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REPRODUCTION OF *BALEA* (*PSEUDALINDA*) *FALLAX* (ROSSMÄSSLER, 1836) (GASTROPODA: PULMONATA: CLAUSILIIDAE) KEPT UNDER LABORATORY CONDITIONS

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ABSTRACT: *Balea fallax* (Rossm.) collected from the Roztocze Upland (SE. Poland) was kept in the laboratory for four years. Observations were conducted between March and October when the snails were kept at room temperature (18–25°C); in winter they were stored at 3°C. The egg-laying period started in late March and lasted till October, with maxima in spring and early autumn. The snails laid oval, gelatinous eggs with separate calcium carbonate crystals in the external envelope (average egg size 1.96 × 1.73 mm). The eggs were deposited in batches (up to 14 eggs at a time) or singly. The number of batches per snail per year ranged between 1 and 4. Snails isolated before maturation laid defective eggs which failed to develop, which suggest that the species is incapable of uniparental reproduction or at least the ability is very limited. The reproduction rate decreased during consecutive years, probably as a result of the aging or the shortage of allosperm in isolated individuals. Compared to batches of typically oviparous clausiliids, eggs of *B. fallax* hatched slightly earlier (interval between oviposition and hatching lasted 8–10 days at room temperature). It is likely that the adults retained developing eggs in the uterus for a short time. The juveniles needed at least 6 months to attain the ultimate shell size.

KEY WORDS: Clausiliidae, land snails, life history, egg-retention, uniparental reproduction

INTRODUCTION

The clausiliid *Balea* (*Pseudalinda*) *fallax* (Rossmässler, 1836) is included in the subfamily Baleinae (FALKNER et al. 2001) and sometimes classified in the genus *Alinda*, subgenus *Pseudalinda* (NORDSIECK 2007). It is a Carpathian-Balkan species (RIEDEL 1988), with few localities in deciduous forests of south-eastern Poland (URBAŃSKI 1958, PIECHOCKI 1990, SULIKOWSKA-DROZD 2005). Owing to the limited distribution the species is on the red list in Poland under the near-threatened category (WIKTOR & RIEDEL 2002).

Until now the reproductive biology of *B. fallax* was unknown. The species was not included in the recent review of clausiliid life histories (MALTZ & SULIKOWSKA-DROZD 2008). The laboratory observations of its life cycle started in the Department of Invertebrate Zoology and Hydrobiology at the University of Łodź in 2007. This paper describes the reproduction mode of *B. fallax*, its fecundity and the time required to reach adult size under laboratory conditions.

MATERIAL AND METHODS

Individuals of *B. fallax* were collected from the Roztocze Upland near Szczebrzeszyn (Szperówka valley, hornbeam forest; 50°43.52'N; 22°54.53'E; 260 m a.s.l.) on 10 November 2007. The shell height of

the adults averaged 17.70 mm (SD 0.62; range 16.4–18.8).

The snails were put into plastic boxes (300 cm^3), each lined with moist tissue paper and containing a piece of limestone. The main observations were conducted between March and October (2008–2011) at laboratory temperatures of 18–25°C. For wintering the snails were kept in a dark, cool store. The temperature was lowered to 12°C in the middle of October, and to 3°C from early November to the end of February; it was increased to 8°C at the beginning of March, and then after a fortnight to room temperature.

During the observation season the boxes were inspected weekly, sprayed with water and cleaned when necessary. The snails were fed on lettuce. All eggs seen were transferred to separate boxes with humid tissue paper. They were checked every three days for hatching. The hatchlings were transferred to new boxes. The eggs (n=208) were measured using a stereomicroscope with a graticule.

Shell growth was recorded for 20 specimens hatched on 23 April 2008. They were kept in three boxes (one with 10 and two with 5 individuals) at room temperature (18–25°C) until the snails formed the lip (shell height: 16–21 mm).

The snails caught in the wild were divided into three experimental groups based on conchological criteria:

1. 20 adult snails (with fully developed closing apparatus and lip) kept in pairs;
2. 10 adult snails (with fully developed closing apparatus and lip) kept singly;
3. 10 juvenile snails, isolated before maturation (closing apparatus not completed), kept singly.

