

Reproduction and imposex in the edible snail *Adelomelon ancilla* from northern Patagonia, Argentina

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Abstract The edible volutid snail *Adelomelon ancilla* occurs on the Argentine coast and may have the capacity to serve as source of support for local fisheries. However, knowledge of its reproduction is lacking. Over 3 years (from September 2000 to July 2004) we studied the oviposition at Golfo Nuevo, Patagonia by diving. Monthly collections were performed to examine gonadal stages histologically during 2002–2003. Gamete development stages corresponded with the expected period for oviposition occurring from July to November, a time of increasing day length. Oviposition also took place in March when water temperature attained a maximum of 18°C. Following the spawning period, remaining gametes were resorpted in both sexes. Males contained sperm throughout the year, parasperm and eusperm forms were found within the same acinus. Oogonia/oocytes ranged from 20 to 240 µm in diameter during oogenesis. Females commenced laying egg capsules from a shell length of 114 mm. Reproduction is compared with other fishable volutid snails from the Argentine shelf, and suggests the need to apply certain fishing restrictions if the resource begins to be utilized commercially. The imposex condition was found in snails from

some shipping areas. The frequency of this condition declined with distance from the port.

Introduction

Volutids are edible snails that are being consumed and commercialized in Argentina. Some species of this group are of commercial importance, constituting about 5% of the gastropod biomass extracted worldwide (FAO 2002). Studies on reproductive biology provide valuable information for fisheries. In particular, the reproductive cycle is a basic component of population dynamic studies in gastropods (Underwood 1979; Branch 1981).

Many species of marine dioecious gastropods present morphological alterations in the secondary sexual characteristics, such as the development of the vas deferens and/or a penis in females. This phenomenon was denominated imposex (Gibbs et al. 1990; Gibbs et al. 1991). The presence of imposex is related to tributyltin (TBT), a compound present in antifouling paints (Bryan et al. 1986; Horiguchi et al. 1994; Oehlmann 1994; Oehlmann et al. 1996), and whose presence is associated with high shipping traffic (Smith 1981; Gibbs et al. 1987). Neogastropods were found to be suitable as bioindicator of TBT contamination in marine ecosystems (Barroso et al. 2000; Gibbs and Bryan 1994; Minchin et al. 1996; Minchin 2003; Stroben et al. 1992). Imposex in volutids can also lead to weight loss and shell malformations (Bigatti and Carranza 2007), which has negative consequences on their viability as economic resources.

In northern Patagonia, regular commercial landings of volutids are still infrequent, with the exception of sporadic small catches (Bigatti and Ciocco 2008). *Adelomelon ancilla* (Lightfoot, 1786), has been identified as part of a

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volutid group that could support an alternative fishery by means of artisanal diving catches in northern Patagonic gulfs (Bigatti and Ciocco 2008). Lasta et al. (2000), mention *A. ancilla* as a species of commercial interest, although landings are scarce. In Chile, catches of *A. ancilla* in 1990 reached 110 tons together with *Odontocymbiola magellanica*; both species are consumed fresh, frozen or canned (Osorio 2002). In the Uruguayan–Argentinean common fishing zone, Carranza (2006) reported shell damage in 30% of the individuals of *A. ancilla* and *O. magellanica* is due to by-catch from other fishing operations. Despite the fact that until now *A. ancilla* is consumed only locally, its potential as a fishery resource requires further reproductive studies in order to suggest fishing policies before it can be commercially exploited.

The “violet snail” *A. ancilla* is distributed from southern Brazil (33°S) to Ushuaia in Argentina, and from 25°S to Magallanes Strait, in Chile (de Castellanos and Landoni 1992; Osorio 2002). Along the Patagonian shore, it inhabits shallow waters (from 5 m depth) on gravels or mixed bottoms. In Golfo Nuevo, the water temperature varies from 9.7° to 18.3°C (spring to autumn, respectively) and the photoperiod varies from 9 to 15 h of light. *A. ancilla* lives most of the time buried in the substrate and juveniles are very cryptic and difficult to distinguish in the field. Its diet consists mainly of mollusks and, to a lesser extent, sea urchins. In 98% of the specimens studied from the Golfo Nuevo, the shells of these snails were

covered by the epibiotic anemone *Antholoba achates* (Bigatti et al. 2009a, b).

The aim of the present study is to provide information about the reproductive cycle, the oviposition season and the existence of imposex in the edible snail *A. ancilla* from Golfo Nuevo, Argentina that could serve as a base to suggest fishery management policies.

