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Annual maturation of the perivaginal gland of *Oxychilus* (*Drouetia*) *atlanticus* (Pulmonata: Zonitidae): morphological and cytological approaches

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The family Zonitidae has a reproductive system, which is characterized by the presence of a perivaginal gland that, generally, surrounds the vaginal duct.[1] The majority of works that make reference to this organ are taxonomic in nature, giving brief descriptions of the external shape of the gland and of its position in relation to other parts of the reproductive system.[2–6] Rigby examined the histological structure of the perivaginal gland for the first time, studying the reproductive system of the zonitid *Oxychilus cellarius* (Müller).[7] She reported that two types of glandular cells form this gland, without providing any structural or cytochemical characterization.

In the perivaginal gland of *Oxychilus atlanticus* (Morelet & Drouët), an endemic species from São Miguel Island, Azores, two types of glandular cells were identified, and were structurally and cytochemically characterized by Rodrigues *et al.*[1] One of these cells, PVGI, whose basal domain is distributed more externally in the gland mass, synthesizes glycogen and protein material, and could be implicated in the production of the outer layers of the eggshell. The other cell type, PVGII, occupies the internal part of the gland. Its secretions are rich in mucopolysaccharides and calcium, and it produces a viscous fluid that lubricates the vagina duct during copulation and egg-laying.[1]

According to Rodrigues *et al.*,[8] *O. atlanticus* has a reproductive cycle consisting of three phases: gametogenesis between January and June; copulation and fertilization between June and September; and egg-laying from September onwards. Species become mature between June and November, with a shell diameter of 7 mm.

The aim of this work is to understand how each cellular type relates to the different phases of the reproductive cycle by studying their relative volumetric density throughout the year and, thus, to better infer the physiological role of the perivaginal gland in the reproductive process. Samples were collected at Abelheira, a locality situated 3 km north of Ponta Delgada, São Miguel. Each month from September 1999 to September 2000, the 10 largest specimens observed were collected. The maximum diameter of the shell was measured and the vagina was dissected out under stereomicroscope.

In order to obtain an indicator of maturation, all the perivaginal glands were classified, under a microscope, according to a morphological maturation scale (MMS): (1) incipient, when the glandular body is not yet observed; (2) medium-developed, when the glandular mass is observed and is whitish in colour; (3) well-developed, when the glandular mass is conspicuous and has a brownish and sandy appearance.

For histology, vaginas were fixed in Bouin's solution[9] and embedded in paraffin wax. Serial sections, 7 μm thick, were stained with Mayer's haemalum and eosin.[10] The relative abundance of each cell type was estimated by stereology, using the M168 Weibel Multipurpose Test System.[11]

Pearson's correlations were determined between the following variables: maximum diameter of the shell, morphological maturation of the perivaginal gland and relative abundance of each glandular cell type.

Data for maximum diameter of the shell (Fig. 1) and morphological maturation of the perivaginal gland (Fig. 2) indicate that: specimens are immature from January/February to April/May; grow and mature from May to June; and are fully mature from June/July onwards. Furthermore, there is a high degree of correlation between these two variables (Table 1).

Our results confirm that the annual variation of the maximum diameter of the shell is a diagnostic characteristic of snail maturation.[8] They also show that snail maturity can be assessed by morphological maturation of the perivaginal gland, since it expresses the phases of the reproductive cycle of *O. atlanticus*, as reported by Rodrigues *et al.*[8]

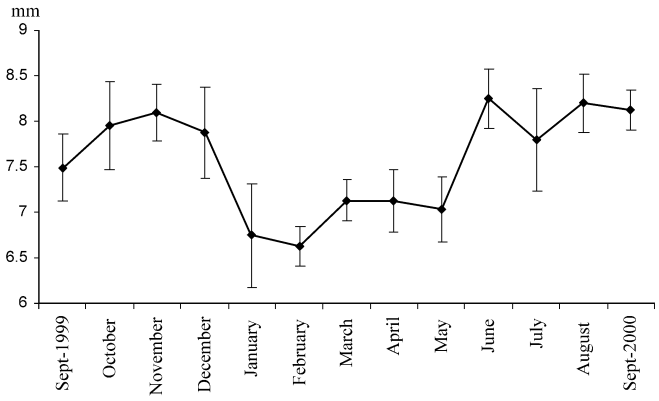

Figure 1. Maximum diameter of the shell of *Oxychilus atlanticus*, from September 1999 to September 2000 (mean values and standard errors).

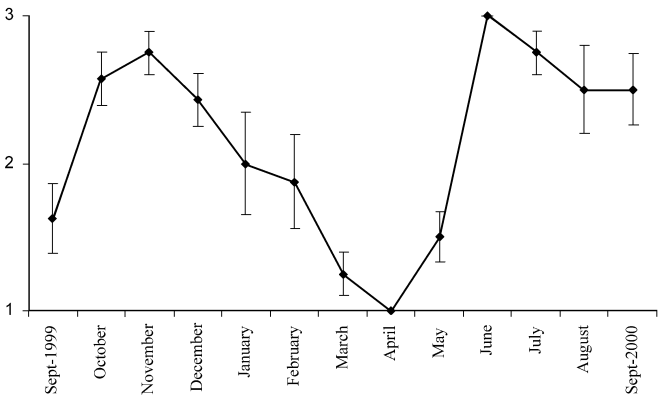

Figure 2. Morphological maturation of the perivaginal gland of *Oxychilus atlanticus*, according to MMS scale described in the text, from September 1999 to September 2000 (mean values and standard errors).

