimations when comprehensively examining the full contents of the entire gut.

By contrast, the round gobies fed on another hard-bodied prey – *Dikerogammarus villosus* – exhibited the slowest gut evacuation rates when continuously fed, which was accompanied by notable mechanical food processing in the sampled digestive tracts. The observed delay in the complete evacuation of crustacean prey (36 h) can be attributed to the resistance of the cuticular exoskeleton to enzymatic breakdown (Bromley 1991; Andersen 1999; Temming and Herrmann 2003), while the subsequent increase in evacuation duration time is correlated with rising ash content within the prey (Couturier et al. 2013). This finding concurs with studies conducted in other fish species that report slower evacuation of prey with structural support than of prey without (Singh-Renton and Bromley 1999; Temming and Herrmann 2003; Gillum et al. 2012). Furthermore, the observed decrease in the evacuation rate of amphipod prey in our studies may suggest greater use of consumed nutrients (Le et al. 2019) since previous research has revealed a reduction in the efficiency of digestion and absorption processes if the gut transit time is shorter (Garber 1983; Lee et al. 2000). Therefore, the prolonged gut evacuation rate coupled with evident food breakdown observed in our study

suggests that *Dikerogammarus villosus* – alongside chironomids – can also serve as a nutritionally valuable food source and prey item providing fatty acid (Sushchik et al. 2006; Maazouzi et al. 2007). These potentially superior nutritional benefits are also supported by studies demonstrating the strong preference of round gobies for amphipod prey, which results in faster growth rates and better overall body condition than in diets based on bivalves (Polačik et al. 2009). This preference may be attributable to prolonged satiety, in which a slower evacuation rate could be an adaptation used during the species' transport and active dispersal to new localities, as the digestive tract capacity of gobies and their temporary food storage function are diminished by their secondary loss of an anatomically distinct stomach (Barton 2007), and lack of intestinal bulb (Trzeciak et al. 2012)—a simple dilatation at the anterior part of the intestine—commonly recognized as a substitute for the stomach in agastric fish (Kapoor et al. 1976; Mokhtar et al. 2021).

In some areas, round goby feed almost exclusively on amphipods, which can constitute up to 70–80% of their diets (Brandner et al. 2013). This finding and our results together suggest that even when round gobies exhibit preferences and prey exclusively on amphipods (Diggins et al. 2002; Polačik et al. 2009; Emde et al. 2014), they may still exert less pressure on this prey group in terms of evacuated biomass quantity per unit of time than on other prey, e.g. the tested chironomid larvae. This implies potentially less predatory pressure on amphipods (even when they are present throughout all seasons in the benthic community) than on other groups of macrozoobenthic organisms whose availability is more seasonally limited (Zinchenko et al. 2017; Pander et al. 2022). However, our suppositions are only supported by our laboratory findings regarding evacuation rates. Since the round goby is recognised as an omnivorous species that feeds on the most readily available prey (Kornis et al. 2012; Nurkse et al. 2016), we believe that – rather than food preferences – local prey abundances together with evacuation rates primarily govern the potential predatory pressure exercised by this species. Hence, dietary analysis and macrozoobenthic availability in local populations will provide more accurate and comprehensive information regarding potential predatory pressure in any particular locality.

We cannot exclude that the differences in gastric evacuation rates between prey types (Food A) were affected also by using different types of second prey (Food B) as our methods included invariable pairs of prey combinations that may occur in nature and the round goby diet (Szydłowska et al. 2024). For example, studies on *N. melanostomus* (Trzeciak et al. 2012) and other gobiid species (Jaroszewska et al. 2008; Wołczuk et al. 2015) have confirmed the presence of an oesogaster with alveolar glands and functions for enzyme secretion and mucus production. These features serve as an effective functional replacement for the conventional stomach, enhancing digestion, particularly evident in the utilization of protein-rich food sources (Wołczuk et al. 2015). Based on this, chironomid larvae, known for their high protein content and used as a subsequent meal (Food B; De la Noüe and Choubert 1985; Nath et al. 2015), could potentially contribute to the rapid evacuation of *Pisidium* sp., as observed in continual feeding mode. However, when considering the analysis of results from the non-continuous feeding mode, which can be regarded as a 'baseline' where each prey type was tested separately, it appears that chironomid larvae were digested and evacuated at different rates depending on the season, while the evacuation rate for *Pisidium* sp. followed by Diptera larvae in continual feeding mode remained the highest regardless of these conditions. Thus, other indicators, such as the size of prey—for instance, even when meal portions are of the same mass, smaller prey are likely to be evacuated and metabolized faster (Nilsson and Brönmark 2000)—are also possibly affecting the evacuation rate of *Pisidium* sp. when tested as Food A. Following that, similar justifications for the

