

Research Article

Reproduction dynamics of the marbled crayfish *Procambarus virginalis* Lyko, 2017 from an anthropogenic lake in northern Croatia

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Citation: Dobrović A, Maguire I, Boban M, Grbin D, Hudina S (2021) Reproduction dynamics of the marbled crayfish *Procambarus virginalis* Lyko, 2017 from an anthropogenic lake in northern Croatia. *Aquatic Invasions* 16(3): 482–498, <https://doi.org/10.3391/ai.2021.16.3.06>

Received: 21 December 2020

Accepted: 14 April 2021

Published: 27 May 2021

Handling editor: Christoph Chucholl

Thematic editor: Ian Duggan

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Abstract

Despite the growing number of established populations in Europe, the reproduction dynamics of parthenogenetic marbled crayfish, *Procambarus virginalis* Lyko, 2017, from populations in the wild is currently understudied. In this study, we performed a systematic seven-month long monitoring of the reproduction dynamics of marbled crayfish population in an anthropogenic lake in continental Croatia. Crayfish were caught monthly by applying the baited stick catch method. We recorded pleopodal fecundity and the number of hatched juveniles in each monthly catch and a random selection of individuals (20 per month) was dissected to determine the ovarian fecundity. Obtained fecundity parameters were correlated with crayfish size (total length, weight and pleon size), body condition (Fulton's condition factor), organosomatic indices (hepatosomatic index: HSI and gonadosomatic index: GSI) and compared with available literature data on marbled crayfish from laboratory-reared or wild populations. Based on the obtained data, we identified two potential reproductive peaks in early summer and mid autumn. However, the continuous presence of individuals with mature ovarian eggs and glair glands throughout almost the entire monitoring period indicates potential reproduction throughout June to November. Ovarian egg number and number of hatched juveniles was significantly correlated with crayfish size and Fulton's condition factor, while GSI exhibited significant variations among analyzed months and was positively correlated with HSI. The number of hatched juveniles in our study was significantly lower compared to literature data for marbled crayfish from populations in the wild and laboratory-reared populations. Collected data offer insights into the understudied reproduction dynamics of marbled crayfish in the wild and represent baseline information for predicting its invasion dynamics and risks of its further spread in this region.

Key words: crayfish invader, parthenogenetic species, fecundity, reproductive cycle, Cambaridae

Introduction

Marbled crayfish, *Procambarus virginalis* Lyko, 2017, is a recently described non-indigenous crayfish species (NICS) with established systematic position but of unknown geographic origin (Lyko 2017; Hossain et al. 2018). The species was discovered in the mid-1990s in the German aquarium trade

(Scholtz et al. 2003) and became popular as a pet (Faulkes 2010; Patoka et al. 2014), which lead to intentional and unintentional releases and subsequent establishment of populations in the wild (Chucholl et al. 2012; Chucholl 2014).

Among the nearly 700 freshwater crayfish species (Crandall and De Grave 2017), marbled crayfish is the only species reproducing *via* obligatory apomictic parthenogenesis (Scholtz et al. 2003; Vogt et al. 2008). Thus, the release of even one female into the wild, under optimal conditions, could be enough to establish a population that can represent a threat to native crayfish (Scholtz et al. 2003). Compared to vulnerable indigenous crayfish species (ICS) *Astacus astacus* (Linnaeus, 1758) and *Austropotamobius torrentium* (Schrank, 1803), but also in comparison to other NICS (Hossain et al. 2018; Vogt 2021), marbled crayfish has a much shorter egg incubation period (20–42 days; Hossain et al. 2018). Also, it features earlier maturation (age at maturity between 5–7 months; Vogt et al. 2021) compared to other NICS and ICS (maturation < 1–3 years for NICS and 3–5 years for ICS) and higher fecundity than ICS and most NICS (Hossain et al. 2018). Marbled crayfish has been confirmed as a vector of *Aphanomyces astaci* Schikora (Pc genotype), the causative agent of the crayfish plague (Keller et al. 2014; Mrugała et al. 2015), which is considered responsible for decline and decimation of numerous populations of ICS throughout Europe (Martín-Torrijos et al. 2019). While its potential to outcompete ICS in agonistic interactions has not been studied, marbled crayfish has exhibited the ability to outcompete and dominate other successful NICS such as *Procambarus clarkii* (Girard, 1852), *Faxonius limosus* (Rafinesque, 1817) and *Faxonius immunitis* (Hagen, 1870) in staged laboratory trials (Linzmaier et al. 2018; Hossain et al. 2019c, 2020).

