

## Reproductive cycle of *Leptaxis caldeirarum*, a locally endangered Azorean land snail

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### Summary

The land snail *Leptaxis caldeirarum* (Morelet and Drouet, 1857), a hermaphrodite Hygromiidae species, endemic from São Miguel Island (Azores), is geographically limited to a range of 31 km<sup>2</sup>, occupying an area of about 14 km<sup>2</sup>. A preliminary study confirmed the locally endangered status of the species, raising the possibility that it could reach the “critically in danger” status, the most serious category in IUCN. A population from Ramal dos Mosteiros was studied all year round in order to elucidate its reproductive cycle and to test the validity of the total weight of the animal and the maximum diameter of the shell as diagnostic parameters for maturation. Gonadal maturation of *L. caldeirarum* proceeds from late winter to early summer. Intense gametogenesis takes place from January until May and the snail is apparently in a reproductive condition to copulate from late spring onwards. The strong correlation observed between the relative volumetric density of mature gametes and the maximum diameter of the shell suggests that the reproductive status of the species can be inferred from the latter parameter. This may be of major importance in future studies, minimizing potential damage to already unstable populations, and facilitating the development of conservation strategies.

**Key words:** *Leptaxis caldeirarum*; reproduction; gametogenesis, endemic, conservation

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### Introduction

Several studies have investigated the maturation process and the reproductive system of land snail species (Lusis, 1961; Smith, 1966, 1967; Runham and Laryea, 1968; Els, 1978; Cuezco, 1990; Rodrigues et al., 1998; Rodrigues and Martins, 2003). Although some studies have been based on the histology of the gonad (Runham and Laryea, 1968; Apley, 1970; Luchtel, 1972a, 1972b; Runham and Hogg, 1979;

Cuezco, 1990, 1993), other authors reported a strong relation between gonadal maturation and the shape and size of the reproductive tract, particularly the seminal vesicle and spermoviduct (Lusis, 1961; Runham, Bailey and Laryea, 1973; Cuezco, 1990; Rodrigues et al., 1998). Several authors reported that maturation of the reproductive system is related to the growth of the animal and Rodrigues et al. (1998) revealed a strong correlation between maturation and the size of the shell

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in *Oxychilus atlanticus* (Pulmonata: Zonitidae). Nevertheless, as pointed out by Cuezco (1993), gonadal development depends on season, age and size of the animal, with the expected life time of the species being an important element in a population's prospects. This presupposes knowledge of the species' life cycle.

Land snails represent an important component of the endemic fauna on the Azores (Backhuys, 1975), about 44% of the 107 species described in the archipelago being endemic. In the Azores, the genus *Leptaxis* comprises five species and two subspecies: *Leptaxis caldeirarum* (Morelet and Drouet, 1857), *Leptaxis terceirana*, (Morelet, 1860), *Leptaxis vetusta* (Morelet and Drouet, 1857), *Leptaxis drouetiana* (Morelet, 1860) *Leptaxis azorica azorica* (Albers, 1852) and *Leptaxis azorica minor* (Backhuys, 1975). All of these are considered as locally endangered, but *L. caldeirarum*, *L. terceirana* and *L. vetusta* are listed by the World Conservation Union (IUCN) red list of threatened species as globally threatened (Hilton-Taylor, 2000).

*L. caldeirarum* is a hermaphrodite Hygromiidae species, endemic on São Miguel Island (Açores). The species is confined to the Sete Cidades volcanic complex, in the western part of S. Miguel Island, the largest island of the Azorean archipelago. This island was formed by two separate islands that coexisted for about 0.550 My and that merged about 50,000 years ago (Forjaz, 1997).

Work from Vieira (2001) stated that this species is geographically limited to a range of 31 km<sup>2</sup>, occupying an area of about 14 km<sup>2</sup>, leading to its locally endangered status, and drawing attention to the possibility that it could reach the "critically in danger" status, the most serious category according to the IUCN. Volcanic activity may be the main historical cause of the species' endangered status, with 17 eruptions during the last 5,000 years, some of them producing deposit layers more than 2 m thick. However, man's activities are probably the most significant present threat to the species, reducing its natural habitat as a result of the enlargement of the area for pastures and logging.

Apart from the work of Morelet and Drouet (1857), dealing with the systematics of the species, and that of Vieira (2001), on its geographical distribution and status, little is known about life cycle of *L. caldeirarum*, especially with reference to its reproductive biology. Thus, the main objective of this work is to study the reproductive cycle of *L. caldeirarum* and to assess the validity of two morphometric parameters; namely, the fresh weight and the maximum diameter of the shell, respectively, as being diagnostic for reproductive maturation, in order to minimize the ecological

impact of future studies and to underpin conservation measures.

