

Different Dynamics of Reproductive Cell Development in Oviparous *Clausilia bidentata* and Ovoviviparous *Ruthenica filograna* Snails

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Most terrestrial snails are oviparous and only some are ovoviviparous. The physiology of the reproductive system and its activity in these two groups have received little attention. We compared the month-to-month dynamics of reproductive cell formation between two species of Clausiliidae snails: *Ruthenica filograna* (an ovoviviparous species) and *Clausilia bidentata* (an oviparous species). Our aim was to test the hypothesis that the seasonal activity of gametogenesis is determined by reproductive strategy (oviparity / ovoviviparity). The results showed that the frequency and number of reproductive cells in different stages varied month-to-month, within each species, and between the two species. *Ruthenica filograna* maintained its reproductive activity throughout the entire observation period, whereas the gonadal activity of *C. bidentata* changed with the seasons (it was temperature dependent). Our results are in tune with the hypothesis of Baur, that ovoviviparity has some advantage over oviparity under harsher environmental conditions.

Key words: Snails, Oviparity, Ovoviviparity, Evolutionary advantage, Reproductive cells.

BACKGROUND

There are 25–30,000 species of terrestrial snail, making them one of the largest taxa among terrestrial animals (Grosberg et al. 2012). Most of the species are oviparous. Our knowledge of the reproduction and embryonic development of Pulmonata is restricted to a few species (Tompá 1979; Tompa et al. 1984; Wirth et al. 1997; Heller et al. 1997; Heller 2001; Kuźnik-Kowalska 1999 2005 2006; Pokryszko 2001; Maltz 2003a b). There is also little data concerning the development of reproductive z cells in other mollusks (Villalejo-Fuerte et al. 2018).

Some research papers, particularly by Sulikowska-Drozd and co-workers, have described the life history and population dynamics of a number of clausiliid species. However, in our opinion, the correlation between habitats and life history has still not been fully analyzed. Detailed information concerning snail reproduction is crucial for studying snail ecology, distribution and protection in the natural environment. This is especially true for species with rare developmental strategies; for example, the phylogeny and evolution of ovoviviparous snails. The family Clausiliidae contains over 1000 species, covering the entire range of reproductive strategies.

There are oviparous, egg-retaining (ovoviviparous), and viviparous species (Maltz and Sulikowska-Drozd 2013). There are Clausilid species that can reproduce both ovoviviparously and oviparously—with egg retention (Mamos et al. 2021). Some aspects of the life cycles of five of these species (*Cochlodina laminata*, *Vestia elata*, *Alinda biplicata*, *Balea perversa* and *Ruthenica filograna*) have been described (Bulman 1996; Kuźnik-Kowalska 1998; Maltz and Sulikowska-Drozd 2008 2011 2012 2014; Sulikowska-Drozd 2009; Sulikowska-Drozd and Maltz 2014; Szybiak 2010).

Three clausilid species occur in the nature reserve “Rezerwat Dębno nad Wartą”: two oviparous species (*Clausilia bidentata* and *C. laminata*) and one ovoviviparous species (*R. filograna*). We decided to focus on the annual life cycle of two species with different reproductive strategies: *C. bidentata*, Ström 1765 and *R. filograna* (Rossmässler 1836). Both are a similar size and found in the same habitat, thus at the same environmental conditions.

Ruthenica filograna is a relatively abundant but not frequently discovered (*i.e.*, appears in scattered but dense populations) species found from Southern Europe (Italy, Bulgaria) to Northern Europe (Estonia). It has been found in France, Austria, Hungary, Switzerland, Czech Republic, Slovakia, Poland and Germany. It is also present in mountains: Harz, Thuringian Forest, Carpathian Mountains, Alps, Franconian and Swabian Jura (Damjanov and Likharev 1975; Falkner 1990; Kerney et al. 1983; Manganelli et al. 1995; Wiktor 2004). It lives in broadleaf and mixed forests, and inhabits ground litter and dead wood (Szybiak 2009 2010). It does not climb plants or abiotic vertical structures, and avoids stones and rocks. *R. filograna* reaches altitudes up to 1500–1700 m above sea level. These snails spend most of their time in the soil and leaves if the temperature and humidity are appropriate (AnimalBase 2012; Baidashnikov 1989).

Clausilia bidentata is a common, frequently discovered snail present in western, southwestern, northern and central Europe: the southern and western boundaries of southern Portugal and northern Spain, northwestern Italy (Liguria), western Switzerland, southwestern and southern central Germany, northern Bohemia, northwestern Poland and Baltic countries near to Baltic Sea (Nordsieck and Neubert 2002). It inhabits humid areas, moderately humid forests, and parks. It tolerates altitudes up to 1000 m above sea level. It can be found on and under rocks, on walls and on tree bark, as well as in litter (AnimalBase 2012). Shell sizes are similar for both species: *C. bidentata*—height up to 10 mm, width 2.0–2.5 mm (Wiktor 2004); *R. filograna*—height up to 10.2 mm, width 1.4–2.6 mm (Szybiak 2010).

The hormone-regulating activity of reproductive organs in animals, including mollusks, is strongly affected by external factors, like photoperiod and temperature. Many molluscan- and vertebrate-specific neuropeptides were described as being present in mollusks (Dorn 2000; Flari and Edwards 2003; Lafont and Mathieu 2007; Stange et al. 2012). The environmental stimuli, via optic tentacles and cerebral ganglia, affect androgen and oestrogen activity, cellular differentiation and growth in accessory organs (for a review, see Flari and Edwards 2003). Although comparing hormone activities between ovoviviparous and oviparous species was not the goal of the present study, we expect that the regulation of hormones and their receptors may differ between species displaying different reproductive strategies.

Little information is available on the life cycle of *C. bidentata* (Frömming 1954; Likharev 1962; Nordsieck 2005; Szybiak et al. 2015), whereas the lifecycle of *R. filograna* was described in detail by Szybiak (2010). Laboratory observations revealed that *R. filograna* litters range from one to four offspring, and 80% are two to three offspring (Szybiak et al. 2015). They produce offspring from April to November, peaking in April and May (75% of individuals laid eggs in those months). In many cases, the individuals reproduced twice a year. *C. bidentata* reproduces from April to October, but these data come from stable, laboratory conditions. However, only 58% of individuals laid eggs, and egg laying peaked in June (47% of individuals laid eggs) (Szybiak et al. 2015).