These traits have enabled successful establishment of numerous marbled crayfish populations in the wild (Vogt 2020). Currently, marbled crayfish records have been reported from 15 European countries, Madagascar, Israel and Japan (Vogt 2020, 2021). Even though laboratory reports have shown that its temperature optimum ranges between 18–25 °C (Vogt 2020), marbled crayfish has a wide thermal niche and viable and reproducing populations have been reported from areas/countries with temperate continental climates (i.e. Germany; Chucholl and Pfeiffer 2010; Slovakia; Lipták et al. 2016, 2017; Czech Republic; Patoka et al. 2016) to those with tropical climate (i.e. Madagascar; Jones et al. 2009). Marbled crayfish is classified as a high-risk species according to the Freshwater Invertebrate Invasiveness Scoring Kit (Chucholl 2016; Vogt 2021) and has been listed among the Invasive Alien Species of Union concern (the Union list), which represents the core of the EU regulation on invasive alien species (EU Regulation No. 1143/2014). Species included on the Union list are subject to restrictions on keeping and prohibited for breeding and placement on the market, and Member States are required to place measures for their early detection, rapid eradication and effective management of established

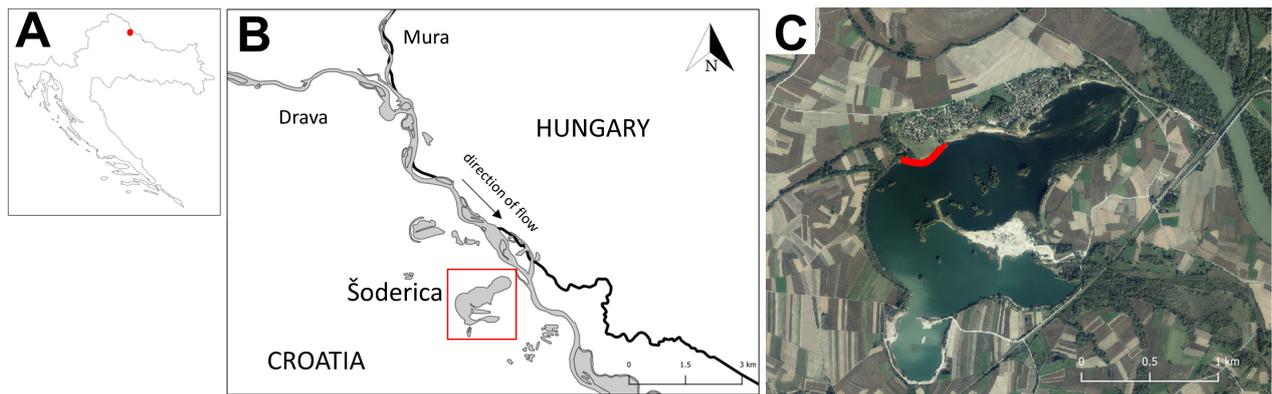


Figure 1. Position of Šoderica Lake – figures A–C show the position of the lake at different scales. In (C) sampling area is marked with the red line.

populations. At the same time, intensive information and communication campaigns are required to minimize both intentional and unintentional introductions of invasive species popular in the pet-trade, such as the marbled crayfish (Patoka et al. 2018).

High reproduction rates, which are typical for parthenogenetic species, are among the key life-history traits of a successful invader (Sax and Brown 2000; Kawatsu 2013) and the understanding of the reproduction dynamics is essential in examining invasion success of a species. As marbled crayfish first appeared in the pet trade (Hossain et al. 2018), most of the knowledge on its reproductive cycle and fecundity comes from laboratory-reared populations (Seitz et al. 2005; Vogt et al. 2004; Vogt 2015; Hossain et al. 2019a). Only a few studies report on marbled crayfish reproduction from established populations in the wild (Jones et al. 2009; Chucholl and Pfeiffer 2010; Lipták 2016, 2017; Andriantsoa et al. 2019) and those are mostly limited to only a short period of field sampling at the same location. This study reports on reproduction dynamics of the established marbled crayfish population in an anthropogenic lake from the continental part of Croatia during a seven-month period (May 2015–November 2015).

Materials and methods

Study area

Šoderica Lake (46°14'20.9"N; 16°54'33.6"E) is an anthropogenic lake formed by sand and gravel exploitation along the right bank of the Drava River near village Botovo in north Croatia, covering approximately 200 ha (Feletar 2016) (Figure 1). Šoderica Lake is fed by Drava River and the water level of the lake corresponds to the water level of the river. Water temperatures of the Drava River measured at Botovo station averaged at 21.4 °C in summer/early autumn (July–September) and 10.3 °C in mid-autumn and winter (October–January). In the northern part of the lake, the average water depth is about 8 meters, although most of the central part of the lake is shallow with water depths ranging from 0.5 to 2 meters. Several larger and smaller islands overgrown with autochthonous shrubby and forest

vegetation have been formed in the northern part of the lake. Water depth in the southern part of the lake reaches up to 20 meters (Kranjčev 2002). Along the northern shore of the lake, there is a tourist settlement with approximately 400 houses, which contributes to an intensive eutrophication of the lake (Feletar 2016).

A native noble crayfish (*A. astacus*) population was recorded in the Šoderica Lake, and the invasive signal crayfish *Pacifastacus leniusculus* (Dana, 1852) population was identified in the Drava River nearby (Hudina et al. 2009; Maguire et al. 2011). The first record of the marbled crayfish in Šoderica Lake was reported in 2013 (Samardžić et al. 2014) and it is the only reported site of marbled crayfish distribution in Croatia to date.

