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Reproductive cycle of the rissoid *Alvania mediolittoralis* Gofas, 1989 (Mollusca, Gastropoda) at São Miguel Island (Azores, Portugal)

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Summary

The reproductive cycle of an *Alvania* species is studied for first time. *Alvania mediolittoralis* Gofas, 1989 is an endemic Azorean rissoid very common in sheltered places and particularly abundant among the algal turf covering the lower half of the intertidal and upper subtidal rocky shores. This species reproduces throughout the year, with two spawning peaks, one during early spring and the other in late autumn. In an attempt to relate to current paleobiogeographical studies, inferences are made regarding the ecological advantages of species with a continuous type of reproduction, on Azorean shores, during glacial episodes.

Key words: *Alvania*, Rissoidae, reproductive cycle, gametogenesis, Pleistocene

Introduction

The Rissoidae are a family of small caenogastropod molluscs with a worldwide geographical distribution. Shells are usually <5 mm long sometimes with elaborate sculpturing on both the protoconch and teleoconch (Ávila, 2005). Rissoidae are dioecious. Males have a simple penis, located behind the right eye, females are monaulic. The majority of species are marine, although some occur in brackish waters, and they are present in the fossil record from the Upper Jurassic of Europe (Chenu, 1859; Ponder, 1985, 1988).

Rissoidae are a principal components of the littoral malacofauna in the Mediterranean and Atlantic shores of Europe, the Madeira archipelago (Watson, 1873), the Canary Islands (Moolenbeek and Faber, 1987a, 1987b, 1987c; Moolenbeek and Hoenselaar, 1989, 1998) and also along west African shores (Gofas, 1999). They are the most represented family in the Azores Islands (Gofas, 1990; Ávila, 2000b; Ávila, 2005), with 24 species belonging to nine genera (*Alvania*, *Botryphallus*, *Cingula*, *Crisilla*, *Manzonia*, *Pusillina*, *Onoba*, *Rissoa* and *Setia*) (Moolenbeek and Faber, 1987b; Gofas, 1990; Knudsen, 1995; Hoenselaar and Goud, 1998; Ávila, 2000a; Ávila, 2005).

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The Azorean rissoids are one of the best studied mollusc families in the region, from MacAndrew (1856) and Drouët (1858) to date. Ávila (2005) summarised all the published information about this family in the archipelago of the Azores, including ecological, taxonomical, palaeontological, and geographical distribution data on all the 24 shallow water species.

Alvania mediolittoralis Gofas, 1989 is a small mollusc (usually with a shell about 2.7 mm long and 1.5 mm wide), with non-planktotrophic larval development, which inhabits the intertidal and upper subtidal algal covered rocky shores (Martins, 2004; Ávila, 2005). It is common and may be present in large numbers in sheltered places, especially under boulders (Ávila, 2000b). It is especially abundant in the lower half of the intertidal zone, among the intertidal algal turf (Ávila et al., 2005) reaching a maximum density of 54,000 individuals/m^2 in the Ilhéu de Vila Franca, São Miguel Island (Bullock, 1995). It is commonly associated with other microgastropods such as *Omalogyra atomus* (Philippi, 1841), *Pisinna glabrata* (Megerle von Mühlfeld, 1824) and *Skeneopsis planorbis* (Fabricius O., 1780), and the bivalve *Lasaea adansoni* (Gmelin, 1791) (Ávila et al., 2005).

Although reported from Madeira by Hoenselaar and Goud (1998) (CANCAP expeditions, Sta.1.D48, 0-22 m/1 shell; Sta.1.K14, in the littoral/1 and Sta. 1.K16, in the littoral/2), we think these reports describe specimens that reached the Madeira archipelago but were not able to establish a viable reproductive population. Recent collecting at Madeira and Porto Santo by Ávila (unpublished data) at appropriate intertidal and sublittoral habitats where *A. mediolittoralis* should occur did not yield a single specimen, thus we believe this species to be endemic to the Azores archipelago. It was reported for the Pleistocene of the Azores (Prainha and Lagoinhas, Santa Maria Island) by Ávila et al. (2002), in outcrops dated from the Marine Oxygen Isotope Substage 5e (MISS 5e) high sea level interval (about 130–120 ka) (Ávila et al., 2008a). Dispersal events (successful or not) are usually rare events. From all the endemic Azorean rissoids, *A. mediolittoralis* is the only species of which shells are reported from sites other than the Azores archipelago (in the present case, the Madeira archipelago, about 900 km apart from the Azores). This provides indirect evidence that speciation occurred long ago, thus surviving to several glacial-interglacial cycles, and making it a particularly interesting species for study.