Materials and methods

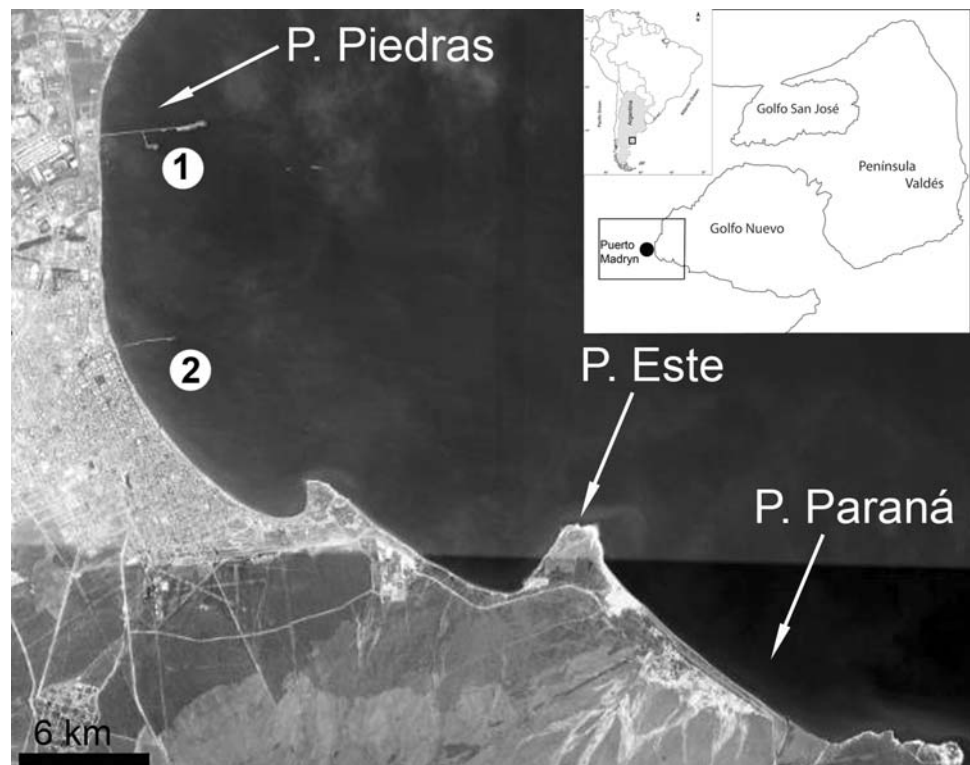
Sampling

Specimens of *Adelomelon ancilla* were captured monthly over 3 years (September 2000–July 2004), by SCUBA diving between 5 and 20 m depth in Golfo Nuevo (42°48'S; 64°54'W) (Fig. 1). Monthly histological studies were carried out on 10–15 mature individuals (2002–2003) for both sexes. Animals were sexed by differences in gonad color and the presence (females) or absence (males) of the pedal and internal sexual glands (albumen and capsule glands). Shell length was measured using a caliper.

Oviposition seasonality

The oviposition seasonality was studied by direct observation of oviposition events (females laying egg capsules). These data were gathered through diving expeditions

Fig. 1 Sampling area. 1 Storni harbour (close to the Parque Piedras site), 2 Piedrabuena harbour



occurring each month from April 2001 to March 2003 in an area of approximately 2 km² in each sampling site (Fig. 1). When laying the egg capsule, volutid females engulf it with their feet (see “Results” for more details).

Gonadal cycle and structure

Individuals utilized in the gonadal cycle study were collected from zones with very low shipping activity (Paraná Beach) (Fig. 1). After removal of the shell, small pieces (3 mm side) of gonads of both sexes were fixed in Bouin’s solution for 8 h, dehydrated in a series of increasing ethanol concentrations and embedded in methacrylate (Historesin[®]) using standard procedures. Serial sections (5 μm) were stained with hematoxylin and eosin to study monthly gametic development and to measure oocyte diameter (only gametes with visible nucleolus). Observation, measurements and photographs of histological slides were taken with a Zeiss Axiostar light microscope.

Histological slides were analyzed by monthly size–frequency histograms for oocyte diameter. For each individual of both sexes (during 2002–2003), a gonadal stage was assigned. To facilitate the understanding of the gonadal cycle, we divided it in five stages: proliferation and growth, pre-evacuation, evacuation, post-evacuation and resorption.

For semi-thin sections, portions of gonad were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 4 h, and post-fixed in 1% osmium tetroxide for 2 h. After this step, they were cut using a Reichert ultramicrotome and stained with uranyl acetate and lead citrate.

We also prepared samples of female’s *bursa copulatrix* for scanning electronic microscopy (SEM, Philips XL 30). Samples were fixed in glutaraldehyde in phosphate buffer for 6 h, treated using the critical point dehydration method, followed by metal coating.

Imposex

To determine the existence of imposex, 334 specimens of both sex of *A. ancilla* were analyzed monthly from 2001 to 2003 from 3 sites: the Parque Piedras site, close to a harbour with high shipping traffic (800–1,000 ships per year); the Punta Este site, an area with intermediate shipping traffic (with presence of SCUBA diving and recreational boating traffic) and Paraná Beach, with very low marine traffic (sporadic ships) (Fig. 1). After sexing individuals based on presence/absence of sexual glands (albumen and capsule gland), penis length was measured in males and imposexed females using a caliper. Relative Penis Length Index (RPLI = [(female penis mean length/male penis mean length) × 100]), sensu Gibbs and Bryan 1994) was calculated in each population. In addition, the percentage of females with imposex [(number of female with imposex/

total number of female) × 100] was calculated for each population. Histological sections were inspected to corroborate the sex. For the Parque Piedras site, measurements of TBT in sediments were performed sensu Delucchi et al. (2007) by GC-MS analysis as follows: the analytical procedure was carried out under DIN124 Norm 38407-F13 (modified) for water, sewage and mud. The samples were mixed with 250 ng (62.5 ng each compound) of an internal standard consisting of tetrapropyltin (TePrT), tripropyltin (TPrT), monoheptyltin (MHT), and diheptyltin (DHT) 100 ng/ml in order to control the effectiveness of the recovery of OTs.

Results

No external shell dimorphism was observed in *Adelomelon ancilla*. In the field, females could be differentiated from males by the presence of a pedal gland located on the anterior portion of the sole females’ foot. This gland is used in the shaping and fixation of egg capsules. Males also exhibit a penis, while females lack it.