The aim of the treatment was to establish if isolated snails were able to reproduce in the absence of mate. Additionally, 30 adults were kept in bigger boxes.

In May 2011 the laboratory-reared snails (397 ind.) were released in the Szperówka valley.

Statistica 6.0 software was used for satatistical analysis.

RESULTS

REPRODUCTION MODE

Under laboratory conditions *B. fallax* deposited eggs on humid tissue paper, usually under stones. Eggs were produced each year (2008–2011) from late March to the end of October, but most were found in April (Fig. 1). The eggs were oval, gelatinous, with separate calcium carbonate crystals in the external envelope (Fig. 2). Their measurements are presented in Table 1, for eggs produced by isolated

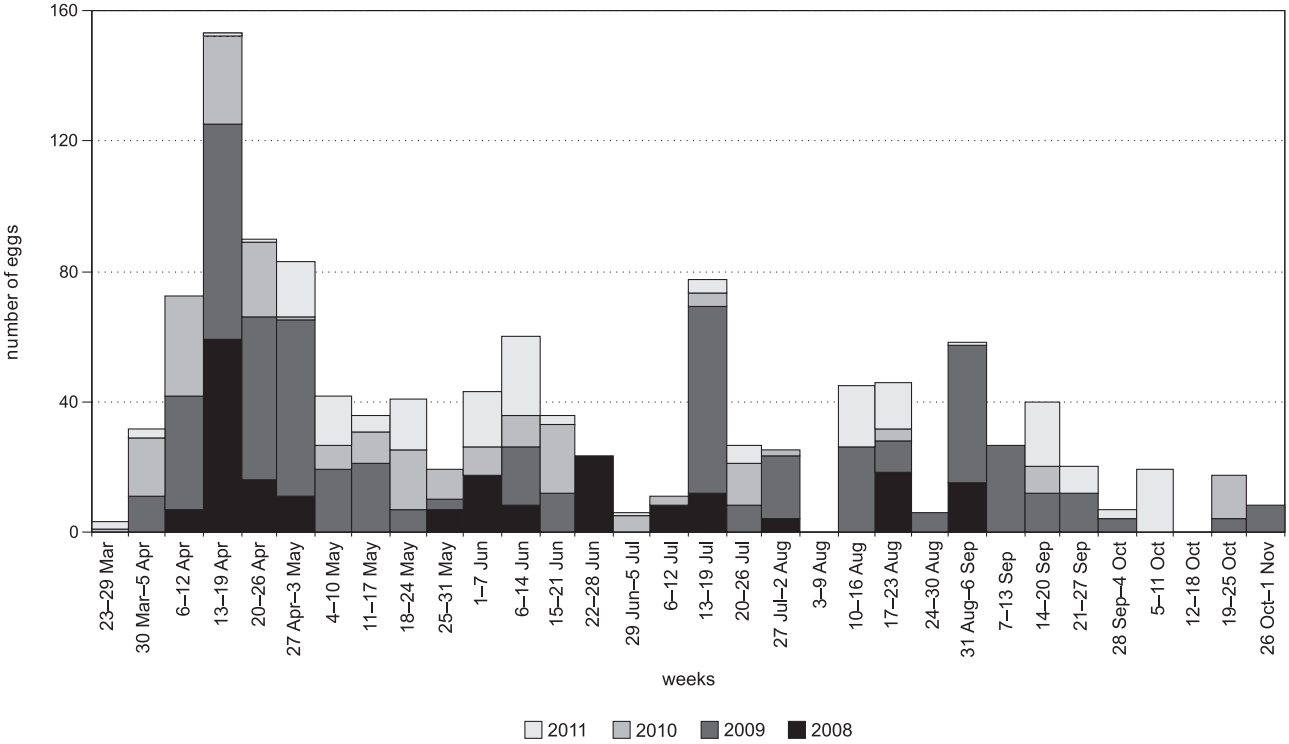

Fig. 1. *Balea fallax* – reproduction in the laboratory: number of eggs recorded during consecutive weeks of observation (2008–2011)

and by paired snails. The eggs hatched 8–10 days after oviposition. Hatching was often asynchronous and egg cannibalism was observed. The hatchling shells consisted of 2.5 whorls (Fig. 2). *B. fallax* laid eggs singly or in batches. The number of eggs in a batch, frequency of egg laying and fecundity are described below, separately for paired and isolated snails.