Materials and Methods

Study area

Caloura (Lat 37º42′30″N, Long 24º30′30″W) is located on the southern shores of São Miguel Island (Azores) (Fig. 1). According to Morton et al. (1998), this biologically diverse site (in both species number and density) is representative of the rocky shore environment in the Azores and has been studied extensively (Hawkins et al., 1990; Costa, 1994; Britton, 1995; Botelho and Costa, 2000). It is particularly suitable for the collection of intertidal species, due to its large and shallow rocky platform, which has a gentle slope towards the sea. The bottom of this relatively protected bay is covered by small boulders, which rest on the underlying lava flow. Most of the boulders possess a dense algal mat that makes the perfect habitat for micro-molluscs such as *Alvania mediolittoralis*.

Sampling and morphometry

During low tide, several rocks were collected, and the upper and lower surfaces brushed inside a plastic container filled with seawater. The residue was then sorted for living individuals. Water temperature was recorded on each sampling date. The largest twenty individuals of *A. mediolittoralis* were sampled once per month, from February 2003 to January 2004. In the laboratory, quantitative measurements of maximum shell length (SL) and shell width (SW) were recorded using a Wild M3 Stereo Microscope with a camera lucida. The length/width ratio was also calculated (SL/SW).

Gonadal maturation state

After measurement, specimens were fixed in Bouin's solution in seawater for 15 h, dehydrated in ethanol and embedded in paraffin wax. Finally, 7 µm thick serial transversal sections cut with a microtome were stained with Mayer's haemalum and eosin (Martoja and Martoja-Pierson, 1970). Observations were made on the histological sections of the gonads of the specimens collected in March, May, July, September and November 2003, and January 2004, and the relative volumetric density of gametes estimated according to the M168 Weibel Multipurpose Test System (Weibel, 1979).

Three stages of development were distinguished during oogenesis (Hill and Bowen, 1976; Rodrigues and Medeiros, 2005; Cúrdia et al., 2005) (Fig. 2): (1) previtellogenic oocytes (PV)—small, rounded cells, with strong basophilic cytoplasm; (2) vitellogenic oocytes (V)—larger than the previous stage, irregular in shape, sometimes with multiple visible nucleoles, and lightly basophilic cytoplasm with slight granulations; (3) maturing oocytes (M)—larger than the vitellogenic oocytes, round in shape with eosinophilic and granular cytoplasm.

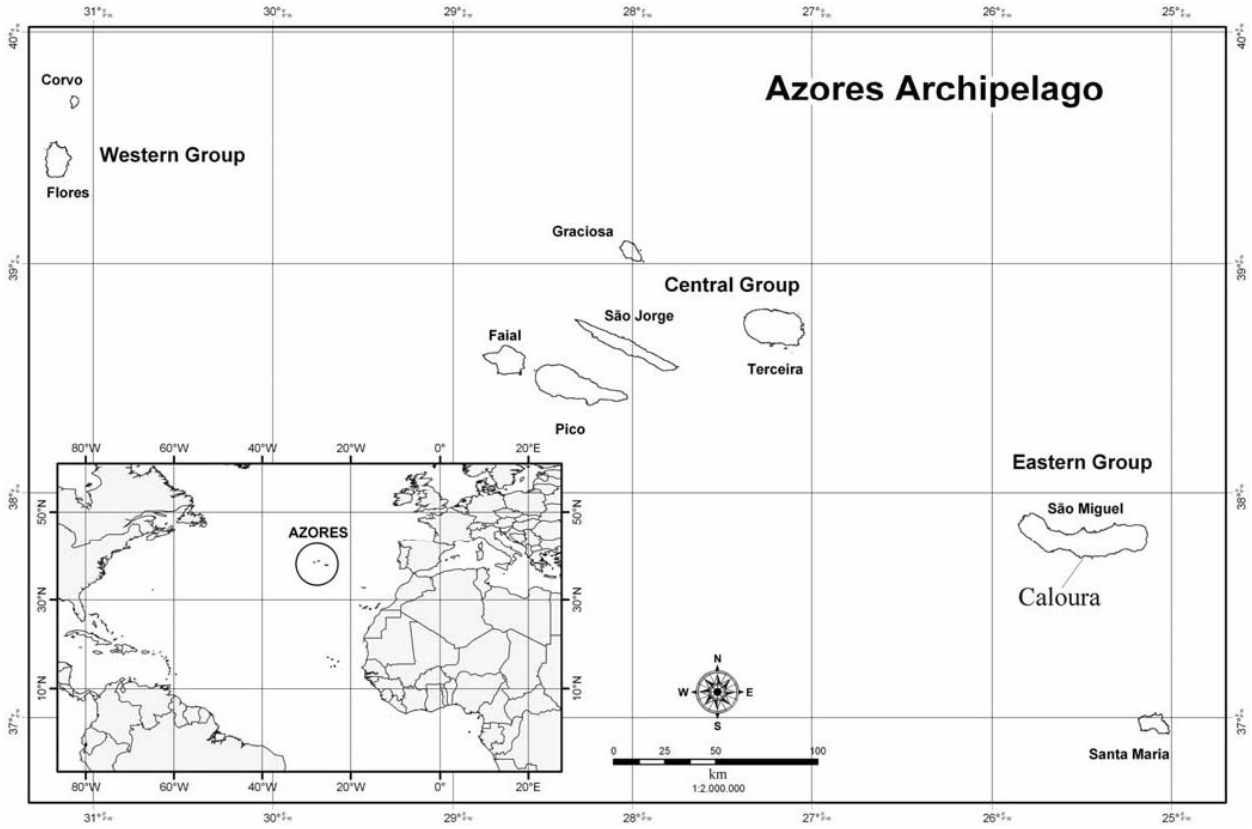

Fig. 1. Azores archipelago and São Miguel Island, with location of the sampling site, Caloura.

