Development and reproduction in *Bulimulus tenuissimus* (Mollusca: Bulimulidae) in laboratory

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ABSTRACT: *Bulimulus tenuissimus* (d'Orbigny, 1835) is a land snail of parasitological importance with a poorly understood biology. The goal of this laboratory study was to determine development and reproductive patterns in *B. tenuissimus*. Recently hatched individuals in seven groups of 10 were maintained in the laboratory for two years. To test for self-fertilization, 73 additional individuals were isolated. After 180 days the isolated snails showed no signs of reproduction. Subsequently, 30 of these snails were paired to test fertility. We noted the date and time of egg-laying, the number of eggs produced, the number of egg-layings per individual, the incubation period and hatch success. This species shows indeterminate growth. Individuals that were maintained with others, as compared to isolated individuals, laid eggs sooner, laid more eggs and had a greater hatching success. This species can self-fertilize, however, with lower reproductive success. *Bulimulus tenuissimus* has a well-defined reproductive period that is apparently characteristic for this species.
KEY WORDS. Growth; land snail; reproduction.

RESUMO. **Padrão de desenvolvimento e aspectos reprodutivos de *Bulimulus tenuissimus* (Mollusca: Bulimulidae) em condições de laboratório.** Apesar de ser uma espécie de importância parasitológica, não existem estudos sobre a biologia de *Bulimulus tenuissimus* (d'Orbigny, 1835). O objetivo desse trabalho foi verificar o padrão de crescimento e aspectos reprodutivos dessa espécie em laboratório. Assim, 70 animais recém-eclodidos foram distribuídos em sete grupos, com 10 moluscos cada, por um período de dois anos. Para estes animais foi verificado o padrão de crescimento. A ocorrência de autofecundação foi observada através do isolamento de 73 animais. Por não ter sido verificado autofecundação em um período de 180 dias, 30 animais que estavam em isolamento foram pareados de modo a confirmar a fertilidade destes. Registrou-se o tempo para início da oviposição, número de ovos e de posturas, intervalo entre posturas e taxa de eclosão. Foi verificado um padrão de crescimento indeterminado para a espécie. Os animais mantidos agrupados iniciaram a oviposição mais cedo, produziram mais ovos e apresentaram maior eclodibilidade do que os demais grupos. A espécie é capaz de realizar autofecundação, porém um menor sucesso reprodutivo é obtido nesse processo. Os indivíduos de *B. tenuissimus* apresentaram um período reprodutivo bem definido que parece ser característico para a espécie.
PALAVRAS-CHAVE. Crescimento; reprodução; molusco terrestre.

The tropical land snail, *Bulimulus tenuissimus* (d'Orbigny, 1835), is the only species in its family with parasitological importance, being an intermediate host for parasitic helminths that infest domestic animals (THIENGO & AMATO 1995). Additionally, it is widespread in Brazil and therefore potentially very important for its medicinal impact (MORRETES 1943). While studied anatomically and histologically (ARAÚJO *et al.* 1960, REZENDE & LANZIERE 1964), its biology and behavior are almost unknown. Studies of development patterns reproduction are needed to inform possible control management of this species and its parasites (D'AVILA *et al.* 2004).

The goal of this study is to describe patterns of growth and the reproductive biology of this species, including fecundity, number of lifetime reproductive events, incubation and hatching rate of *B. tenuissimus*.

MATERIAL AND METHODS

Growth and reproduction was studied in *B. tenuissimus* from May 2005 to May 2007. Recently hatched animals were gathered for the study from cultivation in the laboratory at the Mollusk Biology Laboratory at the Professor Maury Pinto de Oliveira Museum of Malacology, of the Federal University Juiz de Fora, where this study took place.

Seven groups of animals (10 snails in each) were maintained in plastic terrariums (14 cm x 9 cm deep) for two years. Substrate (mulch dirt) was sterilized (at 120°C for one hour) and then, during culture, was humidified once a day (BESSA & ARAÚJO 1995). Animals were fed with commercial rations (for bird courtship), enriched with calcium carbonate at 3:1 (OLIVEIRA *et al*. 1968, BESSA & ARAÚJO 1995). Rations were placed daily in a small plastic container (3 cm diameter) to avoid contaminating the substrate.

