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Reproductive biology and ecology of *Adelomelon brasiliana* (Mollusca: Gastropoda) off Buenos Aires, Argentina

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SUMMARY

Adelomelon brasiliana is an abundant shallow water volutid, which is distributed from Río de Janeiro, Brazil, to Río Negro, Argentina, SW Atlantic. This species has been commercially exploited in Uruguay by small-scale fishery since the early 90's (Riestra & Fabiano 2000). In Argentina this marine snail is very common but is still not systematically exploited.

At this time there is an almost complete lack of information on the biology, ecology and fisheries biology of these black snails *A. brasiliana*. The reproductive biology, and some of the ecologically important aspects of this predator volutid have been studied at a southern locality off Mar del Plata, Argentina, on the South American Atlantic shelf (38° 20'S; 57° 37'W). Twenty animals were captured each month at a depth of 15 m for a period of two consecutive years (2001-2002).

Gonad samples were analysed using histological techniques. Oocyte diameter was recorded to determine the female gonad development stage. The reproductive season of the population extends from September to April (austral spring and summer), showing synchronization with the water temperature cycle. The yolk content of the oocytes increases up to 200 µm in diameter before spawning. In autumn, a resting phase begins, in which no new oocytes develop, while in the unspawned reabsorption occurs. Gonadic development begins during the early winter when new previtellogenic oocytes can be observed.

Growth, age and somatic production were studied through two different methods: internal shell growth marks, which form annually and are visible by cuts and the stable oxygen isotope record in the shell carbonate, which reflects seasonal oscillations in water temperature. Modal shell length (SL) of the population was 140 mm, while modal shell free wet mass was 255 g. A logistic growth function (SL_∞ = 186.28 mm, K = 0.185, t_0 = 4.601) fitted 131 pairs of size-at-age data (25 shells) best. Somatic production of the individuals (P_i) increased up to 46 g$*$year^{-1} (SFWM) at 145 mm shell length, which occurred in the 12th year of life. *A. brasiliana* has a very long life-span, living for up to 20 years of age.

The size of *A. brasiliana* at first maturity, both in females and males, was established by analysis of gonad tissue samples and secondary characteristics. Minimum size for gonad maturity for females was 107 mm, and for males 102 mm in shell length, whereas 50% of the females were mature at 115 mm and males at 107 mm SL (approx. 7 years of age). All females were mature at 125 mm and all males at 115 mm SL (up to 8 years of age), and both remain with a functional gonad for their whole lives.

Imposex – penis development in females- was found in over 30 % of the females in the sampled period, however the relative penis size index (RPSI) was very low and the functionality of the reproductive system was not affected. Tributyltin (TBT) in soft parts decreased from 46 ng/g to 21 ng/g in the study period, while in the sediment it decreased from 240 ng/g to 1.4 ng/g TBT, indicating a major pollution event before the start of the study.

A. brasiliana is a species with a long life span, slow growth rate and long maturation size. These features make this species very vulnerable to exploitation. Accurate management rules are needed before starting commercial exploitation. *A brasiliana* is also very sensitive to pollution.

ZUSAMMENFASSUNG

Adelomelon brasiliana ist eine häufige Flachwasserschnecke der Familie Volutidae, dessen Verbreitungsgebiet sich von Río de Janeiro, Brasilien, bis zum Río Negro, Argentinien, im SW Atlantik, erstreckt. Während ihre Bestände in Uruguay seit den frühen neunziger Jahren durch die Fischerei kommerziell ausgebeutet werden (Riestra & Fabiano 2000), wird diese marine Schneckenart trotz hoher Abundanzen in Argentinien bisher nicht systematisch befischt. Biologie, Ökologie und Fischereibiologie von *A. brasiliana* sind weitestgehend unbekannt.

Im Rahmen der vorliegenden Arbeit wurden die Reproduktionsbiologie sowie einige wichtige ökologische Aspekte dieses räuberischen Volutiden an Exemplaren von der argentinischen Küste (Mar del Plata, 38° 20'S; 57° 37'W) untersucht. Über zwei Jahre hinweg (2001-2002) wurden jeden Monat etwa zwanzig Tiere in einer Tiefe von 15 m gefangen. Gonadenproben wurden durch histologische Verfahren analysiert. Der Durchmesser der Oozyten wurde bestimmt, um die weibliche Gonadenentwicklungsphase zu ermitteln. Die Reproduktionsphase der Population erstreckte sich von September bis April (Frühjahr und Sommer auf der Südhalbkugel) und zeigt eine Synchronisation mit dem Wassertemperaturzyklus. Unmittelbar vor der Eiablage erreicht der Durchmesser des Eidotters der Oozyten einen Wert von bis zu 200 µm. Im Herbst beginnt eine Ruhephase, während der keine neuen Oozyten gebildet werden. Nicht abgelaichte Eier werden reabsorbiert. Erst zu Beginn des Winters setzt die Gonadenentwicklung mit der Produktion von praevitellogenen Oozyten von neuem ein.

