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Reproductive biology of pioneer round gobies (*Neogobius melanostomus* Pallas, 1814) at the edge of their invasion front in three small rivers (Lower Danube Basin, Bulgaria)

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Abstract. Reproductive traits are among the most important biological characteristics that facilitate or impede the establishment of non-native fish into new environments. In several tributaries of the Lower River Danube (Bulgaria), the round goby (*Neogobius melanostomus* Pallas, 1814) is a recent invader. Specimens from the edge of the invasion front were collected monthly (March 2017 to May 2018) from each river. The pioneer individuals displayed relatively small body sizes. First maturation of females occurs at 49 mm total length. Based on the dynamics of oocyte size and GSI, it was established that spawning begins in early spring, at a relatively low water temperature (6–7 °C). The breeding season extended from March to June. Average absolute fecundity was 162 ± 62 oocytes, while average relative fecundity was $94 \text{ oocytes/g} \pm 24$. Female fecundity is linearly dependent on the length and weight. Both of the known male alternative reproductive tactics were observed. Body condition factor was lowest during April for both sexes. The relationship between reproductive traits and invasive potential in *N. melanostomus* is discussed.

Key words: fecundity, size of maturity, sex ratio, GSI, invasive Gobiidae

Introduction

Invasive species are considered to be one of the leading causes of biodiversity loss (McMahon 2002, Ruesink 2005). The Ponto-Caspian round goby (*Neogobius melanostomus*, Pallas 1814) is a successful invader in brackish and fresh waters of large and medium-sized rivers and lakes in Europe and North America (Kornis et al. 2012, Roche et al. 2013, Ojaveer et al. 2015). Its reproductive traits, such as early maturation, relatively high fecundity, batch spawning, and parental care are believed

to facilitate the successful settlement in various environments (Tomczak & Sapota 2006, Balážová-Lavrinčíková & Kováč 2007, Borcharding et al. 2013, Hôrková & Kováč 2014, 2015a, Gertzen et al. 2016, Hirsch et al. 2016a). The round goby's great plasticity and adaptability to a broad spectrum of environmental variables results in its high invasive potential (Polačik et al. 2009, Borcharding et al. 2013, Brandner et al. 2013, Hôrková & Kováč 2014, 2015a, Kornis et al. 2016, Pennuto & Rupprecht 2016). Additionally, *N. melanostomus* is one of the few fish species that can reproduce successfully in

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waters in a wide range of salinities (Bonisławska et al. 2014, Kalamarż-Kubiak & Guellard 2019). Both native and invasive populations have a relatively long breeding season, which includes the warmer months of the year (Kornis et al. 2012, Gertzen et al. 2016, Aydin 2021).

The species is a batch spawner and in one season, females can lay from three to nine batches of eggs (Corkum et al. 1998, Hôrková & Kováč 2014, Gertzen et al. 2016). A single male aerates the eggs of one to several females and defends them from predators (Corkum et al. 1998, Meunier et al. 2009). The existence of alternative male reproductive tactics (ARTs) is also considered a factor contributing to the invasion success of this species (Marentette et al. 2009, Bleeker et al. 2017, McCallum et al. 2018, Cerwenka et al. 2020, Green et al. 2020). Over an entire year non-reproducing males dominate in a population, while at the start of the reproductive season (April, May) parental males are more commonly found, followed by sneaker males, which exhibit sexual mimicry (McCallum et al. 2018, Cerwenka et al. 2020).

In the course of invasion, the reproductive characteristics of the round goby undergo various changes (Tomczak & Sapota 2006, Gutowsky & Fox 2012, Azour et al. 2015, Hôrková & Kováč 2015b, Cerwenka et al. 2018). For example, in recently established populations, females mature earlier and have greater fecundity compared to long-established populations (Balážová-Lavrinčíková & Kováč 2007, Hôrková & Kováč 2014, 2015a, Gertzen et al. 2016, Konečná et al. 2016, Kornis et al. 2016).

Today the round goby continues its successful invasive spread in Europe and North America (Kornis et al. 2012, Roche et al. 2013, Ojaveer et al. 2015, Raab et al. 2018). Relatively recently, *N. melanostomus* has established in several tributaries of the Lower River Danube (northern Bulgaria) and in just a few years, this fish has significantly increased its upstream distribution in the area (Vassilev et al. 2008, Dashinov et al. 2018). This recent invasion of the round goby in the Bulgarian Danube tributaries has not been fully explained. It is possible that the invasion front is moving upstream without forming dense populations. Assisted introduction through boats and nets is not excluded (Hirsch et al. 2016a). To find the drivers of this relatively rapid invasion, we focused on the biological and ecological characteristics of the round goby individuals constituting the invasion