Field sampling

Sampling was performed once a month from May 2015 to January 2016 in the 300 meters long stretch at the northern part of Šoderica Lake (Figure 1). Crayfish were collected at night, by applying baited stick catch (Polcar and Kozák 2005). Baits (pieces of meat, approximately 100 g) were exposed every 5 meters on a 30 cm long stick at a depth of approximately 40 cm and were inspected from the shoreline. Crayfish were collected using hand nets (mesh size 3 mm). The collection effort was approximately two people for two hours for each sampling occasion. Since the shoreline is mostly composed of sand and gravel, and the bottom is overgrown with macrophytes from approximately 50 cm depth, no shelters were available for inspection and only active (freely roaming) crayfish were collected.

Upon capture, all individuals of marbled crayfish were transferred to the laboratory of the Department of Biology at the Faculty of Science in Zagreb for further analyses. Berried females and females with juveniles were transported individually in separate small containers (approximately 15 × 15 cm), while the non-berried females were transported together in large containers (70 × 50 cm). Basic morphometric measurements were taken using a digital caliper (accuracy 0.05 mm): total length (from the tip of the rostrum to the end of the telson; TL), pleon length (from the posterior edge of carapace to the posterior edge of the last pleon somit; PL) and pleon width (width of the first pleon somit; PW) (Sint et al. 2005; Hossain et al. 2019a). All individuals were weighed using a digital scale (accuracy: 0.001 g) and inspected for the presence of glair glands and the molting status according to Lowery (1988).

Body condition indices and fecundity analysis

Morphometric parameters and weight data of individuals were used to calculate Fulton's condition factor (Streissl and Hödl 2002): $FCF = W / TL^3$, where W = weight (g), and TL = total length (mm) of the individuals. Body condition is an important determinant of health and fitness of individual

Table 1. Literature datasets of populations of marbled crayfish in the wild and those reared in the laboratory used for comparisons with the data acquired within this study.

Data type	Population type	Reference	Location
Pleopodal eggs – TL	Laboratory populations	Seitz et al. (2005)	
		Hossain et al. (2019a)	
		Vogt et al. (2019)	
	Wild populations	Jones et al. (2009)	Madagascar
		Lipták et al. (2017)	Slovakia
Juveniles – TL	Wild population	Lipták et al. (2017)	Slovakia

animals (Peig and Green 2010). Following morphometric measurements, in each monthly catch, 20 randomly selected individuals (TL > 40 mm) were dissected and their gonads and hepatopancreas removed. Gonads and hepatopancreas were weighed separately to calculate the gonadosomatic (GSI) and hepatosomatic (HSI) indices (Rodríguez-González et al. 2006):

$$\text{GSI} = (W_{\text{gonads}} / W_{\text{individuals}}) \times 100$$

$$\text{HSI} = (W_{\text{hepatopancreas}} / W_{\text{individuals}}) \times 100, \text{ where } W = \text{weight (g)}.$$

These organosomatic indices are frequently used to determine crayfish condition (Huner et al. 1990; Yamaguchi 2001; Carmona-Osalde et al. 2004; Lucić et al. 2012). The GSI is related to reproductive status of an animal and the HSI is indicative of its energy status (Rodríguez-González et al. 2006, Jin et al. 2019).

After gonad dissection and weighing, selected individuals were further analyzed by counting the number of secondary vitellogenic oocytes in different maturation stages (Abdu et al. 2000; Vogt et al. 2004; hereon referred to as ovarian eggs) in both anterior and posterior ovarian sacs of dissected gonads. For this purpose, gonads of each dissected female were stored separately in a 4% formaldehyde solution and ovarian eggs were counted using a microscope. If individuals carried pleopodal eggs or 1st/2nd stage juveniles (hereon referred to as hatched juveniles) on the pleon, they were carefully removed from the female and/or the individual container in which it was transported and each egg/hatched juvenile was counted separately.

Finally, we compared the relationships between the number of pleopodal eggs and hatched juveniles and total length of crayfish with literature data on wild marbled crayfish populations and those reared in the laboratory (Table 1).

Statistical analyses

Obtained data were tested for normality/homoscedasticity (Zar 2010) to analyze whether they meet the assumptions for parametric tests. Since the data violated these assumptions, their nonparametric analogues were used instead (Kruskal-Wallis ANOVA with post-hoc multiple comparisons of the mean ranks, Mann-Whitney U test, Spearman Rank; Zar 2010).

Morphometric measurements, number of ovarian and pleopodal eggs and number of hatched juveniles were described by standard descriptive statistics (mean, standard deviation, range). Spearman Rho correlation coefficient was used to calculate correlations between crayfish body size and fecundity (ovarian, realized), organosomatic and body condition indices (HSI, GSI and FCF), fecundity, as well as between overall and monthly records of HSI and GSI. Number of ovarian eggs (ovarian fecundity), and the number of pleopodal eggs and hatched juveniles (realized fecundity) were correlated with measured parameters of crayfish size (TL, W, PL, PW) and crayfish condition (FCF). Organosomatic and body condition indices were compared among months using the Kruskal-Wallis ANOVA analysis, and between berried and non-berried females using Mann-Whitney U test. Above-mentioned statistical analyses were performed using Statistica 13.3.0 (TIBCO Software Inc. 2017). In order to test for significance among the number of pleopodal eggs and hatched juveniles and total length of crayfish from our study with literature data on wild marbled crayfish populations and those reared in the laboratory, linear model (lm) analysis was performed using basic R “stats” package in R v. 3.2.0 (R Core Team 2017). Results were visualized using Statistica 13.3.0 (TIBCO Software Inc. 2017).