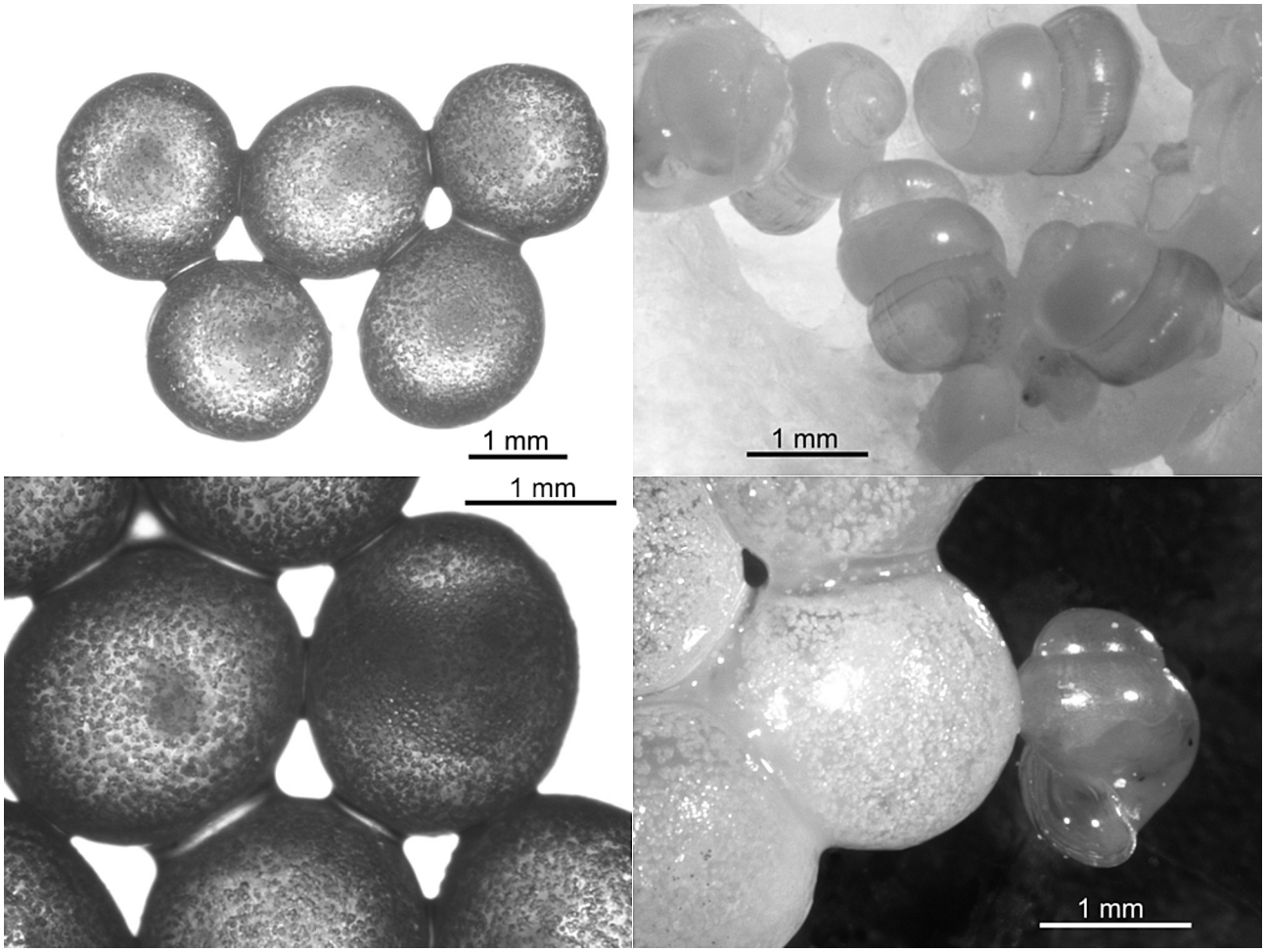

Fig. 2. *Balea fallax* – eggs laid in the laboratory and hatchlings

Table 1. *Balea fallax* – size and shape of eggs laid in laboratory (snails collected in Roztocze and their offspring reared in laboratory)