front of the species in these rivers. We hypothesized that pioneer specimens of the invasion front are likely to differ in some of their characteristics, not only compared to individuals from native populations, but also to those of long-established invasive populations. In general, round gobies from the invasion front in the investigated tributaries exhibit common morphological adaptations with other invasive populations, such as relatively large ventral fins, big eyes and short intra-orbital distances (Dashinov et al. 2020). In addition, these populations have a wide and taxonomically rich trophic niche in comparison to other invasive populations (Dashinov & Uzunova 2020). In order to identify the reasons for the invasion success of *N. melanostomus* in the tributaries of the River Danube, we studied specific aspects of the reproductive biology of the invasion front individuals. Our aims were to study the sex ratio, condition factor, and size at first maturation (L_{50}), annual dynamics of oocytes size and GSI, spawning period, fecundity of females and the frequency of the different male ARTs. These reproductive traits were analysed in the context of the establishment successes and invasive potential of the species in the studied area.

Material and Methods

Study area

Round gobies were collected at the highest points of upstream spread in the Rivers Iskar (N 43.4987, E 24.2424), Yantra (N 43.0760, E 25.5512) and Vit (N 43.4079, E 24.5218) (Dashinov et al. 2018). All three rivers are right-bank tributaries of the Lower Danube and were sampled in sections between 40 and 60 km upstream from the estuary of each river. The River Iskar has a length of 368 km (Hristova 2012). In the sampling area, the bottom substrate is composed mainly of cobbles and pebbles, along with single boulders and more rarely sand. The River Vit is 188.2 km long (Hristova 2012). In the sampled section with the bottom substrate mostly shale, with a few zones covered by cobbles and pebbles. The River Yantra has a length of 285.5 km (Hristova 2012). Single boulders, cobbles, pebbles and sand comprise the bottom substrate. Monthly water temperature varied between 2.3 and 22.3 °C with the lowest temperature observed during January and highest during July (Fig. 1). Mean annual discharge is lower in the River Vit (13.8 m³ s⁻¹) compared to the Rivers Iskar and Yantra – 57.4 and 47.4 m³ s⁻¹, respectively. For all three rivers monthly discharge is highest in May

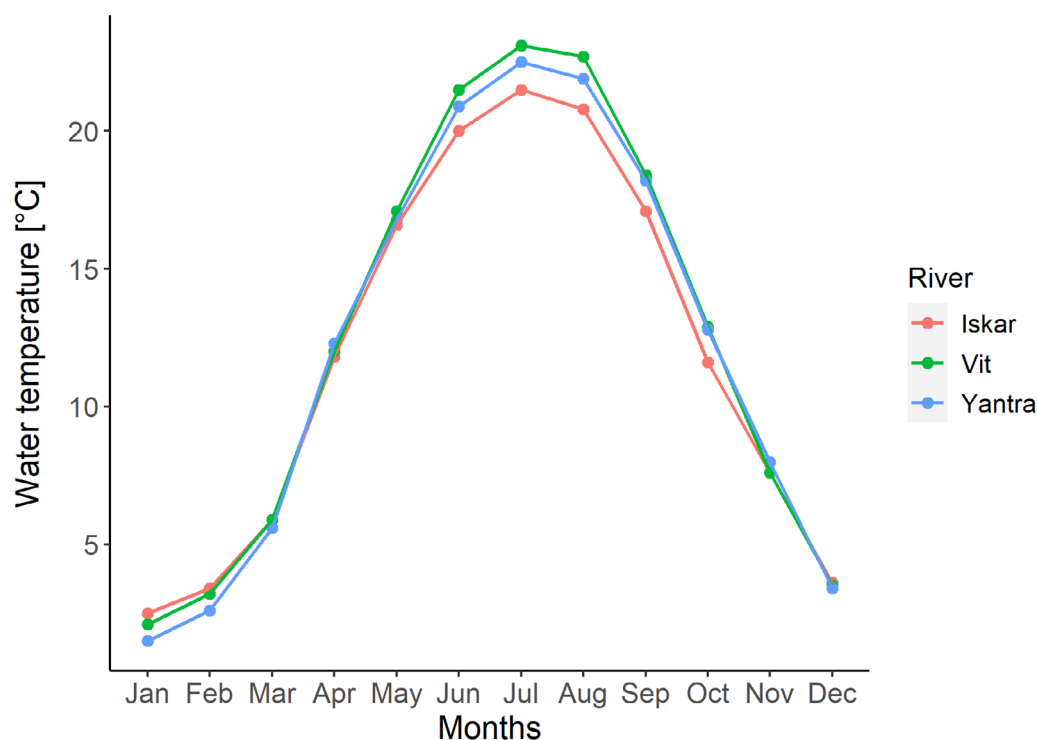


Fig. 1. Average monthly water temperature (°C) of the three sampled rivers (Iskar, Vit and Yantra) according to Hristova (2012).

and lowest in August (the River Iskar) or October (the Rivers Vit and Yantra) (Hristova 2012) (Fig. 2). The fish sampling areas consist mostly of riffles and glides, with intermittent pools and shallows. Water levels (near the riverbank) varied from 0.2 m to 1.0 m depending on the hydrological period. The river width in the sampled zones varied

between 25 and 35 m. Submerged vegetation such as *Myriophyllum* spp., *Potamogeton crispus*, *Najas minor* and *Stuckenia pectinata* were found in all three examined tributaries. The sampled section is dominated mostly by native Cyprinidae fishes, including benthic feeders, such as *Barbus petenyi*, *Vimba vimba*, *Gobio gobio*, *Cobitis* spp.

