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Maturation diagnostic characters in *Oxychilus (Drouetia) atlanticus* (Morelet and Drouët, 1857) (Pulmonata: Zonitidae)

Caracteres identificativos del grado de madurez sexual en *Oxychilus (Drouetia) atlanticus* (Morelet and Drouët, 1857) (Pulmonata: Zonitidae)

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Recibido el 24-VI-1997. Aceptado el 4-XI-1997

ABSTRACT

Oxychilus (Drouetia) atlanticus (Pulmonata: Zonitidae) is an endemic hermaphroditic species from São Miguel island (Açores). Two populations were analysed along one year, in order to know its reproductive cycle and to test the validity of the seminal vesicle and the spermoviduct as maturation diagnostic organs. Concerning gonadal maturation, *O. atlanticus* has a reproductive cycle with three phases: an active gametogenesis occurs between January and May/June with the maturation of oocytes extending until September/October; copula and fertilization take place between June and September; and snails are prepared for egg-laying from September on. Spermoviduct and seminal vesicle undergo considerable morphological changes wich are closely related with gonadal maturation, spermatogenesis and oogenesis. These results suggest that the morphology of spermoviduct and seminal vesicle could be of major importance in the diagnosis of the maturation process.

RESUMEN

Oxychilus (Drouetia) atlanticus (Pulmonata: Zonitidae) es una especie endémica de la isla de São Miguel (Açores). Se han estudiado dos poblaciones diferentes a lo largo del mismo año con el fin de conocer su ciclo reproductor y analizar la validez de la vesicula seminalis y del espermoviducto como órganos identificativos del grado de madurez sexual. En el proceso de maduración gonadal de *Oxychilus atlanticus* pueden definirse tres fases: una activa gametogénesis que tiene lugar entre Enero y Mayo/Junio, con la maduración de los ovocitos prolongándose hasta Septiembre/Octubre; la cópula y fertilización tienen lugar entre Junio y Septiembre; a partir de Septiembre los caracoles están preparados para realizar la puesta. El espermoviducto y la vesicula seminalis sufren cambios morfológicos considerables, los cuales estan estrechamente relacionados con la maduración gonadal, la espermatogénesis y la ovogénesis. Estos resultados sugieren que la morfología del espermoviducto y la vesicula seminalis pueden ser utilizados como caracteres diagnósticos del proceso de maduración.

KEYWORDS: Pulmonata, *Oxychilus*, Maturation, Gametogenesis, Spermoviduct, Seminal Vesicle.
PALABRAS CLAVE: Pulmonata, *Oxychilus*, Maduración, Gametogénesis, Espermoviducto, Vesicula seminal.

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INTRODUCTION

Maturation of the reproductive tract has been the subject of several studies on land snails (LUSIS, 1961; SMITH, 1966, 1967; RUNHAM AND LARYEA, 1968; ELS, 1978; CUEZZO, 1990). In that respect, an important role has been assigned to the seminal vesicle, the middle portion of the hermaphroditic duct, and the spermoviduct in the storage of sperm and in egg formation, respectively (RIGBY, 1963; ELS, 1973; VISSER, 1977; TOMPA, 1984; ZUBIAGA, GOMEZ, MOYA AND ANGULO, 1989; ZUBIAGA, MOYA, GOMEZ AND ANGULO, 1990; HODGSON AND SHACHAK, 1991; HODGSON, 1992).

Some studies (RUNHAM AND LARYEA, 1968; APLEY, 1970; LUCHTEL, 1972; RUNHAM AND HOGG, 1979; CUEZZO, 1990, 1993) have tested the maturity condition of the species after histological observations of the gonad. However, LUSIS (1961) stated that, during the maturation process of *Arion* (*Arion*) *ater* (Linnaeus, 1758), the seminal vesicle becomes wider and more coiled, and the spermoviduct thicker and convoluted. CUEZZO (1990) found that, in *Neohelix major* (Binney, 1837), the coiling degree of the seminal vesicle and the folding of the spermoviduct, as well as the increase in diameter of these organs, are correlated with maturation in the reproductive tract.

