

**NOTES ON THE REPRODUCTION PARTICULARITIES
OF A *Viviparus acerosus* (Linnaeus, 1758)
(GASTROPODA, VIVIPARIDAE) POPULATION FROM
SOUTHERN ROMANIA (DOLJ COUNTY, OLTENIA REGION)**

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Abstract. *We analyzed the fecundity of a Viviparus acerosus population from southern Romania in the autumn period (October 2020). For this, we collected 52 specimens from a channel near Teasc Village, Dolj County. Of these, 13 females were gravid, with a total number of 210 embryos, most in stage III of evolution. The number of embryos was not correlated with the dimensions of the females; therefore, the fecundity of females was not determined exclusively by the dimensions of the females. The studied population reproduces till late autumn, maybe even continuously in the investigated habitat.*

Keywords: *freshwater Gastropods, fecundity, wet areas, climate, Danube.*

INTRODUCTION

Viviparids are snail species widespread around the whole world. They are found in five continents: Europe (e.g., Arias et al. 2020), Australia and New Zealand (e.g., Sheldon & Walker 1993, Carini & Hughes 2006, Torres et al. 2018), Asia (e.g., Gupta et al. 1981, Chung & Jung 1999, Lu et al. 2014, Yesmin et al. 2018, Gu et al. 2019, Saito & Kagawa 2020, Ye et al. 2020), North America (e.g., Kingsbury et al. 2020), South America (e.g., Constanza et al. 2012, Ferreira et al. 2017) and Africa (e.g., Sengupta et al. 2009). In Europe, the family is represented exclusively by the *Viviparus* genus, which is distributed in almost all countries, for example, Ukraine (Ryabceva 2013, Uvaeva 2011, Anistratenko et al. 2019, Yanygina & Volgina 2019, Uvayeva et al. 2021), Turkey (e.g., Arslan et al. 2017),

Poland (e.g., Augustyniuk & Jakubik 2002), Czech Republic (e.g., Beran 2013), Serbia (e.g., Despotovic et al. 2019), Bulgaria (e.g., Glöer & Georgiev 2014, Georgiev & Hubenov 2013), Romania (e.g., Neubauer et al. 2014), Switzerland (e.g., Ribi & Gebhardt 1986). In some regions of the world, the Viviparid species were introduced by humans, and they became invasive in several countries in Europe, for example, Belgium (Van den Neucker et al. 2017), Netherlands (Soes et al. 2011), Rusia (Yanygina & Volgina 2019), or other countries around the world, like Argentina (Constanza et al. 2012) Brazil (Ferreira et al. 2017), Canada (Kingsbury et al. 2020), New Zealand (Torres et al. 2018), and Turkey (Arslan et al. 2017).

In Romania, the *Viviparus* genus was found along the Danube and in the Danube Delta (Cismaşiu et al. 2016, Glöer & Georgiev 2014, Grossu 1973, 1986, Stoica et al. 2014), rivers from mountainous, hill, and plain regions (Cismaşiu et al. 2016, Cioboiu 2007, 2011, Sîrbu 2006, Sîrbu et al. 2010). In the Oltenia region from southern Romania, the following species were mentioned in the literature: *Viviparus acerosus* (e.g., Cismaşiu et al. 2015, Cioboiu & Cismaşiu 2016, Cismaşiu & Cioboiu 2018, Cioboiu et al. 2018) *Viviparus viviparus* (Cioboiu 2006, 2011, 2013, 2014) *Viviparus contectus* (Cioboiu 2006, 2013), *Viviparus hungaricus* (Cioboiu 2006, 2013). Among them, *Viviparus acerosus* has a Danubian distribution (e.g., Glöer 2019). It is characteristic of the middle and lower sectors of the rivers, as it is present both in slow and fast-running waters, of different depths, with or without aquatic vegetation, on muddy or other bottom types (Čejka et al. 2020, Cioboiu & Cismaşiu 2016).

The *Viviparus* species have important ecological roles in their habitats. They have a high capacity for accumulating heavy metals, so they are important for pollution monitoring (Strungaru et al. 2018, Despotović et al. 2019). The industrial, agricultural, and domestic wastes discharged in surface waters can produce cytotoxic damage in the *Viviparus* species (Barsiene 2003) or changes in oxidative stress biomarkers (Despotović et al. 2012).

