



## REPRODUCTION OF *BALEA (PSEUDALINDA) STABILIS* (L. PFEIFFER, 1847) (GASTROPODA: PULMONATA: CLAUSILIIDAE) KEPT UNDER LABORATORY CONDITIONS

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**ABSTRACT:** *Balea stabilis* (L. Pfeiffer) collected from two localities in the Polish Carpathians were kept in the laboratory for more than 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 the middle of September, with the maximum in April–May. The eggs were oval, gelatinous, with separate calcium carbonate crystals in the external envelope (average egg size 1.69 × 1.47 mm). They were deposited in batches, usually of 5–9 eggs (mean 6.9). The number of batches per snail per year was 1–7; the corresponding number of eggs – 3–41. At room temperature the incubation took 15–21 days; the hatching success was 71.9%.

**KEY WORDS:** Clausiliidae, land snails, life history, oviparity

### INTRODUCTION

*Balea stabilis* (L. Pfeiffer, 1847) is a Carpathian endemic species, distributed mainly in the Eastern Carpathians and Transylvania (RIEDEL 1988). It is classified in the subgenus *Pseudalinda*, genus *Balea* (FALKNER et al. 2001) or *Alinda* (NORDSIECK 2007). In Poland, it occurs only in the montane forests up to 1,150 m a.s.l., in the west it reaches the Tatra Mts and Podhale (RIEDEL 1988, SULIKOWSKA-DROZD 2005) and it is red-listed with near-threatened category (WIKTOR & RIEDEL 2002).

Knowledge of life history is required to plan protection measures for any species. We aimed to provide a detailed description of the reproductive biology of

*B. stabilis* (reproductive period, egg size, batch size, number of batches per season, hatching success) under laboratory conditions. Preliminary data concerning the reproduction mode of *B. stabilis* were published by MALTZ & SULIKOWSKA-DROZD (2008). Based on one-year laboratory observations the species was then classified as oviparous. The egg size, batch size, reproductive period and duration of incubation were estimated. In the same paper (MALTZ & SULIKOWSKA-DROZD 2008) we provided an extensive summary of the literature information on clausiliid life history and comparison of reproductive traits of several species recorded from Poland.

### MATERIAL AND METHODS

The laboratory culture of *Balea stabilis* was kept at the Department of Invertebrate Zoology and Hydrobiology, University of Łódź in 2006–2011. The clausi-

liids derived from two localities: Puławy in the Beskid Niski Mts (49°30'04"N, 21°54'41"E; 390 m a.s.l.; alder forest in the Wisłok River valley; five individuals col-

lected on 6 July 2006) and Rzeki in the Gorce Mts (49°35'12''N, 20°13'35''E; 710 m a.s.l.; alder forest in the Kamienica River valley; 32 individuals collected on 1 May 2008). Additionally, 24 offspring of the individuals from Puławy, hatched in 2006 and 2007, were used for the breeding experiments.

The snails were kept singly and in pairs or bigger groups. The primary aim of the treatment was to establish if they were capable of reproduction in the absence of mate and, if so, to assess the individual fecundity. The snails from the Gorce Mts were kept in 22 boxes (12 with isolated individuals and 10 with pairs). The shell height of these specimens averaged 14.11 mm (SD 1.05; range 11.0–15.5). The snails from the Beskid Niski Mts were kept in 17 boxes (10 with isolated individuals, 5 with pairs and 2 with groups of 4–5 snails). The mean shell height of the individuals from the Beskid Niski Mts and their offspring reared in the laboratory was 16.51 mm (SD 0.91; range 14.9–18.4). The snails collected in each site were bred separately to check the possible intra-

specific variation in reproductive traits related to the size of adults.

The snails were kept in plastic boxes (volume 300 cm<sup>3</sup>) lined with moist tissue paper and provided with a piece of limestone. The main observations were conducted between March and October (seasons 2007–2011) in the laboratory at the temperature of 18–25°C. For the wintering, the snails were kept in a dark, cool store. The temperature there was lowered to 12°C in the middle October, and to 3°C at the beginning of November. Then it was fixed at 3°C until 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 every week, sprayed with water and cleaned when necessary. The snails were fed on lettuce. All eggs seen were transferred to separate boxes with damp tissue paper and checked every three days for hatching. The hatchlings were transferred to new boxes. The eggs (n=216) were measured using a stereomicroscope with graticule.