According to LUSIS (1961), maturation of the seminal vesicle of *Arion ater* is more related to spermatogenesis than to oogenesis; maturation of the spermoviduct, on the other hand, is related to gonadal maturation as well as to the oviposition period when the oviducal part shows maximum activity. Studies in *Deroceras* (*Agriolimax*) *reticulatum* (Müller, 1774) confirmed also that morphological changes of the seminal vesicle and spermoviduct are closely related to gonadal development (RUNHAM AND LARYEA, 1968; RUNHAM, BAILEY AND LARYEA, 1973).

Maturation state of the reproductive system is assumed to be related to the growth of the animal (LUSIS, 1961, for *Arion ater*; RUNHAM AND LARYEA, 1968, for *Deroceras reticulatum*) although, SMITH (1966) refuted such relationship for *Arion ater*. CUEZZO (1993), however, confirmed that gonadal development depends on season, age, and size of the animal.

The difficulty in studying reproductive structures in land snails may be due to variations in size related to the change from reproductive to non-reproductive status; consequently, sampling in off-season may lead to misunderstanding about the morphology and function of the various reproductive structures (TOMPA, 1984).

Apart from the works of RIGBY (1963) on *Oxychilus cellarius* (Müller, 1774) and MORDAN (1977) on *Aegopinella nitidula* (Draparnaud, 1805), studies on the maturation of the reproductive system in zonitid species are scarce.

A detailed study of the reproductive cycle of *Oxychilus (Drouetia) atlanticus*, an endemic zonitid from São Miguel Island, Açores, was conducted, and an attempt was made in order to correlate the shape of genital organs with gonadal cycle and maturation. This alternative method will allow us, indirectly, to establish the maturity state of the species, in relation to other laborious methods, such as serial sections of the gonad.

MATERIAL AND METHODS

Samples were collected during 1993, at two sampling sites in São Miguel Island (Azores), Abelheira (AB) and Ramal dos Mosteiros (RM). Monthly, at each site, 15 of the largest specimens were selected for anatomical study.

Specimens used for anatomical and conchological studies were killed by drowning in water with menthol crystals, and then preserved in 70% ethanol.

The shell, seminal vesicle (sv) and spermoviduct (sov) were drawn using a Wild M3 Stereo Microscope with a camera lucida.

Maximum diameter (MD) and total height (TH) of the shell (Fig. 1) were measured.

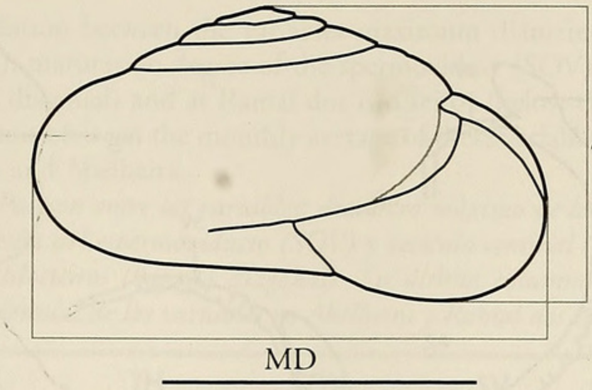

Figure 1. Measurements made on *Oxychilus atlanticus* shells. Maximum diameter (MD) and total height of the shell (TH). Scale bar 5 mm.
Figura 1. Medidas de la concha de Oxychilus atlanticus. *Diámetro máximo (MD) y altura total de la concha (TH). Escala 5 mm.*

The seminal vesicle and the spermoviduct were used to assess the degree of maturity. Three developmental stages of the seminal vesicle were established based on external morphology: 1) narrow and straight; 2) of intermediate thickness and slightly folded (coiled); 3) thick and heavily coiled (Fig. 2).

Four developmental stages were established for the spermoviduct: 1) narrow and smooth; 2) narrow and slightly circonvoluted; 3) of intermediate thickness and circonvoluted; 4) heavily circonvoluted and swollen, along its entire length (Fig. 2).

In order to determine the gonadal maturation state, three snails were collected every two months, measured, and the ovotestis fixed in 10% formalin and embedded in paraffin. Serial sections, 7μm 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).