There are several studies concerning the biometry of *Viviparus acerosus* (e.g., Gural et al. 2019), the ecology of the species within the *Viviparus* genus (Anistratenko et al. 2019, Beran 2013, Uvaeva 2011), the reproduction of *Viviparus contectus* (Jakubik 2009, Ryabceva 2013), *Viviparus sphaeridius* (e.g., Ryabceva 2013), *Viviparus viviparus* (Jakubik 2007, 2011, 2012). To our best knowledge, studies concerning the

reproduction of *Viviparus acerosus* in Romania are absent. Thus, we aimed to fill this gap and began to study the reproduction of *Viviparus acerosus* in southwestern Romania. We hypothesized that in this region of Romania, where the climate is warmer than in the other areas (e.g., Stoenescu et al. 1966, Mândruț 2006), *Viviparus acerosus* reproduce the most intense during summer, but due to the warmer climate, the reproduction period extends until autumn. The objective of our study was to observe if this species is still in the reproductive period in the autumn and if the females' size influences the number of their offspring.

MATERIAL AND METHODS

The samples were collected in 3rd of October 2020 from a stream near Teasc Village, Dolj County, approximately 15 km south of Craiova city. The studied area is in a plain region (Oltenia Plain (Mândruț 2006)). The climate is temperate continental with sub-Mediterranean influences (Mândruț 2006, Mărculeț 2019), with a tendency for climate aridification (Dumitrașcu et al. 2016). The highest temperatures in the summer are between 35,0°C – 43,0°C and the precipitations are scarce during the year (less than 549,6 mm/per year and 173,3 mm/during the summer months). The frequent drought periods in the summer are usually associated with heat waves when the air temperature reaches 41°C (Roșca 2012, Croitoru et al. 2013, 2016). Moreover, from the climate evolution analysis of the last over 55 years, a tendency of increase in the air temperature was observed as the precipitation amount didn't encounter significant changes nor as total amounts, nor as indicators of extreme precipitations (Roșca 2013, Croitoru et al. 2013, 2016, Roșca et al. 2017).

In the studied region, the land is mostly used for agriculture (around 50%), especially for wheat, vineyards, and vegetables (Iordache & Bădiță 2013, Cotea et al. 2016, Ionică 2016).

From a hydrological point of view, the region is part of a protected national interest area named the Lacustrine Complex Adunații of Geormane (code 2392), situated in the Jiu River system (Gavrilescu et al. 2017). The site is characterized by several lacustrine ecosystems: Marica Pond, Victoria Lake, and other surrounding wetlands. The Lacustrine Complex is situated on the Rojișteea terrace on the superior left side of the Jiu River at approximately 25 km south of Craiova in the Oltenia Plain. The Lacustrine Complex is connected to the Jiu by a channel that ensures water circulation (Gavrilescu et al. 2017).

The samples were collected from a channel situated in the western part of Teasc, between the locality and the Jiu River. The channel was narrow, around 3-4

m in width and about 60-90 cm in depth. The substrate comprised a thick layer of mud (more than 50 cm). The channel is full of *Typha* at both banks and has a portion of 1-2 m width in the middle of the channel uncovered, without vegetation.

We have collected a sample of 52 individuals with a hand net. The specimens were preserved in situ in a 4% formaldehyde solution and transported to the laboratory. The following parameters were analyzed: size structure, number of gravid females, the relationship between the shell size, body mass, and fecundity, and the number of embryos in each stage per female.

The shells' height and width were measured with a caliper with an accuracy of 0,1 mm. The shells were distributed in classes depending on their height and width. The snails were weighted with an electronic balance (accuracy 0,001 g). The females were dissected, and their embryos were removed. Following the classification from Jakubik (2007), the embryos were classified into class I embryos with oval transparent egg capsules, class II embryos with an outline of the shells, and class III embryos with fully developed snails with shells.

The shells with body content, the empty shells, and the bodies removed from the shells were weighted using an electronic balance with an accuracy of 0,001 g. The Pearson correlation and the Kruskal-Wallis test for equal medians were calculated using the Past 4.03 program (Hammer et al. 2001).

RESULTS

From the 52 analyzed specimens, we found 13 females with embryos, representing 25% of the investigated individuals. After measuring the height and width of the shells of the gravid females, we found out that all specimens belong to class IV of dimensions – 25.1 mm or higher (according to Jakubik 2006); the height of females varied between 26.8 mm and 34.1 mm (H) and the width between 18.6–25.1 mm (W). The values of the shells' height were grouped into six size classes, with a class interval of 1.2 mm. Table 1 shows the number of individuals and their frequencies from each dimension class for the shell height of gravid females.

The same classification was made for the width of the shells; these were grouped into six size classes with a 1 mm class interval (Table 2).