possible effects could be applied to the other prey combinations tested within our study, depending on the specific example. Thus, the limited findings highlight the necessity for further investigation involving additional prey species, such as Food B, consistent across all three prey types examined in our study. This would be essential for gaining more robust insights into potential field scenarios. Moreover, factors such as surface area to volume ratio (Andersen 1999), shape (Karlsen and Andersen 2012), and energy density might also play a significant role in explaining our results, and their potential effects should be investigated in separate studies.

### Seasonal temperature effects on gut evacuation rate

According to the literature, temperature is probably one of the most studied environmental factors (Shrable et al. 1969; Brett and Higgs 1970; Persson 1979; Bascinar et al. 2016; Dürrani and Seyhan 2019; Horstman 2020) since it has a major effect on metabolism, biochemical reactions and digestion in fish, and so naturally affects evacuation rates (Pääkkönen and Marjomäki 1997; Gillum et al. 2012). We predicted faster evacuation rates at 20 °C than at 14 °C given that this rate is commonly observed to speed up as temperatures increase (He and Wurtsbaugh 1993; De et al. 2016; Mazumder et al. 2020). Indeed, based on comparisons of the yielded coefficients, our study suggests a potential effect of temperature on the round goby evacuation rate. However, contrary to our expectations, no consistent pattern emerged across different prey types between seasons, thereby indicating the effects of temperature as a prey-type dependent. Following that, results confirmed anticipated thermal dependency in round gobies fed on *Dikerogammarus villosus*, with faster evacuation rates observed at higher temperatures in both feeding modes. This agrees with the findings reported by Stehlik et al. (2015) for the clearnose skate *Rostroraja eglanteria* (Bosc, 1800), where similar ranges of evacuation rates, observed at almost the same temperatures as in our experiment, also reflect the typical thermal dependency (Wu et al. 2015; Horstman 2020). Conversely, round gobies fed on *Pisidium* sp. in both feeding modes exhibited faster evacuation at lower temperatures. This observation can possibly find its rationale in the significant seasonal differences in observed consumption probabilities (evident from the graphical representation of the initial relative gut content; Fig. 2). Specifically, round gobies tested in season B (14 °C; spring/autumn conditions) consumed more *Pisidium* sp. than those tested in season A (20 °C, summer conditions), as shown by the observed initial relative gut content. This matches field observations whereby larger round gobies in spring and autumn chiefly base their diet on molluscs (Borcherding et al. 2013). Since the round gobies tested in season B were also slightly bigger (2.06 mm on average) than those tested in season A and the predator and prey body size is regarded as an important variable affecting evacuation rates (Beamish 1972; Fänge and Grove 1979; Andersen 1984; Bromley 1994; dos Santos and Jobling 1995; Seyhan et al. 2020), we cannot completely exclude their possible impact on the effects observed. However, we believe that the detected difference in the size (SL) of the fish used in the two seasons – by a magnitude of just a few mm – although statistically significant, is biologically irrelevant and likely did not affect the actual result of our study in any way.

As the continual feeding mode reflects conditions observed in the wild by simulating different prey type combinations, we can expect similar outcomes in nature. Following that, the inconsistency observed in temperature-related patterns in the evacuation rate of each prey type might be explained not only by the higher relevance of other tested variables but also by the round goby's wide environmental tolerance, as previously documented by several studies (Charlebois et al.