Results

Monitoring was performed from May 2015–January 2016. However, no crayfish were caught during the last two months of monitoring. During the remaining seven months (May 2015–November 2015) a total of 404 marbled crayfish individuals were caught in Šoderica Lake, out of which 35% (140 individuals) were used in the fecundity analysis (20 randomly selected individuals TL > 40 mm from each monthly catch). During the course of the study, one noble crayfish individual was caught.

The size structure of caught individuals is represented in the Figure 2. Mean weight of caught individuals (N = 404) was 5.9 ± 4.0 g (Figure 2a) and ranged from 0.6–22.6 g, while mean total length (TL) was 61.1 ± 13.3 mm (Figure 2b), and ranged from 31.9–96.0 mm.

The highest numbers of individuals were caught in May and September, which represented the peaks of crayfish activity in the lake (Table 2). From May to November, we observed individuals with oocytes in maturation-stage (mature ovarian eggs) and active glair glands which was an indication that individuals were ready to spawn. However, despite regular records of reproductively active females, pleopodal eggs were observed only in June and September in five individuals, while hatched juveniles were observed only in 23 individuals during June, September, October and November (Table 2).

The average number of ovarian eggs per female (N = 140; from 20 randomly selected individuals in each monthly catch with TL > 40 mm) was

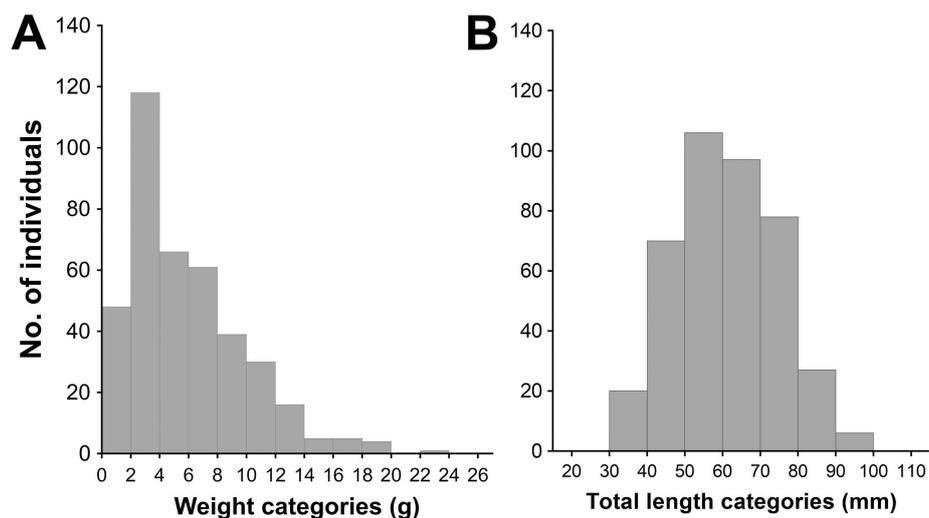


Figure 2. Size structure of marbled crayfish individuals (N = 404) collected from Šoderica Lake using hand nets (May 2015–November 2015): A) weight (g) and B) total length (mm).

Table 2. Reproductive cycle parameters of marbled crayfish population measured during the period of seven months. Recorded parameters are presented as either presence (+) or absence (–) data or as numbers (number of caught crayfish and crayfish with pleopodal eggs or juveniles).

	May	June	July	August	September	October	November
Moulting	–	+	+	+	–	–	+
Active glair glands	+	–	+	+	+	+	+
Ovarian eggs	+	+	+	+	+	+	+
Pleopodal eggs	–	4	–	–	1	–	–
Juveniles	–	4	–	–	2	11	6
No. of caught individuals	181	31	43	30	60	32	27

Table 3. Ovarian and pleopodal fecundity and hatched juveniles in the marbled crayfish population from Šoderica Lake.

	No. of analysed individuals	Average no. of eggs or juveniles	St. dev.	Min	Max	Average female TL [mm]	Min TL [mm]	Max TL [mm]
Ovarian eggs	131	300	187.16	0	860	66.46	33.26	96.04
Pleopodal eggs	5	248	191.69	30	556	74.60	53.14	91.22
Hatched juveniles	23	149	101.06	1	394	74.68	56.03	96.04

Table 4. Spearman rank order correlation coefficients (r_s) between ovarian fecundity (N = 131) or number of hatched juveniles (N = 23) and measured morphometric parameters: weight (W), female size (TL), and pleon size (pleon length: PL, pleon width: PW) of marbled crayfish population from Šoderica Lake. All pairwise correlations were statistically significant at $p < 0.05$.