## RESULTS

During 2006–2011 the individuals kept in the laboratory laid 859 eggs. The snails collected in the Gorce Mts produced a total of 184 eggs (eggs were found in six boxes out of ten with paired snails and in seven boxes out of twelve with isolated individuals). The individuals from the Beskid Niski Mts produced 675 eggs (eggs were found in all boxes with paired snails and in four boxes out of ten with isolated individuals).

The hatching success for the eggs laid by isolated and paired individuals was 71.9% (Gorce population – 73.7%; Beskid Niski population – 71.7%).

*B. stabilis* deposited eggs on the damp tissue paper, usually in sheltered places, e.g. under stones. The eggs were produced each year from late March to the middle of September but most of them were found in April and May (Fig. 1). The eggs were oval, gelatinous

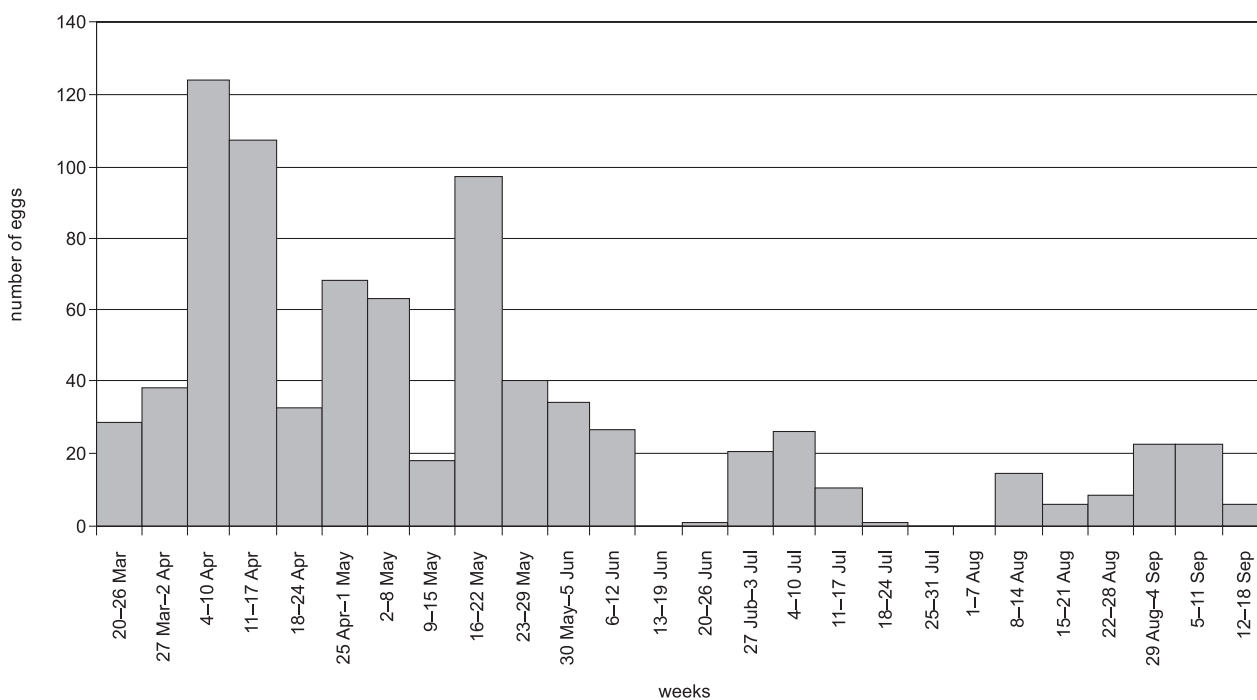


Fig. 1. *Balesa stabilis* – reproduction in the laboratory: number of eggs recorded in 2006–2011 during consecutive weeks of observation