Beginning with the first day of study, every 15 days the shell length of each animal was measured with a caliper (precision of 0.05 mm) to study growth rates for the first 180 days, after which they were measured monthly until the end of the experiment. Daily observations were used to identify the time interval until the first reproduction by finding eggs in the terrarium. Throughout the experiment, all clutches and the number of eggs in each were counted and the day they appeared was recorded.

To test for self-fertilization, 73 snails were kept isolated individually in small plastic terrariums (8 x 6 cm deep), for two years. This group was fed and observed and reproduction recorded as previously described, following OLIVEIRA *et al*. (1968) and BESSA & ARAÚJO (1995).

All egg masses were removed from the terrariums and transfered to small plastic boxes. Eggs from each clutch were counted and kept separate with dirt similar to natural clutches, to determine incubation interval and hatching rate (ALMEIDA & BESSA 2001).

Since egg laying did not occur in the individuals kept separate (self-fertilization) after 180 days, 30 of the 73 were placed together as 15 pairs to test that they were indeed fertile. These individuals were marked to identify their origins and the same reproductive parameters were measured for this group.

This study was carried out at natural temperature, relative humidity and photoperiod. Minimum and maximum temperature and relative humidity were measured throughout the study (Max-min thermometer and wet-dry hygrometer, Inconterm®).

Fecundity and reproduction of the different treatments (isolated and grouped) were compared with Kruskal-Wallis and Student's t-test. Spearmen correlation was used to test for a correlation between egg production, temperature and relative humidity. Significance was considered at $p < 0.05$ and tests were carried out using BioEstat, version 4.0.

RESULTS

Final size of snails averaged 20.60 mm ± 3.75. Growth was slow during the first 15 days (0.74 mm day^{-1}), more rapid up to day 210 (1.34 mm day^{-1}) and declined again after the first egg-laying (0.1 9mm day^{-1}). Growth slowed, but did not stop after egg-laying and so growth is indeterminate in this species (Fig. 1).

Initiation of egg-laying varied between individuals in groups (mean = 210.9 ± 119.5 days, minimum = 180 days) and isolated (454.2 ± 76 days, minimum = 380 days; H = 27.26, p = 0.0001). Isolated individuals had a larger proportion of non-reproducing individuals (23.8%) in two years. For paired individuals after 180 days, the time to first egg-laying was 110.8 ± 89.0 days, with a minimum of 33 days. Only 6.6% of this group never reproduced during the experiment.

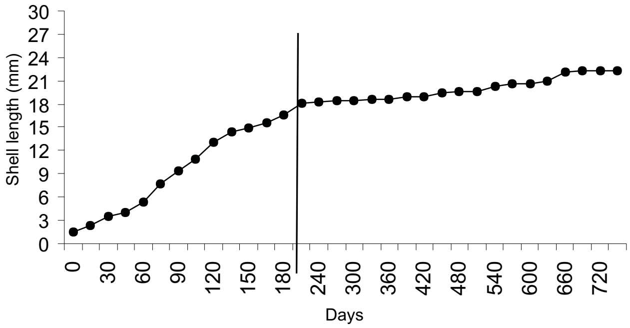

Figure 1. Snail growth during the study. Points show average shell length (mm) for *Bulimulus tenuissimus* in the grouped treatment for two years. Vertical line shows when egg laying began.

Isolated snails produced smaller clutches (647 eggs) than either paired (6240 eggs, t = 143.90, $p < 0.0001$) or grouped (12,642 eggs, t = 166.50, $p < 0.0001$) snails (Tab. I). The interval between egg-laying varied between grouped and paired (t = 25,99, p = 0.0055), grouped and isolated (t = 43.15, p = 0.0003), but not between paired and isolated (t = 17.16, p = 0.07) treatments (Tab. II).

