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ORIGINAL ARTICLE

Reproductive cycle and oviposition in *Zidona dufresnei* (Caenogastropoda: Volutidae) from northern Patagonia, Argentina

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Abstract

This study describes the reproductive cycle in the edible marine snail *Zidona dufresnei* in San Antonio Bay (Patagonia, Argentina), an unusual population that inhabits shallow waters. Histological techniques together with monthly field observations over a two-year period were used to describe the gametogenic cycle, the season of oviposition and the presence of egg capsules. Females were mature from August to April and there was proliferation of the germinal epithelium immediately after evacuation. Growth and resorption of oocytes were observed throughout the study period. Males were reproductively active throughout the year. The histological analysis is consistent with the observations in nature, in which females were recorded depositing egg capsules from late August to late April. The reproductive period seems to be closely related to water temperature and photoperiod. The relatively high seawater temperature in San Antonio Bay may be one of the relevant factors that contributes to the extended period of oviposition and gamete evacuation in *Z. dufresnei*.

Key words: Eggs capsules, gametogenesis, Gastropoda, Mollusca, Patagonia

Introduction

Diverse reproductive characteristics of many Neogastropod species have been widely studied. For instance, there is extensive information on reproductive aspects and gonadal cycles of the volutids *Zidona dufresnei* (Donovan, 1823) (Giménez & Penchaszadeh 2002; Giménez et al. 2008), *Adelomelon brasiliense* (Lamarck, 1811) (Cledón et al. 2005), *Odontocymbiola magellanica* (Gmelin, 1791) (Bigatti et al. 2008); *Adelomelon ancilla* (Lightfoot, 1786) (Penchaszadeh et al. 2009) and *Voluta musica* Linnaeus, 1758 (Rangel et al. 2011; Peralta 2012).

Zidona dufresnei occurs from Rio de Janeiro (Brazil, 22°S) to San Matías Gulf (Argentina, 42°S), occupying sandy substrates from 5 to 110 m depth (Giménez & Penchaszadeh 2003; Carranza et al. 2008; Roche et al. 2011). In the San Matías Gulf there are two distinct populations of this species, one in the

intertidal area inside San Antonio Bay (SAB, 40°45' S, 64°56'W) (Pereyra et al. 2009; Roche et al. 2011, 2013a) and another inhabiting deeper water (up to 120 m) (Medina, 2013). In San Antonio Bay this species is found in a small and shallow channel (4 m maximum depth during low tide), on sandy substrates mixed with boulders and sponges (Roche et al. 2011, 2013a, 2013b). Both sexes reach a similar maximum size (120 mm in shell length, Pereyra et al. 2009) and share the same habitat. *Zidona dufresnei* has direct development and a low dispersal ability (Penchaszadeh & de Mahieu 1976; Giménez & Penchaszadeh 2003), which could result in different population enclaves being locally adapted. Individuals of *Z. dufresnei* spend most of their time buried and females deposit their egg capsules on the substrate (Roche et al. 2011). In SAB, under natural conditions, juveniles of *Z. dufresnei* emerge from the egg capsules after approximately 35 days (Roche et al. 2013a)

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and the crawling juveniles settle in the maternal environment.

Zidona dufresnei is an important fishery resource and is likely to be seriously endangered by overexploitation in the Mar del Plata area (Giménez et al. 2005). In San Antonio Bay this species has been supporting a small-scale artisanal fishery for more than 30 years (Narvarte et al. 2007; Roche et al. 2013b).

The reproductive cycle of *Z. dufresnei* in San Antonio Bay (Patagonia, Argentina) is particularly interesting because several biological characteristics of this species differ from the previously studied populations from open water (Giménez & Penchaszadeh 2002, 2003). Previous studies on *Z. dufresnei* in the area of Mar del Plata show that individuals may reach up to 210 mm (in shell length) and that the oldest individuals can live for more than 17 years, attaining sexual maturity at 128 and 120 mm for females and males respectively (Giménez & Penchaszadeh 2003; Giménez et al. 2004). In San Matías Gulf, individuals from the intertidal population from SAB can reach a maximum size of 120 mm for males and 110 mm for females (Pereyra et al. 2009). Moreover, the females from the intertidal population were found to have wider and heavier shells than males (Pereyra et al. 2009). However, there is no information about the gonadal cycle and size at sexual maturity either for the intertidal or shallow subtidal populations in SAB.

Knowledge about population structure, reproduction and recruitment is essential for management and conservation of fishing resources (e.g. Bigatti & Ciocco 2008). Since environmental factors such as temperature or salinity may affect the gametogenic cycle and hence the reproduction pattern, we also speculate that the reproductive characteristics of the

Z. dufresnei population from the SAB subtidal zone may differ from other sites previously studied.

The aim of this work was to describe the gonadal stages and gonadal cycles of *Z. dufresnei* from San Antonio Bay and to define their relationship with temperature and photoperiod, thus adding new information to the knowledge about the biology of this species. We also carried out some field observations on the timing of oviposition and the presence of egg capsules in order to compare this trait with the reproductive cycle and the histological analysis. The results of this study provide useful information for assessing the artisanal fishery of *Z. dufresnei* in its southernmost limit of distribution.