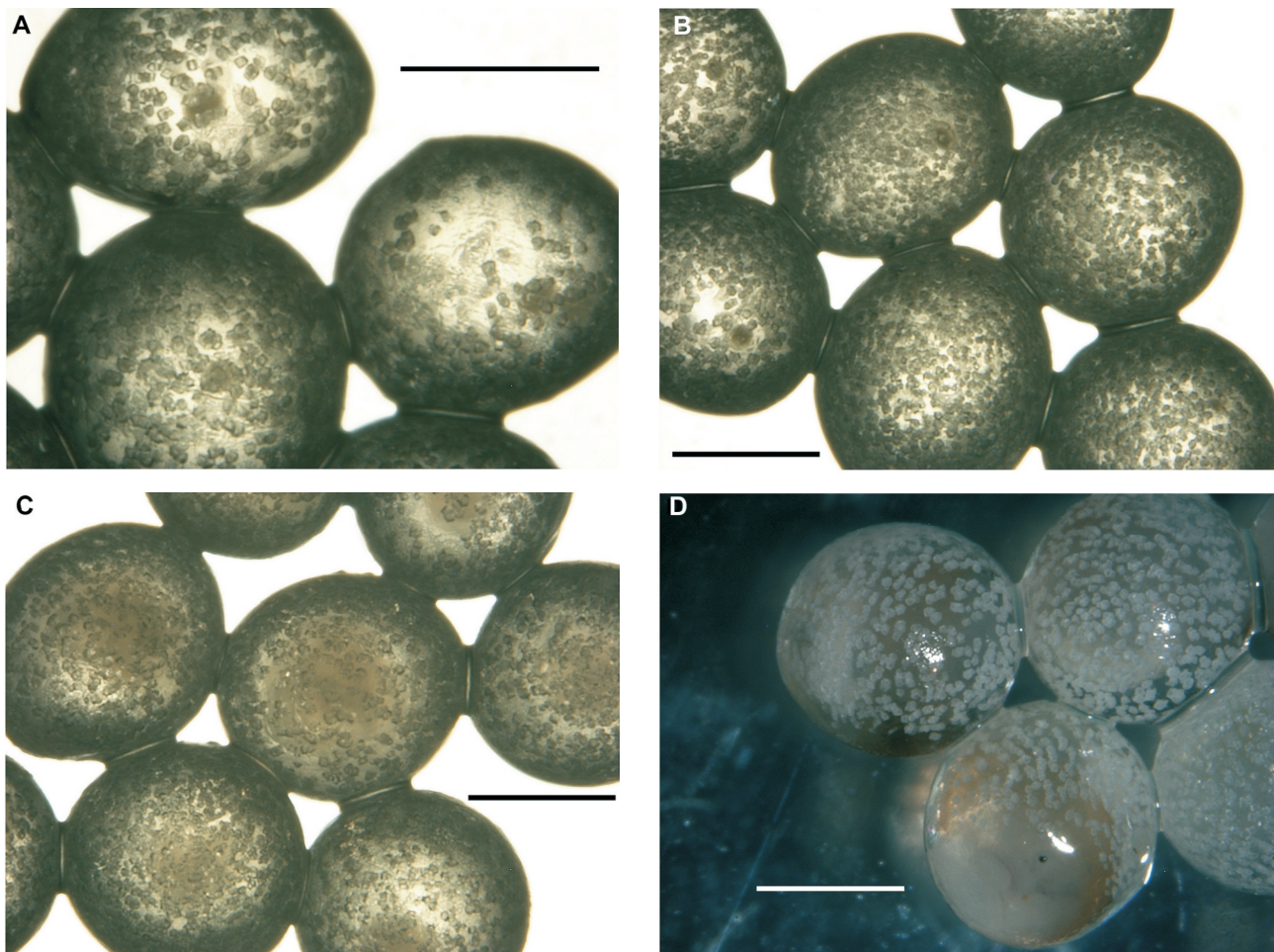


Fig. 2. *Balea stabilis* – eggs laid in the laboratory: A, B – 1-week incubation; C – 2-week incubation; D – 3-week incubation. Scale bar 1 mm.

with separate calcium carbonate crystals in the external envelope (Fig. 2). Immediately after deposition the eggs were transparent, then a small, dark embryo became visible (diameter ca. 100  $\mu\text{m}$ ). After two weeks, the embryo's diameter increased to 0.5–0.7 mm and after three weeks the eggs were ready to hatch – calcium carbonate crystals close to the embryo's head had been utilised (Fig. 2D). The egg mea-

surements are presented in Table 1, separately for eggs produced by snails of different origin.

The observed time between finding the eggs and their hatching was most frequently 15 days (range 12–23,  $n=37$ ) but since the checking was done once a week (see Material and Methods) the additional 1–7 days of incubation must be considered. Thus, the whole incubation period of *B. stabilis* lasted ca. 15–21 days.

Table 1. Size and shape of *Balea stabilis* eggs laid in the laboratory. Snails derived from two populations in the Gorce and Beskid Niski Mts.