Six stages of spermatogenesis were identified based on the classification of GRIFFOND, DADKHAN-TEHERAIN, MEDINA AND BRIDE (1991): 1) spermatogonia, sphaeroidal cell at light microscopy, small in size (8-10 μm in diameter) and with a large nucleus in relation to the small quantity of cytoplasm; 2) spermatocyte, larger than spermatogonia (11-17 μm in diameter) with a more abundant and eosinophilous cytoplasm; 3) early spermatids, small in size (8-10 μm in diameter) sphaeroidal in shape and with the nucleus sphaeroidal in shape, too, but showing frequently basophilous condensations at its poles; 4) late spermatids, the whole cell stretches and elongates showing small tails; 5) viable spermatozoa, with strong basophilous head and long eosinophilous tail, and 6) unviable spermatozoa with eosinophilous head and tail. At light microscopy, no differentiation has been made between spermatocytes I and II, neither between early and mid spermatids.

According to HILL AND BOWEN (1976) three stages of development have been distinguished during oogenesis: 1) previtellogenic oocytes, small, rounded and with strong basophilous cytoplasm (< 1000 μm^3 in volume); 2) vitellogenic oocytes, larger than the previous ones, more flattened and light basophilous (around 15000 μm^3 in volume) and 3) maturing oocytes, rounded and larger than the vitellogenic oocytes and presenting an eosinophilous and granular cytoplasm (> 20000 μm^3 in volume). This last stage also includes the fully mature oocytes.

Scores for volumetric density were summed for each specimen and converted to percentages in order to identify the gonadal maturation state.

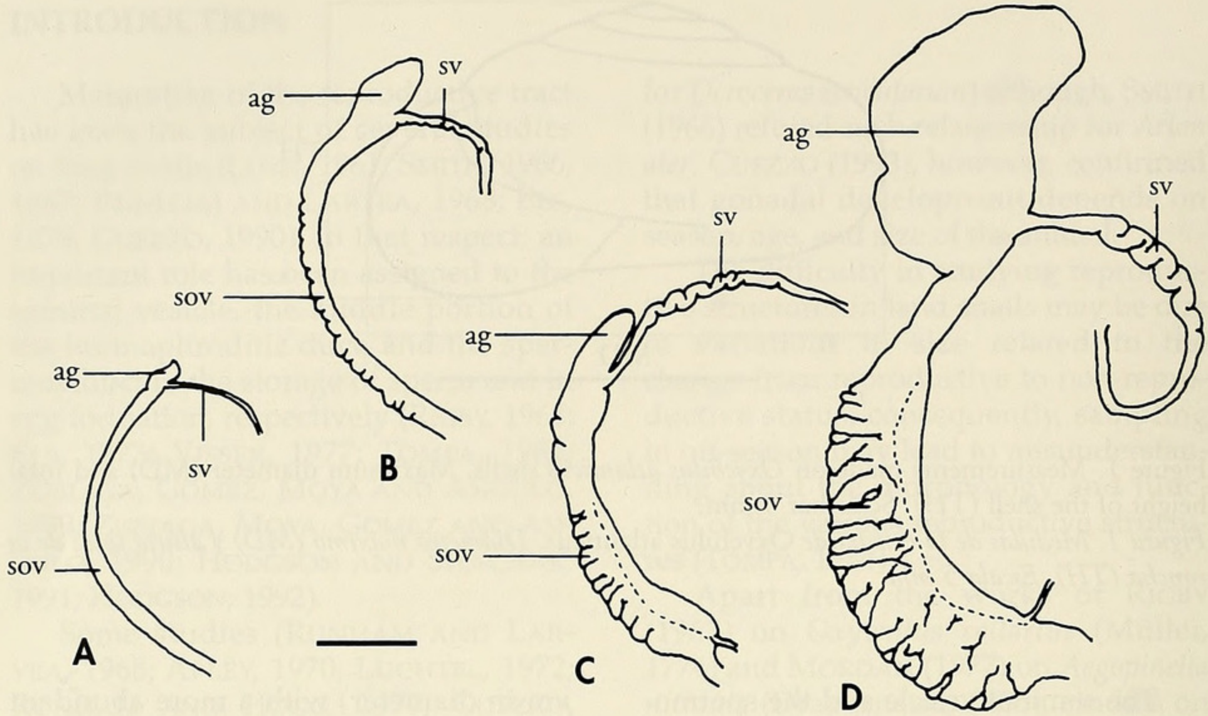

Figure 2. Maturation degrees of the spermoviduct (sov) and seminal vesicle (sv). A: sov degree 1, sv degree 1; B: sov degree 2, sv degree 2; C: sov degree 3, sv degree 3; D: sov degree 4, sv degree 3; ag: albumen gland. Scale bar 1 mm.