Materials and methods

Samples

Zidona dufresnei adults were sampled monthly, between November 2007 and March 2010 from San Antonio Bay (Figure 1). At least five males and 10 females were collected for each sampling date for histological analysis of the gonads. In some months it was not possible to collect samples due to bad weather conditions. In the field, females in the process of laying their egg capsules were also collected. Individuals were selected to cover the whole size range of each population.

Histological analysis of the gonad

In the laboratory, individuals were kept in containers filled with seawater and processed on the day of collection. Sex was identified a priori by the presence of the pedal gland in females, and the presence of a penis in males. This was also confirmed by

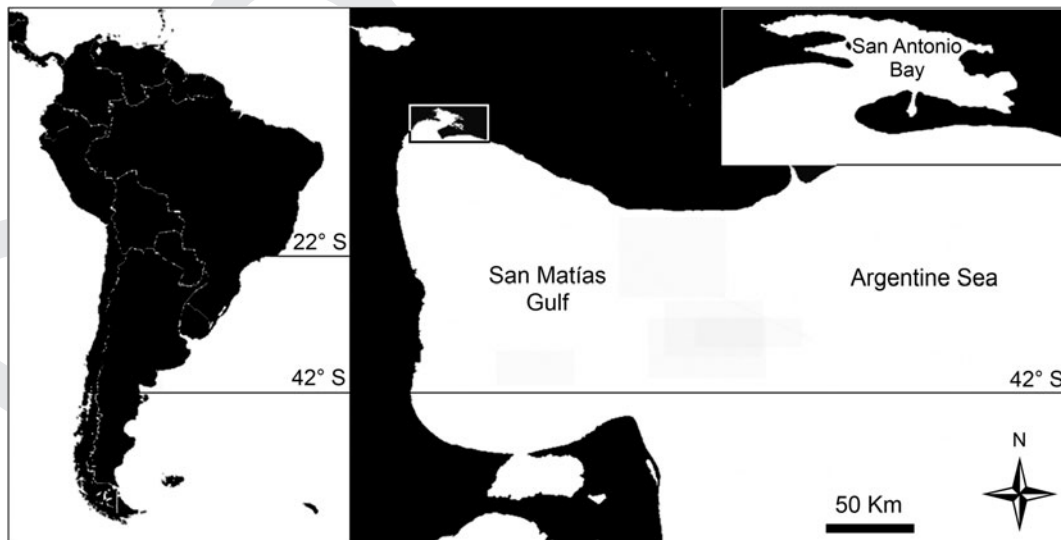


Figure 1. Location of the study site in San Antonio Bay, within San Matías Gulf. Taken from Roche et al. (2013a).

histological analysis of the gonad. When individuals emerge from their shell whilst in containers it was possible to see the presence or absence of the pedal gland. Snails were measured with Vernier calipers (total length in mm, $LT \pm 0.01$ mm).

After removal of the shell, the ovary which was situated in the last whorl could not be distinguished from the digestive gland because of its similar coloration. Gonads were fixed in Davidson's solution for 24 hours at 4°C and transferred to 70% ethanol followed by further dehydration through a series of ethanol and xylol. Subsequently, they were embedded in paraffin wax at 58°C, with sections cut (5–7 µm) with a microtome, stained with Harris haematoxylin and eosin in aqueous solution transferred to synthetic resin, and mounted for microscopy.

The histological sections were observed under a light microscope (Nikon Eclipse E2000). The maximum and minimum diameters of all oocytes for each female were measured and the arithmetic mean between the two variables (average oocyte diameter) was calculated.

To define the gametogenic cycle for both sexes, slides were classified according to the stage of gonad maturation (GMS: I – proliferation, II – maturation, III – evacuation, IV – post-evacuation) (Table I). This is based on the gametogenic pattern for this species described by Giménez & Penchaszadeh 2002.

Seawater temperature and photoperiod

The seawater temperature was recorded throughout the study period. Temperature data were provided from specific records of sea-surface temperature (SST) recorded *in situ* with a multiparameter probe YSI 556 MPS (2008) for each sampling date, and continuous recording with thermo buttons (ibutton) set every 2 hours (2009 and 2010). The data obtained were used to plot and analyse the seasonal

variation over the study period and its relationship with the reproductive cycle. Likewise, the photoperiod cycle (hours of light/day) was also considered as an environmental variable that may influence reproductive activity of *Zidona dufresnei*.

Oocyte diameter was compared statistically with temperature and photoperiod using ANOVA and post hoc comparisons by applying Tukey's test (Zar 1999). The data were previously log transformed in order to meet the assumptions of normality and homogeneity.

Egg capsules in nature

To evaluate the presence of egg capsules at different stages in the field, transects (10 m long and about 5 m apart) were carried out monthly by scuba divers. The numbers of egg capsules present were recorded and their developmental stages classified according to Pereyra et al. (2009) (Table II). In those areas of the bay where egg capsules were in the intertidal zone, similar transects were performed.

Results

A total of 541 adult individuals (319 females and 222 males) were analysed from San Antonio Bay. The maximum size was 128.6 mm for females and 114.9 mm for males, while the smallest size observed was 66.7 mm for females (corresponding to a histologically mature female) and 81.3 mm for males.