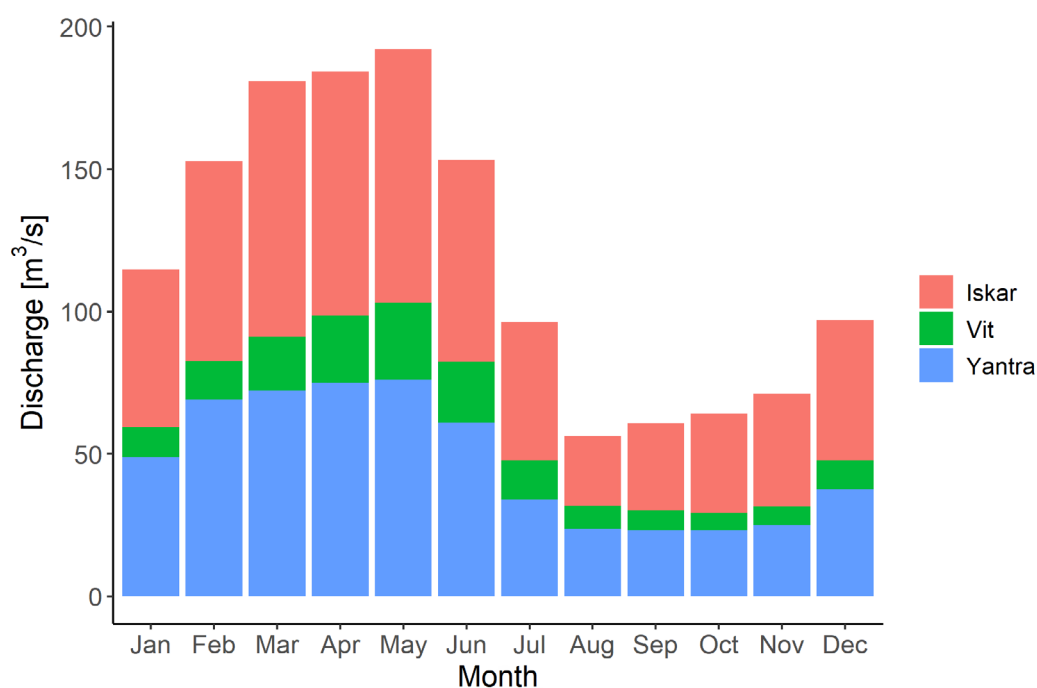


Fig. 2. Average monthly discharge (m³/s) of the three sampled rivers (Iskar, Vit and Yantra) according to Hristova (2012).



Data collection

Round gobies were collected monthly between March 2017 and May 2018 using a DC electrofishing backpack (SAMUS, 200/350 V, 3/12 A, 45-50 Hz). Sampling in the River Iskar was from April 2017 to May 2018, for the River Vit, from March 2017 to January 2018, and from June to October 2017 for the River Iskar. Transect lengths varied from 50 to 250 m depending on local characteristics and hydrological conditions during sampling. In general, the sampled area covered all mesohabitat types (pools, riffles and runs) present in each river section. All captured specimens were euthanized with an overdose of MS 222, fixed and preserved in 10% formalin. Specimens from all three examined rivers (133 from Iskar, 97 from Vit and 47 from Yantra) were pooled in a single sample.

The sex of each fish was determined in the laboratory. Males and females were distinguished using the morphology of the urogenital papilla and macroscopic examination of gonads. Male alternative reproductive tactics were determined based on their external morphology and the gonad structure following Marentette et al. (2009). Parental males are darkly coloured, with relatively wide heads and generally larger in size than sneaker males. The latter also possess developed accessory glands in the testis (Marentette et al. 2009). The total body weight (Wt, g), eviscerated weight (We, g), gonad weight (Wg, g), standard length (SL, mm), and total length (TL, mm) of all round gobies were measured.

For each female a transverse section from the middle of both ovaries was taken. These subsamples were used for oocyte count estimation under a stereomicroscope (Micros Austria $\times 10$ and $\times 30$). The diameter (d) of a random set of 50 oocytes from each female were measured to the nearest 0.05 mm using a stereo microscope with an ocular micrometer. Sexually mature females were divided into groups based on oocyte size. Of all captured females, oocytes were counted in 28 sexually mature females in pre-spawning and spawning period (from January to April).