Figura 2. Grados de maduración del espermoviducto (sov) y la vesícula seminal (sv). A: sov grado 1, sv grado 1; B: sov grado 2, sv grado 2; C: sov grado 3, sv grado 3; D: sov grado 4, sv grado 3; ag: glándula del albumen. Escala 1 mm.

Data were analized with program Exstatix 1.0.1 - Mac (KILLION, 1988) used to calculte average, standard deviation, median and Pearson's correlation, between maximum diameter and total height of the shell, degree of maturation of the spermoviduct and seminal vesicle.

RESULTS

In both populations, the conchological and genital variables are strongly correlated, with special emphasis on the maximum diameter of the shell, and maturation stage of the spermoviduct and the seminal vesicle; these parameters shows equally strong correlation interpopulationally (Table I).

Between January and March the size (maximum diameter and total height of the shell) and degree of maturation (spermoviduct and seminal vesicle) were less conspicuous and reached lowest values in March; size and degree of maturation increased between March and June/July, stabilized thereafter until November/December (Fig. 3).

Both populations reached the highest degree of maturation in July; irrespective of the fact that the Abelheira population showed a smaller shell size.

The stereological study shows that from January to May the previtellogenic and vitellogenic oocytes present a higher relative volumetric density than the maturing oocytes. Such density of the maturing oocytes greatly increases since July, occupying around 20% of the gonadic volume in October and November. One isolated individual from the January sampling presented maturing oocytes that occupied more than 60% of the gonadic volume (Fig. 4).

The relative volumetric density of the spermatogonia was dominant in January, decreasing after this month to reach its lowest value in May. The highest volumetric density of the sper-

Table I. Pearson's correlation between the variables maximum diameter of the shell (MD), total height of the shell (TH), maturation degree of the spermoviduct (SOV) and seminal vesicle (SV), at Abelheira (above the diagonal) and at Ramal dos Mosteiros (below the diagonal). Last column shows Pearson's correlation between the monthly average of these variables between the populations of Ramal dos Mosteiros and Abelheira.

Tabla I. Correlación de Pearson entre las variables: diámetro máximo de la concha (MD), altura total (TH), grado de maduración del espermoviducto (SOV) y vesícula seminal (SV), en Abelheira (sobre la diagonal) y Ramal dos Mosteiros (bajo la diagonal). La última columna muestra la correlación de Pearson entre la media mensual de las variables en Abelheira y Ramal dos Mosteiros.

	MD	TH	SOV	SV	Correlation
MD	-	0.95	0.86	0.81	0.939
TH	0.87	-	0.82	0.77	0.937
SOV	0.71	0.60	-	0.83	0.914
SV	0.70	0.60	0.87	-	0.964

