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Aspects of biology of *Megalobulimus paranaguensis* (Gastropoda, Acavoidea) in the coastal plain of the Brazilian southeast

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ABSTRACT. We studied the reproductive biology of *Megalobulimus paranaguensis* (Pilsbry & Ihering, 1900), a large and long-lived land gastropod from the Atlantic Rainforest of Brazil. The study was conducted at an urban park in the city of Santos, state of São Paulo. For 4 years, we counted the egg postures and annual eclosion rate of 32 captive snails and looked for associations between egg posture and the climatical variables of the period. The annual mean posture of 8.7 eggs per snail obtained in our results is a small number, but typical of Brazilian macromollusks. The annual eclosion rate was 31%. The beginning of the annual activity period of snails occurred in the middle of March, and lasted 33.97±3.02 weeks. The dormancy period started in the beginning of November, and lasted 18.39±3.11 weeks. There were two egg posture peaks, a minor peak between March and May, and a major peak between August and November, with greater values in September. *Megalobulimus paranaguensis* has a well-defined seasonal reproductive pattern influenced by environmental temperature and temperature range. Furthermore, in this snail, reproduction is negatively influenced by temperature increasing and temperature range.

KEYWORDS. Aruá-do-mato, egg posture, land snail, Atlantic Rainforest, reproduction.

RESUMO. Aspectos da biologia de *Megalobulimus paranaguensis* (Gastropoda, Acavoidea) na planície costeira do sudeste brasileiro. Nós estudamos a biologia reprodutiva de *Megalobulimus paranaguensis* (Pilsbry & Ihering, 1900), um grande e longevo gastrópode terrestre da Floresta Atlântica na costa sudeste do Brasil, em um parque urbano na cidade de Santos, Estado de São Paulo, ao longo de quatro anos, pelo número de posturas e taxa de eclosão anual de 32 caracóis cativos. Relacionamos estes dados com variáveis climáticas do mesmo período. Obtivemos uma média anual de posturas de 8.7 ovos por caracol, um número baixo, típico da fauna de macromoluscos terrestres brasileiros. A taxa de eclosão anual foi de 31%. O início do período anual de atividade ocorreu em meados de março e dura 33.97±3.02 semanas; e o início da dormência ocorreu no começo de novembro; com duração de 18.39±3.11 semanas. Existem dois picos de postura, um menor entre março a maio e um pico maior antes da estivação, entre os meses de agosto a novembro, com maiores valores em setembro. *Megalobulimus paranaguensis* possui um padrão sazonal reprodutivo bem marcante influenciado pela temperatura do ambiente e amplitude de temperatura. A reprodução é influenciada negativamente pelo aumento da temperatura e da amplitude de temperatura.

PALAVRAS-CHAVE. Aruá-do-mato, posturas de ovos, caracol terrestre, Mata Atlântica, reprodução.

Megalobulimus Miller, 1878, which includes species popularly known as "aruá-do-mato", is a group of giant Neotropical land snails that belong to the subfamily Megalobuliminae (Strophocheilidae) (Bouchet & Rocroi, 2005). The genus has great diversity, with 83 described species, 63 of which occur in Brazil (Simone, 2006, 2012; Borda & Ramirez, 2013; Fontenelle *et al*., 2014). Like other land snails, the species of *Megalobulimus* are nocturnal (Bequaert, 1948). During the day, or during dormancy periods, individuals bury themselves in the soil or leaf litter (Bequaert, 1948). They are iteroparous hermaphrodite snails with annual periodicity and great longevity, can live up to 35 years (Pinto-de-Oliveira *et al*., 1984; Horn *et al*., 2005; Fontenelle & Miranda, 2012), and generally occur in low densities and irregular distribution (Simone, 1999; Eston *et al*., 2006; Miranda *et al*., 2015). Like other Brazilian pulmonates, they have low reproductive potential, spawning 2-5 eggs per batch (Sobreira & Molina, 2002).

One third of the known species of *Megalobulimus* in Brazil occur in the coastal plain and mountain ranges parallel to the coast, between the South and Southeast (Bequaert, 1948), in the Atlantic Rainforest. This biome is one of the richest in the world in terms of numbers of species, but unfortunately it has been gradually destroyed (Mansur & Leme, 1996; Myers *et al*., 2000). The populations of many species of *Megalobulimus* from this area, for instance *M. fragilior* (Ihering, 1901), *M. parafragilior* Leme & Indrusiak, 1990, *M. grandis* (Martens, 1885), *M. lopesi* Leme, 1989, *M. proclivis* (Martens, 1888) and *M. paranaguensis* (Pilsbry & Ihering, 1900), have been reduced, and some may be threatened (Leme, 1989; Leme & Indrusiak, 1990; Mansur & Leme, 1996; Fischer & Colley, 2005; Miranda *et al*.,

2015). Officially, the Brazilian authorities only list one species of *Megalobulimus* in their list of threatened species, *M. cardosoi* (Morretes, 1952), which is endemic to the state of Alagoas, and is critically endangered (Santos *et al*., 2015).