2001; Christensen et al. 2021), which is reflected in its broad temperature range (Moskal'kova 1996). Laboratory research has further corroborated these findings and demonstrated metabolic adaptability and ability of the species to thrive under disparate conditions, including temperatures ranging from 2.3 to 5.3 °C (Walsh et al. 2007; Silva et al. 2019) and its active feeding year-round even at low temperatures under natural conditions (Dashinov and Uzunova 2020). However, it is important to remember that seasonal changes in the effectiveness of prey use and diet are not only temperature-dependent but are also affected by particular prey types and their seasonal dynamics within the macroinvertebrate assemblages and thus by variations in the availability of food resources (Didenko et al. 2017). Therefore, the outcomes of our analysis underscore the importance of considering the interaction between all the abovementioned and tested conditions.

### Comparison of food processing efficiency between the round goby and local native species

Compared to local native species such as ruffe *Gymnocephalus cernua* (Linnaeus, 1758) in the estuary of the river Elbe (Hölker and Temming 1996) and the river St. Louis (Henson and Newman 2000), our study found that, under the same thermal conditions, the round goby evacuates chironomid larvae up to three times faster. While the abovementioned studies primarily focussed on gastric evacuation rates (which assess stomachs), our findings relate to the gut evacuation rate that encompasses the whole alimentary tract due to its different structure. This variance in digestive anatomy may affect the comparability between species of evacuation rates. Nevertheless, this disparity might still indicate the superior efficiency of the round goby in acquiring food and competing for resources, consistent with findings from the experimental studies conducted by Bergstrom and Mesinger (2009), as the evacuation rates were found to regulate food intake rates (Aas et al. 2013; Hedden et al. 2020). This efficiency, combined with its feeding plasticity and ability to feed on the most abundant local prey (Carman et al. 2006), could be linked to the observed depletion (Lauer et al. 2004; Rakauskas et al. 2013; Ramler and Keckeis 2019) – or even in some particular cases collapse – of indigenous fish populations (Jůza et al. 2018) and negative trends in macrozoobenthic assemblages (Pennuto et al. 2010; van Deurs et al. 2021).

On the other hand, research by Lagrue and Bollache (2006) into the persistence of gammarids in European bullhead *Cottus gobio* Linnaeus, 1758 stomachs over 24–30 hours at 14 °C suggests that the round goby may in fact be less efficient at food processing than other analogous native fish species that prey on amphipods. This implies a potential lack of superiority of a non-native predator over its native counterparts in competing for food resources in localities where they occur sympatrically (Janáč et al. 2018; Roje et al. 2021). Our study corroborates this observation given that, among all the offered prey types, the slowest evacuation rate while continuously feeding at the 14 °C exhibited by round gobies was for crustaceans. However, Lagrue and Bollache (2006) noted that the persistence time was strongly influenced by temperature and prey type (e.g. the invasive *Gammarus roeseli* Gervais, 1835 had significantly greater persistence at 14 °C than its native counterparts), which highlights potential variations in outcomes if *Dikerogammarus villosus* would be included given its more robust and thicker exoskeleton and resistance (Błońska et al. 2015). This raises questions regarding our previous statement, as the inclusion of *D. villosus* could influence the persistence time in native fish predators. However, it is important to emphasise that while the gut evacuation rate is a good predictor and plays a primary role in



regulating feeding rates (Hedden et al. 2020), the overall efficiency and impact of a predator depend on a series of factors including fish capture effectiveness and foraging behaviour (Gebauer et al. 2018; Franta et al. 2021), as well as the abundance of local prey populations.