	W [g]	TL [mm]	PL [mm]	PW [mm]
No. of ovarian eggs	0.42	0.36	0.37	0.38
No. of juveniles	0.52	0.54	0.53	0.49

300 ± 187.2, and the smallest female with ovarian eggs had total length of 33.26 mm (Table 1). The average recorded pleopodal fecundity (N = 5) was 248 ± 191.7 eggs, while the average number of recorded hatched juveniles per female (N = 23) was 149 ± 101.1 (Table 3). Ovarian egg number and the number of hatched juveniles were significantly correlated with all measured morphometric parameters (TL, W, PL, PW; Table 4; Figure 3a). Finally, the number of ovarian eggs and the number of hatched juveniles were significantly correlated with body condition parameter FCF (Spearman rank correlation: $r_s = 0.349$, and $r_s = 0.546$; $p < 0.05$), while pleopodal fecundity

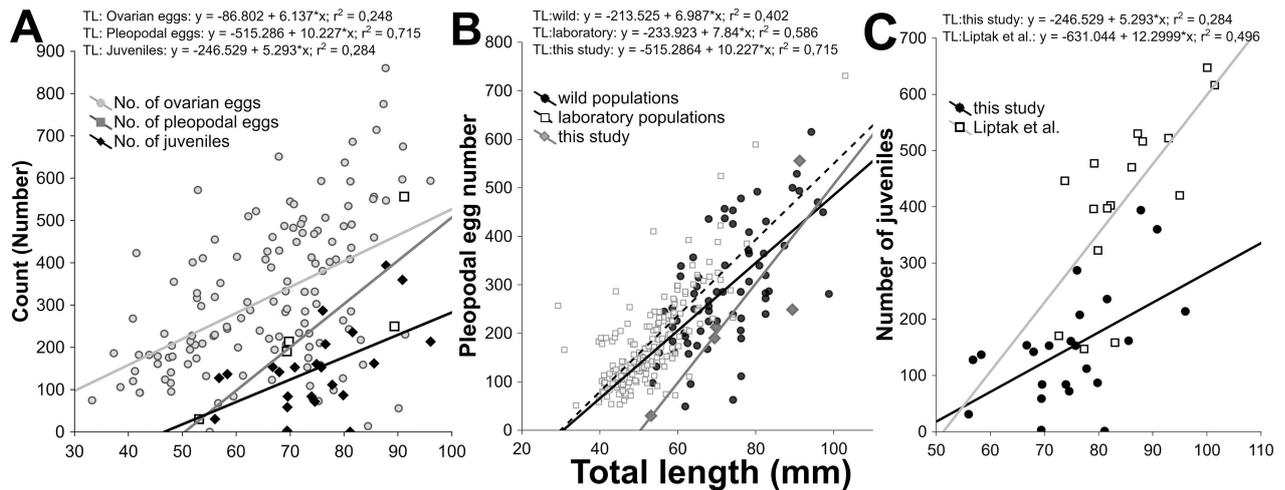


Figure 3. Relationship between: (A) female size (TL) and number of ovarian eggs, pleopodal eggs and hatched juveniles, (B) female size (TL) and pleopodal fecundity from literature data from laboratory and wild marbled crayfish populations and this study and (C) female size (TL) and number of hatched juveniles from this study and from literature data.

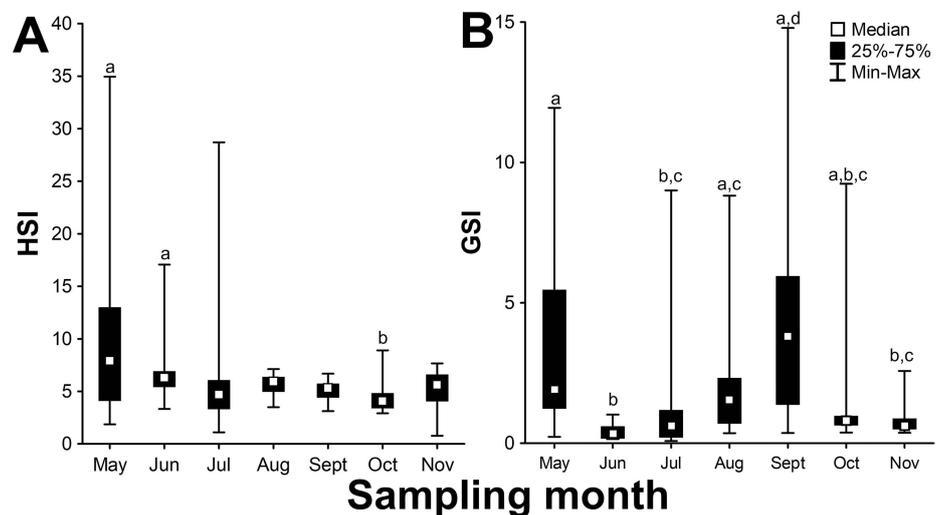


Figure 4. Monthly changes in the marbled crayfish gonadosomatic indices: A) HSI and B) GSI examined using Kruskal-Wallis ANOVA with post-hoc multiple comparisons of mean ranks. Different letters indicate significant differences ($p < 0.05$) in GSI and HSI.

also tended to increase with FCF, but the correlation was not statistically significant ($r_s = 0.026$, $p > 0.05$). None of the measured parameters of fecundity (number of ovarian or pleopodal eggs and number of hatched juveniles) were significantly correlated with measured organosomatic indices (HSI and GSI).