## Materials and Methods

To perform this study we choose a site (Ramal dos Mosteiros: latitude 37°52'26" N; longitude 25°49'30" W) where the human influence is already very evident — a small valley near to a national road and almost without native vegetation — in order to minimize our impact on the habitat of the species. Every 2 months, from January to November 2003, 7–10 of the largest individuals found during a collecting period lasting 2 h were transported alive to the laboratory (January, n = 10; March, n = 8; May, n = 10; July, n = 10; September, n = 9; November, n = 7). The remaining individuals were returned to the environment. In the laboratory, the maximum diameter of the shell and the total fresh weight were measured before excision of the gonad for histology.

In order to determine the state of gonadal maturation, the ovotestis were fixed in 10% formalin and embedded in paraffin. Serial sections, 7-µm thick, were stained with Mayer's haemalum and eosin (Martoja and Martoja-Pierson, 1970). The relative volumetric density of the gametes was estimated using the M168 Weibel Multipurpose Test System (Weibel, 1979).

Five stages of spermatogenesis were identified on the basis of the classification of Griffond et al. (1991): (1) spermatogonia, small spheroidal cells (8–10 µm diameter), with a relatively large nucleus and scanty cytoplasm; (2) spermatocytes, larger than spermatogonia (11–17 µm in diameter), with more abundant and eosinophilic cytoplasm; (3) early spermatids, small in size (8–10 µm diameter), spheroidal in shape, but frequently showing basophilic condensations at their poles; (4) late spermatids, with the whole cell elongated and with small tails; (5) spermatozoa, with strongly basophilic head and long eosinophilic tails. As with *Oxychilus atlanticus* (Pulmonata: Zonitidae) (Rodrigues et al. 1998), no unambiguous distinction could be made between spermatocytes I and II, nor between early- and mid-spermatids, with light microscopy.

In accordance with Hill and Bowen (1976) and Rodrigues et al. (1998), three developmental stages of oogenesis were identified: (1) previtellogenic oocytes, small, rounded cells with strongly basophilic cytoplasm; (2) vitellogenic oocytes, larger than the previous stage, more flattened and lightly basophilic; and (3) maturing oocytes, larger than the previous, rounded in shape and with an eosinophilic and granular cytoplasm. This last stage also includes the fully mature oocytes.

In order to identify the gonadal maturation state, scores for volumetric density were summed for each specimen and converted to percentages. The data were subject to statistical analysis using SPSS 11.5 for Windows (SPSS Inc., 2002). Pearson's correlations were determined between the following variables: maximum diameter of the shell, total fresh weight and relative volumetric density of each gametogenic stage. One-way analysis of variance (ANOVA) was performed to compare morphometric variables and relative volumetric density of each stage of oogenesis and spermatogenesis during the collection period. Gametogenic data was arcsin-transformed prior to analysis, while morphometric data were transformed into  $\sqrt{x + 0.5}$ . When significant differences were observed, the least significant difference (LSD) test was used for pair-wise comparisons among samples.

### Results

The profiles of changes in the maximum diameter of the shell and the total fresh weight, respectively, are very similar during the sampling period, showing some growth between January and May, with larger specimens occurring at the end of spring (May) and during the summer (July) (Fig. 1). In September a significant decrease was observed in total fresh weight compared to May ( $P = 0.008$ , LSD test) and in the maximum diameter of the shell compared to May ( $P = 0.024$ , LSD test) and July ( $P = 0.037$ , LSD test).

Stereological study showed that the gonadal volume occupied by maturing oocytes increased from

January to July, when it reached a relative volumetric density over 10%, significantly higher than in January ( $P = 0.01$ , LSD test) and September ( $P = 0.045$ , LSD test) (Fig. 2A). Individuals from September revealed the lowest relative volumetric density of maturing oocytes, a situation similar to that of January. Apart from January and September, maturing oocytes occupied more than 5% of the gonadal volume throughout the year (Fig. 2A).

The relative volumetric density of spermatozoa shows that they occupy more than 30% of the gonadal volume throughout the year, reaching the highest values in May, July and September (Fig. 2B). Individuals from September show a relative volumetric density of spermatozoa which is significantly higher ( $F = 3.382$ ;  $df = 5, 48$ ;  $P = 0.011$ ) than those from January, March, May and November; and they have the lowest volume of the gonad occupied by the immature stages of the spermatogenesis, as well as the lowest volumetric density of maturing oocytes (Fig. 2B).