	Roztocze			Laboratory	Total
	isolated adults	paired adults	juv. isolated before maturation	paired adults	
number of measured eggs	59	115	5	28	207
egg major diameter (mm)					
mean ± SD	1.89 ± 0.15	1.96 ± 0.14	2.58 ± 0.90	1.97 ± 0.35	1.96 ± 0.24
range	1.56–2.36	1.48–2.39	1.98–4.10	1.56–2.93	1.48–4.10
egg minor diameter (mm)					
mean ± SD	1.68 ± 0.15	1.76 ± 0.12	1.76 ± 0.25	1.73 ± 0.26	1.73 ± 0.16
range	1.37–1.98	1.44–2.05	1.56–2.20	1.37–2.36	1.37–2.36
egg shape (minor diameter/major diameter)					
mean ± SD	0.89 ± 0.07	0.90 ± 0.05	0.72 ± 0.14	0.88 ± 0.10	0.89 ± 0.07
range	0.69–1.00	0.68–1.00	0.54–0.86	0.51–1.00	0.51–1.00

REPRODUCTION OF SNAILS KEPT IN PAIRS

Eggs were found in boxes with paired individuals in 2008–2011. During that period five snails died, which resulted in a smaller number of boxes used for statistical analysis (8 in 2009, 7 in 2010 and 5 in 2011). The paired individuals produced 431 eggs in total. The number of eggs encountered on each control in a single box was 1–20 (mean 7.6; SD=3.8; n=57). The number of eggs per batch decreased during consecutive seasons (Table 2, Fig. 3). The number of batches per pair per season ranged from 1 to 4. The fecundity varied between 1 and 42 eggs per season (mean 16.1 per pair; SD=11.1; n=26) and decreased from year to year (Table 2). The total number of eggs laid in a box with two individuals during the study period ranged from 31 to 64 (mean 49.8; SD=12.3; n=5). The hatching success was 72–86% in 2008–2010. In the last year of observations 25% of the eggs hatched successfully.

REPRODUCTION OF INDIVIDUALS ISOLATED AFTER MATURATION

All observed snails reproduced in 2008, while in 2009 and 2010 only seven out of ten produced eggs. In 2011 three individuals laid eggs. The isolated individuals produced 385 eggs in total. The eggs were laid in batches of 1–14 eggs (mean 6.75; SD=3.61; n=57). The number of eggs per batch decreased during consecutive seasons (Table 3, Fig. 4). In 2008 batches of 4–11 eggs were most frequent, since 2009 eggs laid singly were more common. The snails laid eggs once, twice, three or four times during the season. At the beginning of the experiment most snails produced 2–4 batches per season, in 2010 and 2011 – only one. The fecundity varied between 1 and 36 eggs per season (mean 14.5; SD=10.06; n=27). The number of eggs decreased from year to year (Table 3). The total number of eggs per snail during the study period ranged between 5 and 62 (mean 38.5; SD=19.0; n=10). The

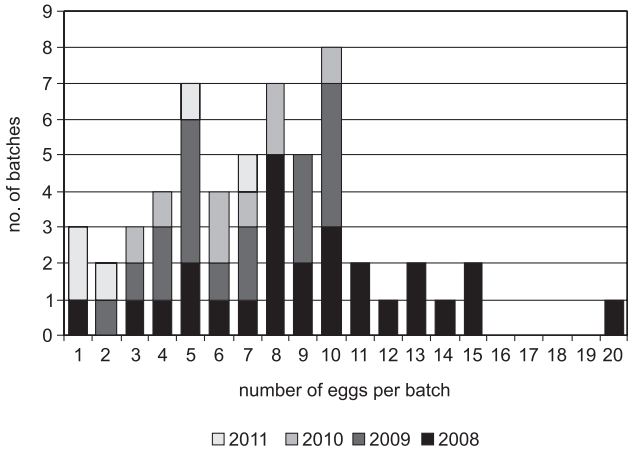

Fig. 3. *Balea fallax* – number of eggs per batch laid by pairs of snails kept under laboratory conditions in consecutive seasons (2008–2011)