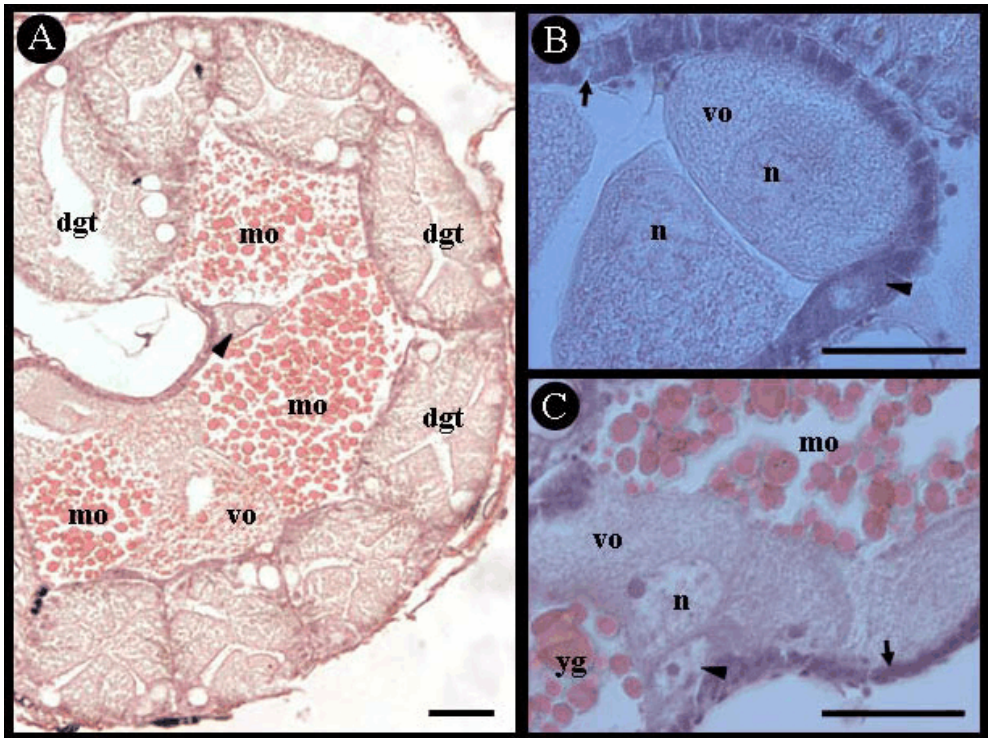

Fig. 2. Histological section of a *Alvania mediolittoralis* female gonad. A. General view; B, C. Highlight of the oogenic stages. Arrows, germinal layer; arrowheads, previtellogenic oocytes; dgt, digestive tubule; n, nucleus; mo, maturing oocytes; vo, vitellogenic oocytes; yg, yolk granules. Scale bars: 50 µm.