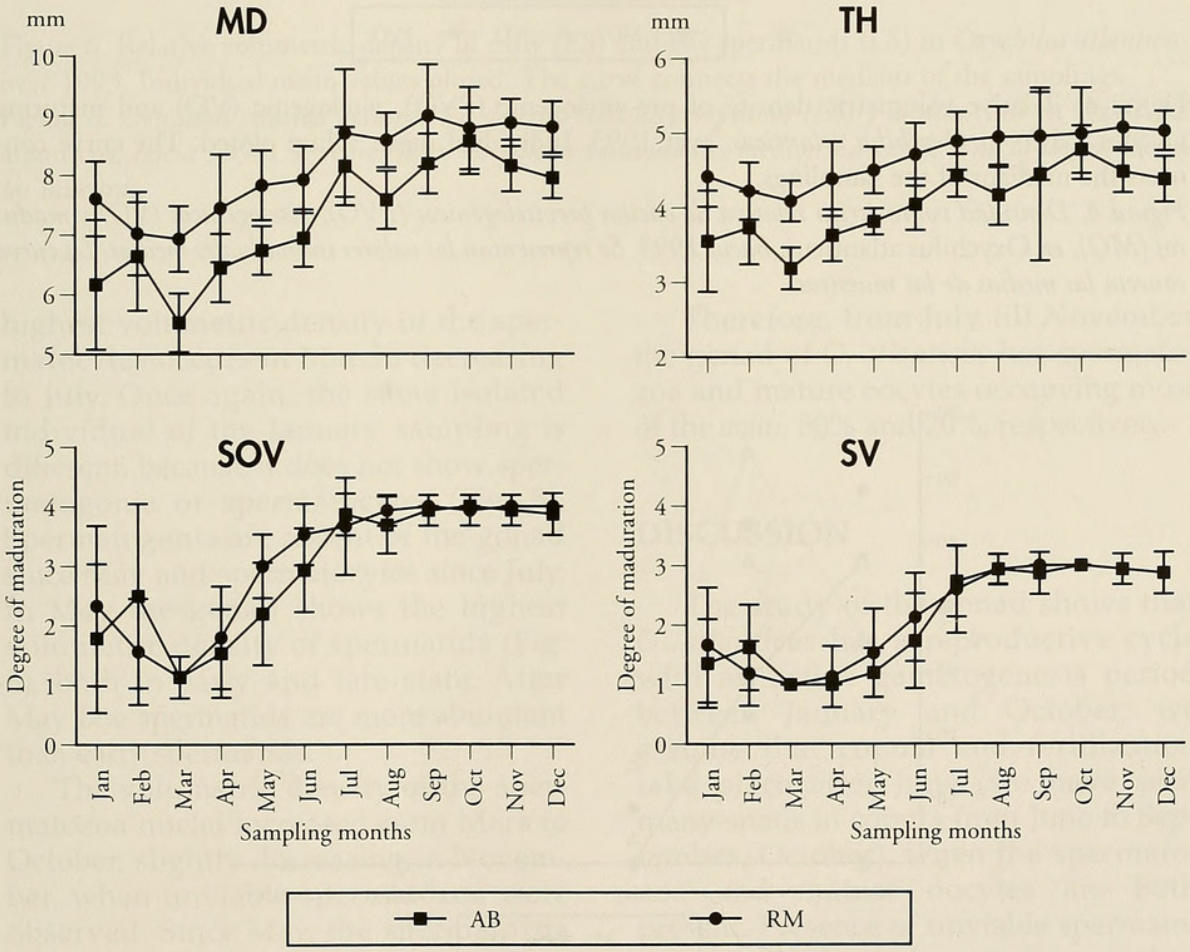

Figure 3. Graphic representation of mean value and standard deviation of maximum diameter of the shell (MD), total height of the shell (TH), spermoviduct (SOV) and seminal vesicle (SV), over 1993, at Abelheira (AB) and Ramal dos Mosteiros (RM).

Figura 3. Representación gráfica del valor medio y la desviación estandar del diámetro máximo de la concha (MD), altura total de la concha (TH), espermoviducto (SOV) y vesícula seminal (SV), hacia 1993, en Abelheira (AB) y Ramal dos Mosteiros (RM).