	Gorce	Beskid Niski	Total
egg major diameter (mm)			
mean ( $\pm$ SD)	1.73 ( $\pm$ 0.12)	1.67 ( $\pm$ 0.11)	1.69 ( $\pm$ 0.12)
range	1.37–2.01	1.37–2.05	1.37–2.05
egg minor diameter (mm)			
mean ( $\pm$ SD)	1.45 ( $\pm$ 0.14)	1.48 ( $\pm$ 0.09)	1.47 ( $\pm$ 0.10)
range	1.22–1.73	1.14–1.75	1.14–1.75
egg shape (minor diameter/major diameter)			
mean ( $\pm$ SD)	0.84 ( $\pm$ 0.08)	0.89 ( $\pm$ 0.06)	0.88 ( $\pm$ 0.07)
range	0.67–1	0.67–1	0.67–1
number of measured eggs	51	165	216

Table 2. Number of *Balea stabilis* eggs per batch for isolated and paired snails. Snails derived from two populations in the Gorce and Beskid Niski Mts.

	Gorce		Beskid Niski		Total
	isolated	paired	isolated	paired/group of 4–5	
mean ( $\pm$ SD)	5.12 ( $\pm$ 2.4)	8.11 ( $\pm$ 2.67)	5.83 ( $\pm$ 2.46)	7.60 ( $\pm$ 3.44)	6.91 ( $\pm$ 3.22)
range	1–11	5–14	1–10	1–17	1–17
number of clutches	19	9	23	70	121

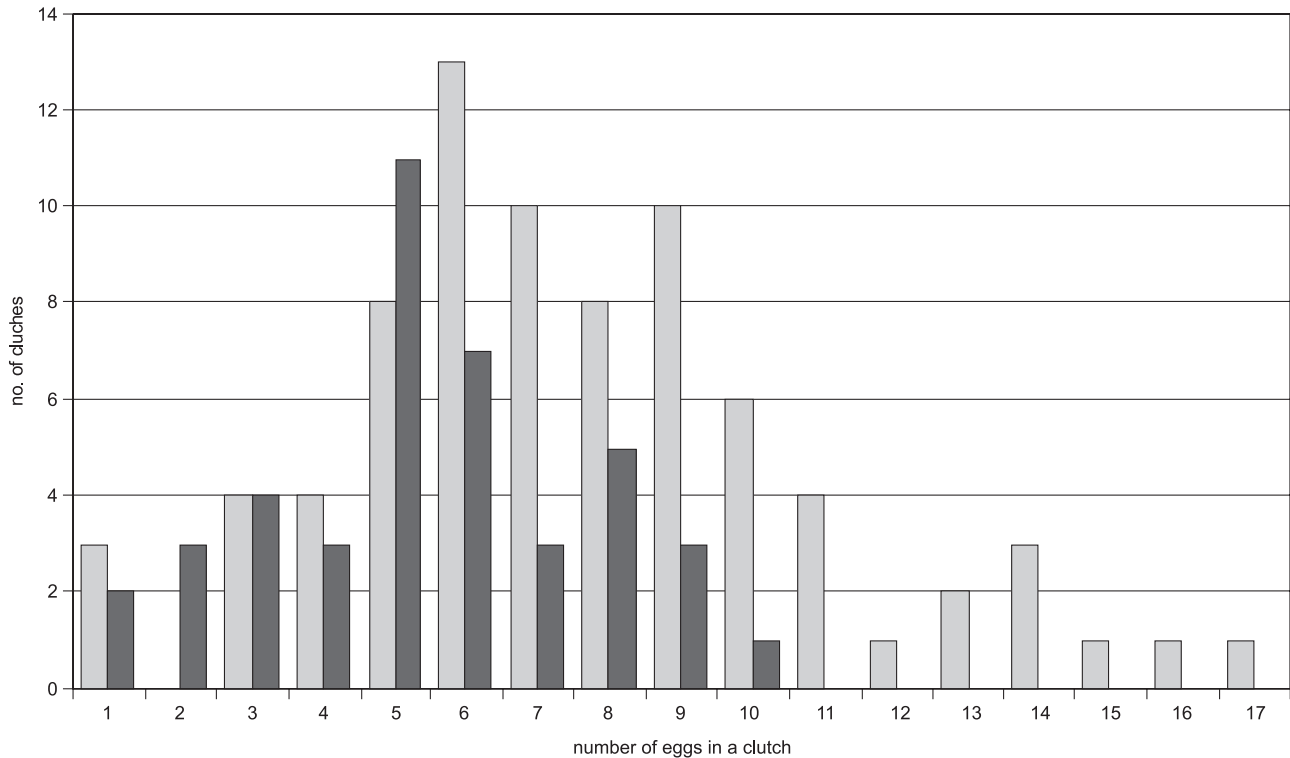


Fig. 3. *Balea stabilis* – reproduction in the laboratory: number of eggs per batch in paired (grey) and isolated (black) snails