GSI and HSI exhibited significant variations among the analyzed months (Kruskal-Wallis ANOVA with multiple comparisons of mean ranks: GSI $H_{(6,132)} = 43.93$, $p < 0.001$; HSI $H_{(6,132)} = 21.24$, $p = 0.002$; Figure 4). The GSI was the highest in May and September and the lowest in June and November, i.e. in periods when females with pleopodal eggs and hatched juveniles were recorded. The HSI was the highest in May, followed by June and remained relatively constant in the upcoming months (Figure 4). A significant positive correlation between HSI and GSI was established ($r_s = 0.249$, $p < 0.05$) during the whole monitoring period, as well as for specific months (May and

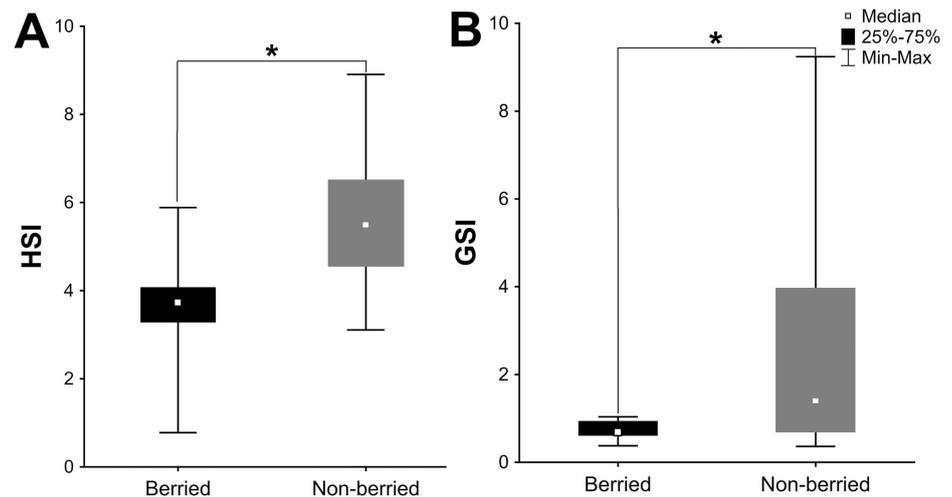


Figure 5. Differences in A) HSI and B) GSI between berried females (i.e. females carrying eggs or juveniles) and non-berried females examined using Mann-Whitney U test. Berried females had significantly lower values of both organosomatic indices. Statistically significant differences ($p < 0.05$) are marked with *.

July: $r_s = 0.597$ and $r_s = 0.853$, $p < 0.05$, respectively). Finally, we compared the HSI and GSI of berried vs. non-berried females and found significantly lower HSI (Mann-Whitney U test: $U = 100$, $p < 0.001$, $N_1 = 20$, $N_2 = 40$) and GSI ($U = 198$, $p = 0.003$, $N_1 = 20$, $N_2 = 40$) in females with pleopodal eggs or juveniles (Figure 5).

Finally, we tested for significance among the number of pleopodal eggs and hatched juveniles and total length of crayfish from our study with literature data on wild marbled crayfish populations and those reared in the laboratory. Results of linear model (lm) showed significant differences ($F_{(2,251)} = 58.60$, $p < 0.001$) among all examined groups (laboratory, wild populations and this study) in pleopodal fecundity and TL relationship (Figure 3b). Also, the number of hatched juveniles related to TL exhibited significant differences ($F_{(1,36)} = 64.7$, $p < 0.001$) between our study and literature data on wild populations (Lipták et al. 2017).

Discussion

The reproductive cycle and reproduction dynamics in wild marbled crayfish populations is only partially studied (Lipták et al. 2016, 2017; Hossain et al. 2018) and our study reports, for the first time, systematic seven-month long monitoring of the marbled crayfish reproductive cycle in a wild population from an anthropogenic lake in northern Croatia. Šoderica Lake features the only known marbled crayfish population in Croatia. However, due to proximity of the Drava River and similar gravel pit lakes, it is highly possible that the species has already spread to nearby waterbodies (Samardžić et al. 2014). In Šoderica Lake, marbled crayfish co-occurs with the native *A. astacus*; however, the impacts of the marbled crayfish on *A. astacus* populations have not been studied. The *A. astacus* abundance in the lake is considered to be low and only one individual was caught in the lake throughout all of our monitoring campaigns.

Out of all caught marbled crayfish (404 individuals), the highest number of individuals were caught in May (181 individuals) and September (60 individuals), which represented the peaks of marbled crayfish activity in the lake. We were unable to compare these activity peaks to literature data since the sampling period in the majority of other studies lasted over one or two months (Chucholl and Pfeiffer 2010; Lipták et al. 2017; Ercoli et al. 2019). The size range of caught individuals corresponded to those reported in other studies (Jones et al. 2009; Lipták et al. 2017; Andriantsoa et al. 2019; Ercoli et al. 2019; Vogt 2021), with the average size of caught individuals (61.1 mm TL) being smaller compared to other European populations (Lipták et al. 2017; Ercoli et al. 2019; Vogt 2021) and the most similar to the Madagascar populations (69 mm TL; Jones et al. 2009; 30–80 mm TL and 1–10 g W; Andriantsoa et al. 2019).