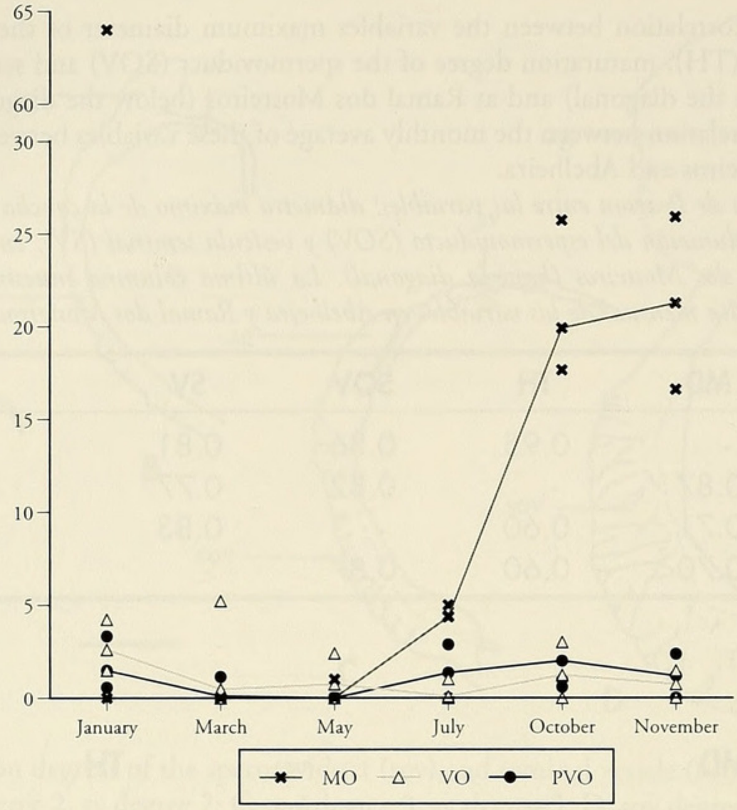

Figure 4. Relative volumetric density of pre-vitelogenic (PVO), vitelogenic (VO) and maturing oocytes (MO) in *Oxychilus atlanticus*, over 1993. Individual mean values ploted. The curve connects the medians of the samplings.

Figura 4. Densidad volumétrica relativa de oocitos previtelogénicos (PVO), vitelogénicos (VO) y maduros (MO), en Oxychilus atlanticus, *hacia 1993. Se representan los valores individuales medios. La curva conecta las medias de las muestras.*

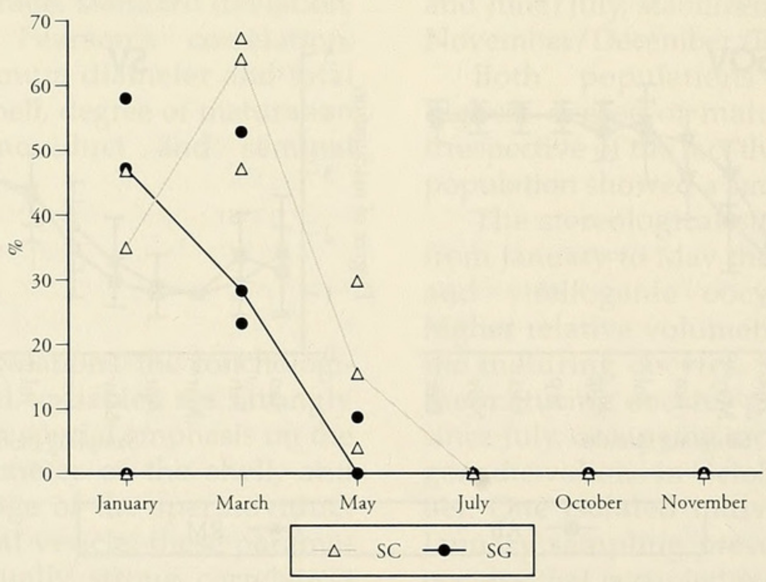

Figure 5. Relative volumetric density of spermatogonia (SG) and spermatocytes (SC) in *Oxychilus atlanticus*, over 1993. Individual mean values ploted. The curve connects the medians of the samplings.

Figura 5. Densidad relativa volumétrica de espermatogonias (SG) y espermatocitos (SC) en Oxychilus atlanticus, *hacia 1993. Se representan los valores individuales medios. La curva conecta las medias de las muestras.*