One interesting aspect of the two species is the limitations and possible benefits of each's reproductive strategy. The differences in geographical range, altitude and habitats raise the question whether (and to what extent) ovoviviparity has benefits over oviparity in terrestrial snails in harsher environments. Perhaps, as suggested by Baur (1994), ovoviviparity minimizes mortality within eggs and protects them from predators or unfavorable environmental conditions. If this is true, then ovoviviparous species would be found more frequently or at higher numbers in areas and habitats unavailable for oviparous species, like in some reported cases (Heller et al. 1997; Shine 2004; Maltz and Sulikowska-Drozd 2013). In terms of habitat, *C. bidentata* and *R. filograna*'s geographical range and—primarily—altitude above sea level may support this statement. This suggests that developmental strategy may correlate with gonad activity, reproductive behavior and environmental conditions. More extreme habitats—*e.g.*, higher altitudes, borders of the geographical range or harsh seasons—enforce the reproductive strategies that involve protecting the developing juvenile stages. This, in turn, allows the mature snail to reproduce

during the short periods of favorable conditions or in the conditions when oviparity is not possible. This may be an important reason why egg-retaining species are found in such habitats, while the distribution of oviparous species is limited to the more hospitable areas.

In the present study, we investigated the seasonal dynamics of reproductive cell production in the gonads of two syntopic clausilid species in a nature reserve in western Poland. By comparing the seasonal dynamics and distribution ranges between the oviparous (*C. bidentata*) and the ovoviviparous species (*R. filograna*), we hoped to find indications for a correlation between reproductive strategies and ecology.

MATERIALS AND METHODS

Collection of snails

Adult specimens of *R. filograna* and *C. bidentata* were collected at random from the nature reserve “Rezerwat Dębno nad Wartą” along the river Warta in western Poland (92 m above sea level, 52°05'N, 17°28'E, c. 20 ha); the reserve is in a riparian forest with oaks, alders, ashes and elms and is periodically flooded. The research was carried out from August 2011 to October 2012, with samples collected roughly every four–six weeks, with the exception of January 2011, when the weather conditions were poor and samples could not be collected. On each sampling occasion, five specimens with a fully developed shell were collected from each species by chance and taken for histological examinations. It is also worth mentioning that parts of the nature reserve periodically flood in spring, which may significantly affect the survival of eggs, embryonic development and hatching success of the early-laid eggs.

Histological observations

The specimens were anesthetized under CO₂, sectioned and then investigated under a light microscope. The gonads were dissected in Ringer’s solution (5.87 g of NaCl, 0.73 g of KCl, 1.99 g of CaCl₂, 1.87 g of NaHCO₃, 2.63 g of MgCl₂, 0.22 g of KHCO₃ in 1000 ml of distilled water) under a stereomicroscope. They were fixed in Bouin’s fluid for 24 h. Then they were rinsed in 70% ethanol several times. Following dehydration, samples were embedded in paramat (paraffin wax blended with synthetic polymers (BDH)) and serially sectioned at 5–6 μm. Tissue sections were stained with hematoxylin and eosin. All the serial sections were examined using a light microscope. The

sections are currently being stored at the Department of General Zoology of the Adam Mickiewicz University in Poznań.

The following stages of spermatogenesis were described: sc1 - primary spermatocytes in meiotic I (chiefly in prophase): the most prominent spheroidal cells 10–12 μm in diameter with a large nucleus surrounded by a narrow band of cytoplasm. sc2 - secondary spermatocytes in meiosis II: cells 7–8 μm in diameter with a more abundant quantity of cytoplasm. st1 - early spermatids: small cells 4–5 μm in diameter with no flagellum. st2 - late spermatids: with flagella. sp - mature sperm cells forming bundles.

The stages of oocyte development were distinguished during oogenesis (Griffond and Bolzoni-Sungur 1986; Maltz and Sulikowska-Drozd 2010): po - previtellogenic oocytes: the lowest cells (30–40 μm in diameter), with basophil cytoplasm with no visible lipid droplets. vo - vitellogenic oocyte: extensive cells (90–120 μm in diameter) with numerous lipid droplets. mo - mature oocyte: rounded cells (about 27 μm in diameter, cytoplasm filled with numerous inclusions and lipid droplets).

Processing of data

The stages of individual reproductive cells were estimated based on the method of Maltz and Sulikowska-Drozd (2010). The number of the cells in a particular stage were classified into the following ranks: 0 - no cells found. 1 - occasional cells in 1–2 lobules. 2 - cells were numerous in 3–5 lobules, but the number of cells does not exceed 50% of the lobule. 3 - cells were very numerous (more than 50% of the lobule) in more than five lobules.

The mean number of the rank and the mode for each sectioned gonad were calculated. Then, the mean of these values for the gonads collected on the same day was calculated. Since the charts for means and modes looked very similar, we decided to use means to avoid potentially underestimating the presence of low numbers of cells within a particular point—in the case of low values, the mode may suggest there were no cells present. The mean values for adult gonads were analyzed, as indicators of the months when the fertilization might take place. When all stages were present, the rank was defined as > 1.5.

The environmental temperatures were obtained from the webpage of the Polish Institute of Meteorology and Water Management - National Research Institute (2019). Table 1 shows the mean, maximum and minimum temperatures for the closest measurement geographical point at the day the sample was collected. In addition, the temperature change tendencies in the

days prior to the collection of snails were analyzed (Table 1).

RESULTS

The frequencies and number of particular stages of reproductive cells varied among months, both within each species and between the two species. The stages of reproductive cells found in both species are presented in figures 1 and 2.