Table I. Number of eggs per clutch in *Bulimulus tenuisimus* in groups, paired and isolated during two years.

Treatment	Number of eggs clutch-1				
	Minimum	Maximum	Mode	Mean ± SD*	CV (%)
Grouped	6	252	32	59.9 ± 42.48	70.91
Paired	6	134	24	48.3 ± 26.53	54.86
Isolated	1	53	7	14.7 ± 12.76	86.79

* The isolated treatment produced fewer eggs per clutch (Kruskal-Wallis $p < 0.05$).

Table II. Interval between egg-laying in *Bulimulus tenuissimus* in groups, paired and isolated during two years.

Treatment	Interval between egg-laying				
	Minimum	Maximum	Mode	Mean ± SD	CV (%)
Grouped	1	160	2	17.45 ± 33.38	191.28
Paired	1	226	8	31.66 ± 57.37	180.63
Isolated	1	39	18	18.75 ± 9.06	49.58

The interval between egg-laying was lower in paired, however, not differ in isolated and grouped treatments (Kruskal-Wallis $p < 0.05$).

Average time to hatching was 23.62 ± 0.89 days. Hatching rate varied between treatments: 39.0% for grouped, 32.6%

for paired and 15.8% for isolated (H = 19.80, p = 0.0001) with a significant difference between grouped and isolated (t = 80.00, p < 0.0001) and paired and isolated (t = 60.71, p = 0.0015). Additionally, eggs from 59.0% of the clutches from isolated individuals were shells only.

There was an apparent seasonality to reproduction in *B. tenuissimus* due to a greater production during the months August-December, in both years of study, in both, grouped and paired treatments (Fig. 2). While this seasonality was during the months with greater temperatures and humidities, there was no correlation between egg production and either environmental variable (humidity: rs = 0.24, p = 0.35, temperature: rs = 0.35, p = 0.18; or hatching rate and humidity: rs = -0.09, p = 0.73).

Reproduction was similar during the two breeding seasons (Z = 24.50, p = 1.0) but the hatching rate was greater during the first year (Z = 2.61, p = 0.009).

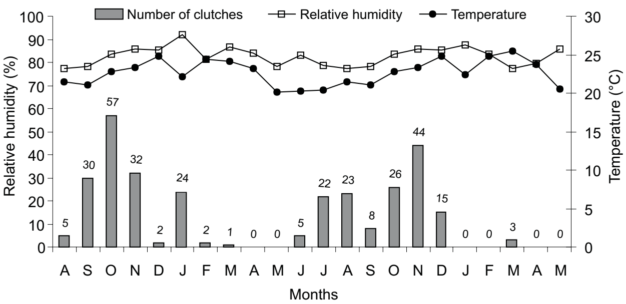

Figure 2. Average temperature and relative humidity and number of clutches laid each month by *Bulimulus tenuissimus* in groups from August 2005-May 2007. Numbers above bars indicate the number of clutches.

DISCUSSION

Indeterminant growth in *B. tenuissimus* represents continuous energetic investment in growth after the beginning of egg production, even though growth declines with age (Cichon 1999). Other pulmonates also show this pattern, such as *Achatina achatina* (Linné, 1758) (Achatinidae) (Hodasi 1979), *Subulina octona* (Brugüìere, 1792) (Subulinidae) (Bessa & Araújo 1995, D'avila & Bessa 2005) and *Bradybaena similaris* (Férussac, 1821) (Bradybaenidae) (Almeida & Bessa 2001).

Indeterminate growth is apparently optimal when productivity increases with shell size. Thus, growth after the initiation of egg-laying does not reduce reproductive success since it is compensated for, since fecundity increases with increasing body size (Heller 2001). For example, body weight, shell length and egg production are all correlated in *S. octona* (D'ávila & Bessa (2005). *Bulimulus tenuissimus* is long lived and egg production is relatively delayed, which suggests that these animals gain reproductive success by breeding when they have reached a larger size.