Oviposition

The major oviposition events (when the number of females laying eggs was at its maximum) in the field occurred from July to November (Fig. 2). This season coincides with the increase in photoperiod, from the winter solstice (June 21) to the summer solstice (December 21). However, a less marked oviposition peak was registered in March. This peak in oviposition occurred soon after the maximum water temperature (Fig. 2) and was observed for all sampled years. The minimum shell length of females laying egg capsules was 114 mm. When laying egg capsules, females covered them with their feet and fixed the capsule on the

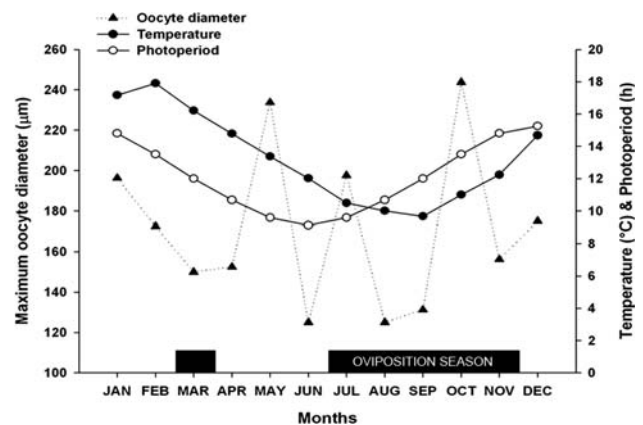


Fig. 2 Oviposition events (shaded bars along x-axis), photoperiod, surface water temperature, and maximum oocyte diameter

concave surface of stones, egg capsules of elasmobranchs, or shells of dead bivalves; then, they used the same pedal gland to shape the egg capsule. These were soft and smooth with a proteic appearance, without a calcareous cover. The time of each oviposition event could not be established.

Gonadal structure

Gonads of both sexes were situated dorsally to the digestive gland and embedded in it, varying in size within individuals or seasons.

Females

The ovary is orange-brown and is disposed intrusively in the digestive gland, so it was not possible to calculate a gonadosomatic index. The ovary was composed by many tubules where the process of oogenesis is carried out. The proliferation was characterized by the presence of oogonia in growth, which was joined to the membrane tubule and surrounded by companion cells (Fig. 3a). The nucleus and nucleolus of oocytes were visible and well developed (Fig. 3). While the proliferation phase advanced, the oocyte diameter grew in size (Fig. 3b) and the vitellum entered into the cytoplasm. At this phase, oocytes were still attached to the follicular wall by one or two peduncles (Fig. 3c). During the evacuation, oocytes were liberated into the lumen, and ready to be evacuated. The resorption stage was characterized by empty follicles, and the few remaining oocytes began degradation and consequent

resorption by nutritive phagocytes. Subsequently, new pre-vitellogenic oocytes appeared, starting the active stage of the next cycle (Fig. 3d).

Males

The male gonad was easy to distinguish after shell removal because the testis was light brown to dark brown and conspicuous. The seminal vesicle was contiguous to the testis, full of sperm or empty, depending on the season. We registered the complete spermatogenesis cellular series in the same tubule for all sampling months (Fig. 4).

The spermatogonia were situated close to or in contact with the tubule membrane, while the spermatocytes (distinguished by a bigger nucleus) were located near the lumen (Fig. 4a, d). As development of the typical spermatid proceeded, the nucleus increased in length and decreased in width (Fig. 4a, d). The spermatozoa were located in small clusters connected to Sertoli cells by cytoplasmic projections until their development finished (Fig. 4b). Meanwhile, the mature spermatozoa were situated free in the lumen of the tubule (Fig. 4b, c).

Another type of spermatozoa, the paraspermatozoa, was observed in the same tubule (Figs. 4d, 5a). Dispersal remnants of nuclear material, denominated cariomerites, were observed inside the cytoplasm.

Among the spermatogenic tubules, the seminiferous tubules or seminal vesicle were distinguished. In this vesicle, the mature sperm was collected and subsequently released towards the vas deferens (Figs. 4c, 5a).

Fig. 3 Histology of female gonad. **a** Developing oocyte (Oo) attached to the tubule membrane and surrounded by companion cells (cc). **b** Proliferation and growth: oocytes grow and are attached to companion cells. Note the nucleus (N) and nucleolus (Nu) **c** Pre-evacuation: oocyte grows attached to the follicular membrane by peduncles. **d** Resorption and proliferation: atresic oocytes are in degradation and subsequently new oocytes start to grow from the follicular membrane *fm*, follicular membrane