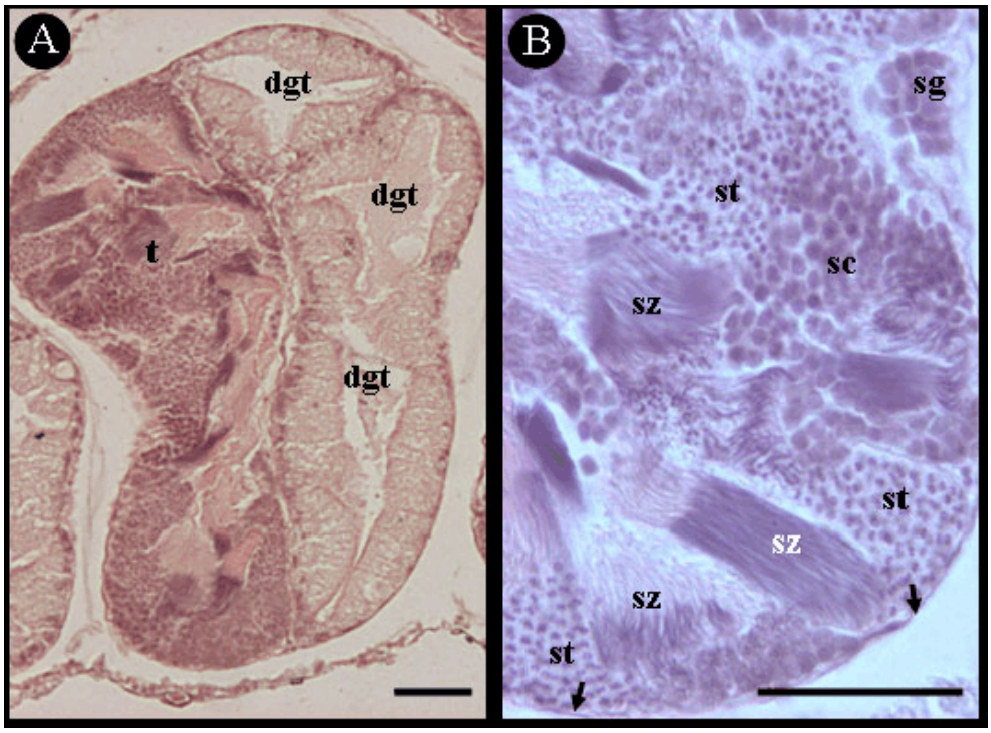

Fig. 3. Histological section of a *Alvania mediolittoralis* male gonad. A. General view. B. Highlight of the spermatogenic stages. Arrows, germinal layer; dgt, digestive tubule; sc, spermatocytes; sg, spermatogonia; st, spermatides; sz, spermatozoa; t, testis. Scale bars: 50 µm.

Four stages of spermatogenesis were identified (Griffond et al., 1991; Rodrigues and Medeiros, 2005; Cúrdia et al., 2005) (Fig. 3): (1) spermatogonia — medium-sized cells, with a large nucleus in relation to the quantity of cytoplasm, always located near the acinar epithelium; (2) spermatocytes — larger than spermatogonia with basophilic cytoplasm; (3) spermatids — smaller than spermatocytes, spheroid in shape and slightly more basophilic than spermatocytes; (4) spermatozoa — with strong basophilic head and esosinophilic tail. Under light microscopy, no differentiation was made between spermatocytes I and II.

Statistical analysis

Scores for volumetric density were summed for each specimen and converted to percentages, in order to identify the gonadal maturation state. Data were analyzed with the statistical package PRIMER (version 6β) (Plymouth Routines in Multivariate Ecological Research, Plymouth Marine Laboratory) and with MINITAB Release 11 statistical package. The mean and standard error of each gonadal maturation state were calculated. Relative volumetric density of each oogenesis and spermatogenesis stage was compared using a one-way ANOVA. Multivariate analyses were undertaken to assess for seasonal patterns. Data were transformed into a lower triangular resemblance matrix using the Euclidean distance similarity index, and a dendrogram was constructed with the UPGMA method. A total of 117 specimens of *A. mediolittoralis* were analysed (71 females and 46 males).

The mean gonad index (MGI) (Brown, 1982) was also calculated for each sampling date according the formula:

$$MGI = \{\Sigma[1\,(\text{no. ind. Stage 1}) + 2\,(\text{no. ind. Stage 2}) + \ldots + n\,(\text{no. ind. Stage } n)]\} \,/\, (\text{total number of animals in the considered sampling period})$$

This index is associated with the reproductive condition of the population at a particular time. A decreasing index is associated with spawning, whereas an increasing index in successive samples indicates that the gonad is developing.

Results

Oogenesis

The relative volumetric densities of previtellogenic oocytes ranged between 10% in November 2003 and about 17% in January 2004. The percentage volume of vitellogenic cells showed a statistically significant

Table 1. One-way ANOVA results for each gametogenic stage

Source of variation	*df*	SS	MS	*F*	*p*	
Previttellogenic oocytes	5	211.1588	42.2318	1.7088	0.1451	NS
Vitellogenic oocytes	5	1447.1708	289.4342	2.8016	0.0237	*
Maturing oocytes	5	1158.7307	231.7461	1.4177	0.2297	NS
Spermatogonia	5	19.0708	3.8142	2.0476	0.0925	NS
Spermatocytes	5	198.2827	39.6565	0.8045	0.5533	NS
Spermatids	5	226.4310	45.2862	3.3322	0.0131	*
Spermatozoa	5	240.7188	48.1438	0.6612	0.6549	NS

NS, not significant. *Significant at $p < 0.05$.