The development of mature reproductive cells in *R. filograna* is shown in figure 3. Neither sperm cells nor oocytes showed prominent seasonal peaks. One can observe that there were fewer mature cells in autumn and winter, but these mature cell numbers also differed among individuals. An interesting observation is that the curves for both male and female cells followed the same pattern, but sperm cells matured about one month ahead of oocytes. In addition, the majority of immature stages are present at almost every sampling date. In the case of both male and female reproductive cells, the number of particular stages resembles pyramids, with the youngest stages being the wide base of the pyramid. The premature stages were numerous (prevalence > 1.5) in more months than the mature oocytes and sperm cells (Fig. 4). In fact, the mature sperm cells never reached a prevalence > 1.5. The detailed dynamics of all tested types of reproductive cells are presented in table 2.

Seasonal changes in *C. bidentata* reproductive cells showed a different pattern than in *R. filograna*. During autumn, winter and spring, the number of mature cells was relatively low (Fig. 5). However, as temperatures increased, the number of these cells increased, too, peaking in summer. Like in the case of *R. filograna*, we noted that the oocyte numbers increased one month after sperm cells. Also, the prevalence of

immature cells was significantly higher in the summer months than winter and spring ones. The prevalence chart does not resemble a pyramid, as the production intensity for all stages stayed more or less constant across the months. Also of note is the difference in the number of the adult reproductive cells between August and September 2011 and 2012 (Fig. 6). Details on the dynamics of all tested types of reproductive cells are presented in table 3.

DISCUSSION

The main finding of our research is that the two snail species with different reproductive modes show temporally different patterns of reproductive cell development. Previously, we had not found any significant anatomical and histological changes in reproductive organs differences in the structure of reproductive tracts between *C. bidentata* and *R. filograna*, except for a periodic transformation of the oviduct's distal part in *R. filograna*, which folds due to muscle contractions, and may widen to form a kind of a brood bag (Szybiak et al. 2013). In addition, Maltz and Sulikowska-Drozd (2013) did not find significant differences in the anatomy or histology of the spermoviduct and free oviduct sections between oviparous and viviparous species of snails from the clausilid subfamily Baleinae. Therefore, the dynamics of sperm and egg production seem to be particularly interesting, and important for comparing reproductive strategies. Brooding in ovoviviparous and oviparous snails has already been studied. For example, *A. biplicata* was found to switch its reproductive strategy towards oviparity under the influence of an oceanic climate (Sulikowska-Drozd et al. 2013). Incidental cases of *A. biplicata* laying eggs, as reported by

Table 1. Official environmental temperatures at the measurement point closest to the Dębno

Date	11.08	15.09	15.10	12.11	07.12	08.02	20.03
Mean temperature [°C]	14.5	11.9	2.2	-3.3	0.3	-13.5	5.5
Max temperature [°C]	21.2	20.2	7.2	4.7	3.2	-8.5	12.3
Min temperature [°C]	6.7	5.6	-1.5	-9.1	-1.5	-17.4	0.4
Tendency	↓	↓	↓	↓	↓	↑	↓
Date	30.04	02.06	12.07	23.08	20.09	18.10	
Mean temperature [°C]	19.7	11.7	19.1	20.8	9.7	7.6	
Max temperature [°C]	29.6	16.8	25.1	27.1	12.3	17.8	
Min temperature [°C]	8.2	7	13.3	16.9	8.4	1.5	
Tendency	↑	↓	↓	↓	↓	↓	

↓ - decreasing tendency of temperature changes in the days before collection of snails; ↑ - increasing tendency of temperature changes in the days before collection of snails; ↓ - no clear tendency of temperature changes in the days before collection of snails.

Frömming (1954) and Fechter and Falkner (1990), might be triggered by some short term environmental cue influencing parturition. For the genus *Vestia*, interspecific variation and between-season variation

were observed. The largest number of eggs were observed in the species that retain eggs for a shortest time, and the major diameter of eggs was about 0.25 mm longer in the longest-retaining species than in