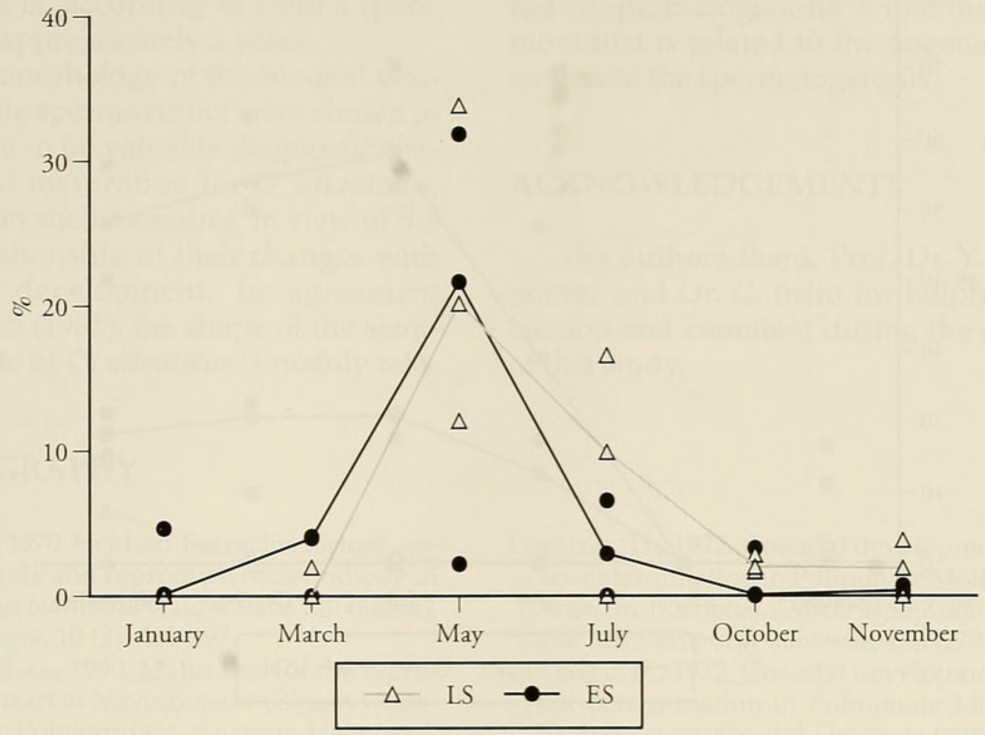

Figure 6. Relative volumetric density of early (ES) and late spermatids (LS) in *Oxychilus atlanticus*, over 1993. Individual mean values ploted. The curve connects the medians of the samplings.
Figura 6. Densidad relativa volumétrica de espermátidas tempranas (ES) y tardías (LS) en Oxychilus atlanticus, *hacia 1993. Se representan los valores individuales medios. La curva conecta las medias de las muestras.*

highest volumetric density of the spermatocytes occurs in March, decreasing to July. Once again, the same isolated individual of the January sampling is different, because it does not show spermatogonia or spermatocytes (Fig. 5). Spermatogonia are absent of the gonad since May and spermatocytes since July. In May the gonad shows the highest volumetric density of spermatids (Fig. 6), both in early and late state. After May late spermatids are more abundant than early spermatids.

The volumetric density of the spermatozoa nuclei increased from Mars to October, slightly decreasing in November, when unviable spermatozoa were observed. Since May, the spermatozoa tails occupy most of the *acini*. Once again the same individual from January had already 20% of the gonadal volume occupied by spermatozoa nuclei (Fig. 7). The whole spermatozoa, head and tail, nearly occupy 80% of the gonad, from July to October.

Therefore, from July till November the gonad of *O. atlanticus* has spermatozoa and mature oocytes occupying most of the *acini*, 80% and 20%, respectively.

DISCUSSION

The study of the gonad shows that *O. atlanticus* has a reproductive cycle with an active gametogenesis period between January and October; we assume that copula and fertilization take place after June (we have seen many snails in copula from June to September/October), when the spermatozoa and mature oocytes are both present. Presence of unviable spermatozoa after November may reveal a gonadal degenerating period (RODRIGUES, 1995).

The morphology of the seminal vesicle and spermoviduct clearly show that in both studied populations, *O. atlanticus* specimens are immature