*B. stabilis* laid eggs in batches. Most frequently there were 5–9 eggs per batch (Fig. 3). For exact values for egg batches laid by isolated snails and snails kept in pairs or groups see Table 2. Adult *B. stabilis* kept singly

never laid more than 11 eggs at a time. In boxes with 2–5 snails sometimes 16–17 eggs were found together. The difference between isolated and paired individuals in the mean number of eggs laid at a time was statistically significant (pooled data for snails of different origin Mann-Whitney U-test,  $p < 0.001$ ).

During the first observation season (2008) isolated snails from the Gorce Mts laid 3–20 eggs in 1–4 batches. In the following seasons the number of eggs decreased considerably. Thus, from 2008 to 2011 the overall fecundity ranged between 5 and 27 eggs (mean for reproducing individuals 14.4; SD 8.1). Isolated individuals from the Beskid Niski Mts produced 18–41 eggs in 1–7 batches during the 2009 season. During 2009–2011 isolated snails produced 5–46 eggs (mean for reproducing individuals 32.0; SD 19.3).

Hatching lasted 2–3 days. Shells of hatchlings consisted of 2.5–3.0 whorls (Fig. 4). According to our observations *B. stabilis* showed no egg cannibalism. Rarely the hatchlings damaged defective eggs, which showed no signs of development.



Fig. 4. *Balea stabilis* – hatchlings and damaged undeveloped eggs in the laboratory culture. Scale bar 1 mm

## DISCUSSION

*B. stabilis* reproduced in the laboratory, however the reproduction rate was low; especially in the population derived from the Gorce Mts. Compared to a critically endangered Carpathian clausiliid *Vestia elata* (Rossmässler, 1836), kept in the same laboratory, fewer individuals of *B. stabilis* started to breed (see SULIKOWSKA-DROZD 2008). It is likely that the laboratory boxes did not contain preferable egg-laying substrata for the studied species. In natural habitats *B. stabilis* occurs in leaf litter but also climbs logs and tree trunks (SULIKOWSKA-DROZD 2005), which are probably its egg-laying sites. The lack of fresh bark in the breeding boxes might decrease the reproductive ability of this clausiliid. According to MALTZ & SULIKOWSKA-DROZD (2008) other clausiliids such as *Charpentieria ornata* (Rossmässler, 1836), *Macrogastera latestriata* (A. Schmidt, 1857) and *Clausilia parvula* Férussac, 1807, laid eggs only in moss tufts, *Cochlodina laminata* (Montagu, 1803) and *M. tumida* (Rossmässler, 1836) preferred bark, while *Laciniaria plicata* (Draparnaud, 1801) showed no preferences. A strong affinity to bark as an egg-laying site was observed in laboratory-kept *Helicodonta obvoluta* (O.F. Müller, 1774) (MALTZ 2003). For *Bulgarica cana* (Held, 1836) freshly collected bark was the preferable source of food (MARZEC, pers. com.).

In *B. stabilis* egg incubation in the laboratory lasted 15–21 days, and thus longer than it was estimated during preliminary observations (see MALTZ & SULIKOWSKA-DROZD 2008). For most oviparous clausiliids the incubation time at room temperature ranges from 10 to 16 days but tends to be shorter in the species producing smaller eggs (e.g. *Clausilia parvula*). In a

cold-adapted population of *Macrogastera badia* (C. Pfeifer, 1828) it was 16–19 days (MALTZ & POKRYSZKO 2009). On the other hand, incubation shorter than 10 days may suggest that the snail retains developing eggs in the uterus, as observed in *Vestia gulo* (E. A. Bielz, 1859) and *Balea fallax* (Rossmässler, 1836) (MALTZ & SULIKOWSKA-DROZD 2008, SULIKOWSKA-DROZD & MALTZ 2012). The duration of incubation for several laboratory-kept clausiliids is compared in Figure 5. High intraspecific variation in the duration of embryonic development is probably related to the temperature changes in the laboratory, since the studied clausiliids were not kept in a climatic chamber. It seems that egg batches are prone to fungal infections at temperatures higher than the optimum.