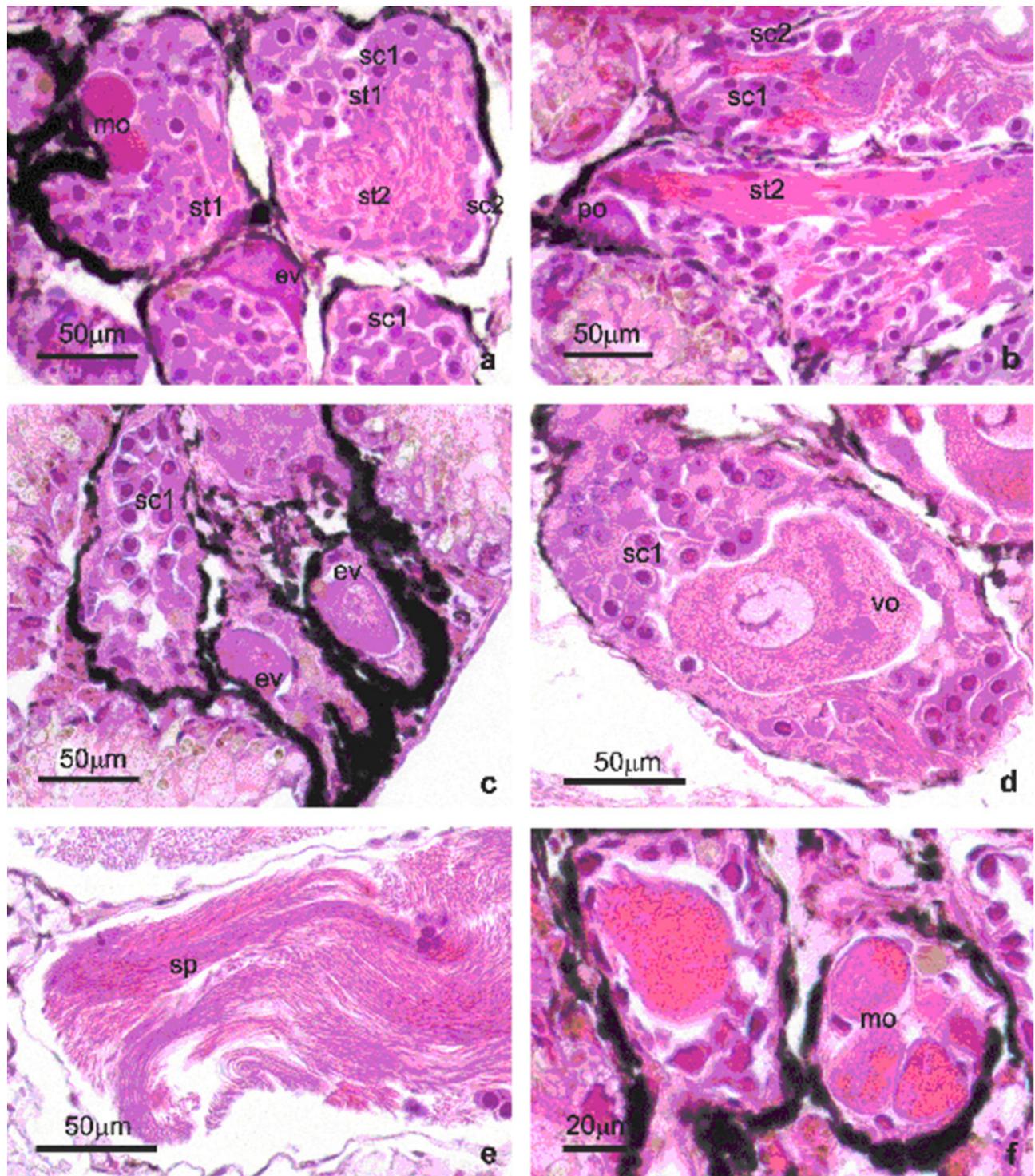


Fig. 1. Reproductive cells of *R. filograna*. 1°spermatocytes (sc1, a–d), 2° spermatocytes (sc2, a, b), 1°spermatids (st1, a), 2° spermatids (st2, b), mature sperm cells (sp, e), previtellogenic oocytes (po, b), early vitellogenic oocytes (ev, a, c), vitellogenic oocyte (vo, d), mature oocyte (mo, a, f).

the shortest-retaining one. (Sulikowska-Drozd 2009). *Vestia turgida*, which retains eggs for a the longest time, occurs at higher altitudes than *Vestia gulo*, which shows shorter egg-retention time.

Our study is, to the best of our knowledge, the first to report on the seasonal dynamics in the formation of reproductive cells of the two studied species. The seasonal dynamics and development of

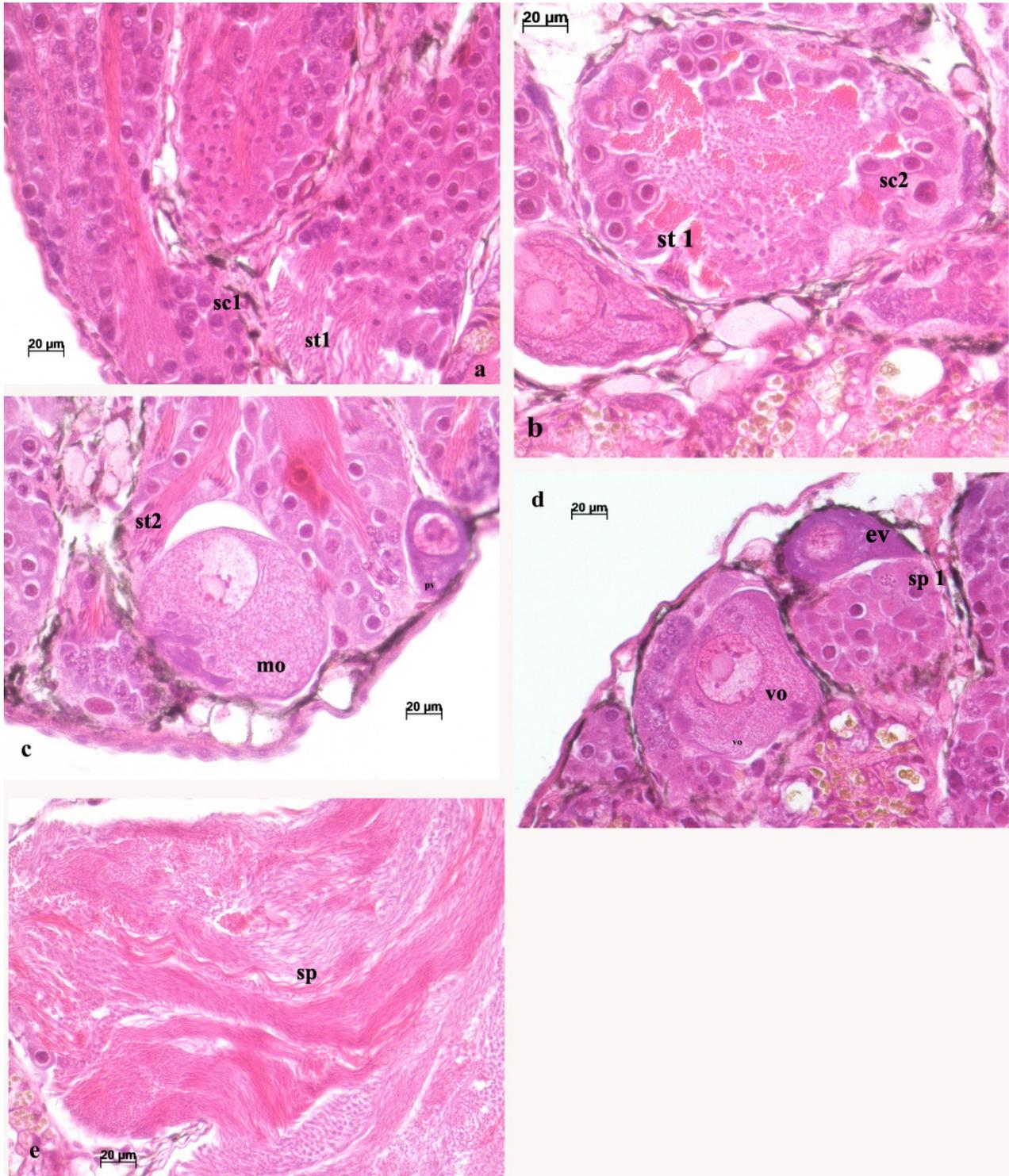


Fig. 2. Reproductive cells of *Clausilia bidentata*. 1° spermatocytes (sc1, a, d), 2° spermatocytes (sc2), 1° spermatids (st1, a, b), 2° spermatids (st2, c), mature sperm cells (sp, e), previtellogenic oocytes (po, c), early vitellogenic oocytes (ev, d), vitellogenic oocyte (vo, d), mature oocyte (mo, c).