The total weight of the females was between 4.52 g and 9.92 g, and the weight of the body mass varied between 1.39 g and 5.57 g. In the uteri of the 13 females, we found 210 embryos in each of the three developmental stages. We found 24 embryos in stage I, 59 embryos in stage II, and 127 embryos in stage III. The biggest female had only two embryos in stage III,

and the smallest female had 15 embryos, two in stage I, three in stage II, and 10 in stage III.

Table 1. Dimension classes for the shell height of the gravid females

Class (mm)	26.8-28.0	28.1-29.3	29.4-30.6	30.7-31.9	32.0-33.2	33.3-34.1
No. of females	3	1	5	1	2	1
Frequency (%)	23.08	7.69	38.46	7.69	15.38	7.69

Table 2. Dimension classes for the shell height of the gravid females

Class (mm)	18.6-19.6	19.7-20.7	20.8-21.8	21.9-22.9	23.0-24.0	24.1-25.1
No. of females	2	0	4	3	2	2
Frequency (%)	15.38	-	30.77	23.08	15.38	15.38

The number of embryos of each stage/female depending on their height was the following: for the female from class 26.8-28 mm, we found two embryos in stage I, 36 embryos in stage II, and 27 embryos in stage III, totalizing 65 embryos for the females from this class. For the females from class 28.1-29.3 mm we found five embryos from stage I, three embryos from stage II, and 10 embryos in stage III, a total of 18 embryos in the females from this class. The females from class 29.4-30.6 mm had the highest number of embryos in their uteri - 80, distributed as follows: 12 in stage I, 14 in stage II, and 54 in stage III. The females from class 30.7-31.9 mm had 19 embryos, of which two were in stage I, three in stage II, and 14 in stage III. In the next class, 32-33.2 mm, we found 26 embryos, most of them in stage III - 20, three embryos in stage II, and two in stage I. In the last class of dimensions 33.3-34.1 mm, we found the smallest number of embryos, only two in stage III.

The Pearson correlation between the size of the shell and the weight of the specimens was positive ($r = 0.51$ for the body mass and shell weight and $r=0.97$ for the total weight and shell weight), but the correlation between the height of the shell and the number of embryos was negative ($r=-0.23$), as well as the correlation between the body mass and the number of embryos ($r=-0.56$). The Kruskal-Wallis test for equal medians between the body mass and the number of embryos $H(\chi^2)=14.6$, and

between the shell height and the number of embryos H ($\chi^2=18.78$) shows a significant difference between sample medians ($p<0.001$).

DISCUSSION

The proportion of gravid females was relatively small (only 25%) compared to the data known on *Viviparus viviparus* (Jakubik 2007, 2011, 2012), *Viviparus contectus* (Jakubik 2012), *Viviparus sphaeridius* (Ryabceva 2013) and *Viviparus ater* (Ribi & Gebhardt 1986), where the proportion of gravid females in the population exceeded 50% sometimes. Our specimens were collected in October; therefore, we expected a smaller proportion of gravid females because the reproduction is the most intense during the spring and summer, and the number of gravid females during the autumn was also small in other *Viviparus* species (Jakubik 2007, 2011, 2012, Ryabceva 2013, Ribi & Gebhardt 1986). To our best knowledge, no studies have been published on the reproduction of *Viviparus acerosus* yet, so we don't know its exact reproduction period. Because it is congeneric to other European Viviparid species, we suppose that it has a similar reproductive period. The ecology and the habitat of this species are the same as those of other *Viviparus* species, so there is no reason for a different reproductive season.

The collected females belong to dimension class IV according to several authors (Jakubik 2012, Ribi & Gebhardt 1986, Augustyniuk & Jakubik 2002), although the literature mentions that the smaller females can also carry embryos (Jakubik 2012). The proportion of embryos in different stages is similar to the data from other populations of other *Viviparus* species (Jakubik 2012, Ribi & Gebhardt 1986). In the literature, a high number of embryos in stage III during the autumn was also mentioned, as the reproduction season comes to an end (Jakubik 2012, Ribi & Gebhardt 1986).

Seven of the 13 gravid females had more than 15 embryos; this proportion of females with a high number of embryos exceeded the data from the literature in the same period of the year (Jakubik 2009, Ribi & Gebhardt 1986). The data published by Jakubik (2009) was made in Poland on *Viviparus contectus*. The differences may be due to the location of the study; Poland is located much further north and has a colder climate than Oltenia. In the study published by Ribi & Gebhardt (1986), *Viviparus ater* has different reproductive behavior depending on the habitat. In one of

the lakes (Lake Zurich), the reproduction has two peaks, one in May and another in August, and the population from Lake Maggiore had only one peak in July (Ribi & Gebhardt 1986). According to the authors, these differences are generated by the environmental conditions in the two lakes, as Lake Zurich has better environmental conditions (temperature, food), so the gastropods can reproduce twice a year (Ribi & Gebhardt 1986).