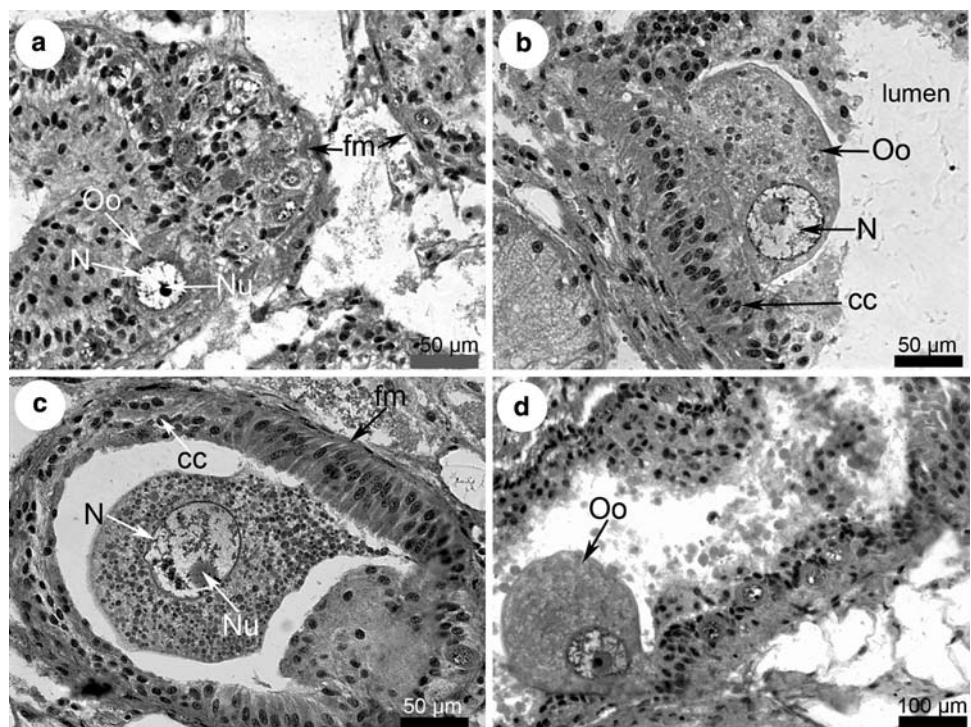
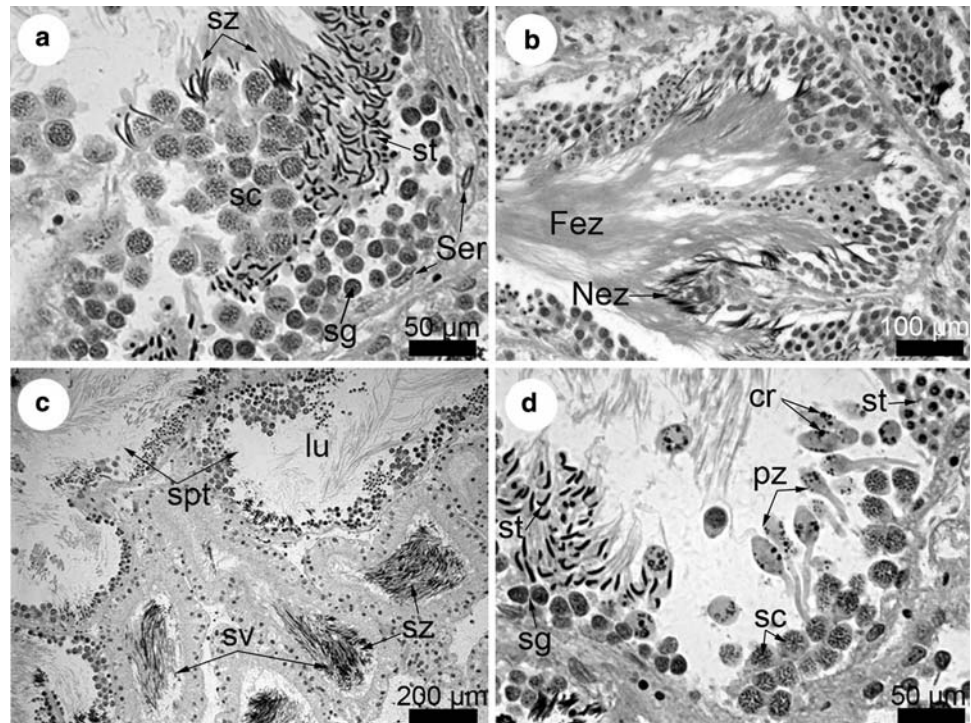


Fig. 4 Histology of male gonad. **a** Tubule with all the spermatogenic series and the Sertoli cell (*Ser*). Note the spermatogonia (*sg*), the spermatocyte (*sc*), the spermatids (*st*) and the mature spermatozoa (*sz*). **b** Pre-evacuation: tubule with mature spermatozoa free into the lumen. Note the nucleus (*Nez*) and the flagella (*Fez*) of spermatozoa. **c** Evacuation and development in the same tubule; note the seminal vesicle (*sv*) full of sperm to be evacuated. **d** Detail of the spermatogenic tubule showing the paraspermatozoa (*pz*). Note the cariomerites (*cr*) into the cytoplasm; *lu* lumen



Using SEM, we observed clusters of sperm attached to the epithelium of the bursa copulatrix, where males had deposited gametes into the females (Fig. 5b). We were unable to distinguish whether this sperm was composed only of euspermatozoa or whether it also included paraspermatozoa. Living spermatozoa were also observed under microscopy from fresh bursa copulatrix tissues.

Gonadal cycle

Females

All the individuals captured were mature with a maximum shell length of 168 mm and minimum of 102 mm. Five stages of the gonadal cycle were established: proliferation and growth (oogonia growing from the acinus wall), pre evacuation (oocytes <180 μ m, growing attached to the follicular membrane by peduncles, with vitellum into the cytoplasm), evacuation (oocytes free into the lumen; diameter >180 μ m), post evacuation (empty lumen without oocytes) and resorption (degradation of matures oocytes with presence of nutritive phagocytes). Different gonadal stages were present all year long. Oocytes were released from the gonad from June to November (Fig. 6), concordant with the oviposition season observed in the field (Fig. 2). During March, oocytes were also evacuated, corresponding to another oviposition event. The number of oocytes that reached the size of maturity (>180 μ m) were few, so the amount of oocytes evacuated were very low. The resorption

stage occurred almost all of the year, in particular, after the liberation of gametes. However, this phenomenon was not registered in February, May and November. Females developed oocytes (proliferation and growth stage) all the year round, mainly from summer (December) to the beginning of winter (July), when oocyte release and oviposition season begins (Fig. 6).