reproductive cells are closely linked, and reproductive cell development is a basis for continuous or seasonal reproduction. The continuous process, in which extrauterine eggs are gradually released, was described

for *A. biplicata* by Sulikowska-Drozd et al. (2013). These authors stated that this phenomenon is closely related to demanding, harsh climatic conditions—like higher altitudes, colder climates or unpredictable

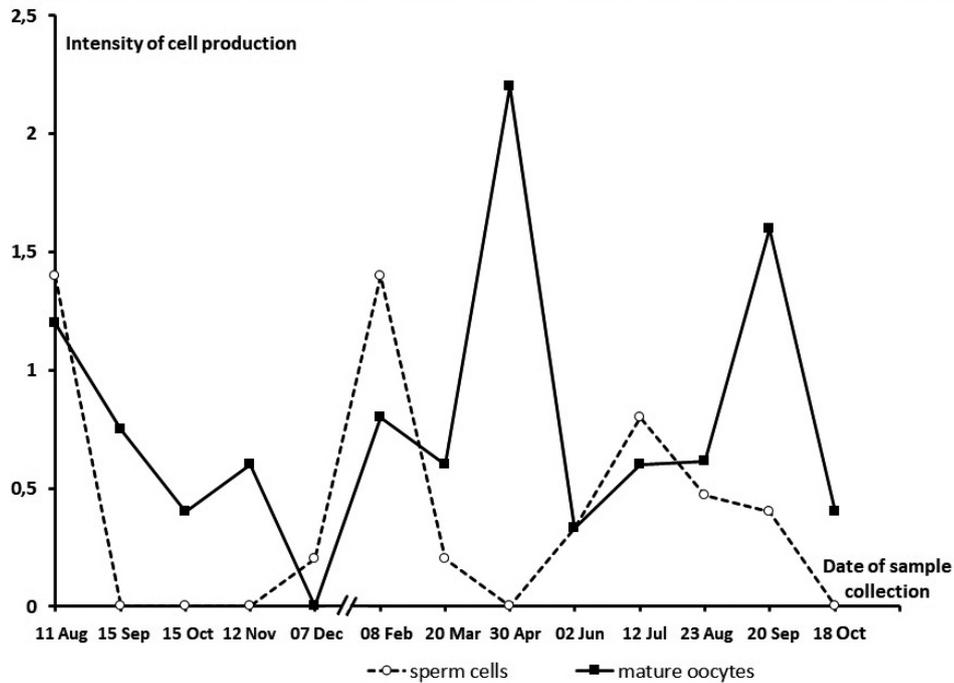


Fig. 3. Dynamics of appearance of mature reproductive cells in *R. filigrana*. Data are means. See table 2 for values and SD for other stages of cells.

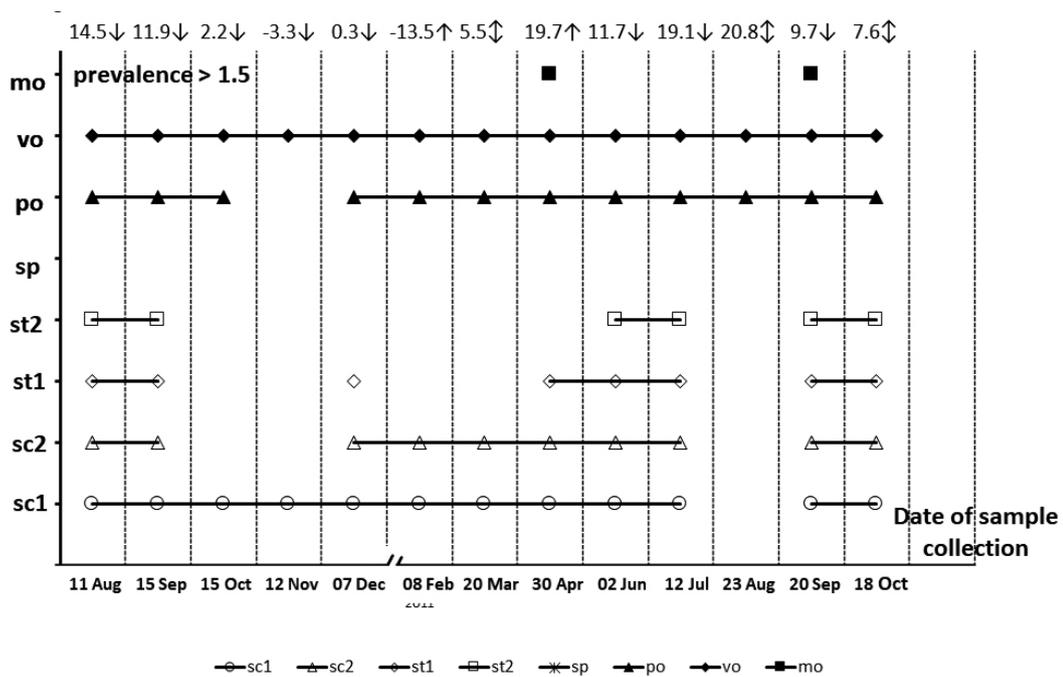


Fig. 4. Prevalence (value > 1.5) of all stages of *R. filigrana* reproductive cells for different collection months. Mean temperatures and tendencies of temperature changes in the days before the collection of snails are shown at the top of the picture ↓ - decreasing tendency; ↑ - increasing tendency; ↓ - no clear tendency.

changes in the seasons—and gives an advantage over oviparous species. Also, non-oviparous Baleinae species are found in mountain areas (Maltz and Sulikowska-Drozd 2013). On the other hand, Mamos et al. (2021)

state that, although the egg-retaining strategy may not have a clear advantage at the macroclimatic scale, but may affect the distribution within microhabitats. Environmental factors, like photoperiod, humidity