Only one of the gravid females had just two embryos, both in stage III. The females probably have already laid down most of their embryos in the late summer as other species of *Viviparus* (Jakubik 2009, Ribi & Gebhardt 1986). The number of embryos of all stages in the sampled gravid females was the highest in the 29,4-30,6 mm dimension interval (80 embryos), followed by the 26,8-28 mm class (65 embryos). These were all females of class IV, but the smallest and the medium ones. This is a high number of embryos/females compared to the data from the literature, where the maximum number mentioned was 34 embryos/female at *Viviparius sphaeridius* (Ryabceva 2013), or a variable number from author-to-author in the case of *Viviparus viviparus*: 45 (Alyakrinskaya 1969), 12-74 (Levina, 1992), 64 (Miroshnitshenko 1958), 85 (Zhadin 1928). It seems that the investigated population has high fecundity, probably due to favorable environmental conditions (high temperature of the water and adequate food resources (Ribi & Gebhardt 1986)). Still, because there are no mentions in the literature about the number of embryos/females in this species, this can also be a particularity of *Viviparus acerosus*.

The Pearson correlation was negative both between the height of the shell and the number of embryos, as well as between the body weight and the number of embryos, indicating that there is no connection between the dimension of the females from class IV of size and the number of embryos they produce. In the case of *Viviparus ater*, the shell dimensions of the females and the number of embryos were correlated (Ribi & Gebhardt 1986). The correlation was positive for the populations living in favorable conditions, and it was negative for those living in a less favorable environment (Ribi & Gebhardt 1986). It seems that in the population from southern Romania, the environment does not determine the species to adopt one or another strategy.

The results of the Kruskal-Wallis test show significant differences between the medians, which confirm the results of Pearson correlation, namely that there is no direct relationship between the females' dimensions and the number of offspring they produce. Fecundity can be characteristic

of the individual, not necessarily correlated with the body dimensions. Ribi & Gebhardt (1986) observed at *Viviparus ater* that older females produce fewer but bigger offspring, and younger females produce more numerous offspring of smaller dimensions.

Embryos from stages I and II probably will not be laid before winter, but they will stay in the uteri of the females and will be laid in the following spring before starting a new reproductive season (Rybaceva 2013). Some data from the literature mentions an increased number of embryos/females during the autumn at *Viviparus viviparus* and *Viviparus sphaeridius* (Rybaceva 2013), even with the maximum fecundity in the winter at *Viviparus viviparus* (Rybaceva 2013). Contrarily, other authors encountered a reduction in the number of embryos/females during autumn (Jakubik 2009). These differences are probably due to the environmental factors in which the mollusk populations live. The population from Oltenia has a behavior that looks more like those which have a high fecundity in the winter (Rybaceva 2013). This can result from the living conditions such as the long warm period, mild winters, a constant volume of water, and abundant aquatic vegetation. According to the existing data, compared to other regions of Romania, in this region, the temperatures in the autumn can be very high (Roşca 2012, Croitoru et al. 2013, 2016), and the winters short and mild (Drugescu & Geacu 2004). Thus, in the region, southern species are present and largely distributed (Covaciu-Marcov et al. 2020). The high air temperatures will also maintain high temperatures in water, allowing the gastropod population to be active late in the autumn, as other populations of *Viviparus viviparus* and *Viviparus sphaeridius* from two different rivers in Ukraine (Rybaceva 2013).

The vegetation in the channel is abundant, as it covers almost all of its width, so it offers optimal feeding conditions to the gastropod population. In optimal conditions, the reproductive period can be extended until the colder period of the year, as in other species of *Viviparus* (Ribi & Gebhardt 1986). The channel and the lacustrine system from nearby are fed by groundwater, which does not allow the channel to dry out in the summer and freeze in the winter (Gavrilescu et al. 2017). This fact is also favorable for the gastropod population and can positively affect their breeding period. The presence of numerous springs in the area positively influences another aquatic species with high conservative and biogeographic importance, namely *Umbra krameri* (Cupşa et al. 2021).

The reproductive period of the studied population extends till the end of

autumn at least, and maybe it can be continuous due to favorable environmental conditions. This particularity can offer an advantage to the populations by their ability to produce a higher number of offspring than a population with a shorter reproductive period. Because there is not enough data concerning the reproductive biology of *Viviparus acerosus*, it will be necessary to extend the study throughout the year to establish the exact breeding period.

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