Wachstum, Alter und somatische Produktion wurden mit zwei verschiedenen Methoden untersucht:

1.) Auswertung jährlich entstehender Wachstumsringe in den Schalen der Tiere, welche durch Schalenschnitte sichtbar gemacht werden können.

2.) Analyse stabiler Sauerstoffisotope im Schalenkarbonat, die jahreszeitliche Oszillationen in der Wassertemperatur reflektieren.

Die modale Schalenlänge (SL) der mit einer Maschenweite von 70 mm befischten Population betrug 140 mm, während das modale Gewicht ohne Schale (SFWM) 255 g betrug. Das Erreichen der Geschlechtsreife wurde bei weiblichen und männlichen Individuen von *A. brasiliana* anhand von Gonadenproben und sekundären Kennzeichen bestimmt. Die Mindestgröße für die Gonadenreife weiblicher Schnecken war 107 mm und für männliche 102 mm. Bei einer Schalenlänge von 115 mm waren 50% aller weiblichen Tiere geschlechtsreif,

bei 107 mm 50% aller männlichen (ungefähr 7 Jahre alt). Alle weiblichen *A. brasiliana* waren reif bei einer Länge von 125 mm und alle männlichen bei 115 mm (ca. 8 Jahre alt). Die Gonaden beider Geschlechter bleiben für den Rest des Lebens aktiv.

Imposex – Penisentwicklung bei weiblichen Schnecken - wurde in über 30 % der Exemplare im Untersuchungsgebiet gefunden, der relative Penisgrößenindex (RPSI) war jedoch sehr niedrig, und die Funktionsfähigkeit des Reproduktionssystems wurde nicht beeinträchtigt. Die Konzentration von Tributylzinn (TBT) in den Weichteilen der Schnecke nahm im Laufe des Untersuchungszeitraumes von 46 ng /g auf 21 ng/g ab. Gleichzeitig sank die Konzentration im Sediment von 240 ng/g auf 1,4 ng/g. Diese Ergebnisse deuten auf hohe TBT-Werte vor Beginn der Studie hin, die möglicherweise auf Sedimentbewegung beim Strandausbau zurückzuführen.

A:brasiliana ist eine Spezies mit einer hohen Lebenserwartung, verlangsamter Wachstumsrate und später Reife. Diese Kennzeichen machen die Art sehr anfällig für Ausbeutung. Genaue Richtlinien sind notwendig, bevor mit der gewerblichen Nutzung begonnen wird. *A. brasiliana* regagiert außerdem sehr empfindlich auf TBT-Verschmutzung.

1. INTRODUCTION

1.1. THE FAMILY VOLUTIDAE

Unlike other evolutionary branches of gastropods, neogastropods have not invaded the land and only few have penetrated into fresh water, but they have adapted to almost every marine environment (Ponder 1973).

Different authors placed neogastropod origin between the late Mesozoic and early Cretaceous. Knight et al. (1954) indicated that they have derived from the extinct Subulitacea in the Mesozoic, this superfamily divided into Muricacea, Buccinacea and Nerineacea. The latter one gave rise to Volutacea and Conacea, so the four recent superfamilies existed in the early Cretaceous (Sohl 1964).

Ponder (1973) suggested that the cretaceous neogastropods arose from an archeogastropod, because they have many anatomical similarities.

The neogastropods (Fig.1) are characterized by an elongated siphonal canal of the shell. The order Neogastropoda is divided in two suborders, the Toxoglossa and the Rachiglossa (radular denticles disposed in lines of 2 or 3, respectively), which has divided in three superfamilies, Buccinacea, Muricacea and Volutacea (Fig. 1).

Etymology of Volutidae: from Latin *voluta*, from feminine past participle of *volvere*, to turn, roll.

Classification
Class: Gastropoda
Subclass: Orthogastropoda (or Prosobranchia)
Superorder: Caenogastropoda
Order: Neogastropoda (or Stenoglossa)
Suborder: Rachiglossa Gray, 1853
Superfamily: Volutacea Rafinesque, 1815
Family: Volutidae Rafinesque, 1815

The Volutidae are a large family of extremely diverse gastropods (Fig.1). There are approximately 43 genera and more than 200 living species. The family inhabits seas worldwide particularly in the southern hemisphere from shallow to abyssal depths, though most live and burrow in sand in sublittoral areas (Darragh & Ponder 1998).