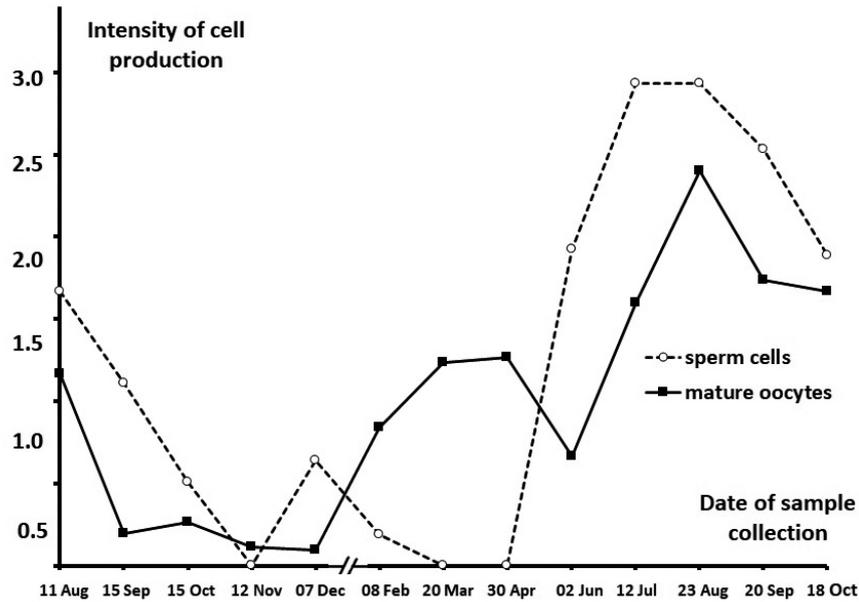


Fig. 5. Dynamics of when mature *Clausilia bidentata* reproductive cells appear. Data are means. See table 3 for values and SD for other stages of cells.

Table 2. Dynamics of production for all tested stages of reproductive cells within the *R. filograna* reproductive system

Stage \ Date	01 Aug.	15 Sept.	15 Oct.	12 Nov.	07 Dec.	08 Feb.
sc1	3.0 ± 0.0	3.0 ± 0.0	2.6 ± 0.49	2.8 ± 0.40	2.6 ± 0.48	2.4 ± 0.80
sc2	2.6 ± 0.49	1.5 ± 1.12	1.0 ± 0.89	0.4 ± 0.49	2.6 ± 0.80	2.2 ± 0.39
st1	1.6 ± 0.80	1.75 ± 4.43	0.2 ± 0.40	0.0 ± 0.0	2.4 ± 0.79	1.4 ± 1.02
st2	2.6 ± 0.49	2.25 ± 0.43	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 1.27	0.8 ± 1.17
sp	1.4 ± 1.02	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.40	1.4 ± 1.02
po	2.2 ± 0.75	2.5 ± 0.50	3.0 ± 0.0	1.4 ± 0.49	2.6 ± 0.80	2.6 ± 0.48
vo	3.0 ± 0.0	2.75 ± 0.43	2.2 ± 0.4	2.6 ± 0.51	2.2 ± 0.75	3.0 ± 0.0
mo	1.2 ± 1.47	0.75 ± 0.43	0.4 ± 0.49	0.6 ± 0.81	0.0 ± 0.0	0.8 ± 0.75

Stage \ Date	20 Mar.	30 Apr.	02 June	12 July	23 Aug.	20 Sept.	18 Oct.
sc1	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	1.2 ± 1.47	2.8 ± 0.40	2.8 ± 0.40
sc2	2.4 ± 0.50	2.6 ± 0.78	2.7 ± 0.47	3.0 ± 0.0	1.4 ± 1.21	2.0 ± 0.63	2.6 ± 0.78
st1	1.0 ± 1.09	1.6 ± 0.47	2.7 ± 0.47	2.8 ± 0.40	1.4 ± 1.12	2.0 ± 0.63	1.6 ± 0.81
st2	0.6 ± 1.20	0.4 ± 0.82	2.3 ± 0.48	2.8 ± 0.40	1.4 ± 1.0	2.8 ± 0.38	2.4 ± 0.90
sp	0.2 ± 0.40	0.0 ± 0.0	0.33 ± 0.47	0.8 ± 0.75	0.47 ± 0.29	0.4 ± 0.80	0.0 ± 0.0
po	2.6 ± 0.80	1.8 ± 0.40	2.33 ± 0.49	2.2 ± 0.70	1.2 ± 0.85	1.8 ± 0.74	2.4 ± 0.49
vo	3.0 ± 0.0	2.8 ± 0.38	2.7 ± 0.42	3.0 ± 0.0	1.3 ± 1.26	3.0 ± 0.0	3.0 ± 0.0
mo	0.6 ± 0.49	2.2 ± 1.17	0.33 ± 0.46	0.6 ± 0.50	0.6 ± 0.29	1.6 ± 0.49	0.4 ± 0.45

Data are means ± SD.

and temperature, affect the activity of reproduction-regulating hormones, e.g., the egg-laying hormone, male tentacular factor, and maturation hormone, and sex hormones (Sokolova et al. 1984; Takeda 1989; Flari and

Edwards 2003; Ter Maat et al. 2012). The activity of these hormones must differ between *R. filigrana* and *C. bidentata*; this is certainly worth studying in the future.