Both isolated and paired individuals of *B. stabilis* were able to reproduce. Some of the adults kept singly produced viable eggs during all three seasons. This indicates the capability of sperm storage. Reproduction of isolated snails was observed also in other clausiliids kept in the laboratory, e.g. *Vestia elata*, *Bulgarica cana* and *Balea fallax* (SULIKOWSKA-DROZD 2008, MARZEC 2010, SULIKOWSKA-DROZD & MALTZ 2012). The reproductive ability of *B. stabilis* isolated before maturation (uniparental reproduction) has not been examined yet.

The number of eggs per batch varied. The isolated adults produced significantly smaller egg batches (up to 11 eggs) than the paired individuals (up to 17 eggs). The difference might have two sources. Firstly, isolated snails might have smaller fecundity since they had to use the decreasing supply of allosperm stored from the previous mating. Secondly, paired snails might lay eggs in the same place, one shortly after an-

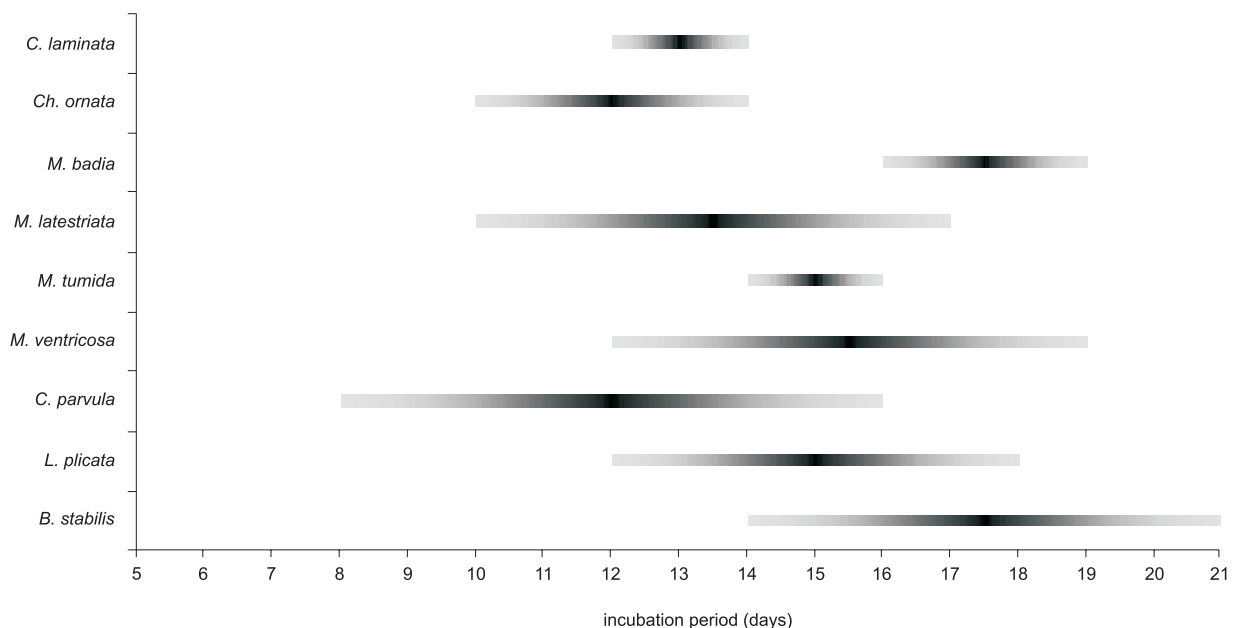


Fig. 5. Duration of incubation in oviparous Clausiliidae kept under laboratory conditions according to MALTZ & SULIKOWSKA-DROZD (2008), MALTZ & POKRYSZKO (2009) and this paper