Data analysis

To account for variation in body condition, the Fulton's condition factor (K) was estimated, using the formula: $K = 100 \times Wt/TL^3$, where Wt is total weight and TL is total length. The monthly values of the gonadosomatic index (GSI) were used to identify the spawning period of *N. melanostomus*.

The GSI was estimated using the equation: $GSI = Wg/Wt \times 100$, where Wg and Wt are the gonad weight and the total body weight (g) (West 1990).

Females with poorly developed gonads that were yellow or pink-coloured, or that were indistinct, were classified as juveniles (Tomczak & Sopota 2006). Females with ovaries in which oocytes had accumulated vitellogenin were considered reproductive. Three size classes of oocytes were differentiated in the gonads of female round gobies: oocytes type A with a diameter $d < 0.9$ mm and spherical form, type B with diameter $0.9 < d < 2$ mm and an irregular form (vitellogenic oocytes) and type C oocytes with a $d > 2$ mm and an elliptic form. Oocyte size distribution, including size class discrimination, was evaluated within the context of the annual gonadal cycle.

The length at first maturity was calculated using only females captured from the beginning of the year until the start of the reproductive season – from January to April; 48 females in total, which were divided in ten size groups (at 5 mm TL intervals). The frequency of mature females was used as the response variable, and standard length was used as the explanatory variable in a logistic regression (Dinh 2018). The estimated mean body length at first maturity (L_{50}) represents the length at which 50% of the fish are mature, whereas L_{100} represents the length at which all fish are mature. The suitability of the logistic model was assessed using the Wald test.

Absolute fecundity (F_{abs}) was estimated based on the number of all oocytes (regardless of size, shape or presence of vitellogenin) for 28 mature females collected during the pre-spawning period (based on the monthly dynamics of GSI). The number of oocytes in the subsample was extrapolated for the whole gonad. This estimate assumes that the subsample has oocyte counts proportional to the weight of the subsample (and the value is thus extrapolated based on the full ovarian weight). Then the extrapolated and the non-extrapolated F_{abs} were plotted as a linear regression with the former as the dependent and the latter the independent variable. Relative fecundity was determined as the number of oocytes in a female divided by the total weight of that female. Batch fecundity (F_{btc}) was estimated based on the number of vitellogenic oocytes (type B) in the ovary.

Both batch and absolute fecundity were plotted as dependent variables against the total weight

and the total length. Monthly differences in the GSI were demonstrated statistically with a t-test. All statistical analyses were performed using R (R Core Team 2013, Wickham 2016).

Results

A total of 277 *N. melanostomus* specimens were analysed (160 females and 117 males). The sex ratio was 1.37:1, which was not significantly different ($\chi^2 = 3.20$, $df = 1$, $P = 0.074$) from the expected 1:1 ratio. Total length of the investigated males ranged from 2.77 to 12.49 mm (mean 6.79 cm \pm 1.85 SD), body weight from 0.86 to 28 g (mean of 5.42 g \pm 5.08 SD) and eviscerated body weight from 0.74 to 25.9 g (mean 4.71 g, SD \pm 4.09). Total length of females ranged from 3.82 to 10.66 cm (mean of 6.47 cm \pm 1.44 SD), body weight ranged from 0.8 to 16.9 g (mean of 4.54 g \pm 3.19 SD), the eviscerated body weight – from 0.7 to 15 g (mean of 4.04 g, SD \pm 2.92). A total of 109 males were classified as non-reproducing (NRM) based on the macroscopic characterisation of the gonads, GSI ($< 1\%$) and external body morphology. Out of all the 117 collected male round gobies, only eight individuals were reproductive males, which were caught between March and May (20% of all the males captured during these months). Both of the two known alternative reproductive tactics of this species were present. Three parental male individuals (PMs) were caught (one in March, one

in April and another in May), while five sneaker males (SMs) were all caught in March.

Size at first maturation (L_{50}) of female gobies was estimated at 4.93 cm TL or 3.71 cm SL, while the L_{100} was estimated at 6.52 cm TL or 5.06 cm SL according to the logistic function: $P = 1/(1 + e^{-2.965(TL - 4.925)})$, where P is the proportion of mature females in a size class. Body length was a statistically significant predictor of maturity (Walt test = 12.54, $df = 1$, $P < 0.001$). The highest frequency of females with developed gonads was observed in February and March. Since there were only eight reproductive male round gobies in the samples, L_{50} or L_{100} could not be estimated.