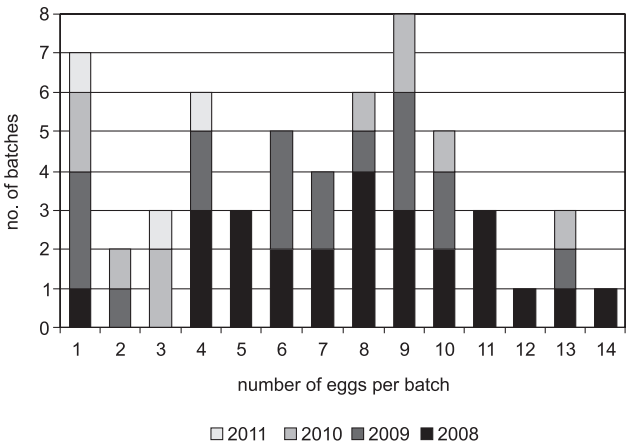

Fig. 4. *Balea fallax* – number of eggs per batch laid by isolated snails kept under laboratory conditions in consecutive seasons (2008–2011)

Table 2. *Balea fallax* – reproduction of pairs of snails kept under laboratory conditions in successive seasons (2008–2011)

Season	2008	2009	2010	2011
Number of batches				
mean ± SD	2.6 ± 1.2	2.7 ± 0.8	1.3 ± 0.5	2.0 ± 1.4
range	1–4	2–4	1–2	1–4
number of egg-laying pairs	10	6	6	4
Number of eggs in a batch				
mean ± SD	9.4 ± 4.2	6.3 ± 2.6	6.5 ± 2.3	3.3 ± 2.4
range	1–20	2–10	3–10	1–7
number of egg batches	26	16	8	7
Number of eggs produced by a pair of snails				
mean ± SD	24.3 ± 12.3	16.8 ± 2.9	8.7 ± 5.1	6.0 ± 5.9
range	8–42	12–20	3–5	1–14
number of egg-laying pairs	10	6	6	4

Table 3. *Balea fallax* – reproduction of individuals isolated after maturation in successive seasons (2008–2011)

Season	2008	2009	2010	2011
Number of batches				
mean ± SD	2.6 ± 1.1	2.6 ± 1.3	1.4 ± 0.8	–
range	1–4	1–4	1–3	1
number of egg-laying snails	10	7	7	3
Number of eggs in a batch				
mean ± SD	7.9 ± 3.2	6.3 ± 3.5	5.9 ± 4.4	2.7 ± 1.5
range	1–14	1–13	1–13	1–4
number of egg batches	26	19	10	3
Number of eggs produced by an individual				
mean ± SD	20.5 ± 9.3	16.1 ± 10.4	8.4 ±5.1	2.7 ±1.5
range	4–36	4–26	1–16	1–4
number of egg-laying snails	10	7	7	3

hatching success was 67–69% in 2008–2010. In the last year of observations none of the eggs hatched.

REPRODUCTION OF INDIVIDUALS ISOLATED BEFORE MATURATION

Only eight out of 10 snails isolated before lip formation survived till 2011 and four of them (50%) produced eggs in 2009–2011. The number of eggs per individual per season ranged from 2 to 8. Usually 1–2 eggs were laid at a time (mean 2.18; SD 1.63; max 7). The eggs were deposited 2–3 times during the season. The total number of eggs per isolated individual ranged from 2 to 18 (mean 9.25; SD 7.54). The eggs were often very elongated (Table 1, Fig. 5). The small number of measured eggs in this experimental group precluded any statistical comparison with the eggs laid by paired snails. No embryos at advanced development stages were observed inside these eggs and none of them hatched.

SEASONAL CHANGES IN BATCH SIZE

Egg batches laid in spring (April – early May) were usually larger than those deposited in summer and autumn. The difference was significant for isolated *B. fallax* in 2009 (Mann-Whitney U-test, $p<0.05$): large egg batches were laid in April and early May (mean 8.6 eggs) and small batches were laid from June till the end of the season (mean 4.8 eggs). For paired snails the respective mean values were 8.4 and 4.9 (Mann-Whitney U-test, $p<0.05$).