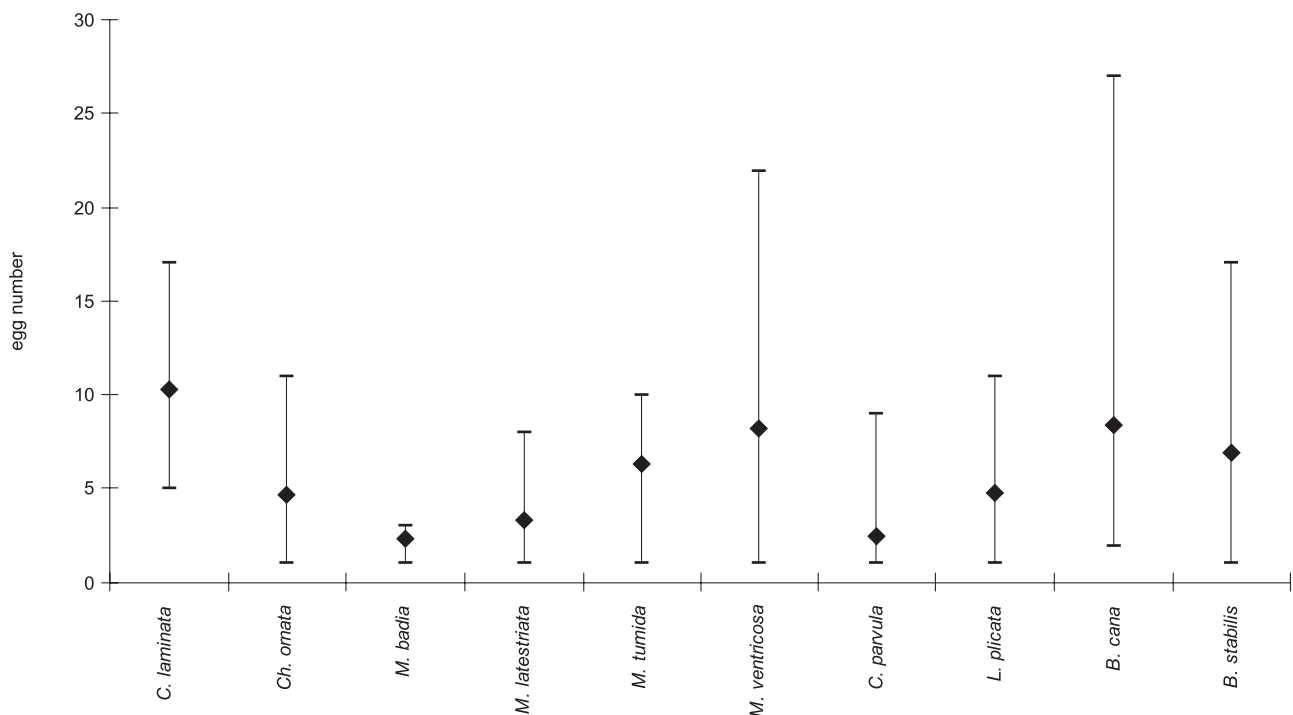


Fig. 6. Number of eggs per batch produced by oviparous Clausiliidae according to MALTZ & SULIKOWSKA-DROZD (2008), MALTZ & POKRYSZKO (2009), MARZEC (2010) and this paper. Black diamonds indicate mean values, whiskers – range between minimum and maximum records

other, consequently two batches could be recognised as a single large batch. The latter possibility cannot be rejected, as the number of places suitable for oviposition in the laboratory boxes was limited. The absence of egg cannibalism may favour simultaneous egg laying in *B. stabilis*.

Earlier observations of MALTZ & SULIKOWSKA-DROZD (2008) and MARZEC (2010) also showed considerable intraspecific variation in batch size among laboratory-kept clausiliids (Fig. 6). For most oviparous clausiliids the number of eggs laid at a time ranged between 1 and 11. Larger batches were found only in *Macrogastra ventricosa* (Draparnaud, 1801), *Cochlodina laminata* and *Bulgarica cana*, which are among the biggest (in terms of shell height and shell volume) clausiliids of Central Europe. The maximum number of eggs per batch tends to correlate positively with the size of adults. However, the mean batch size is usually much smaller, for example in *B. cana* 75% of observed egg batches contained fewer than 10 eggs

(MARZEC 2010). The strategy to produce smaller but more frequent batches during the season is probably the better choice for animals having to cope with unpredictable environment.

In the laboratory snails derived from the two Carpathian populations of *B. stabilis* were kept separately, but the observed differences in life history traits between these groups were rather minor (the range of egg size and batch size widely overlapped), even if the adult shell height was significantly different. The same breeding conditions in the laboratory might decrease the variation and the small number of offspring in the later seasons excluded statistical comparisons between the groups.

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