The size of the oogonia/oocytes ranged from 22 μ m in March to 240 μ m in October (maximum registered). The oocytes free in the lumen and ready to be evacuated measured >180 μ m (Fig. 7) and no remnants of these larger sizes oocytes were registered after the oviposition season. Although few oocytes >180 μ m were developed in the oviposition season, the proliferation and growth stage was constantly observed throughout all the months sampled (Figs. 3, 6) and no resting season was apparent.

Males

All the individuals captured were mature with a maximum shell length of 151 mm and a minimum of 110 mm. The same five gonadal stages displayed by the females were also found in the males: growth and proliferation (presence of all cellular stages of spermatogenesis), pre evacuation (full lumen with mature sperm and cluster of spermatozoids attached to the membrane acinus), evacuation (free mature sperm in the lumen), post evacuation (remaining sperm in the almost empty acinus) and resorption (degradation of mature sperm with presence of nutritive phagocytes and yellow bodies).

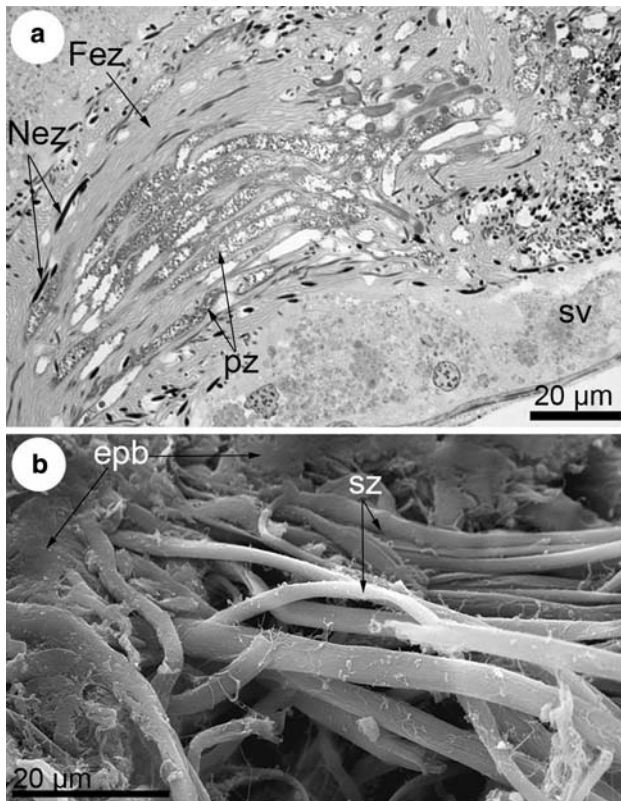


Fig. 5 Mature spermatozoa of *Adelomelon ancilla*. **a** Into the seminal vesicle (sv). Note the two types of spermatozoa, the paraspermatozoa (pz) and the euspermatozoa showing the nucleus (Nez) and the flagellum (Fez). **b** SEM picture showing spermatozoa into the bursa copulatrix of the female. Note the attachment of the spermatozoa (sz) to the epithelium of the bursa (epb)

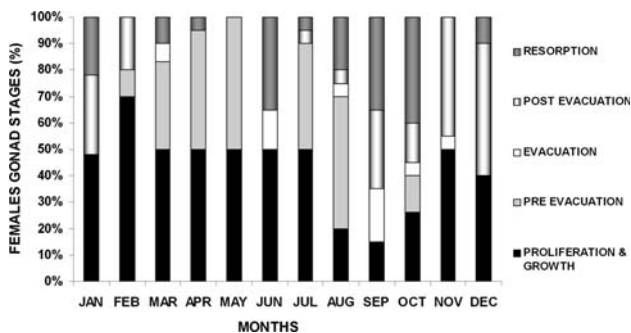


Fig. 6 Gonadal stages from histological analysis of females

In contrast to the females, males showed a fully active gonad and gamete liberation all year round (Fig. 8). In all cases, the spermatogenic tubules contained all cellular stages (spermatogonia, spermatocytes 1° and 2°, spermatids, and spermatozoid). The release of mature sperm was registered during every month of sampling. After gamete liberation, the tubules remained almost empty. Resorption of gametes by nutritive phagocytes was observed in March, April and October (Fig. 8).

Imposex

A total of 334 *A. ancilla* individuals, both males and females, were analyzed in order to determine the presence of imposex in 3 populations from Golfo Nuevo (Table 1). The individuals collected near to the commercial harbour (Parque Piedras site) showed an imposex frequency between 66 and 100%. The concentration of TBT in this area was 1.7 ng Sn/g. At Punta Este, a zone of SCUBA diving and recreational boating traffic, the occurrence of imposex ranged from 0 to 75%. In Paraná Beach, where the gonadal cycle of *A. ancilla* was studied, the frequency of imposex was zero. Histological sections confirmed the sex of the females with imposex, and no male gonad proliferation was observed. Moreover, females with imposex did not have vagina blockage by the vas deferens and individuals laying egg capsules were observed.