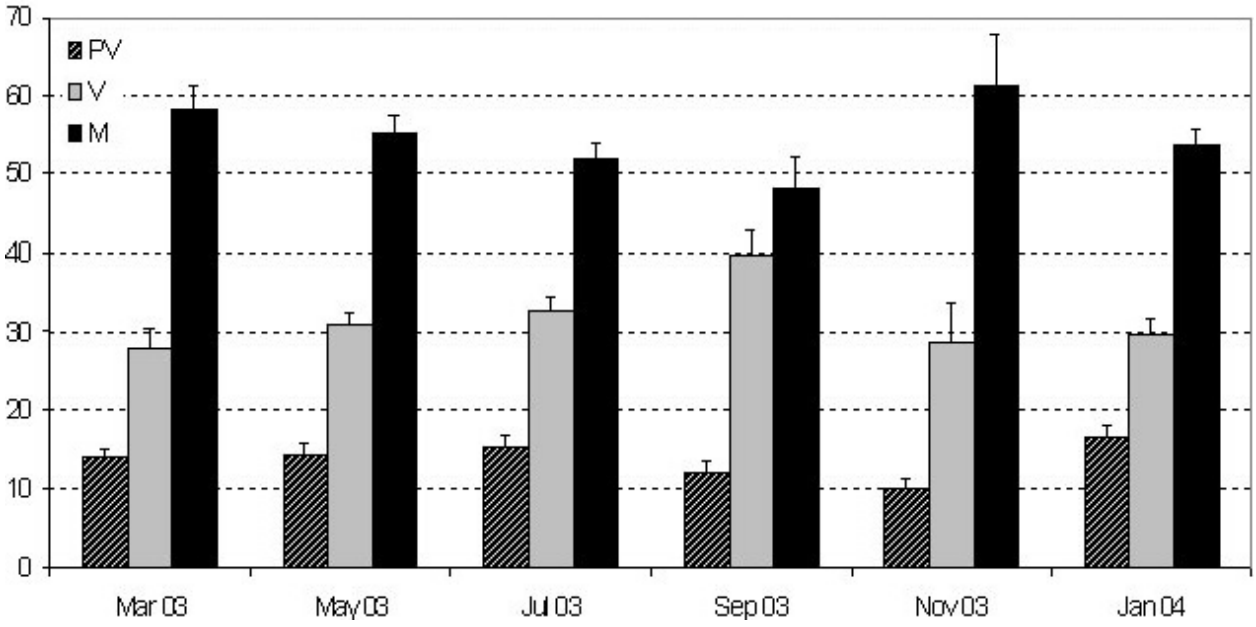

Fig. 4. Mean values and standard errors of the previtellogenic (PV), vitellogenic (V) and maturing oocytes (M) for females of *Alvania mediolittoralis*.

difference between sampling periods (ANOVA, $p<0.05$, Table 1). The values of this parameter were higher in September 2003 (40%) and about 30% during the rest of the sampling period. It was also in September that the maturing oocytes reached the minimum value (48%), but most of gonadal volume throughout the entire sampling period was occupied by maturing oocytes. Maximum values were found in March (58%) and November 2003 (61%) (Fig. 4). Previtellogenic and maturing oocytes did not show statistically significant differences between sampling periods (Table 1).

Cluster analysis does not show a seasonal trend, with specimens of different sampling periods mixing in all 3 main groups (not shown).

Spermatogenesis

The percentage volume of spermatogonia was always very low, ranging from about 3% in November 2003 to about 5% in both May and July 2003. Spermatocytes have a fairly constant value of about 34 to 36% in all the 2003 samples, decreasing to about 31% in January 2004. Spermatids show a statistically significant difference between sampling periods (ANOVA, $p<0.05$, Table 1), with a maximum value of about 20% in July and November and a minimum of 13% in May. Mean spermatozoa percentage volumes were consistently higher than 40% during the whole sampling period. The lower values were about 42% in May, July and November 2003, and the higher values were in January 2004 (49%) (Fig. 5). Spermatogonia, spermatocytes and spermatozoa did not show statistically significant differences between sampling periods (Table 1).

Cluster analysis does not show a seasonal trend, with specimens of different sampling periods mixed in all three main groups (not shown).