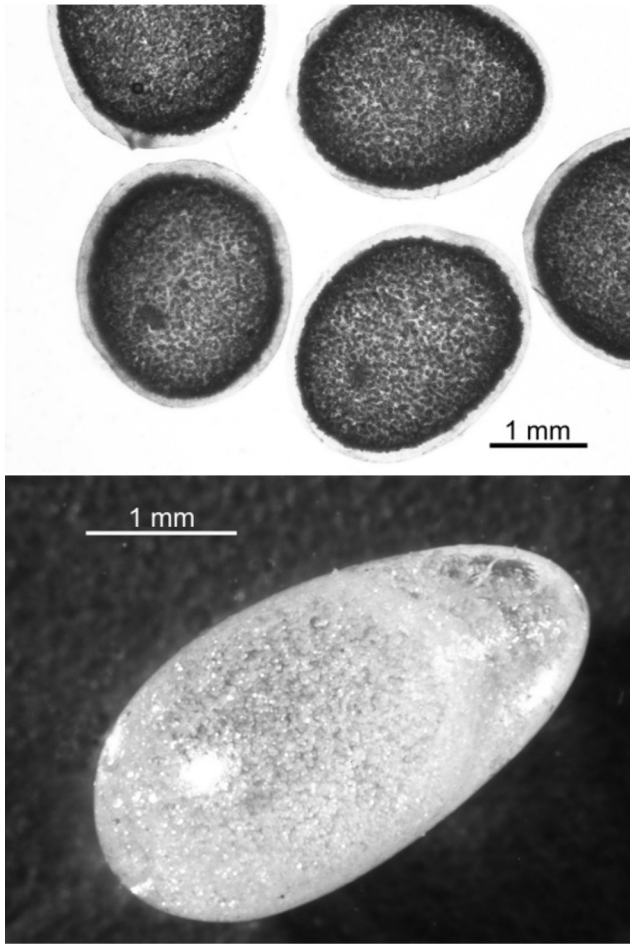

Fig. 5. *Balea fallax* – eggs laid by individuals isolated before maturation

SHELL GROWTH AND REPRODUCTIVE MATURITY

The snails hatched on 23 April 2008 completed their growth between 27 October 2008 and 7 May 2009, most of them in January 2009. Thus, the time needed for growth completion ranged between 6 and 12.5 months. The snails kept in a group of ten grew more slowly (>8 months) – the first reached its ultimate size on 5 January 2009. The first eggs were laid on 1 April 2009 (box with 5 individuals). During 2009 and 2010 these snails laid eggs several times but only two juveniles hatched (4 May 2009, 13 April 2010). In April 2011, 15 eggs hatched and all juveniles survived.

DISCUSSION

In Poland *B. fallax* is a rare and threatened species, even if locally it may be a dominant in beech forest malacocoenoses (PIECHOCKI 1990, CAMERON et al. 2010). Thus, the successful breeding in laboratory provides data which are not only valuable for the studies on the evolution of reproductive strategies, but also are of high conservation importance.

In the laboratory *B. fallax* lays eggs, however their large size and the interval before they hatch suggest that the strategy of short egg retention should be considered here. The eggs major and minor diameter was 1.96 and 1.75 mm, respectively (data for paired snails). These dimensions are more similar to those of eggs of *Vestia gulo* (E. A. Bielz, 1859) (average 1.84 × 1.67 mm; reproductive mode – short egg retention) or *Vestia elata* (Rossmässler, 1836) (average 2.08 x 1.80 mm; reproductive mode – long egg retention) than to the congeneric *Balea stabilis* (L. Pfeiffer, 1847) (1.7 × 1.47 mm; oviparous) (see MALTZ & SULIKOWSKA-DROZD 2008, SULIKOWSKA-DROZD 2008). The occurrence of large eggs means greater parental investment per offspring, and often involves also some degree of parental care to enhance the juveniles' fitness. On the other hand, the differences in egg size between *B. fallax* and *B. stabilis* might also reflect the differences in the parental body size. According to WIKTOR (2004) the shell height in *B. stabilis* ranges from 14 to 16 mm, which does not overlap with the size range recorded for *B. fallax* collected for this study.