Our observations demonstrate that in females of *A. ancilla* affected by imposex, a vas deferens developed as a first step and then penis neoformation occurred. Penis development in females was always smaller than the male penis (Fig. 9a, d). Some females and males at the Parque Piedras site presented malformations such as multiple penis neoformation (Fig. 9b, c), while at the Punta Este site all the females examined only had a vas deferens (without a penis neoformation).

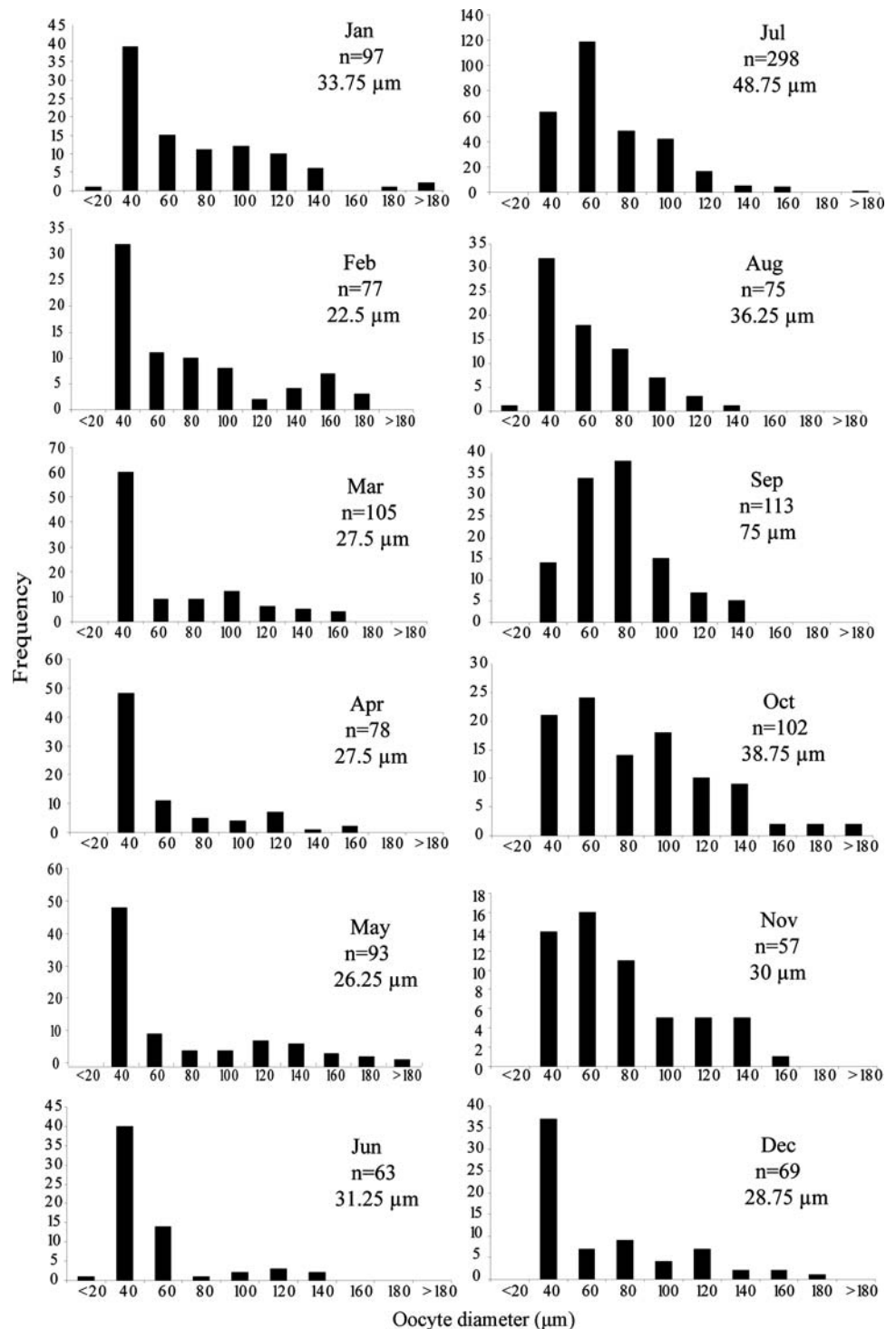
Discussion

Specimens of *Adelomelon ancilla* show macroscopic characteristics that allow distinction between both sexes, such as the pedal gland, the secondary glands (albumen and capsule) in the female and the conspicuous penis in the male. The male gonad is tubular and its colour varies from dark-brown to light-brown depending on the reproductive season, while the female gonad is orange-brown. The reproductive anatomy of *A. ancilla* is similar to other volutids from South America (Ayçaguer 2002; Bigatti 2005; Bigatti et al. 2008; Cledón et al. 2005; Giménez and Penchaszadeh 2002).

Oviposition

The mean peak of oviposition coincides with the increasing day length. Gastropods are sensitive to changes in the local environment and their activities exhibit seasonal and circadian variations (Chase 2002). However, gastropods not only respond to changing conditions, they are also able to anticipate those using internal timing mechanisms (Chase 2002). The other oviposition peak registered in March occurs after the maximum water temperature, although the length of day decreases in this season (equivalent to

Fig. 7 Monthly oocyte diameter histograms during 2002. n total number of oocytes measured each month; the modal monthly oocyte diameter is shown below n