The two different reproductive strategies of the

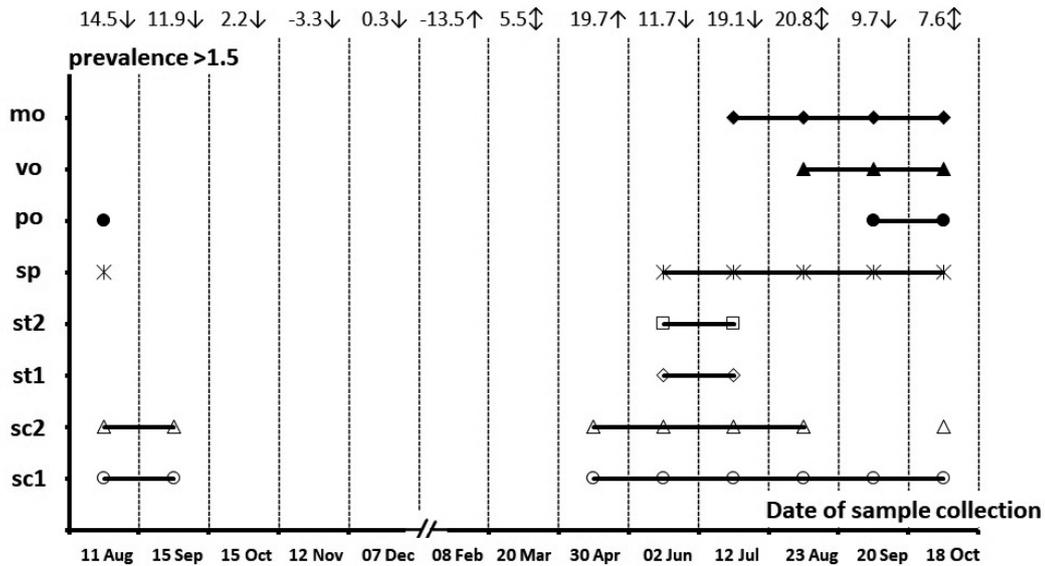


Fig. 6. Prevalence (value > 1.5) of all stages of *Clausilia bidentata* reproductive cells for different collection months. Mean temperatures and temperature change tendencies in the days before the snails were collected are shown at the top of the picture. ↓ - decreasing tendency; ↑ - increasing tendency; ↕ - no clear tendency.

Table 3. Dynamics of production for all tested stages of reproductive cells within the *C. bidentata* reproductive system

Date	01 Aug.	15 Sept.	15 Oct.	12 Nov.	07 Dec.	08 Feb.
Stage						
sc1	2.5 ± 0.65	1.5 ± 0.77	1.2 ± 0.3	0.62 ± 0.518	1.1 ± 0.30	1.18 ± 0.34
sc2	2.7 ± 0.55	1.7 ± 0.90	0.9 ± 0.25	0.68 ± 0.519	1.4 ± 0.56	1.09 ± 0.398
st1	1.0 ± 1.03	0.8 ± 0.33	0.4 ± 0.35	0.0 ± 0.0	0.3 ± 0.69	0.1 ± 0.233
st2	1.2 ± 0.12	0.9 ± 0.76	0.5 ± 0.36	0.06 ± 0.124	0.4 ± 0.58	0.19 ± 0.467
sp	1.7 ± 1.21	1.1 ± 1.07	0.5 ± 0.69	0.0 ± 0.0	0.6 ± 0.7	0.19 ± 0.467
po	1.5 ± 0.25	0.7 ± 0.28	0.9 ± 0.28	0.87 ± 0.56	0.9 ± 0.69	1.02 ± 0.43
vo	1.3 ± 0.49	0.3 ± 0.23	0.3 ± 0.15	0.69 ± 0.65	0.4 ± 0.34	0.77 ± 0.44
mo	1.2 ± 0.51	0.2 ± 0.11	0.3 ± 0.35	0.11 ± 0.16	0.1 ± 0.23	0.8 ± 0.83

Date	20 Mar.	30 Apr.	02 June	12 July	23 Aug.	20 Sept.	18 Oct.
Stage							
sc1	1.0 ± 0.19	2.23 ± 0.2	1.7 ± 0.67	2.4 ± 0.39	1.8 ± 0.50	2.5 ± 0.65	2.1 ± 0.57
sc2	1.1 ± 0.32	2.43 ± 0.33	2.3 ± 0.49	2.33 ± 0.3	1.8 ± 0.47	1.0 ± 0.56	2.1 ± 0.57
st1	0.0 ± 0.0	0.0 ± 0.0	2.4 ± 0.35	1.53 ± 0.27	0.6 ± 0.39	0.6 ± 0.39	0.9 ± 1.0
st2	0.0 ± 0.0	0.0 ± 0.0	2.5 ± 0.31	2.3 ± 0.37	1.1 ± 0.36	1.1 ± 0.13	0.7 ± 0.70
sp	0.0 ± 0.0	0.0 ± 0.0	1.9 ± 0.43	2.93 ± 0.13	2.9 ± 0.13	2.5 ± 0.34	1.9 ± 0.79
po	0.8 ± 0.32	0.93 ± 0.25	1.0 ± 0.63	1.03 ± 0.43	1.0 ± 0.67	1.6 ± 0.39	1.6 ± 0.63
vo	0.8 ± 0.33	1.1 ± 0.49	0.9 ± 0.38	1.0 ± 0.31	1.4 ± 0.38	1.9 ± 0.40	1.9 ± 0.34
mo	1.2 ± 0.44	1.3 ± 0.57	0.7 ± 0.43	1.6 ± 0.39	2.4 ± 0.65	1.7 ± 0.57	1.7 ± 0.27

Data are means ± SD.

tested Clausiliidae snail species reported in our paper appeared to be correlated with different dynamics of reproductive cell development and gonad activities. In Poland, the warm season is limited to four to five months; spring and autumn are characterized by high daily temperature fluctuations, and winter temperatures are close to or below 0°C. Ovoviviparity may render higher reproductive success by allowing the organism to reproduce under the less friendly environmental conditions—*e.g.*, low temperatures, seasonal flooding—when the development of laid eggs would otherwise not be possible and reproductive success would be low. It may decrease cannibalism (Sulikowska-Drozd 2009), too. Even though egg retention produces additional costs, these costs may be compensated by higher survival and a longer reproductive season. In addition, constantly reproducing throughout the season leads the population to grow faster.

Sulikowska-Drozd (2005) described *R. filograna* as a species that reaches “the moderately cool climatic zone with an annual average temperature higher than +4°C”. The northern geographical range is similar for both species (Scandinavia, Estonia), but *R. filograna* is able to live at higher altitudes than *C. bidentata*. To the best of our knowledge, there are no detailed data on population density at the margins of a population’s range. This suggests that this species is better adapted to the harsh environment of high mountains, with their low temperatures, high temperature fluctuations and short vegetation period, than the oviparous species. Similarly, gonads of an ovoviviparous *Lauria cylindracea* are highly active throughout the whole winter (rainy) season (Heller et al. 1997). However, these data are hard to compare with ours because they are based on completely different characteristics of climatic seasons.