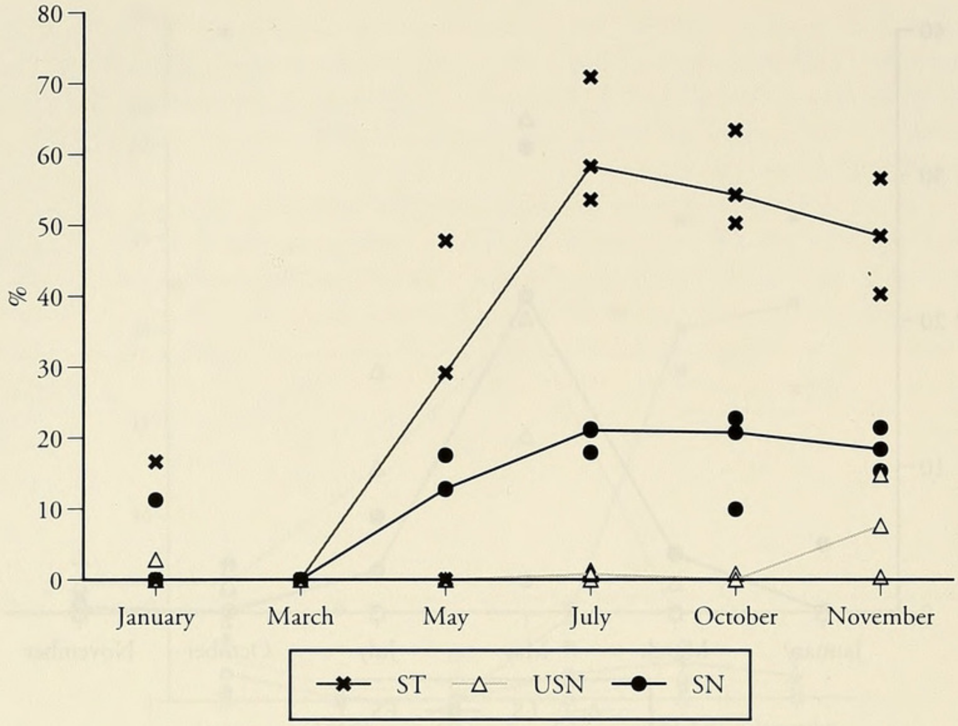

Figure 7. Relative volumetric density of the viable spermatozoa nuclei (SN), spermatozoa tails (ST) and unviable spermatozoa nuclei (USN) in *Oxychilus atlanticus*, over 1993. Individual mean values ploted. The curve connects the medians of the samplings.
Figura 7. Densidad relativa volumétrica de nucleos de espermatozoides viables (SN), colas de espermatozoides (ST) y nucleos de espermatozoides inviables (USN) en Oxychilus atlanticus, *hacia 1993. Se representan los valores individuales medios. La curva conecta las medias de las muestras.*

between January and March/April. The first mature specimens are formed in April, while the highest percentage of mature snails occurs in July, remaining nearly constant until November/December.

The coexistence of mature and immature specimens in January and February, revealed by the morphology of seminal vesicle and spermoviduct, was also observed at the gonadic level. This may indicate that a gonadic degenerating period, referred below, could be extended until January/February in some specimens of the same population. Morphological changes of seminal vesicle and spermoviduct in *O. atlanticus* are closely related to gonadic cycle; the same relationship was observed by RUNHAM *ET AL.* (1973) in *Deroceras reticulatum*. According to Lusis (1961) anatomical and physiological changes of the seminal vesicle of *Arion ater* are more related to the spermatogenesis process than to oogenesis. The same author states that spermoviduct maturation is related not only with gonadic maturation but also with the oviposition period, when the oviducal portion shows its maximum size.

Shell parameters are primarily related with snail growth and secondly with the maturation process. CUEZZO (1993) stated that gonadic development depends not only on the season but also on the size of the animal. In disagreement with SMITH (1966), LUSIS (1961) and RUNHAM AND LARYEA (1968) showed that maturation degrees of the reproductive system are related to the growth phases of the animal. CUEZZO (1990) for *Neohelix major* pointed out that there is a relationship between shell diameter and the degree of maturity reached by the reproductive tract. The high correlation between shell parameters and genital maturity could be supported by the fact that the life cycle of *O.*

atlanticus is, according to Cunha (pers. comm.), approximately 2 years.

The morphology of the seminal vesicle and the spermoviduct were shown in this study to be valuable diagnostic characters of maturation for *O. atlanticus*, better than shell measures, in view of the close relationship of their changes with gonadic development. In agreement with LUSIS (1961), the shape of the seminal vesicle of *O. atlanticus* is mainly related to spermatogenesis, while the spermoviduct is related to the oogenesis, as well as to the spermatogenesis.

ACKNOWLEDGEMENTS

The authors thank Prof. Dr. Y. Marigómez and Dr. C. Brito for helpful discussion and comment during the course of this study.

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