In Šoderica Lake, individuals bearing eggs or hatched juveniles were caught in June and from September to October, while individuals with maturing ovaries (i.e. secondary vitellogenic oocytes in different maturation stages) were recorded over the longer period (May–November) and comprised 93.6% of the individuals used in the ovarian fecundity analysis. The smallest size of the marbled crayfish with mature ovaries was recorded at 33.26 mm TL, which is in line with the literature data. Ovary in marbled crayfish is structurally complete at body size around 20 mm TL (Vogt et al. 2004). The oocytes mature in the following life stages and pleopodal eggs in laboratory-reared marbled crayfish usually appear at around 40 mm TL (Vogt and Tolley 2004; Vogt et al. 2004) and only exceptionally in smaller individuals (Seitz et al. 2005; Vogt et al. 2018). Individuals longer than 40 mm TL caught in Šoderica Lake comprised 95% of the total catch, and the smallest individual caught with pleopodal eggs was 53.14 mm TL. Therefore, the majority of caught crayfish in our study were sexually mature. The observed body size distribution in this study is probably the result of the applied trapping method: although hand catch has been considered less biased in relation to size of the captured individuals compared to crayfish traps (Hilber et al. 2020), catch by hand nets has also been shown to lead to size bias since larger and adult crayfish are easier to spot (Rabeni et al. 1997; Davis and Huber 2007). Larger average size of caught individuals in other European populations might also be the consequence of the applied trapping method: we applied baited stick catch in shallow parts of the lake in our study, while other studies combined either manual search or artificial refuge traps with the application of crayfish traps (Lipták et al. 2017; Ercoli et al. 2019), which are known to be size-selective towards larger individuals (Rabeni et al. 1997).

Based on the gathered data, we assume that marbled crayfish has two reproductive peaks in Šoderica Lake: first in late spring/early summer and second in mid-autumn, which is in line with the observations from laboratory populations (Vogt 2015) and populations from Madagascar (Jones et al. 2009;

Vogt 2015). In wild populations of central Europe, ovigerous females can be found throughout most of the year (Vogt 2020). In the Moosweiher Lake in southern Germany, heterogeneous ovary development stages of marbled crayfish were observed, and berried females were found from early June to late July (Chucholl and Pfeiffer 2010). In the same lake, individuals carrying pleopodal eggs or juveniles were also found in December and January (Vogt 2020). In the Czech Republic, females with well-developed glair glands and oocytes have been reported in March (Patoka et al. 2016), while in Slovakia, individuals with pleopodal eggs or juveniles were found in September and October (Lipták et al. 2017). This suggests that in other waterbodies in Europe, reproduction peaks probably occur in late spring/early summer and in autumn/winter; however, systematic and regular monitoring of reproductive cycles is lacking for these sites, and no comparisons with the Šoderica Lake population could therefore be made. Even though marbled crayfish exhibits high ecological plasticity and may colonize diverse habitats with variable water and habitat characteristics (e.g. Veselý et al. 2015; Andriantsoa et al. 2019), site-specific conditions (i.e. temperature regime, availability of shelters and food, population density, presence of other congeners and water quality) might significantly affect its reproduction dynamics (Marenkov et al. 2017; Hossain et al. 2019b, 2021), as is the case in other crayfish species (Jones and Ruscoe 2001; Reynolds 2002; Alcorlo et al. 2008; Harlioğlu and Farhadi 2017). The small number of caught berried females in this study (7% of the total catch: five individuals with pleopodal eggs and 23 individuals with juveniles) in comparison to other studies (berried females comprised 69% of the total catch in Slovakia; Lipták et al. 2017; and 12% in Madagascar; Jones et al. 2009) might also be the result of the applied trapping method. Baited stick catch in shallow parts of the lake, as performed in our study, is potentially biased towards active (freely roaming) crayfish, while berried females are largely inactive and usually retreat into shelters (Reynolds 2002; Vogt et al. 2018, 2019). Due to morphology of the lake (shoreline with sand/gravel with macrophyte vegetation from approximately 50 cm depth) we were unable to sample crayfish in shelters, which has probably introduced a sampling bias toward non-berried crayfish.