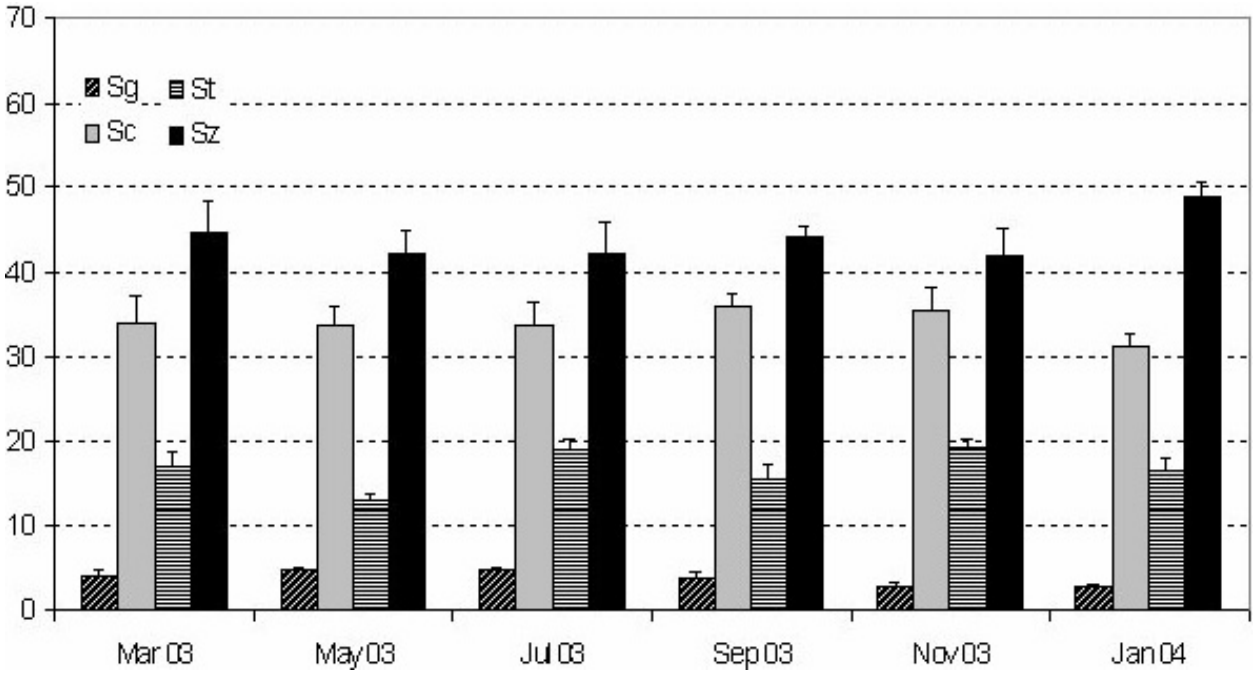

Fig. 5. Mean values and standard errors of the spermatogonia (Sg), spermatocyte (Sc), spermatid (St) and spermatozoa (Sz) for males of *Alvania mediolittoralis*.

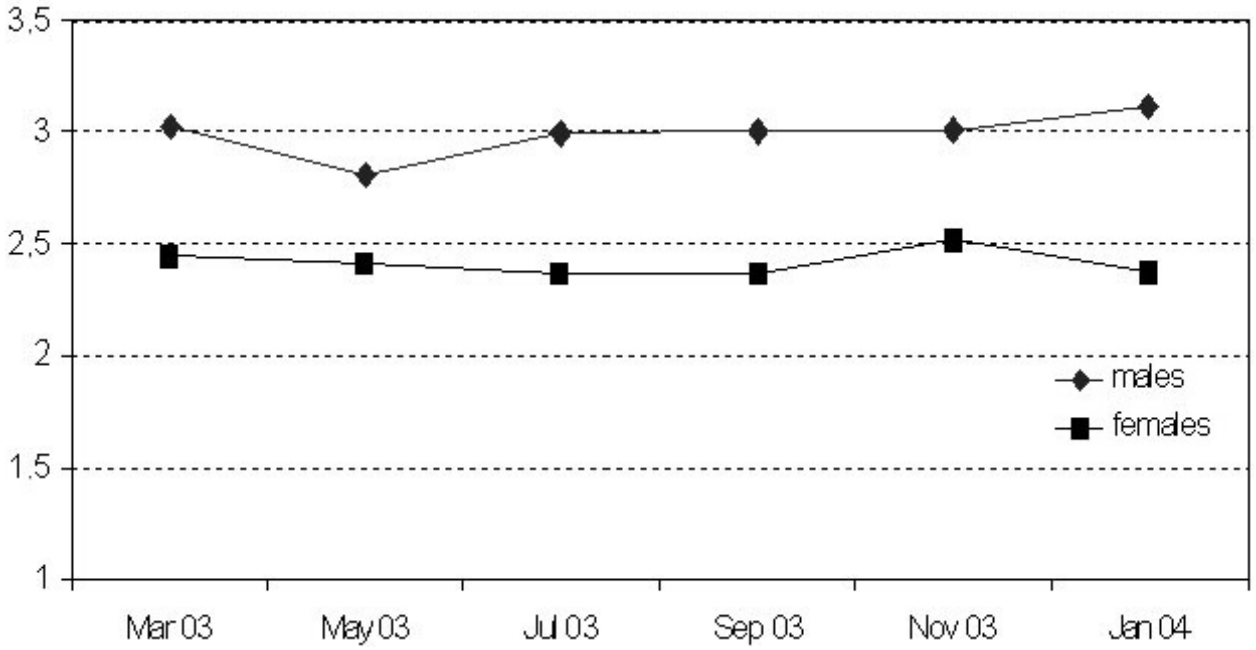

Fig. 6. Mean Gonad Index for the males and females of *Alvania mediolittoralis*.

Mean Gonad Index

The mean gonad index variation over the sampling period is shown in Fig. 6. No seasonal trend is apparent, as both males and females show a very constant value for this index, about 3.0 and 2.5, respectively for males and females.

Morphometric parameters

Females were consistently larger then males both in shell length and width, a difference statistically significant for shell length (ANOVA, $p = 0.00550$) and shell width (ANOVA, $p = 0.00018$) (Figs. 7 and 8).