Gonad maturation stages

Females were reproductively active throughout the study period. Four gonad maturation stages were recognized: proliferation, growth, evacuation and post-evacuation (Figure 2, Table I). The most frequent range of sizes for oocytes in proliferation

Table I. Microscopic features of each gonad maturation stage for females and males in *Zidona dufresnei*.

Gonad stage	Females	Males
Proliferation	Ovary contains oogonia and previtellogenic oocytes with diameter less than 30 µm. Oocytes less than 40 µm initial vitellogenesis	Spermatogenic tubule contains a thicker layer of spermatogonia and primary spermatocytes
Mature	Oocytes between 40 and 80 µm are in vitellogenesis (yolk granules are evident in the cytoplasm). Oocytes larger than 80 µm complete the vitellogenesis. Gonad increases in size	Spermatogenic tubule contains spermatozoa in the lumen. The layer of spermatogonia and primary spermatocytes decreases in thickness
Evacuation	Presence of oocytes larger than 90 µm (cytoplasm uniformly filled with yolk granules). Oocytes free in the lumen. Oocytes decrease in abundance in the ovary	Few relict spermatozoa are in the spermatogenic tubule that looks empty. Spermatid duct are filled with spermatozoa
Post-evacuation	Empty ovary follicles with oocyte remains unreleased, atresic oocytes and phagocytes. Follicles begin to decrease in size and connective tissue begins to proliferate. Previtellogenic oocytes present	Resorption process is evident, phagocytes surround the spermatid duct. Spermatocytes and spermatides are in spermatogenic tubule

Table II. Description of the egg capsule developmental stages (taken and summarized from Pereyra et al. 2009).

Stage	Description
E0	newly laid, female still laying, whitish, capsule wall still soft
E1	recently laid, externally not different from the previous stage but a female was not observed
E2	capsule wall tough, capsular fluid with large clear areas
E3	embryos clearly visible, fluid transparent
E4	capsule with hole or slit-like hatching opening, with or without embryos

stage was 26–30 μm (Figure 2a, Figure 3). Oocytes over 30 μm had companion cells. Yolk granules were evident in the cytoplasm of the oocytes larger than 80 μm (Figure 2b, Figure 3). Oocytes over 90 μm were observed loose in the lumen for an individual in the evacuation stage (Figure 2c). Overall, during the evacuation stage very few mature oocytes per follicle were present. In each episode of gamete release, previtellogenic oocytes were observed near the follicle wall (Figure 2c). The ovary during post-evacuation was in a relaxed state with some previtellogenic oocytes and ‘phagocytes’ which would be responsible

for the resorption of the oocytes that were not evacuated (Figure 2d).

Thirteen females in the oviposition stage were collected in the field, but two of them could not be histologically analysed. The females in the oviposition stage had oocytes in previtellogenic and growth stages, together with ‘phagocytes’. The smallest females in the oviposition stage were 87.90 mm and the largest 114.20 mm. The mean size of females in the oviposition stage was 98.30 (± 8.2) mm TL.

The male gonad of *Zidona dufresnei* comprises numerous spermatogenic tubules separated by connective tissue (Figure 4a). During the proliferation stage, spermatogonia and spermatocytes were observed within the spermatogenic tubules (Figure 4a,b). When the male reaches maturity, the centre of the spermatogenic tubule is filled with spermatozoa. Spermatocytes and spermatids are close to the periphery of the tubule (Figure 4c,d). During the evacuation stage, only spermatozoa and spermatids were evident inside the spermatogenic tubules (Figure 4e). Also, at the same stage of evacuation, the spermatic ducts are filled with spermatozoa (Figure 4f). During the post-evacuation stage, the spermatogenic tubule contains some spermatocytes