During the year, two peaks in the GSI were observed for female round gobies – in February (GSI, mean 8.22% \pm 2.66 SD) and in May (GSI, mean 7.2% \pm 4.19 SD) (Fig. 3). Spawning lasted until June, after which the mean GSI was 1.88% \pm 1.39 SD. There were no significant differences in the GSI from February to June, though there was between June and July ($t = 2.58$, $df = 6.06$, $P = 0.041$) and between July and August ($t = -2.66$, $df = 8.92$, $P = 0.026$). The GSI did not differ significantly from August to November, but there were significant differences between November and December ($t = -3.11$, $df = 8.28$, $P = 0.014$). There were no differences, between December and January, while January differed significantly with February ($t = -2.95$,

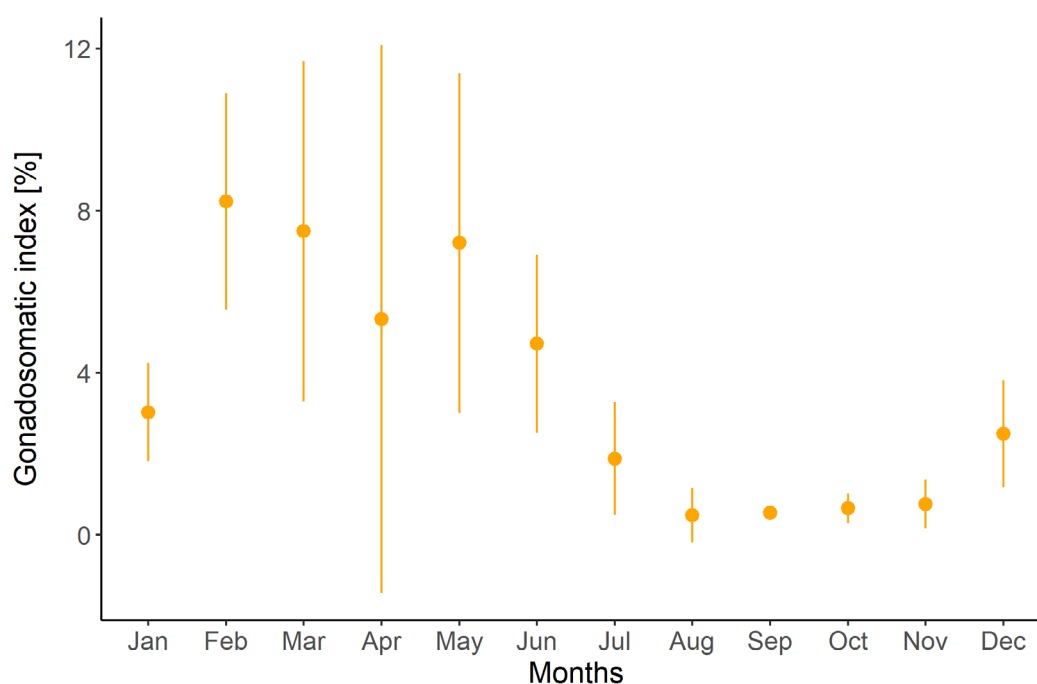


Fig. 3. Seasonal variation of the Gonadosomatic Index (GSI, %) (mean values with dots, standard deviation as vertical lines) of female round gobies ($n = 160$) from the Danube tributaries (the Rivers Iskar, Vit and Yantra).

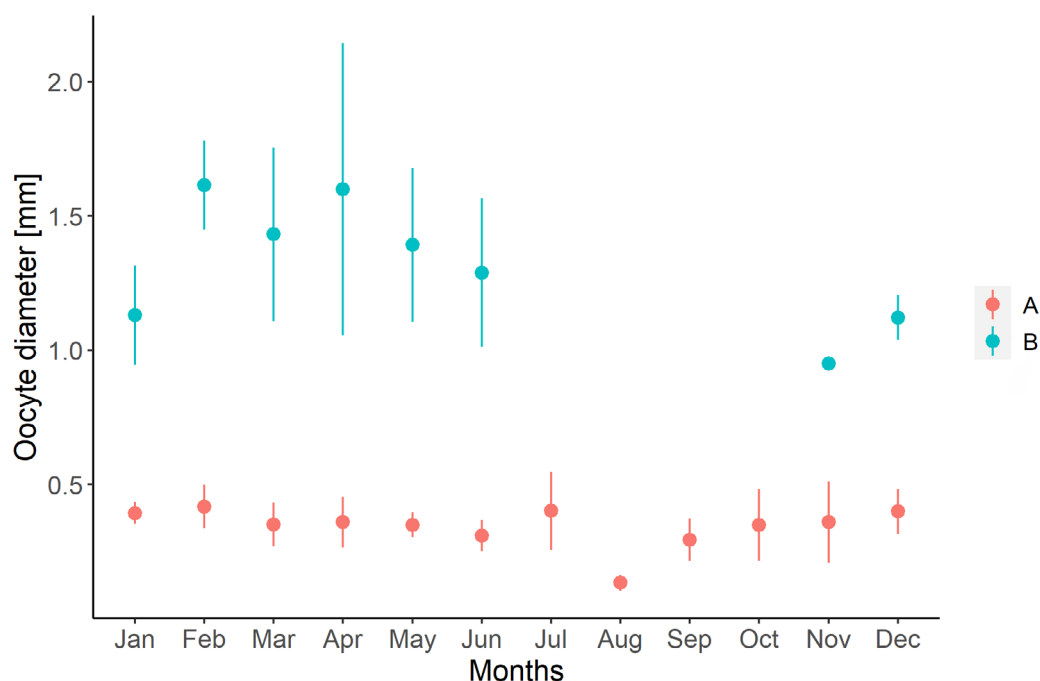


Fig. 4. Seasonal variation in size of Type A and B oocytes of the round goby (mean values with dots, standard deviation as vertical lines) ($n = 160$) from the Danube tributaries (the Rivers Iskar, Vit and Yantra).