Megalobulimus paranaguensis (Pilsbry & Ihering, 1900) was described from the city of Paranaguá (state of Paraná), and is distributed in the coastal plain of the Atlantic Rainforest, between Guaratuba, state of Paraná, and Itanhaém, state of São Paulo (Morretes, 1954). There have been records also between the Island of São Vicente, state of São Paulo (Miranda *et al*., 2015) and the northern coast of the state of Santa Catarina (Agudo-Padron, 2014), coinciding with a geomorphology characterized by large coastal plains between Serra do Mar, and long beaches interspersed by estuaries (Muehe, 1998). After the introduction of the alien snail *Achatina fulica* Bowdich, 1822 many individuals of *M. paranaguensis* have been accidentally killed in control campaigns (Colley & Fischer, 2009).

Recently, aspects of the ecology and distribution of some species of *Megalobulimus* were studied in more detail (Beltramino, 2013, 2014; Beltramino *et al*., 2015; Miranda *et al*., 2015; Miranda & Fontenelle, 2015; Miranda & Pecora, 2016), providing important information about their biology and conservation. On the other hand, data about the reproductive biology of *Megalobulimus* species are still scarce, and restricted to the studies of Pinto-de-Oliveira *et al*. (1984), on *Megalobulimus bronni* (Pfeiffer, 1847), Sobreira & Molina (2002), on *Megalobulimus* **aff**. *gummatus* (Hidalgo, 1870) and Horn *et al*. (2005), on *Megalobulimus abbreviatus* (Bequaert, 1948). Information obtained by these studies have increased our knowledge of their ecology and can be used to develop conservation and management plans for *Megalobulimus* spp. The objective of this study was to analyze the activity and oviposition of captive *M. paranaguensis* at Parque Zoobotânico Orquidário Municipal, and to evaluate which environmental variables affect this pattern.

MATERIAL AND METHODS

This study took place at "Parque Zoobotânico Orquidário Municipal de Santos" (PZOMS), a small 22,000 m^2 park located in the municipality of Santos (UTM: 23K E 362672.57, 7348904.53 N). PZOMS presents flat topography, arboreal phytophysiognomy with understory formation and litter accumulation. Its area is fragmented, and has malls, animal enclosures, a 4,300 m^2 lake and rainwater drainage channels.

Between January 2007 to December 2010, 32 individuals of *M. paranaguensis* were captured at the PZOMS, kept captive in boxes and placed in the shade, in understory areas at the park. The boxes were 50 x 80 x 80 cm and had a 0.5 inch screen. Four snails were maintained in each box, which was lined with substrate containing 15 cm of soil + sand and 10 cm of leaf litter. Food was provided *ad libitum* and it was similar to what is consumed by the free population of the PZOMS, consisting of *Malvaviscus*, *Spathiphyllum*, *Piper* and "jurubeba" (*Solanum paniculatum*). The leaf litter level was completed weekly, litter was changed monthly and the number of postures (eggs/individual) was counted once a month and divided by the number os specimens in each box. Each egg ovoposed were measured (n=255) in length and width. For the years of 2009 and 2010, the annual eclosion rate were obtained. Voucher specimens of the population studied are deposited in the mollusk collection of the Museu de Zoologia da Universidade de São Paulo (lot MZSP 54629). In addition, the beginning and length of activity and dormancy periods of each snail were monitored. The following environmental variables were obtained for the duration of the experiment, from the website http://www.ciiagro.sp.gov.br/ciiagroonline/#Monitoramento (maintained by EMBRAPA – Agronomic Institute of Campinas) for the municipality of Santos, SP: mean temperature, potential evapotranspiration, number of rainy days, precipitation and temperature range. The temperature range values were obtained by subtracting the mean minimum temperature from the mean maximum temperature from January 2007 to December 2010. A stepwise regression, using AICc as the variable selection criterion between environmental variables and number of postures, was used to evaluate which variables influenced the number of postures. In the results, we only show the environmental variables that have affected the number of postures.