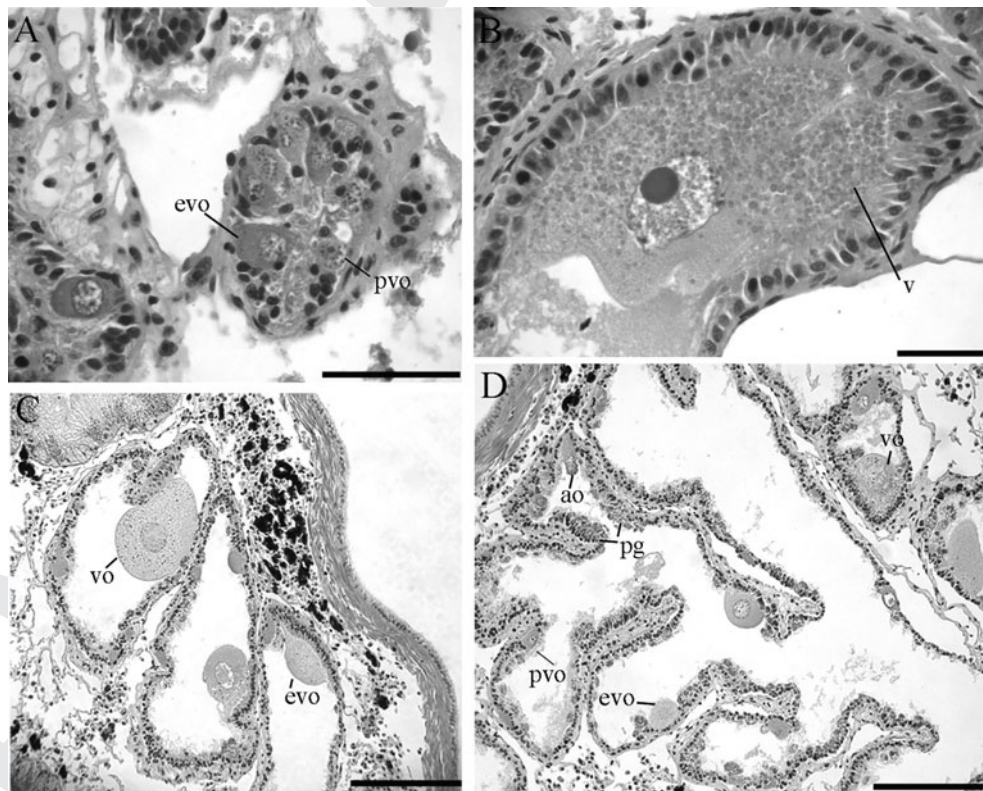


Figure 2. *Zidona dufresnei*. Photomicrographs of histological sections showing the main gonad maturation stage in females. (a) Proliferation stage; (b) Mature stage (note oocyte at complete vitellogenesis, rounded with accessory cells); (c) Evacuation stage, vitellogenic oocytes free in the lumen; (d) Post-evacuation stage. Lax ovary, presence of atresic oocytes and phagocytes which would be participating in the resorption process with some previtellogenic, early oocytes and atresic oocytes. Abbreviations: ao, atresic oocytes; evo, early vitellogenic oocyte; og, oogonia; pg, phagocytes; v, yolk in the cytoplasm; vo, vitellogenic oocyte. Scale bars: a, b, 50 μm ; c, d, 200 μm .