Under semi-natural conditions only about 50% of *C. bidentata* snails lay eggs, whereas all adult *R. filograna* bore juveniles in spring, with some reproducing again in autumn. On average, *C. bidentata* laid 5.2 eggs per clutch, and about 59% survive (Szybiak et al. 2015). That makes the reproductive success close to three individuals per parent. *R. filograna* produces one to four juveniles (average 2.5), of which on average 1.35 survive. However, the high number of eggs are laid in April–June only, whereas neonates appear in high numbers from April to November (Szybiak et al. 2015). These data correlate with our findings concerning the presence of mature reproductive cells within the study period. Eventually, population growth in the laboratory conditions was estimated to be 105% (year-to-year) for *C. bidentata* and 140% for *R. filograna* (Szybiak et al. 2015). This suggests that, under harsh conditions, the ovoviviparous species may develop better. Of course, long-term studies on the same area may yield more data

supporting this statement. Research on the survival of the juveniles would also be important, but we do not have such data on the two study species.

We are aware of the limitations of our study, specifically that it focuses mostly on histological observations of populations obtained from a single population. Therefore, it is not our ambition to draw general conclusions concerning advantages and disadvantages of specific developmental strategies. However, our results support the hypothesis of Baur (1994), that ovoviviparity may have an advantage over oviparity in that it enables more successful development in harsher habitats. The gonads of *R. filograna* remained active throughout our entire observation, thus enabling the species to constantly be reproductive; the gonads of *C. bidentata*, on the other hand, were related to the seasons, their temperature and day length, and thus its reproduction was limited to the more favorable seasons. Our observations are also in tune with those of Shine (2004), who pointed out that only viviparous species of reptiles may inhabit higher altitudes, whereas oviparous species are only present at lower altitudes.

Our observations on the oviparous and ovoviviparity (a mid-step between the two above mentioned phenomena) in mollusks are in tune with the above mentioned observations. Although systematically distinct, snails and reptiles are both poikilotherms. Therefore, their development strongly depends on external conditions. In our previous study, we reported that the percentage of surviving offspring was similar in *C. bidentata* and in *R. filograna* (Szybiak et al. 2015). This observation suggests that the effort put into intrauterine development can limit the number of eggs produced by snails, decrease the risk of embryo death due to predation, parasitism and environmental conditions, and therefore increase developmental success over oviparous species. Perhaps if *R. filograna* was not ovoviviparous, it would have been extruded from the studied biocenosis by *C. bidentata*.

The relatively low prevalence of mature sperm cells and oocytes, together with the more frequent presence of the premature stages suggests, that the process of fertilization and embryo development may occur constantly, and, thanks to ovoviviparity, can be accomplished even when the environmental conditions are not hospitable. On the other hand, *C. bidentata* strongly depends on the climate, since their eggs develop outside of the parent’s body. Therefore, the development of germ cells is much more intensive in the summer and inhibited during the winter months. During this season, the number of used compounds and energy expenses for gamete production would be very high. In addition, the reproductive success of the next generation would be much lower in the winter and

early spring months. For oviparous snails, the strategy “once and for all” seems to be much more effective than the “little bit at a time” strategy used by ovoviviparous species.

The dynamics of development of reproductive cell within the oviparous species may also depend on temperature—the temperature in August 2012 was ca. 6°C higher than in August 2011. Also, the maximum and minimum temperatures were much closer in 2012 (*i.e.*, the fluctuations were lower) and temperatures showed an overall increasing trend in August 2012 and decreasing trend in 2011. The temperatures in September were similar in both years, but there was a decreasing tendency in the first year that we collected data and increasing in the second year. Both of these differences affected the reproduction of *C. bidentata* but not *R. filograna*. Perhaps the environmental conditions strongly affect the development of eggs through their effects on the hormonal regulation (mentioned above) and, in consequence, to speed up or to slow down development of reproductive cells. Next, the egg development process is more strongly affected by environmental conditions than the hatched juveniles.

Interestingly, both species showed a similar mode of gamete production: peak sperm production precedes peak egg production. This may be a mechanism that minimizes the possibility of self-fertilization, a phenomenon found in some snail families, including Clausiliidae (Schmera et al. 2016).

Our results indicate that climate plays a very important role in the regulation of reproduction in oviparous snails, whereas ovoviviparous snails seem to be able to reproduce when the environmental conditions are not hospitable. The strong dependence of *C. bidentata* on the weather conditions may explain its lower vertical range of distribution. Generally, the richness of the mollusk community decreases with altitude (Jacobsen 2004). Mollusk richness was positively associated with the wetland area and negatively associated with altitude (Maltchik et al. 2010). Another ovoviviparous species, *Pyramidula pusilla*, is found at 2600 m above sea level (Kirchner et al. 2016). Higher altitudes have much lower temperatures and shorter warm seasons. At the same time, ovoviviparity enables *R. filograna* to reproduce in the less favorable climate conditions. Therefore, *R. filograna* may reach higher altitudes and have a northern range farther north than that of *C. bidentata*. In addition, Pedersen et al. (2014) claim that environmental temperature, as well as its changes, may be among the main factors determining the spatial distribution of snails. This, of course, raises new questions around the effects of global warming on snail populations. The mode of reproduction and development may

play a crucial role in how snails respond to changing environmental conditions.

CONCLUSIONS

In conclusion, we described the differences in the dynamics of gonad activity in ovoviviparous species and suggest that these dynamics may play an important role in colonizing harsher habitats. This would make gonad activity a crucial physiological process that led ovoviviparous species to have an evolutionary advantage over oviparous ones in harsher, colder environments.

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List of abbreviations

sc1, primary spermatocytes.
 sc2, secondary spermatocytes.
 st1, early spermatids.
 st2, late spermatids.
 sp, mature sperm cells.
 po, previtellogenic oocytes.
 vo, vitellogenic oocyte.
 mo, mature oocyte.

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