$df = 2.88$, $P = 0.063$), when the GSI reached its highest mean value.

Type A (unvolved) oocytes were present in the ovaries of females throughout the entire year. The mean monthly d of type A oocytes varied between 0.18 and 0.54 mm, with a maximum observed in February and a minimum in August (Fig. 4). Type B oocytes were not found during August, September and October in any of the females; mean monthly d ranged between 1.03 and 1.60 mm with maximum values were observed during March and May. After that time, egg size decreased and a minimum size was recorded in the period November-January (Fig. 4). The absolute fecundity of females varied from 159 to 773 oocytes (mean of 360, 95% CI between 310 and 410 oocytes), batch fecundity was from 32 to 507 oocytes (mean of 162, 95% CI between 127 and 199 oocytes) and relative fecundity ranged from 55 to 134 oocytes (mean of 94, 95% CI between 84 and 103 oocytes). There was a linear relationship between absolute fecundity and total length ($F_{abs} = 95.83TL - 256.9$, $R^2 = 0.63$) with a SE of 94.02 for the intercept and 14.42 for the slope of TL. The equation $F_{abs} = 43.07Wt - 175.72$, $R^2 = 0.75$ explained the linear relation between F_{abs} and total weight with a SE of 24.14 for the intercept and 4.86 for the slope. Batch fecundity was linearly dependent both on total length ($F_{bfc} = 71.77TL - 299.65$, $R^2 = 0.69$, SE 61.85 and 9.49 for the intercept and TL, respectively) and body weight ($F_{bfc} =$

$34.282Wt - 15.73$, $R^2 = 0.93$, SE 9.35 and 1.88 for the intercept and Wt, respectively).

Fulton's condition factor (K) for female round gobies varied among months, with a maximum in February (1.75 ± 0.3 SD) and minimum in April (1.25 ± 0.11 SD). For male specimens the highest values of K were observed during May (1.54 ± 0.32 SD) and the lowest in April (1.25 ± 0.13 SD).

Discussion

The round goby population investigated here from the invasion front in tributaries of the Lower Danube shows characteristics that differentiates them from native or early-established invasive populations. For example, there are reported differences in external morphology (eye diameter, interorbital distance, head width, ventral fin width) (Dashinov et al. 2020). Furthermore, this species has a wide and diverse food niche and feeds during the entire year (Dashinov & Uzunova 2020). In the present study, we add to the understanding of these populations with data on reproductive traits. The studied river sections have similar hydromorphological, hydrological and temperature regimes according to the hydrological observation over a 50-year period (Hristova 2012) and belong to the same river type (semi-mountainous rivers) (Cheshmedjiev et al. 2013). Similar mesohabitats were observed in all three study areas. These commonalities justify



pooling of specimens from the three rivers into a single sample. Nevertheless sample pooling masks fine differences between the three examined populations and is a limitation of our study.

The round goby specimens from the studied rivers were relatively small in size compared to the native and to most non-native populations. In general, larger body size of *N. melanostomus* is an exception for invasive populations (Skóra & Stolarski 1996, Sokołowska & Fey 2011). A maximum total length of 35 cm for *N. melanostomus* was reported from its native range (Ak et al. 2009). Small-sized round gobies can be viewed as an adaptation to a relatively strong current velocity, since small-bodied individuals are better suited to upstream movement (Tierney et al. 2011, Pennuto & Rupprecht 2016, Dashinov et al. 2020). On the other hand, the potential influence of freshwater on the development of the round goby must be considered. The effect of freshwater on osmotic regulation and embryonic development in the round goby is manifested in smaller eggs, shorter duration of embryogenesis, as well as the size of larvae and their developmental progression (Bonisławska et al. 2014). As observed by Moiseyeva (1983), larvae hatching after 18 days of embryonic development in seawater were 0.5–0.8 mm longer than hatchlings obtained in freshwater. The same tendency was mentioned by MacInnis & Corkum (2000): round goby individuals aged 1, 2, and 3 years from the marine environment were longer than those caught in brackish and freshwater areas. Smaller body sizes of non-native populations compared to native have also been described for other species, such as the pumpkinseed (*Lepomis gibbosus* L.) in Europe (Copp et al. 2014). It has been suggested that these populations of *L. gibbosus* may have originated from a source population of relatively small body size.