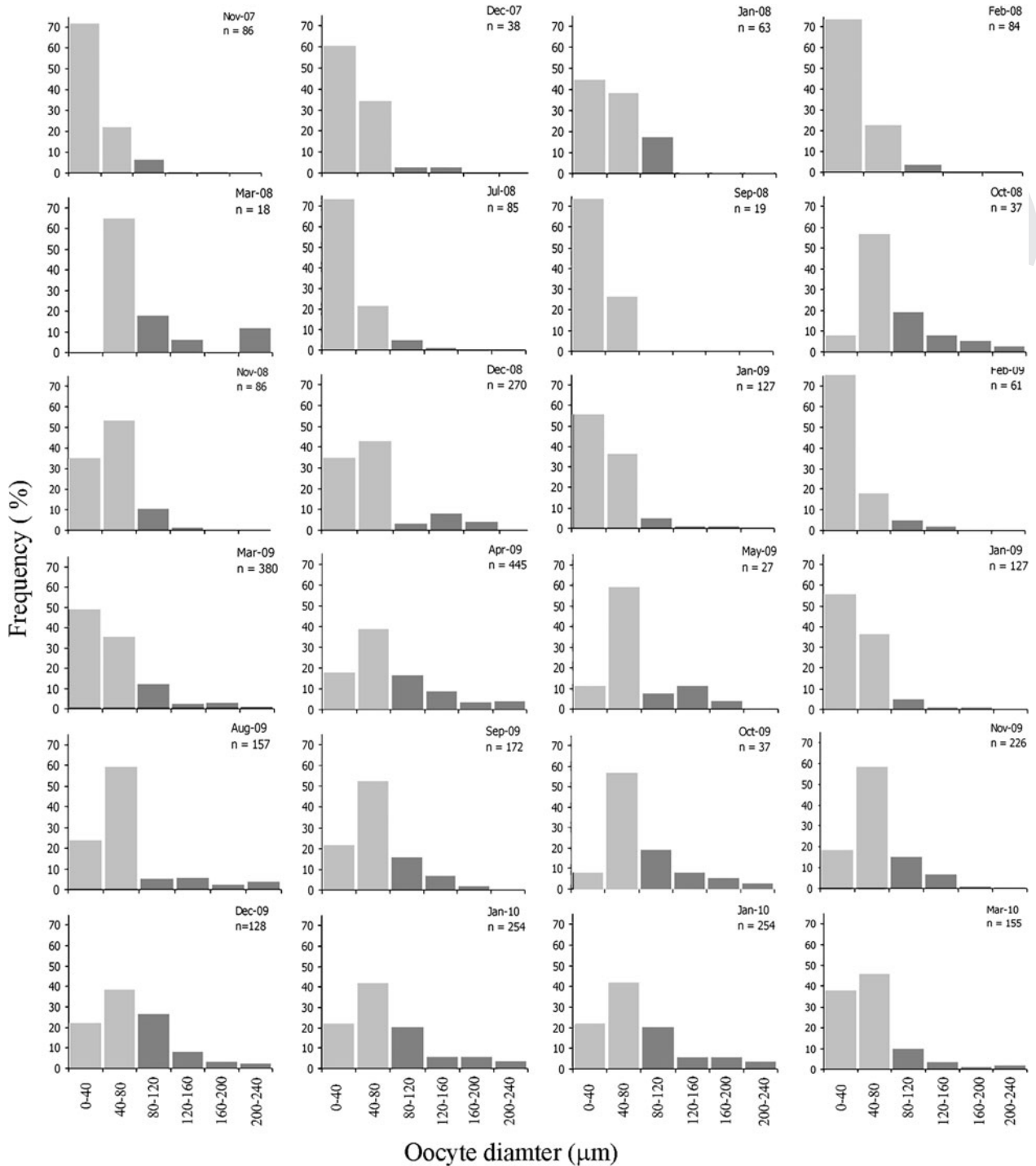


Figure 3. Frequency distribution of the oocyte diameters (Nov 2007–March 2010) at San Antonio Bay. *n* = total number of oocytes. Light grey bars: previtellogenic oocytes; Dark grey bars: vitellogenic oocytes.

and spermatids (Figure 4g). In the post-evacuation stage, phagocytic granules were present in the wall of the spermatic duct (Figure 4h).

Reproductive cycle

The gonadal stages were observed in different proportions throughout the year (Figure 5). Females

in the evacuation stage were found from August 2009 to April 2010, when the temperature increased at the end of the winter and the beginning of spring continuing to the autumn. Only one female was collected in May 2009 and the histological analysis showed that although there were growing oocytes (larger than 100 µm) they were possibly mature oocytes that had not been evacuated. In all months