The assumption of two reproductive peaks in the Šoderica Lake population is further supported by the obtained GSI values, which were the highest in May and September, i.e. months that preceded the reproductive peaks. The GSI was the lowest in June and November, i.e. when the highest number of individuals with pleopodal eggs and hatched juveniles were recorded. Ovarian fecundity tended to increase with the GSI, which was expected since mature ovary increases in size as the oocytes proliferate and increase in diameter during yolk incorporation (Laufer et al. 1998). Interestingly, unlike in a number of decapod crustacean species, there was a significant positive correlation between GSI and HSI. In decapod

crustaceans, hepatopancreas is considered to provide transfer of energy reserves to gonads for gametogenesis, which usually results in inverse correlation between the two organosomatic indices (e.g. Beatty et al. 2003; Rodríguez-González et al. 2006; Peruzza et al. 2015; Farhadi and Harlioğlu 2019) since vitellogenin, the main egg yolk protein, is produced in the hepatopancreas (Tseng et al. 2001). In the case of the marbled crayfish in Šoderica Lake, HSI was the highest in May, followed by June and remained relatively constant in the remaining monitoring period. Therefore, the changes of HSI increment could not be directly linked to gonadal development, as is the case in other crayfish species. This could be due to the impact of other environmental factors such as water temperature, photoperiod length or food availability, which are also known to affect the dynamics of HSI increment (Farhadi and Harlioğlu 2019). However, relatively constant HSI values from June to November may indicate the constant energy transfer from hepatopancreas to gonad development. Finally, we observed significantly lower HSI (and GSI) values in females carrying pleopodal eggs and juveniles (berried females) compared to non-berried females, which may be related to the fact that in berried females transfer of energy reserves to gonads has occurred (as mentioned above) and berried females are less active and feed less (Vogt et al. 2018, 2019), which impacts their energetic status and consequently HSI.

Although we observed two distinctive reproduction peaks, females with mature ovarian eggs were continually observed in the population from May to November and individuals with active glair glands (developed in reproduction season prior to spawning; Vogt 2018) were observed through almost the entire monitoring period (Table 2), which indicates that reproduction in marbled crayfish may be constant during this period in climatic conditions of continental Southeastern Europe. This could also explain the above-mentioned relatively constant HSI values observed throughout the monitoring. Also, this is in line with the current knowledge of species reproductive biology, which suggests that if the conditions are favourable, single marbled crayfish can reproduce throughout the whole year (Vogt et al. 2004; Seitz et al. 2005; Vogt 2015); the results of laboratory-reared populations further suggest a potential fusion of two reproductive peaks to one broader summer/autumn peak in wild populations of central Europe (Vogt 2015). Thus, it is also possible that one broader reproductive peak (June–November) exists in the Šoderica Lake population, but was not recorded during July and August due to above mentioned sampling bias towards non-berried females and also since the crayfish that carry juveniles retreat to deeper parts of the lake (as in other crayfish species, e.g. Tulonen et al. 2008) and were therefore inaccessible for hand catch.

As expected, fecundity was correlated with TL as in other crayfish species (Abrahamsson 1971; Eversole and Mazlum 2002; Maguire et al. 2005; Kozák et al. 2006; Yue et al. 2010; Hudina et al. 2011; Martinsson 2011;

Chybowski 2013; Pârvulescu et al. 2015) and other marbled crayfish populations (Seitz et al. 2005; Jones et al. 2009, Lipták et al. 2017; Hossain et al. 2019a; Vogt et al. 2019). Pleopodal fecundity did not show significant correlation with crayfish size (TL) in the Šoderica Lake population, most likely due to the small sample size (five individuals). Significant differences in pleopodal fecundity and TL relationship were observed between wild and laboratory populations from the literature and our study. These differences could be the consequence of the small sample size of females with pleopodal eggs from the Šoderica Lake population, but also due to: i) differences in environmental and ecological conditions between wild and laboratory populations, and ii) size differences between these groups. In laboratory-reared populations, females with pleopodal eggs had a body size up to 65 mm TL, while wild populations comprised larger mature females with about one third of individuals up to 65 mm TL.

In comparison with literature data, the regression slope for the number of hatched juveniles and TL in our study was significantly lower than the one for the marbled crayfish population in Slovakia, recorded by Lipták et al. (2017). This may also be attributed to the different size distribution of captured individuals between these two populations, but it is possible that the population found in the dead-end artificial canal in Bratislava, Slovakia (Lipták et al. 2017) has more favorable habitat or environmental conditions than the population in Šoderica Lake.

High reproduction rates are one of the key life-history traits of the successful invaders (Sax and Brown 2000), especially in combination with parthenogenetic reproduction (Stelzer 2011; Kawatsu 2013), as is the case with the marbled crayfish. This study reports, for the first time, systematic monitoring of marbled crayfish reproduction from a wild population in continental Croatia. Such data are of paramount importance for understanding the understudied reproduction dynamics of the marbled crayfish in the wild and for gaining new insights into the species' invasive potential in waterbodies throughout Europe, as well as for predicting the dynamics and risks of its further spread.

Acknowledgements

Authors would like to thank Mišel Jelić, Gordan Hudina, Mirjana Hudina, Tomislav Devčić and Marija Vuk for their help during fieldwork. We also thank the anonymous reviewers and the editor for helpful comments and suggestions on earlier versions of the manuscript.

Authors' contribution

AD – data analysis and interpretation, writing – original draft, writing – review & editing; IM – sample design and methodology, investigation and data collection, writing – review & editing; MB – investigation and data collection; DG – data analysis and interpretation; SH – research conceptualization, sample design and methodology, investigation and data collection, data analysis and interpretation, writing – original draft; writing – review & editing.

Funding declaration

Ana Dobrović has been funded by Croatian Science Foundation Grant DOK-09-2018. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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