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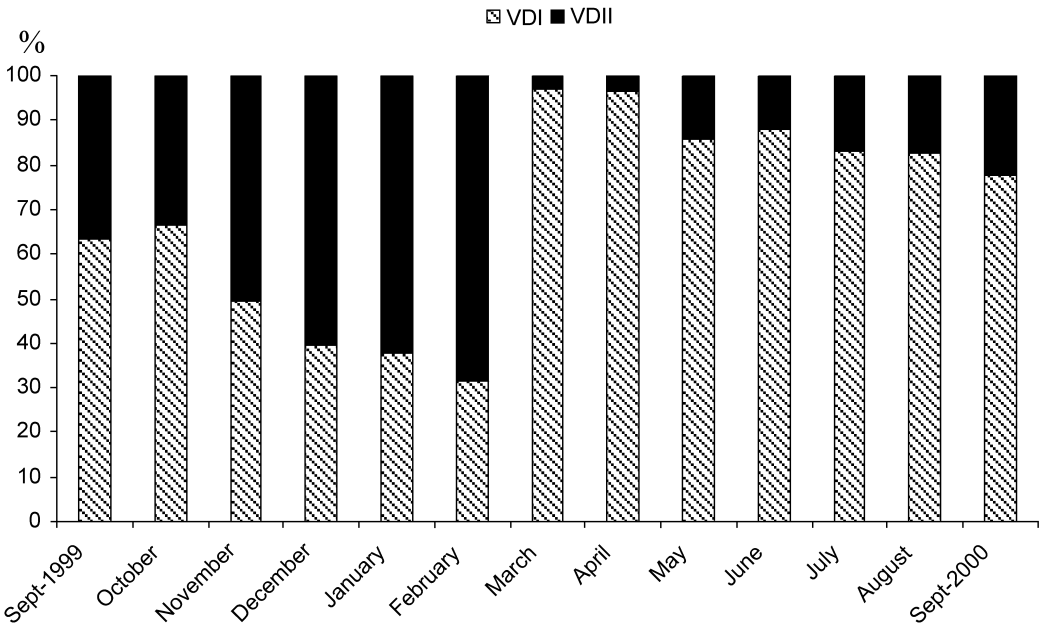

Figure 3. Relative volumetric density of PVGI (VD I) and PVGII (VD II) in *Oxychilus atlanticus*, from September 1999 to September 2000 (mean values).

Table 1. Pearson's correlation between the following variables: maximum diameter of the shell (MD); morphological maturation of the perivaginal gland (MMS); relative volumetric density of PVGI (VD I); relative volumetric density of PVGII (VD II).

	MD	MMS
MD	–	
MMS	0.546* ($n = 102$)	–
VD I	0.334* ($n = 98$)	0.292* ($n = 98$)
VD II	0.037 ($n = 98$)	0.400* ($n = 98$)

*Correlation is significant at $\alpha = 0.01$.

Concerning the relative abundance of the glandular cells, PVGI prevails from March to September, while PVGII increases gradually, reaching the higher values (ranging between 33 and 68%) from September/October to February. Although PVGII dominates (>50%) from November to February, PVGI is the dominant cellular type during the rest of the year (Fig. 3). PVGI is the first cellular type to arise, constituting almost the entire gland during March and April (Fig. 3), when glands are still incipient (Fig. 2).

Based on the maturation of the perivaginal gland, sexual maturity is reached from June onwards (Fig. 2), when the relative abundance of the PVGII cellular type is over 17% (Fig. 3). This is confirmed by a positive correlation between these variables (MMS and PVGII; Table 1). During the period, between June and December/January, *O. atlanticus* specimens copulate and lay eggs,[8] suggesting that the perivaginal gland is directly related to these processes. This relationship becomes more evident when we observe the predominance of PVGII during the egg-laying period, supporting the roles of this cellular type previously suggested by Rodrigues *et al.*:[1] a lubrication function through the synthesis of a viscous fluid rich in mucopolysaccharides, during copulation and egg-laying; and participation in the final phase of the eggshell building process.

On the other hand, Rodrigues *et al.*[1] reported that the PVGI cellular type produces glycogen and protein material. Therefore, the prevalence of this cellular type during May/June to September (the copulatory phase), together with the nature of its secretions,[1] suggests a possible role for the gland in the nourishment of allosperm. A relative abundance of PVGI over 30% during the egg-laying period could also suggest participation in eggshell formation, through the synthesis of cystine,[1] a role attributed to the free oviduct wall of *Deroceras reticulatum* (Müller).[12] According to Dasen,[13] in Ariophantidae, the secretion of a capsule around the eggs is provided by an oviducal gland, named the capsular gland. A stimulatory function has been suggested for the perivaginal gland,[14,15] but Hausdorf[16] has shown that in zonitids the stimulator is located in the penial complex and that the two structures are not homologous.

In summary, morphological development of the perivaginal gland of *O. atlanticus* throughout the year tracks the different phases of the reproductive cycle of the species, providing useful diagnostic characteristics of maturation stages. The perivaginal gland reaches maturity from June/July onwards, when copulation and egg-laying occur. Given the period when the gland is fully developed in terms of morphology and cytology, and the nature of the products secreted by the two glandular cell types, we assume that lubrication and eggshell production are the most important functions of this organ.

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