September–October). Both oviposition peaks were registered when light hours were identical (12 h) and water temperature was maximum (March) and minimum (September). Therefore, it is likely that day length is the main factor influencing the oviposition in *A. ancilla*. Egg laying season, coinciding with the increase in photoperiod, was also observed in the sympatric species *Odontocymbiola magellanica* in the same sampling zone (Bigatti et al. 2008). In

addition, in intertidal populations of *Trochon geversianus* from Golfo Nuevo the photoperiod seems to be the main factor that regulates the spawning of egg capsules (Cumplido 2009). However, temperature is often mentioned as a very important factor in gonadal development in caenogastropods (Giese and Pearse 1974), because gastropods have no internal means of regulating their body temperature (Chase 2002). In the volutes inhabiting northern coasts of

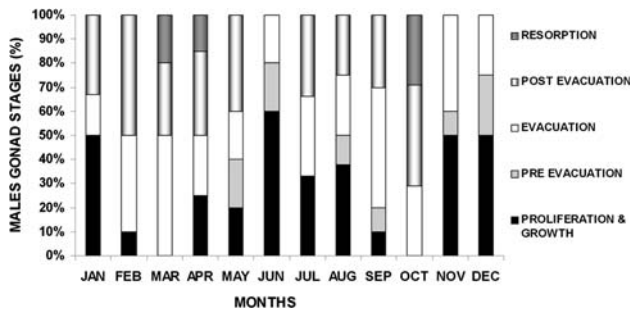


Fig. 8 Gonadal stages from histological analyses of males

Argentina, as is the case of *A. brasiliensis* and *Z. dufresnei*, the temperature seems to be the main factor affecting the liberation of gametes (Giménez and Penchaszadeh 2002; Cledón et al. 2005). The relation between oviposition and the detection of light by volutes, as well as the associated circadian rhythms must be studied for *A. ancilla* under experimental conditions.

Gonadal structure

The gonadal structure of both sexes of *A. ancilla* is similar to other volutids such as *O. magellanica* (Bigatti et al. 2008), *Z. dufresnei* (Ayçaguer 2002; Giménez and Penchaszadeh 2002) and *A. brasiliensis* (Cledón et al. 2005). As the gonad is situated intrusively within the digestive gland, a gonadosomatic index is not possible to calculate. The companion cells in the female most likely provide support and/or nutrition until oocyte evacuation occurs.

The presence of two types of sperm (euspermatozoa and paraspermatozoa) in *A. ancilla* has been previously reported (Zabala 2007; Zabala et al. 2009) and their morphology shows similarities with other members of the Volutidae family like *O. magellanica* (Bigatti et al. 2008) and *Z. dufresnei* (Giménez et al. 2008). Results indicated that spermatozoa are present and attached to the female *bursa copulatrix* wall; however, the period of time in which they remain there could not be quantified. In *O. magellanica*, copulation occurs all year round and sperm is stored in the *bursa copulatrix* at least for 4 months, allowing a multiple sperm contribution (Bigatti et al. 2008). We did not distinguish between paraspermatozoa and euspermatozoa inside the *bursa copulatrix*, whether they were free or

in an euspermatozoa–paraspermatozoa association (spermatozeugmata). Many caenogastropods, such as *Littorina* sp. (Buckland-Nicks 1973; Buckland-Nicks and Chia 1977) and some mesogastropods (for example Epitoniacea Melone et al. 1978, Tonnacea Buckland-Nicks et al. 1982), exhibit this association. Possible functions of paraspermatozoa are to transfer nutrient stores to the female during mating (including lysosomes and secretory products) and to create a hostile environment for incoming rival sperm through the formation of sperm plugs (Buckland-Nicks 1998). More exhaustive studies of electronic microscopy and histology of the bursa copulatrix are needed to elucidate this hypothesis in *A. ancilla* and other volutids.

Gonadal cycle

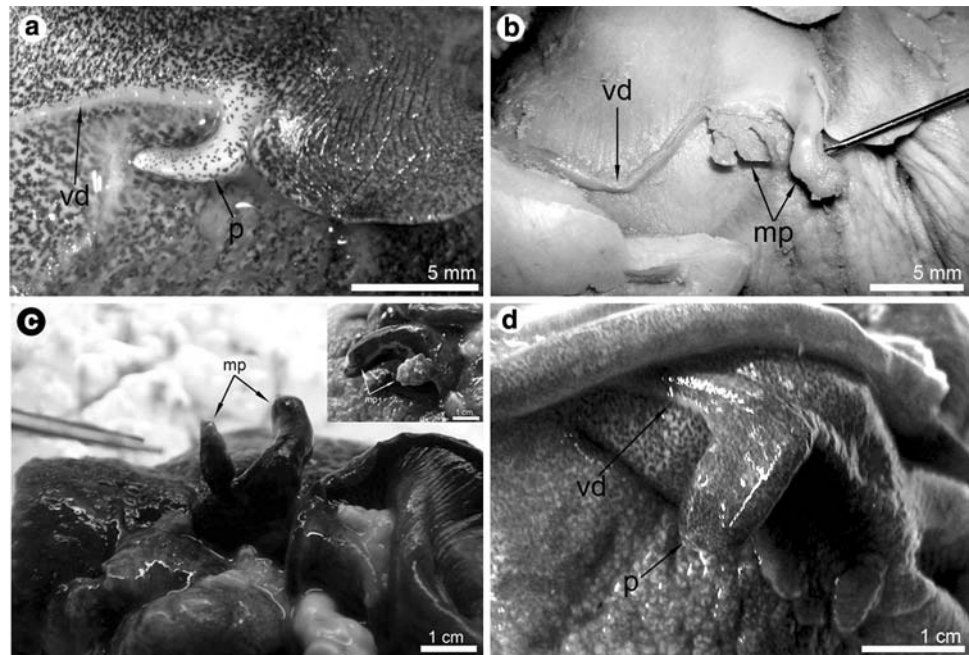
The greatest gametogenic activity in females takes place from June to November. Despite the large amount of proliferating and growing oogonia, few mature oocytes are evacuated. This phenomenon could explain the fact that the number of embryos registered inside each egg capsule is also low (2–8 embryos) (Penchaszadeh et al. 1999). Protection of eggs inside a capsule could be a strategy that permits the increasing of embryo survival, and diminishes of energy requirements. Oocytes that do not develop to the mature size (>180 μm) are probably resorpted and the energy reutilized. This seems likely because the resorption stage occurs throughout almost the entire year, with the most intensive period occurring during and after the oviposition season. The presence of proliferating oocytes all year long in *A. ancilla* denotes the absence of a resting stage in the gametogenic activity, as occurs in *O. magellanica* (Bigatti et al. 2008); while other South American volutids, such as *Adelomelon brasiliensis* and *Zidona dufresnei* (Giménez and Penchaszadeh 2002; Cledón et al. 2005) enter into a resting stage.