The sex ratio of round gobies from the edge of the invasion front in the studied rivers did not deviate from unity. A balanced sex ratio is more common for stable, native (Kovtun 1978, 1980, Aydin 2021) or long-established invasive populations (Hempel et al. 2012, Azour et al. 2015). Controversially, some studies have reported that males were more abundant than females in both the native (Gözler et al. 2003, Gümüş & Kurt 2009) and invasive range of *N. melanostomus* (Corkum et al. 2004, Tomczak & Sapota 2006). A female-biased sex ratio was observed in an isolated round goby population in the upper Elbe (Janáč et al. 2019). Of note was

the observation that male fish were unbalanced in terms of the presence of reproducing and non-reproducing males. A comparatively low number of reproductively active males was also observed in other round goby populations, such as those in the Baltic Sea region (Green et al. 2020). Among reproductive males both alternative reproductive tactics were observed: sneaker and parental males. Parental care morphs were less numerous than sneakers and have the largest body size. Contrary to our observations, other studies found numerical domination of the parental male morphs (Bleeker et al. 2017, Bose et al. 2018, McCallum et al. 2018, Cerwenka et al. 2020). The low number of reproductive males, may arise from sampling bias, since these males are weakly mobile during the nest guarding stage (Všetičková et al. 2015). Presumably, stunned males from the electrofishing would remain hidden in their nests and are, therefore, less likely to be collected.

Green et al. (2020) found that sperm viability of *N. melanostomus* is low in the freshwater environment compared to brackish conditions. Authors suggest that in such disadvantageous conditions males are dependent on acquiring and defending a nest site in order to reproduce successfully. Therefore, males have to invest more in somatic growth and fat reserves, compared to fish from brackish waters, resulting in larger males with higher energy reserves. Green et al. (2020) also suggest that male round gobies start out by reproducing through sneaking and go on to adopt a nest-holding strategy when large enough. The influence of salinity may consequently play a role in determining reproductive tactics, but also fish density, invasion phase, availability of sufficient and suitable nesting sites may all play a role in selection for one or other morph. The morphology of the riverbeds in the studied area is complex, which suggests the presence of suitable sites for nesting. On the other hand, as the species spreads upstream, high water velocity could be a significant limiting factor for the successful reproduction in stream conditions. Stream velocity is recognised as a variable that can restrict the spread of *N. melanostomus* into small and mid-size tributaries of large rivers (Jakubčinová et al. 2018, Raab et al. 2018).

In the Danube tributaries, female round gobies mature at a smaller size (37.1 mm SL) compared to indigenous populations in the Black Sea, where maturation occurs mostly in females longer



than 63.6 mm SL (Aydin 2021). Other invasive populations (such as those from the Danube Basin) are also composed of females that mature at a larger body size (> 40 mm SL) (Lavrinčíková & Kováč 2007, Hôrková & Kováč 2014, Gertzen et al. 2016). Round gobies with smaller length at first maturity (37 mm TL) have been reported only in the Lower River Rhine (Gertzen et al. 2016). Konečná et al. (2016) found no significant differences in size at maturity between native and non-native round goby populations in the Middle Danube.

In the Lower Danube tributaries, spawning in *N. melanostomus* starts earlier compared to other populations (native or invasive) (see Gertzen et al. 2016 for an overview). The dynamics of GSI and oocyte size over the course of a year show that female fish are ready to breed as early as March and the breeding period lasts until June. A similar range and duration of breeding is seen in *N. melanostomus* populations from the River Danube (Hôrková & Kováč 2014, 2015b), Baltic Sea (Tomczak & Sapota 2006) and Lower Rhine (Gertzen et al. 2016). For most invasive populations, reproduction continues until late summer (August, September) (Tomczak & Sapota 2006, Gertzen et al. 2016). In their native range, spawning takes place between May and September (Aydin 2021), but is also prolonged in some invasion populations (Tomczak & Sapota 2006). The onset of spawning and duration of the breeding period in fish usually depends on temperature and photoperiod. However, some studies have shown that breeding events in the same water body vary significantly between years and no direct link can always be established with these environmental parameters (Gertzen et al. 2016). Cerwenka et al. (2017) found little association of biological traits, such as condition factor and growth, with environmental variables. The highest GSI values of female *N. melanostomus* were typically reported at the beginning of May and again in June (Gertzen et al. 2016). In our study, we observed a rather shortened spawning period from March to June. At the beginning of the spawning season of *N. melanostomus* in the Danube tributaries, water temperatures were between 5.6–5.9 °C (Hristova 2012). So, the first batch of the eggs is probably released when the water temperature is slightly lower than the reported range (7–26 °C) (Kornis et al. 2012). From laboratory observations it is known that females release a batch of eggs every 18–20 days (Gertzen et al. 2016). Therefore, the reproduction period is approximately 153 days and females can deposit up to six or seven separate batches of eggs,

each with an average of 163 oocytes. However, we did not identify clear breeding pauses, similarly to previous studies (Gertzen et al. 2016). Our data for the beginning of the spawning season at relatively low temperatures show that the species has the capacity to reproduce in the upper reaches of these rivers while maintaining approximately the same breeding duration. However, neither the early onset of the breeding season of *N. melanostomus* nor the long growing season have an effect on the mean body size of the fish, which are relatively small.