The time between egg-laying and hatching in *B. fallax* ranges between 8 and 10 days. It is shorter than in typically oviparous clausiliids (usually 12–14 days) (MALTZ & SULIKOWSKA-DROZD 2008). The development of batches of egg-retainers *V. gulo* and *V. elata* in the laboratory takes 7–10 days and 2–4 days, respectively (SULIKOWSKA-DROZD 2009). Comparison of these data places *B. fallax* among ovoviviparous snails sensu lato but direct evidence from dissection of specimens collected in the wild is still needed.

Based on the observations of isolated individuals (matured in the wild) the size of egg batches in *B. fallax* ranges between 1 and 14 eggs. Similarly, egg batches of paired snails usually do not exceed 15 eggs. According to MALTZ & SULIKOWSKA-DROZD (2008) in other Central Europaean clausiliids the number of eggs laid at a time ranges from 1 to 11, with the exception of large species such as *Macrogastra ventricosa* (Draparnaud, 1801), which produce up to 23 eggs. In egg retaining species of the genus *Vestia* the maximum number of eggs in a batch is 10 in *elata* and 19 in *gulo* (SULIKOWSKA-DROZD 2009). Apparently, the batch size does not discriminate between oviparous and egg-retaining species.

During the study period the mean number of eggs per batch decreased from 7.9 in 2008 to 5.9 in 2010 and 2.7 in 2011 for isolated *B. fallax*. Assuming an incapability of uniparental reproduction in the species (see below), the isolated snails gradually used up the allosperm acquired during mating in the wild (the snails were sampled in autumn 2007) which resulted in decreasing egg production. However, decreasing fecundity was observed also in the boxes with paired snails. The senile age of snails (age 5+) or the lack of stimuli for courtship in the laboratory may have decreased their reproductive ability.

Under laboratory conditions *B. fallax* can produce eggs during the whole season but a significant increase in the reproductive activity was observed in April, ca. one month after the temperature in the room was risen, and a less pronounced increase - in August-September. A second reproduction peak in the late summer season was observed in most laboratory-kept clausiliids (MALTZ & SULIKOWSKA-DROZD 2008). In December 2007, five weeks after *B. fallax* was collected and transferred to the laboratory, eggs were found in a box kept at room temperature and in February 2008 mating was observed (Fig. 6). It is unknown when the species lays eggs in the wild, but the laboratory data suggest that the temperature rather than photoperiod regulates the process. The batch size in *B. fallax* was found to vary seasonally. For both isolated and paired snails the batches laid in spring (April-early May) contained significantly more eggs than those produced in summer or autumn.

The capability of uniparental reproduction, as recorded by WIRTH et al. (1997) in a natural population of *Balea perversa* (Linnaeus, 1758), is not necessarily a common feature in the family Clausiliidae. In the laboratory culture of *B. fallax,* the snails isolated before maturation laid eggs (2–18 per individual) which failed to develop. The elongated shape and very large size of such eggs (maximum diameter 4.1 mm) are common. Based on the laboratory observations, uniparental reproduction in *B. fallax* is either nonexistent or much reduced (malformations of eggs probably cause small hatching success).

Under laboratory conditions (high humidity, temperature ca. 20°C, ad libitum food) clausiliids need 3.5–8 months to attain their ultimate size (MALTZ & SULIKOWSKA-DROZD 2008). Here, juvenile *B. fallax* took 6–8 months to grow and complete the closing apparatus. Thus, the species belongs to the slow-growing clausiliids, together with *B. stabilis, Alinda biplicata* (Montagu, 1803) and *Laciniaria plicata* (Draparnaud, 1801). The first eggs were laid five months after shell growth completion – such a delayed maturity was also observed in other clausiliids (MALTZ & SULIKOWSKA-DROZD 2011). Unexpectedly, the majority of eggs laid by laboratory-reared snails in the first and second year