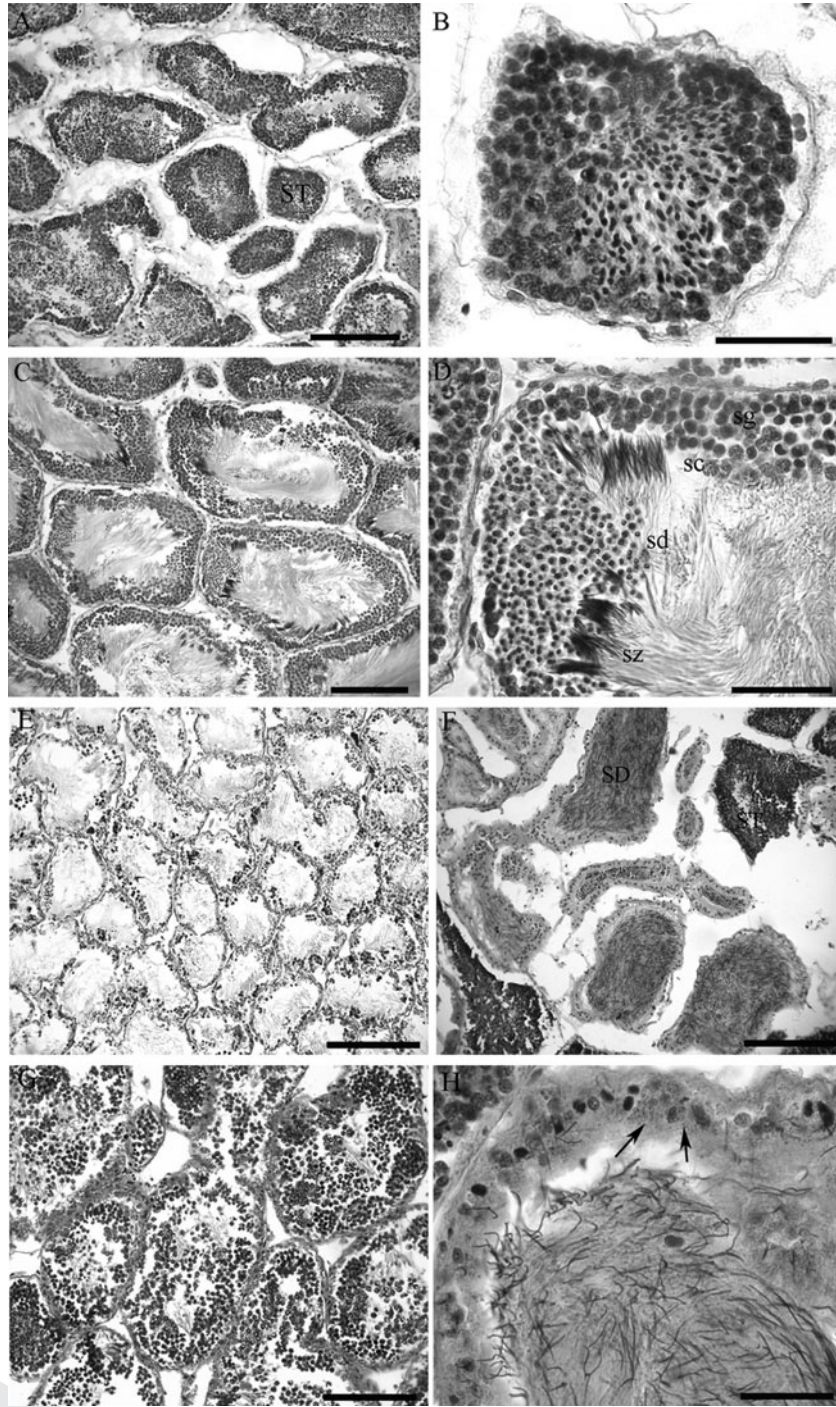


Figure 4. Male gonad maturation stages of *Zidona dufresnei*. (a) Proliferation stage, general view; (b) Proliferation stage detail of the spermatogenic tubule; (c) Mature stage, general view; (d) Mature stage, detail of spermatogenic tubule; (e) Evacuation stage. General view of empty spermatogenic tubule, with some relict sperm; (f) Evacuation stage, detail of the spermatid duct filled of spermatozoa; (g) Post-evacuation stage, spermatogenic tubule, spermatogenesis begun, presence of spermatocytes and spermatids; (h) Post-evacuation stage. Phagocytes are present in the wall of the spermatid duct. Arrows indicate the presence of yellow-brown granules associated with the resorption phenomenon. Abbreviations: sc, spermatocyte; SD, spermatid duct; sd, Spermatid; sg, spermatogonia; ST, spermatogenic tubule; sz, sperm. Scale bars: a, c, e, f, 200 μm ; b, d, h, 50 μm ; g, 150 μm .

of the post-evacuation stage analysed, the resorption process was evident (Figure 5).

The histological analysis showed that males were active throughout the year. Only in November 2008, May and August 2009 was gamete release observed.

In most specimens, there were different stages of gonad maturation (Figure 6).

Minimum temperature recorded *in situ* was 6.47°C in August and the maximum was recorded in January (23.10°C) during summer. The day-length

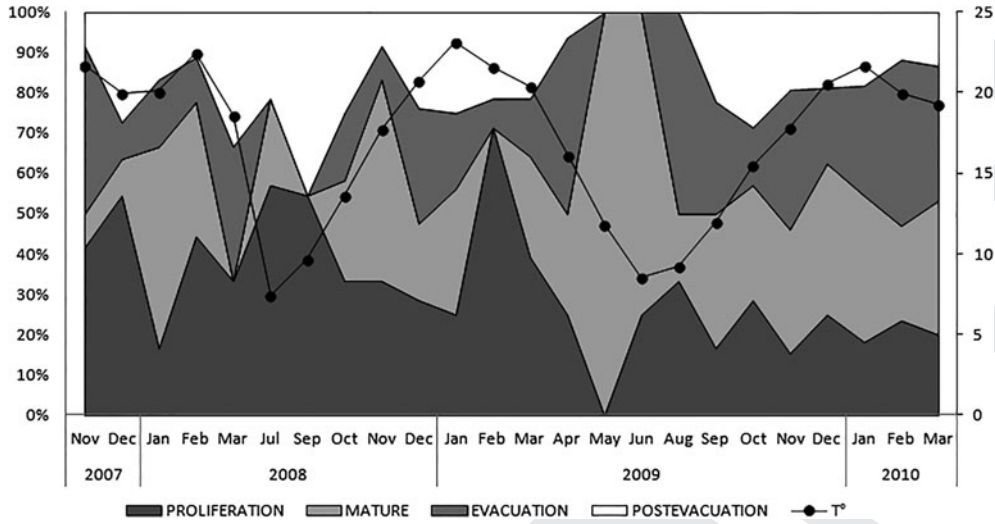


Figure 5. Monthly frequency of gonad maturation stages in females of *Zidona dufresnei*.

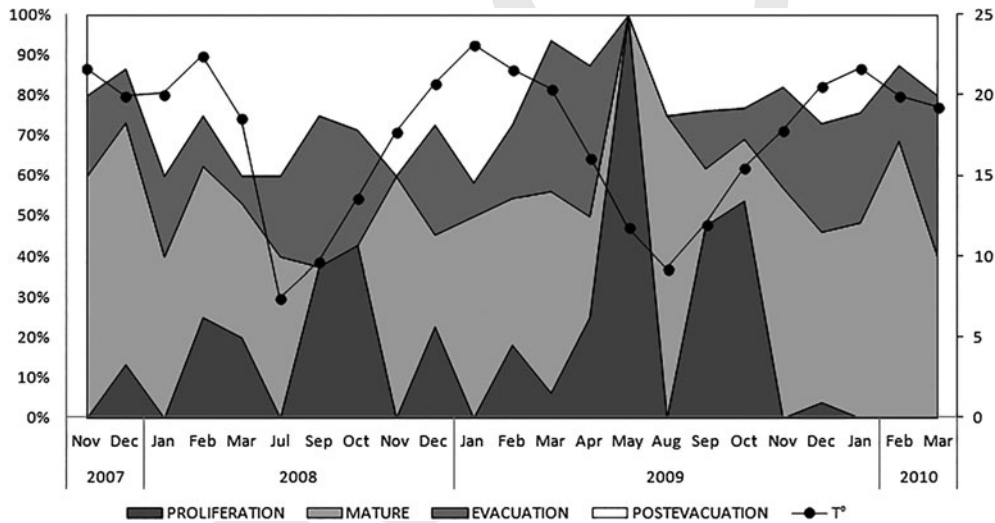


Figure 6. Monthly frequency of gonad maturation stages observed in males of *Zidona dufresnei*.

was minimum in June (9 h) and maximum in December (15 h) (Figure 8). The reproductive activity coincided with an increase of day-length during winter and with the increase of temperature and also with a decrease of temperature and daylight hours during the autumn.