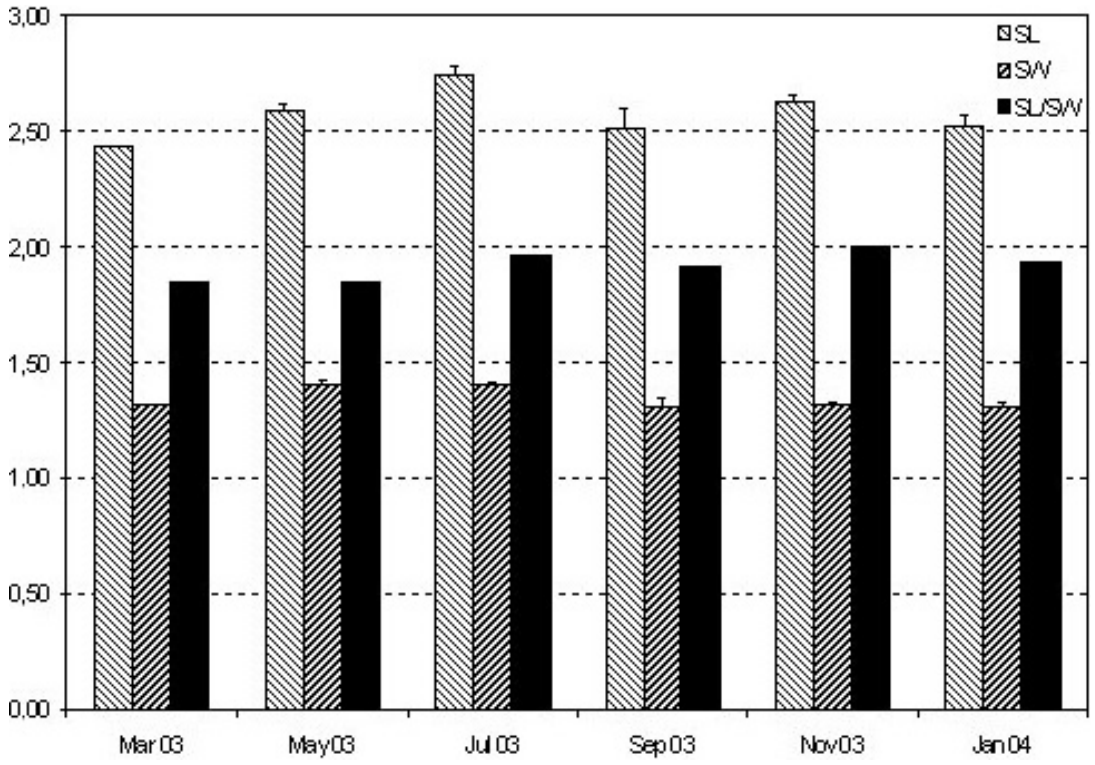

Fig. 7. Mean values and standard error (in mm) for the shell length (SL), shell width (SW) and SL/ SW ratio of the females of *Alvania mediolittoralis*.

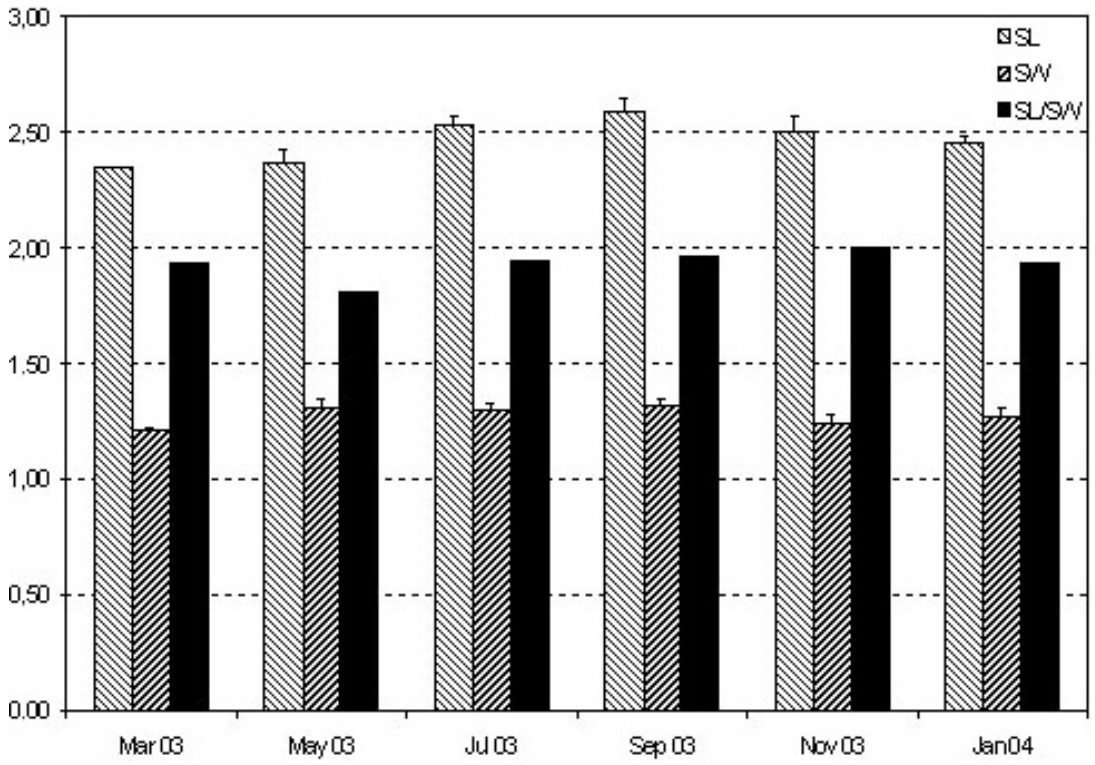

Fig. 8. Mean values and standard error (in mm) for the shell length (SL), shell width (SW) and SL/ SW ratio of the males of *Alvania mediolittoralis*.