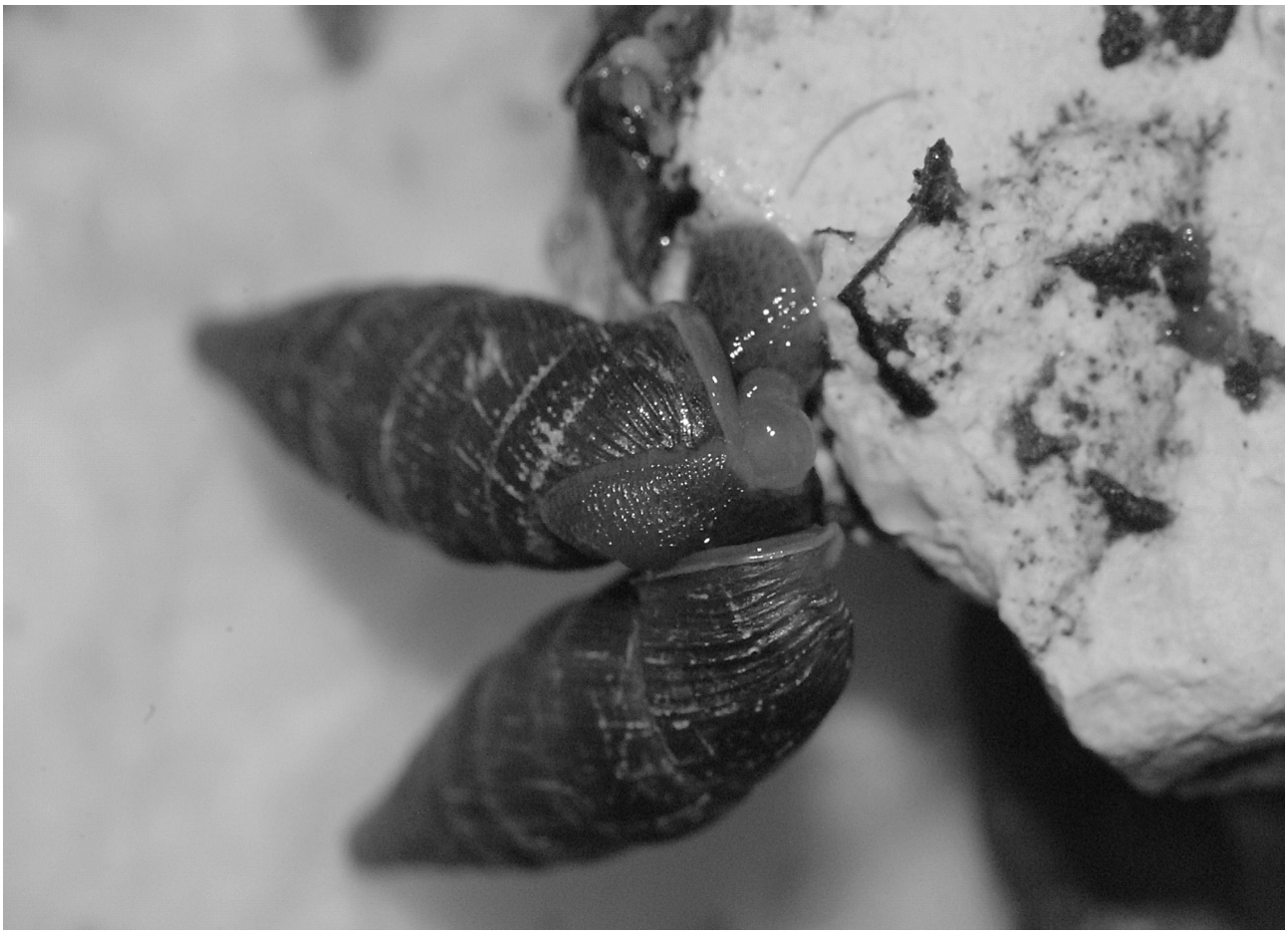

Fig. 6. *Balea fallax* – mating in laboratory (15 Feb 2008)

of the study failed to hatch; the reasons remained unknown.

The following questions should be addressed in the future studies on the reproductive biology of *B. fallax*:

1. Is the egg retention obligatory or facultative (e.g. adjustment to weather/laboratory conditions)?;
2. In which season do the snails retain eggs in the wild?;
3. What is the developmental stage of retained eggs/embryos?;
4. How many eggs can be retained at a time?

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