RESULTS AND DISCUSSION

We observed the beginning of the annual activity period in the mid-March, lasting 33.97±3.02 weeks; and the beginning of dormancy in the beginning of November, lasting 18.39±3.11 weeks in captive individuals. The annual mean egg posture was 8.7 eggs per snail. There were two egg posture peaks, a minor peak between March to May, and a major peak between August and November, with greater values in September (Fig. 1). The annual eclosion were 31% for both years analysed. The eggs are white, calcareous, elliptical, and are 26.3 ± 1.82 mm length per 20.1 ± 1.16 mm width (Fig. 2). They are partially buried in litter. At birth,

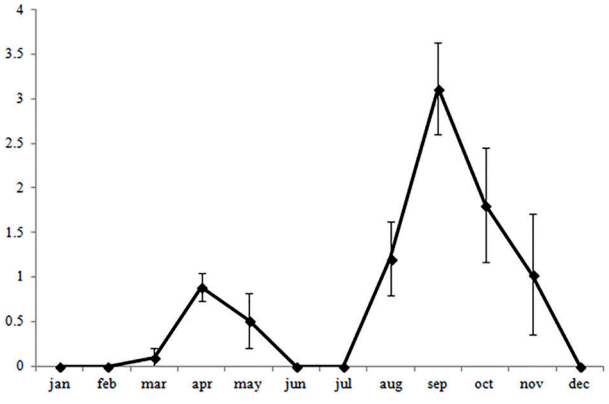

Fig. 1. Mean number of postures of *Megalobulimus paranaguensis* (Pilsbry & Ihering, 1900) in Parque Zoobotânico Orquidário Municipal de Santos (PZOMS).

newly-born individual sometimes eat the eggs from which they have hatched.

Mean temperature values were greater during summer months (from November to February) and lowest in winter months (from May to August), and these fluctuations were well marked during the year (Fig. 3). The temperature range presented greater values in February, June and July, and minor values in May, September and October (Fig. 4). The stepwise regression mainted the variables mean temperature and temperature range in the model. The stepwise regression revealed that mean temperature and temperature range are negatively correlated with both variables (mean temperature: $t = 1.93$, p = 0.05, b = - 0.10; temperature range: $t = 2.41$, p - 0.01, b = - 0.30).

Megalobulimus paranaguensis has a well-marked seasonal reproductive pattern, influenced by temperature and temperature range. The number of postures decrease with increasing temperatures. Gomot (1990) showed that an increase in temperature, related with photoperiod variations, reduces the number of weeks in the reproductive cycle of *Helix pomatia* Linnaeus, 1758. The abundance and activity of *M. paranaguensis* are influenced by humidity and evapotranspiration in the environment (Miranda & Fontenelle, 2015; Miranda *et al*., 2015), which indicate that an increase in the temperature and temperature range result in decreased activity of individuals and, consequently, a decrease in egg production.

Generally, gastropods react to changes in environmental conditions, but also have a circadian cycle and seasonal behavior synchronized by an endogenous biological clock, which anticipate variations in temperature, water and light throughout the year (Lazaridou-Dimitriadou & Saunders, 1986; Iglesias *et al*., 1996). Their characteristic dormancy during the summer months, called aestivation, happens when it is hot or dry (Boss, 1974). The main function of this dormancy, which is very common among land and freshwater mollusks, is to limit water loss by evaporation (Cáceres, 1997). In *M. paranaguensis*, aestivation occurs in response to an increase in evapotranspiration (Miranda & Fontenelle, 2015) and decrease in humidity (Miranda *et al*., 2015) between November and March, which increases water loss to the environment.

The number of postures of *M. paranaguensis* confirms the observation of Miranda & Fontenelle (2015) that this species is iteroparous. Their study was based on mark-recapture estimates, and spawning peaks in their data coincided with the activity and recruitment peaks obtained by Miranda *et al*. (2015) and Miranda & Fontenelle (2015) for the same species. The iteroparous pattern also occurs in *M. abbreviatus* (Horn *et al*., 2005), which indicates that this must be a pattern in the genus. Moreover, the presence of aestivation and a period with no reproductive activity is a characteristic of *M. paranaguensis* that differs from *M. abbreviatus*, and it is more similar to the patterns of temperate zone species (Bailey, 1981).

Megalobulimus paranaguensis has low egg production, a pattern that is common in other Brazilian land snails. Jurberg *et al*. (1988) reported between 10 to 12 eggs per batch in *Thaumasthus taunaisii* (Ferrusac, 1822). Within *Megalobulimus*, 1.44 eggs per batch were recorded for *Megalobulimus* **aff**. *ovatus* (Müller, 1774), 2.87 eggs per

Fig. 2. Egg posture of *Megalobulimus paranaguensis* (Pilsbry & Ihering, 1900) in leaf litter (1b). Scale = 30 mm.