Grouped animals reproduce earlier than either paired or isolated, as in *Bradybaena similares* (Almeida & Bessa 2001). In *B. similares* sexual maturity for either grouped or isolated individuals occurred at 70 d after hatching, even thought grouped individuals laid eggs sooner (Furtado *et al*. 2004). A similar trend is possible with *B. tenuissimus*, since the time interval to egg laying after mating is very short. However, this was not tested histologically in this study. Grouping and pairing apparently stimulate egg production, as shown by the lower production of snails kept in isolation, as demonstrated with *Theba pisana* (Müller 1774) (Helicidae) (Lazaridou-Dimitriadou & Daguzan 1981) and *Bradybaena similares* (Almeida & Bessa 2001).

Variation in clutch size is common in other bulimulids as well, such as *Bostryx conspersus* (Sowerby, 1833) (Bulimulidae) (Ramírez 1988), and other terrestrial pulmonates, such as *Helix pomatia* Linnaeus, 1758; *Helix aspersa* (Müller, 1974) (Helicidae) (Hyman 1967) and *B. similaris* (Almeida & Bessa 2001). Variable clutch size in terrestrial mollusks is apparently due to many factors, such as age, size and environmental conditions and seasonality (Heller 2001, Almeida & Bessa 2001). The interval between clutches for grouped snails also shows greater production in this treatment, such as in *Subulina octona* and *B. similaris* (Bessa & Araujo 1995, Almeida & Bessa 2001). Age was unrelated to egg production in this study, although it is possible that it was related to hatching rate during the two years of study.

Incubation period in *B. tenuissimus* (19.56 ± 1.68 d) is similar to that of other species in the family, such as *Bostryx conspersus* (Ramírez 1988). In *Protoglyptus carlucioi* Pilsbry, 1897 (Orthalicidae) (Rezende *et al.* 1972) the incubation period was 21 d, with hatching rate of 97.62%, also in the laboratory.

The low hatching rate of eggs produced by self-fertilization in *B. tenuissimus* is also found in other terrestrial pulmonates such as *Bradybaena similaris* (Almeida & Bessa 2001) and *Arianta arbustorum* Linnaeus, 1758 (Helicidae) (Baur *et al*. 1998). Thus, while self-fertilization is possible, it does not confer a large reproductive success and is perhaps only important in unfavorable conditions, such as those of isolation.

Reproductive seasonality similar to that shown here was found in *Trochoidea simulata* (Ehrenberg, 1831) (Helicidae), which has very well defined seasonal reproduction, apparently generated by environmental conditions of temperature and humidity (Ward & Slotow 1992). *Mastus olivaceos* (Philippi, 1845) and *Mastus crelensis* (Pfeiffer, 1846) (Bulimulidae) also have well-defined reproductive periods, also due to variable environmental conditions. Reproduction in these species is coincident with the wet season (Parmakelis & Mylonas 2002).

Egg laying is dependent up favorable environmental conditions, such as temperature (Dimitrieva 1975) and humidity (Heller 2001). In *B. tenuissimus* however, egg laying is not dependent upon either temperature or relative humidity. Yet, individuals had well-defined reproductive periods, which suggests that other environmental or endogenous factors trigger reproduction, such as in *Sphincterochila zonata* (Bourguignat,

1852) (Sphincterochilidae) and *Sphincterochila prophetarum* (Bourguignat, 1853) (Sphincterochilidae) (Alon *et al*. 2007). Apparently hormonal changes, including testosterone, progesterone and estrogen, show seasonal variation (Alon *et al*. 2007). We suggest that similar studies be carried out with *B. tenuissimus* to better understand the reproductive cycle in this species.

ACKNOWLEDGMENTS

We thank Juliane F.S. Lopes for the suggestions and the statistical analysis, and Sthefane D'Ávila for her critique and suggestions for the manuscript, and CAPES, for the fellowship.

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Submitted: 09.X.2007; Accepted: 06.VI.2008.
Editorial responsibility: Georgina Bond-Buckup