In males of *A. ancilla*, the gametogenic activity occurs all year round. Spermatogenesis is evident throughout all the studied period, and every cellular stage can be observed during this process. Sperm are released every month; however, during the oviposition season this process is more intensive. It could be stimulated by the presence of egg laying females in the population as occurs in *Z. dufresnei* (Giménez and Penchaszadeh 2002), but must be tested in

Table 1 Imposex results from sampling sites

Sampling site	Description site	Imposex mean % \pm SD	RPLI	Total (N)	Females (N)
Parque Piedras	Heavy marine traffic (harbour traffic)	92.29 \pm 13.78	24.35	76	40
Punta Este	Intermediate marine traffic (recreational marine traffic)	44.63 \pm 19.19	5.56	108	59
Paraná Beach	Low marine traffic	0	0	150	81

Fig. 9 Specimens of *Adelomelon ancilla*. **a** Female with the presence of the *vas deferens* (*vd*) and the penis (*p*). **b** Female with the occurrence of multiple penises (*mp*). **c** Male with double penis. *Inset* Detail of the double penis. Note the papilla of the neoformation. **d** Male without imposex showing a normal penis to compare with the imposexed female penis



A. ancilla. The resorption stage in males happens only in autumn and in October, after the highest peaks of evacuation of spermatozoa.

Imposex

Our results reveal the existence of imposex in *A. ancilla* in two different sites in Golfo Nuevo. The incidence of imposex in the Parque Piedras site is likely to be related to the TBT concentrations registered in the harbour zone (1.7 ng Sn/g) with high shipping traffic, a factor that has been discussed in several papers for other neogastropods (Bech 2002; Bryan et al. 1986; Gibbs and Bryan 1994; Maguire et al. 1986; King et al. 1989; Krone et al. 1989; Shim et al. 2002). This finding is supported by other studies of imposex performed in this area (Bigatti and Penchaszadeh 2005; Bigatti and Carranza 2007; Bigatti et al. 2009) and by studies of other volutids from Argentina (Cledón et al. 2006; Goldberg et al. 2004). Although we did not measure TBT concentrations in Punta Este, this site showed a lower incidence of imposex than the harbour zone, which may be attributable to its lower exposure to marine traffic, as stated in other studies (Bech 2002). Different sensitivity to TBT was registered in *Nucella lapillus* and *Littorina littorea* (Oehlmann et al. 1998). Though no gonad structure alterations were registered histologically in this work, the neoformation of multiple penises in females and males may indicate a higher sensitivity of *A. ancilla* in comparison to other snails studied in Golfo Nuevo. *Odontocymbiola magellanica* showed similar imposex percentage and lower RPLI values in Parque Piedras, while *Trophon geversianus* presented low imposex % and RPLI (Bigatti et al. 2009).

The high sensitivity of *A. ancilla* could be used as a way to infer TBT pollution in the zone, without depending on direct TBT concentration analysis.

Fishing recommendations

A. ancilla and other volutids species are being consumed locally in north Patagonia, and the stocks present in some areas of the Chubut Province are compatible with commercial catches (Bigatti and Ciocco 2008). Even though volutids present high sizes and somatic production, its slow growth, the late sexual maturity and a direct development without free-swimming larvae make this group extremely vulnerable to over-exploitation (Bigatti et al. 2007). The volutid *Z. dufresnei* has been exploited for the last 30 years in the northern coast of Argentina with landings of up to 1,300 tons in 1997 and signs of overfishing (Giménez et al. 2005). The whelk *Buccinanops globulosus*, very common in northern Patagonian, is the target of a new fishery in San Matías Gulf (Narvarte 2006). *A. ancilla* is not yet established as a commercial fishery resource in Argentina, but as this potential develops, it is important that the results from the present paper are taken into account when implementing fishery policies.

The most sustainable method of harvesting volutids is through diving; this has a lower impact on the populations as well as their environment (Bigatti and Ciocco 2008). We suggest to capture *A. ancilla* by diving, to take individuals bigger than the minimum mature sizes registered in this work (114 mm for females and 110 mm for males) and to ensure the protection of the egg capsules and the females found (by the observation of the pedal gland) in the

oviposition season. Likewise, studies on the embryonic development, first maturity, number of egg capsules laid per female/year, age and growth, as well as densities of the species compatible with commercial catches are mandatory to determine if the populations can support an artisanal fishery.

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