## Conclusions

Overall, our study shows that the round goby exhibits higher efficiency and effectiveness in food processing when feeding on chironomid and mollusc prey and, contrary to expectations, can maintain high evacuation rates even at low temperatures (when after feeding on bivalves). These findings suggest a potentially higher consumption rate and predatory pressure on the aforementioned types of prey, which are the main components of the round goby's diet (Phillips et al. 2003; Weimer 2003; Carman et al. 2006; Polačik et al. 2009; Kirilenko and Shemonaev 2012; Matern et al. 2021; van Deurs et al. 2021), as well as less impact on amphipod prey (Lederer et al. 2008). Moreover, our study confirmed the generally observed rule that the evacuation rate significantly increases in situations of greater prey availability. This indicates higher potential consumption efficiency under these conditions allowing to maximise yields from available resources, which could seriously affect native macrozoobenthic and fish assemblages (Barton et al. 2005; Rakauskas et al. 2013; Mikl et al. 2017) and potentially lead to decreased resource availability. We believe that further investigations focusing on modifying feeding scenarios and prey combinations should be prioritized to better understand food processing efficiency and, hence, possible predatory pressure on specific prey taxa.

Our research takes the first step toward accurately quantifying consumption and assessing the predatory impact of round gobies in colonized regions. By integrating laboratory-derived gut evacuation rates with field gut samples (Bernreuther et al. 2009), we can accurately calculate daily consumption (Elliot and Persson 1978). This, combined with data on macrozoobenthic assemblage densities, can offer comprehensive insights into predator-prey dynamics and consumption patterns in invasive species. Considering the environmental conditions used in the presented studies (primary food resource types, seasonal temperatures, and diverse prey availability scenarios), we believe our findings apply not only within local contexts but also to other colonized regions, where monitoring local populations can allow to assess predatory pressure and consumption rates estimations at both *per capita* and population levels. Following Stehlik et al. (2015), our findings will help quantify the consumption rate of round gobies in ecosystems, and we recommend that future research prioritizes the study of evacuation rates to improve estimates of prey use and predator efficiency.

## Authors' Contribution:

S.W., L.R., B.D., P.F. research conceptualization; P.F., B.D., sample design and methodology; N.S., P.F. investigation and data collection; M.L., N.S. data analysis and interpretation; M.B. funding provision; N.S. writing - original draft; M.B., B.D., S.W., M.L., N.S. writing – review & editing.

## Funding Declaration

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## Ethics and Permits

All research pertaining to this article was carried out in accordance with the ethical guidelines of the Czech Republic and obtained formal approval from the applicable ethics committee.

All animal handling was adhered to the legal requirements in the Czech Republic (§ 7 Law No. 114/1992 on The Protection of Nature and Landscape and § 6, 7, 9, and 10 Regulation No. 419/2012 on the Care, Breeding, and Use of Experimental Animals).

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## Supplementary material 1

### Consumption probability

Authors: Natalia Z. Szydlowska, Marek Let, Pavel Franta, Miloš Buřič, Susanne Worischka, Luise Richter, Bořek Drozd

Data type: pdf

Explanation note: Effect of prey type 1. Pis = *Pisidium* sp.; 2. Chiro = chironomid (*Chironomus* sp.) larvae; 3. Dikero = *Dikerogammarus villosus*, season (season A represents T = 20 °C and season B represents T = 14 °C) and prey availability: 1. Non-con = non-continual feeding mode; 2. Con = continual feeding mode; on the probability of being consumed by round goby (*Neogobius melanostomus*) individuals. Whiskers represent 95% confidence intervals around mean estimates (food consumption probability) within each tested factor level.

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## Supplementary material 2

### Change in the relative gut content (*GI*) in time in the round goby (*Neogobius melanostomus*) represented by different treatments

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Data type: pdf

Explanation note: Season A represented by T = 20 °C (dot); Season B represented by T = 14 °C (triangle). Different prey species: 1. *Pisidium* sp. (red); 2. *Chironomus* sp. larvae (green); 3. *Dikerogammarus villosus* (blue). Feeding modes: 1. Non-con = non-continual feeding mode (shown by dashed lines) and 2. Con = continual feeding mode (shown by solid lines). Relative gut content expressed as a ratio of the dry weight (DW) of the tested food (food A) for each treatment at the time of sampling vs. fish dry body weight [g.g<sup>-1</sup>].

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## Supplementary material 3

### Standard length (SL) differences in the round goby (*Neogobius melanostomus*) individuals used in experiments in the two seasons (A and B), with different prey type and prey availability

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Data type: docx

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