The absolute fecundity of the pioneer round gobies in the Danube tributaries was lower than that of the native as well as some of the invasive populations of the species (Kovtun 1978, Hôrková & Kováč 2014, Gertzen et al. 2016). Similarities in this reproductive trait with some invasive populations (the Rivers Upper Detroit and Middle Danube) are evident (MacInnis & Corkum 2000, Konečná et al. 2016). As previously mentioned, individuals from the studied invasion front mature at a smaller body length, which might explain the observed differences in absolute fecundity. Relative fecundity of round gobies from the invasion front in the Danube tributaries is close to that found in gobies in the River Danube (Lavrinčíková & Kováč 2007, Konečná et al. 2016), and the Baltic Sea (Gulf of Gdansk) (Tomczak & Sapota 2006). In the studied river sections, factors such as significant seasonal differences in the hydrological regime may also influence the fecundity of female *N. melanostomus*. In the River Danube, female round gobies, after a strong environmental perturbation, increase their absolute as well as relative numbers of oocytes compared to round gobies from a period with low anthropogenic pressures (Hôrková & Kováč 2015a, b). Both absolute and batch fecundity had a linear relationship with body length and body weight. In other invasive populations, fecundity was exponentially dependent on fish size (Konečná et al. 2016, Hempel et al. 2018). Again, this is most probably linked to the relatively small body size of the examined individuals.

Our results for the Fulton condition factor are similar to findings for other round goby populations, such as those from the River Rhine and the upper sections of the River Danube (Borcherding et al. 2013, Brandner et al. 2013). However, round gobies inhabiting the Lower Danube have a higher body condition than individuals from the Danube tributaries (Polačik et al. 2009). This difference



might be due to the more suitable conditions in the River Danube (food base, temperature and flow regime, bottom substrate) in comparison to inflowing tributaries. Furthermore, the fact that *N. melanostomus* in the tributaries is at an early phase of the invasive process probably also influences body condition. An opposite tendency is reported for the Baltic Sea and the River Rhine where round gobies at the invasion fronts have a higher body condition than early-settled non-native populations (Borcherding et al. 2013, Azour et al. 2015). In general, fish from established and high-density round goby populations are slow growing and display a poorer condition compared to round gobies from the recently invaded locations (Azour et al. 2015). The condition factor of both sexes was low during April, which might be due to energy depletion during the onset of reproduction.

For the Upper Danube population in the period 2010-2015 the median condition factor has not changed significantly, though its variance increased after initial introduction (Cerwenka et al. 2017). The same is true for other individual population characteristics and it has been suggested that the traits of certain individuals contribute highly to the invasion success of the whole population, a prediction of the individual trait utility hypothesis (Cerwenka et al. 2017). In our study, this is the case for the condition factor, which varies more strongly, compared to observations from the Lower and Middle Danube population (Polačik et al. 2009). Observed specialisation of particular round goby individuals to certain macrozoobenthic groups probably accounts for differences in nutrient uptake and hence in body condition (Dashinov & Uzunova 2020).

In conclusion, some features of round gobies from the invasion front could be attributed to a modified life-history in the invasive populations. This effect might include a shift in allocation of resources to reproduction and offspring care rather than to somatic growth (Gruľa et al. 2012), though reproductive traits in novel areas seems more likely

to result from shifts in environmental conditions (Konečná et al. 2014). Environmental factors, such as relatively high water velocity and flow level fluctuation in the upstream sections of the studied rivers, may restrict the future spread of the species (Jakubčinová et al. 2018, Raab et al. 2018). Water pollution is another factor that could play a role in the invasion success of *N. melanostomus*. Bonisławska et al. (2014) considered that industrial pollution resulting in an increased salinity of Polish rivers might contribute to the creation of conditions enhancing reproduction by the round goby. The role of reproductive traits in the invasion success of the round goby is not fully understood and seems to vary at an ecosystem level (Hirsch et al. 2016b). Although our study was focused on pioneer specimens in a new environment, they showed a number of features of long-established populations, such as balanced sex ratio. The overall small size of individuals and early sexual maturity are characteristic of a recently invasive population. The almost complete absence of male reproductive individuals cannot be adequately explained but is potentially an artefact of sampling. Our results confirm the observation that *N. melanostomus* has a high degree of phenotypic plasticity and is able to adapt to novel environments.

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