The mean diameter of oocytes varied significantly with temperature and photoperiod during the seasons ($F_{(3,2994)} = 86,650.98$; $P < 0.001$). In autumn when the temperature and the photoperiod decreased (Figure 8) the values of mean oocyte diameter were greater than for the other seasons ($P < 0.0001$). In spring when the temperature and the photoperiod began to increase, the oocyte diameters were greater than in summer ($P < 0.001$).

Meanwhile the values between summer months and winter months showed no significant differences ($P > 0.05$).

Oviposition

First egg capsules of *Zidona dufresnei* (Figure 7) observed within the SAB were in late August. The presence of recent egg capsules (E0–E1) was constant from the beginning of the breeding season until mid-April, with a peak in February and October 2008 and again in January 2009 (Figure 8). These stages always exceeded 50% of the observations. Also, there seems to be a clear correlation between the oviposition events and the evacuation processes;

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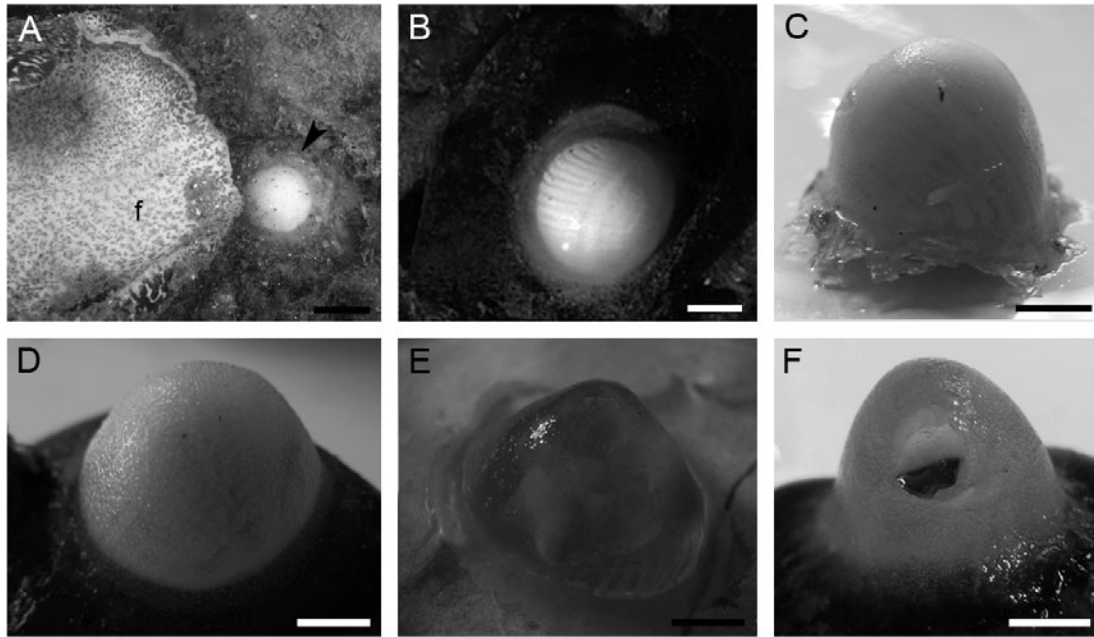


Figure 7. Egg capsule stages from *Zidona dufresnei* during intracapsular development. (a) Egg capsule just laid, note the presence of foot (f) besides the egg capsule recently laid (E0); (b) Detail of the egg capsule in E0 stage; (c) E1 stage; (d) E2 stage; (e) E3 stage; (f) E4 stage, empty egg capsule. Scale bars: a, 20 μm ; b-f, 10 μm .

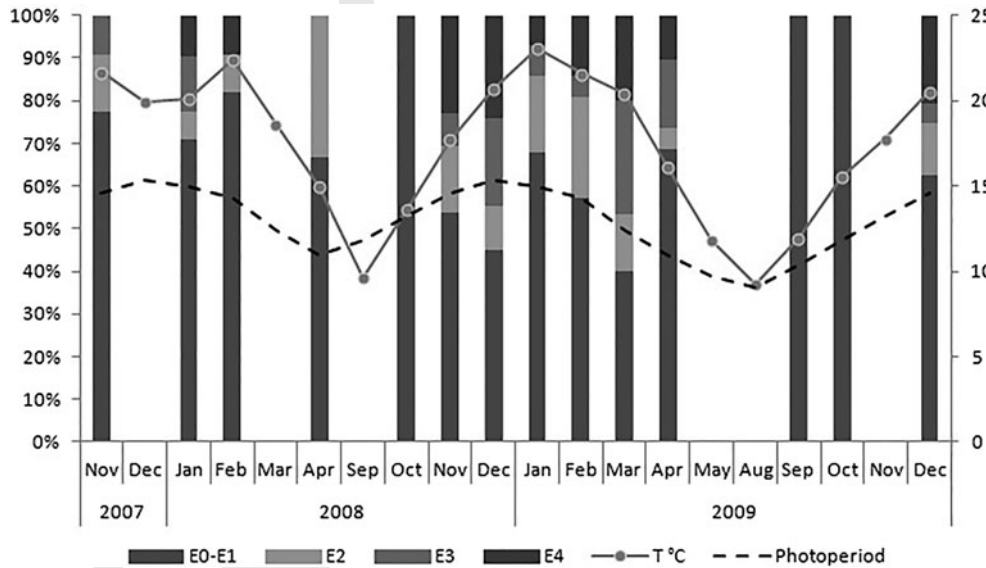


Figure 8. Oviposition events and egg capsule stages of *Zidona dufresnei* from spring 2007 to spring 2009.

both processes match the changes in water temperature and photoperiod (Figure 8).