The morphometric variables of shell diameter and fresh weight are strongly correlated ( $n = 54$ ,  $r = 0.917$ ;  $P < 0.001$ ), and the maximum diameter of the shell shows a significant correlation ( $n = 54$ ,  $r = 0.307$ ;  $P = 0.024$ ) with the gonadal volume occupied by mature gametes (spermatozoa and maturing oocytes considered together). The relative volumetric density of spermatocytes is negatively correlated with the maximum diameter of the shell ( $n = 54$ ,  $r = -0.323$ ;  $P = 0.017$ ) and total fresh weight ( $n = 54$ ,  $r = -0.312$ ;  $P = 0.022$ ).

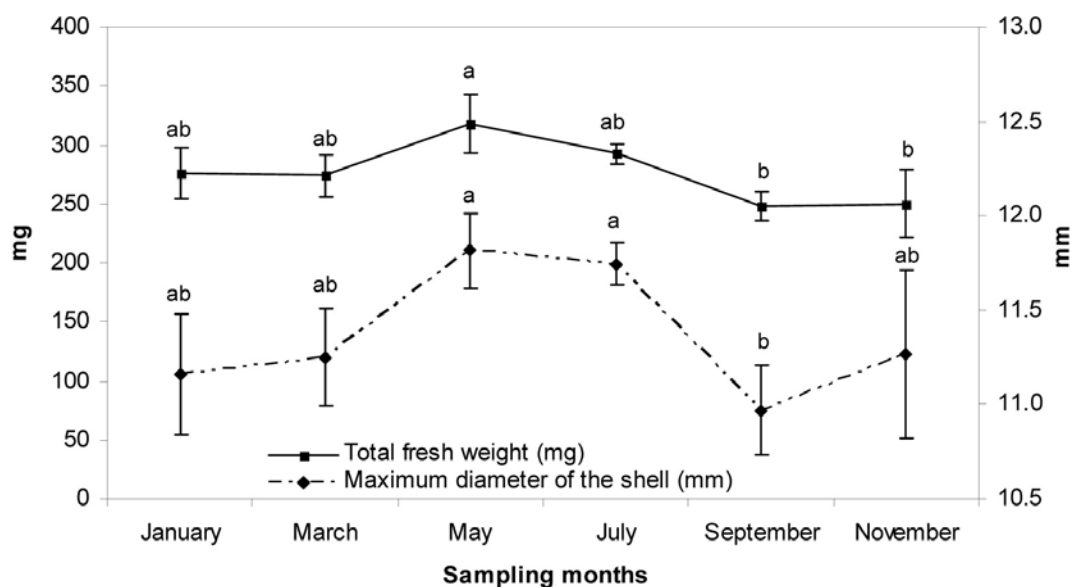


Fig. 1. Total fresh weight (mean  $\pm$  SE) and maximum diameter of the shell (mean  $\pm$  SE) of *Leptaxis caldeirarum*, from January to November 2003. Different lower cases indicate significantly different values, ANOVA ( $P < 0.05$ ).