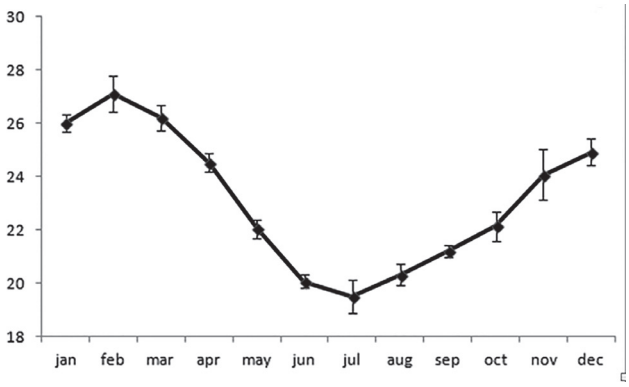

Fig. 3. Seasonal variation of mean temperature per month in municipality of Santos during period sampled.

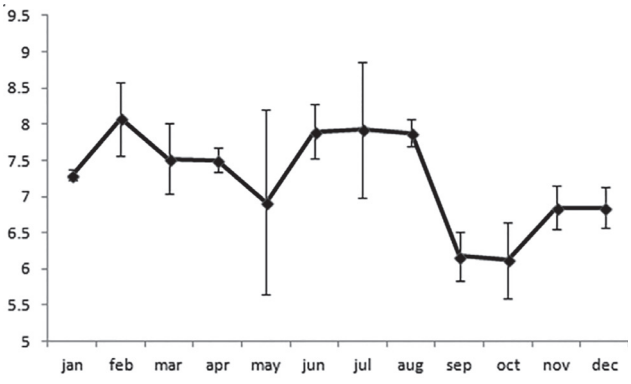

Fig. 4. Seasonal variation of temperature range per month in municipality of Santos during period sampled.

batch for *Megalobulimus* sp. and 3.71 eggs per batch for *M. gummatus* (Sobreira & Molina, 2002; Rios *et al*., 2004). The first behavior performed by newly-born individuals is to eat the egg case. This behavior is important because the shell of young individuals is rather fragile (Jurberg *et al*., 1988), and calcium is a limiting element for mollusk survivor, being absorbed by the diet and by animal foot sole (Fournier & Chétail, 1984).

The eclosion rate found in our study was low, when compared with another species in literature for another species of genus. Sobreira & Molina (2002) found 52.4% eclosion rate for *Megalobulimus gummatus* (Hidalgo, 1870). Rios *et al*. (2004) found for *Megalobulimus* **aff**. *ovatus* (Muller, 1774) and eclosion rate of 57.9% for specimens introduced at t the Zoological Park of São Paulo, and for F1 generation it was found 34.5% eclosion rate of egg obtained by cross fertilization and 28.6% of the eggs obtained by self-fertilization.

Egg posture peaks in *M. paranaguensis* occur in the same period as the activity pattern recorded by Miranda & Fontenelle (2015) in natural conditions and same period, but in a reverse way: when there is a major activity peak, egg laying slows down. This pattern indicates a major reproductive activity period, when energy is allocated for reproduction, decreasing the activity of individuals. An interesting characteristic of the egg posture cycle of *M. paranaguensis* in the study area is that the major peaks occur right before the aestivation period, which occurs between November and March (Miranda *et al*., 2015; Miranda & Fontenelle, 2015). In *Megalobulimus bronni* (Pfeiffer, 1847) from Juiz de Fora (state of Minas Gerais), three egg posture peaks were recorded, a major peak between December and February, followed by a second pre-aestivation peak between April and May, and a post-aestivation peak in September. This differs from the pattern of *M. paranaguensis* due to environmental differences between both areas. The major peaks in the end of the year can be explained by the major photoperiod in summer months, which increases egg production in pulmonates (Gomot-de-Vaufleury, 2001). Moreover, the young individuals that are born in this period generally aestivate and die because they do not have sufficient energy reserves. In general, in newer cohorts there are high mortality rates (Heller, 2001), which can be more than 94% in *M. mogianensis* Simone & Leme, 1998 (Romero, 2004). The occurrence of this egg laying pattern can be an important factor in the mortality rate of wildlife populations.

In this paper, we concluded that *M. paranaguensis* has a well marked seasonal pattern of activity and oviposition. This pattern also directly influendes the surviovorship and activity and juveniles, and can generate a high mortality in younger conhorts.

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