Discussion

An extensive reproductive period, from late winter to early autumn, was observed for *Zidona dufresnei* in San Antonio Bay (Patagonia, Argentina), with major peaks of evacuation towards the end of summer and the beginning of autumn. This is also seen in other

populations of *Z. dufresnei* (Giménez & Penchaszadeh 2002) and other volutids in Argentina (e.g. *Adelomelon brasiliana*, Cledón et al. 2005; *Odontocymbiola magellanica* Bigatti et al. 2008), where gametogenesis was constant throughout the year. The histological examination showed that both males and females were gametogenically active and showed gonadal maturity all year round, thus the individuals of this population did not present a stage of gonadal rest. However, the cellular diversity observed in the

follicles throughout the year did not allow us to identify key stages in the gametogenic development.

The mean oocyte size of mature oocytes (about 170 μm) was consistent with that seen by Giménez & Penchaszadeh (2002) for the same species and was within the range of other volutids in the Argentine Sea (Cledón et al. 2005; Bigatti et al. 2008; Penchaszadeh et al. 2009). The low number of mature oocytes ready to be evacuated, and the cell diversity observed throughout the year (i.e. without predominant gametogenic stages) has also been reported for the Caribbean volutid *Voluta musica* (Peralta 2012).

According to Giménez & Penchaszadeh (2002), the gonadal resorption stage is mainly characterized by the presence of yellow-ochre bodies with oocytes which had not been spawned. These authors mentioned that the presence of yellow-ochre bodies was always observed after spawning periods. In our study, these 'phagocytes' were observed in different proportions during all the months studied. The constant observation of cells compatible with 'phagocytes', and the proliferation of cells after the evacuation of gametes, could be due to an over-production of oocytes, that cannot be maintained within the egg capsule. As a consequence, the process of phagocytosis of the remaining oocytes seems crucial to reuse the energy in the same reproductive period. In other volutids such as *V. musica* and *Adelomelon ancilla* (Penchaszadeh et al. 2009; Peralta 2012), this phenomenon was also observed throughout the year.

In males, the observation of different gametogenic stages in the same individual is consistent with the observations made by Giménez & Penchaszadeh (2002) in the same species and by Cledón et al. (2005) in *A. brasiliensis*, Bigatti et al. (2008) in *O. magellanica* and Penchaszadeh et al. (2009) in *A. ancilla*.

Since *Z. dufresnei* is commercially important, the reproductive cycle of oviposition events was established, however, this information should be treated with caution when designing management measures for this species in SAB. Further studies have to be carried out in order to find the size at first sexual maturity in this population of SAB. On the other hand, the absence of individuals smaller than 65 mm in total length in the samples could be related to the cryptic habits of this species. Several authors have highlighted this habit in other volutids (Clench & Turner 1970; Poppe & Gotto 1992; Pereyra et al. 2009; Bigatti et al. 2010; Peralta 2012). In this regard, the lack of samples or the low number of individuals collected in some autumn-winter months was due to the inaccessibility and the difficulty of observing the snails as the substrate was completely covered by algae during these months.

The observations of oviposition in nature between late August and April, jointly with the histological observations, showed that the reproductive events of *Z. dufresnei* in SAB take place from early spring to early autumn. Also, the presence of recent egg capsules through the breeding season seems to indicate that oviposition is a continuous process during the reproductive season. In this study, the oviposition period is longer than that described earlier in SAB (November to April; Pereyra et al. 2009), and for the species in the subtidal waters of Buenos Aires (October to March, from histological observations of the gonad; Giménez & Penchaszadeh 2002). The duration of the reproductive period seems to be closely related to water temperature (which in SAB is closely associated with increased photoperiod). Previous authors have already mentioned this relationship for *Z. dufresnei* in northern populations (Penchaszadeh & Giménez 2002), and for other volutids (Cledón et al. 2005; Penchaszadeh et al. 2009). However, this clearly differs in *O. magellanica*, as Bigatti et al. (2008) identified a relationship with photoperiod, but not with the increase in water temperature in high latitude sites near to this study area. The particularly high temperature of the seawater possibly is a factor that contributes to the extended period of oviposition and evacuation of gametes in *Z. dufresnei* in San Antonio Bay.

Considering that *Z. dufresnei* is of commercial importance in San Antonio Bay (Narvarte et al. 2007; Roche et al. 2013b), and that the higher period of collection occurs from November to May, which overlaps with the oviposition season (August to April) (Roche et al. 2013b), and that the females observed in oviposition have a mean length of 98 mm, it seems that the most vulnerable and susceptible individuals correspond to mature females. In consequence a precautionary approach should be considered when managing this resource.

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