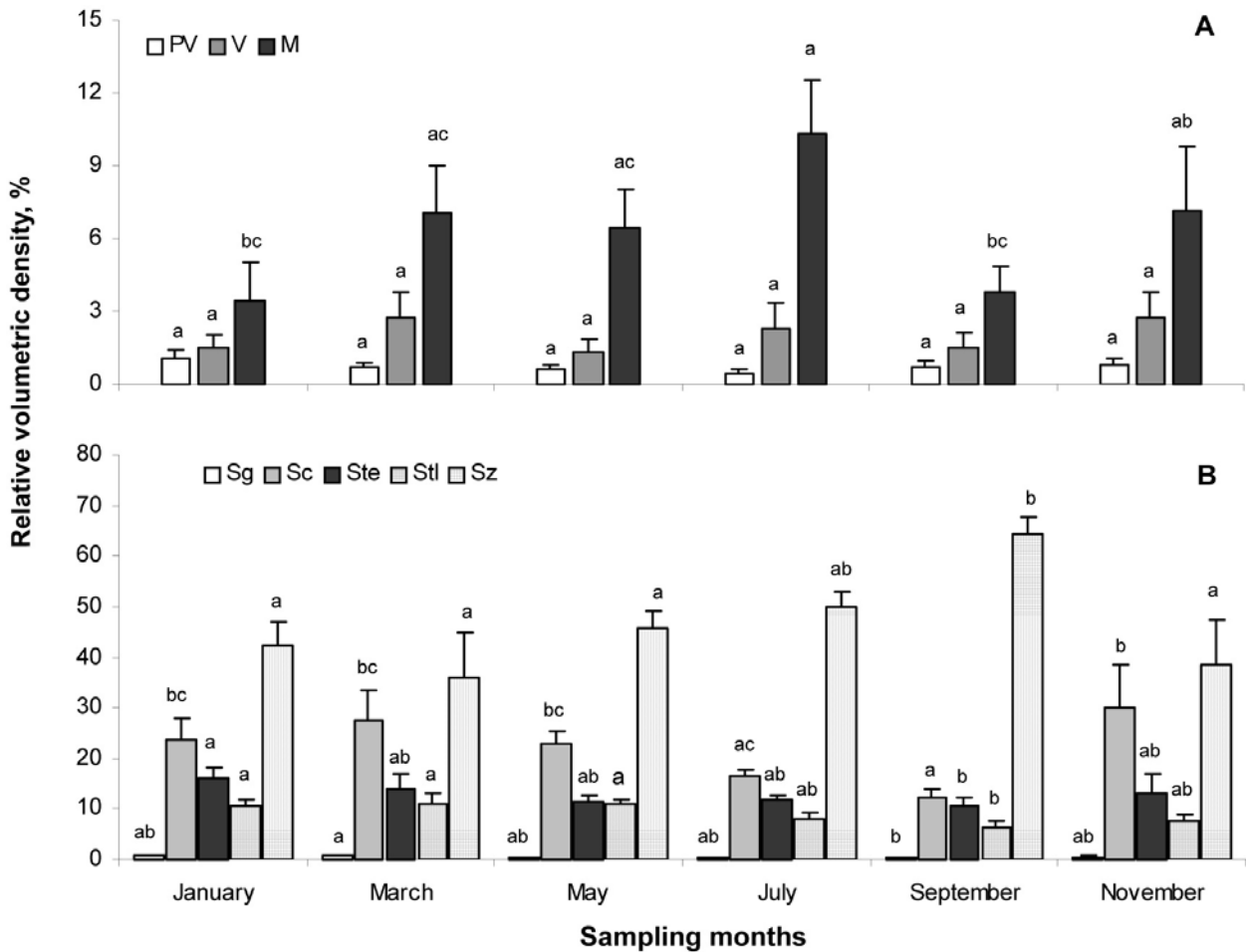


Fig. 2. Relative volumetric density (mean  $\pm$ SE) of the gametogenic stages in *Leptaxis caldeirarum*, from January to November 2003. A. Oogenesis: pre-vitellogenic (PV), vitellogenic (V) and maturing oocytes (M). B. Spermatogenesis: spermatogonia (Sg), spermatocytes (Sc), early spermatids (Ste), late spermatids (Stl) and spermatozoa (Sz). Different lower cases above columns, within a stage of reproductive maturation between sampling dates, indicate significantly different values, ANOVA ( $P < 0.05$ ).

### Discussion

Morphometric data indicate a single generation each year with mature snails reproducing and dying in summer, and juveniles growing in the autumn, winter and spring. The occurrence of many large empty shells in September is a result of the post-reproductive death and supports this hypothesis.

Our observations on gonadal maturation show that *L. caldeirarum* matures from late winter to early summer, accumulating gametic resources to copulate from late spring onwards, with more than a half the gonadal volume in summer being occupied by spermatozoa, and with a relative volumetric density of maturing oocytes above 10% (Fig. 2).

A protandric tendency of the species is supported by the fact that spermatozoa occupy more than 35% of

the gonadal volume throughout the year, even in March and November, when spermatocytes reach the highest relative volumetric densities and maturing oocytes are uncommon. Whilst considering simultaneous hermaphroditism as characteristic of pulmonates, Gómez (2001) noted the existence of an initial, predominantly masculine phase, followed by one where female function is more evident. Morton (1967) also drew attention to the protandric tendency of molluscs, suggesting that male gametes will be produced at the beginning of the reproductive season, or when the animal is younger, and this is in accordance with the size advantage model of Ghiselin (1969). According to the latter, large body size is adaptive for female function, given the fact that eggs are larger than the sperm.

There is a positive correlation between sexual maturity, as expressed by the volume occupied by both spermatozoa and maturing oocytes combined, and the maximum diameter of the shell. These results are in accordance with those of Cuezco (1990, 1993) and Rodrigues et al. (1998). In late spring and in summer, we categorize all *L. caldeirarum* individuals with a shell diameter larger than 11.5 mm as being fully mature, since more than 50% of their gonadal volume is occupied by mature gametes. Shell diameter could thus be important in assessing the reproductive status of this endangered pulmonate, since it minimizes damage to a vulnerable population.

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