Discussion

The endemic Azorean Rissoidae *A. mediolittoralis* reproduces throughout the year with maturing oocytes and spermatozoa occupying more than 50% and 40%, of the total ovary and testis volume, respectively. However, the relative volume occupied by vitellogenic oocytes increased significantly in September 2003 to about 40%, decreasing to less than 30% in November 2003, when maximum percentage of maturing oocytes was registered, thus suggesting that the production of new oocytes may occur during autumn, and that females of *A. mediolittoralis* may preferably spawn in the autumn, but also during early spring (Fig. 4). Males show a similar pattern, possibly reproducing during the whole year. The relative volume occupied by spermatids increased significantly between May and July, as well as between September and November, but no seasonal trend is apparent, notwithstanding a maximum volume of spermatozoa in January 2004 (see Fig. 5). The scrutiny of another quantitative analysis of the reproductive cycle, using the MGI (Brown, 1982) reinforces our interpretation that *A. mediolittoralis* is able to reproduce during the whole year, because no seasonal trend was found, both males and females displaying a very constant index value of about 3.0 and 2.5, respectively for males and females (Fig. 6).

No information regarding reproductive cycle for species of the genus *Alvania* was found in the literature, therefore comparisons could not be established.

Temperature is an important abiotic factor affecting marine invertebrate reproduction (Fretter and Graham, 1994). In the Azores, average sea surface temperatures range between 15ºC in the winter, and about 23–24ºC in the summer (Table 2). As there is no seasonal trend in reproduction, this means that this endemic Azorean rissoid is able to reproduce during the entire year, over a 8ºC temperature range and with average minimum temperatures of 15ºC in winter, a fact that may have important palaeobiogeographical and evolutionary implications.

Ávila (2005) and Ávila et al. (2002, 2008a, 2008b, accepted) have established that certain species, present in the Pleistocene fossil record of Santa Maria Island, were affected by the last glaciation and locally disappeared from the Azorean shores. Those species were the warm-water species with west-African/Cape Verde and Caribbean affinities, such as the gastropods *Bulla amygdala*, *Cantharus variegatus*, *Zebina vitrea*, *Conus ambiguus*, *C. ermineus*, *C.* cf. *miruchae*, *C.* cf. *roeckeli*, *C. ventricosus*, *C. venulatus*, *Trachypollia nodulosa*, *Zonaria picta* and *Polynices lacteus*, as well as the endolithic bivalve *Myoforceps aristatus*, and littoral endobenthonic bivalves usually associated to fine sandy substrata, such as *Ensis minor* (Chenu, 1843) and *Lucinella divaricata* (Linnaeus, 1758). It is worth noting that none of the endemic Azorean species, of which the minute Rissoidae account for almost half, were extirpated from the Azorean malacofauna by the drop of sea-surface temperatures and the associated sea level fall (Ávila, 2005).

The drop of the sea surface temperatures during the last glaciation in the region of the Azores is still a matter of debate, but the most consensual estimate reports average temperatures between 2–3ºC lower than the present ones (Crowley, 1981). This would mean that if *A. mediolittoralis* nowadays reproduces during all the year, a fall of 2–3ºC during the peak of the last glaciation would have probably just provoked a shift of the reproductive period to the warmest months of the year, with minor consequences in the reproductive cycle of this species. Taking this in consideration, one can expect that many of the Azorean species common in the Pleistocene outcrops of Santa Maria and living intertidally, in the same habitat of *A. mediolittoralis*, e.g., the rissoid *Cingula trifasciata* (Adams J., 1798), the anabathrid *Pisinna glabatra* (Megerle von Mühlfeld, 1824) and the minute skeneopsidae *Skeneopsis planorbis* (Fabricius O., 1780), and that have survived the last glaciation, are also probably able to reproduce during the whole year. This will be an evident ecological competitive advantage in times of lower sea surface temperatures as those occurring during glaciations, and it may be a plausible explanation for the survival of these species to glacial episodes. We intend to further work on *A. mediolittoralis*, expanding similar studies on the reproductive cycles to other Azorean endemic rissoids.

Table 2. Water temperature recorded from Caloura, São Miguel Island (Azores)

Date	Temperature, °C
21 March 2003	15.2
19 May 2003	17.1
15 July 2003	20.8
24 September 2003	23.7
20 November 2003	18.0
26 January 2004	16.7

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