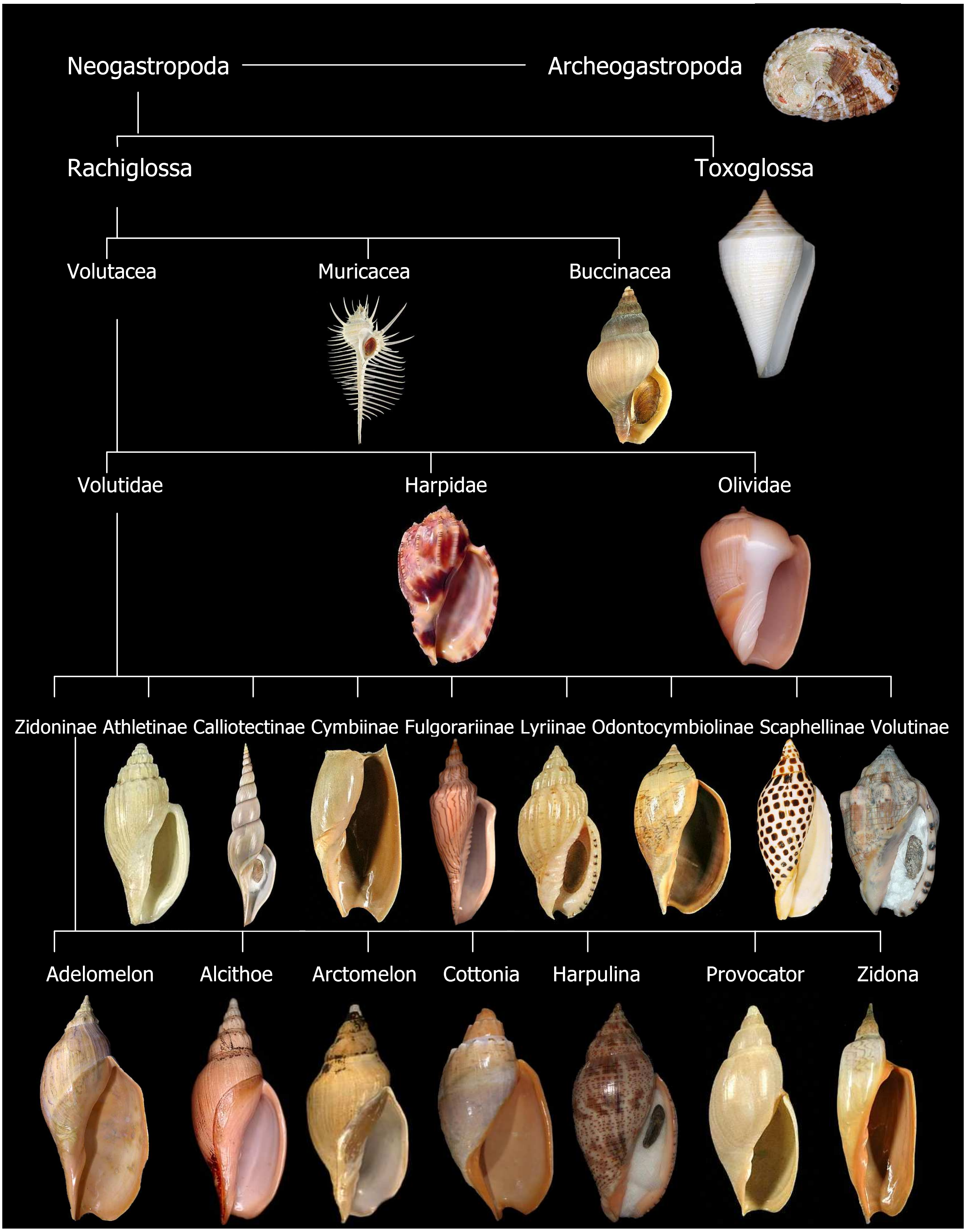

Figure 1: Taxonomical classification of Neogastropoda with representative examples of each group. Species and picture origen from up to down: Archeogastropoda (*Haliotis unilateralis* from Femorale), Toxoglossa (*Conus atractus* from Femorale), Muricacea (*Murex pecten* by G. & Ph. Poppe), Buccinacea (*Pareuthria plumbea* by G. & Ph. Poppe), Harpidae (*Harpa Harpa* by A. Pina), Olividae (*Olivancillaria urceus* by K. Sangiouloglou), Athletinae (*Athleta gilchristi* by G. & Ph. Poppe), Calliotectinae (*Calliotectum tibiaformis* by G. & Ph. Poppe), Cymbiinae (*Cymbium cymbium* by G. & Ph. Poppe), Fulgorariinae (*Fulgoraria ericarum* by G. Sangiouloglou), Lyriinae (*Lyria beauii* by G. & Ph. Poppe), Odontocymbiolinae (*Odontocymbiola magellanica* by G. & Ph. Poppe), Scaphellinae (*Scaphella junonia* by A. Dickson), Volutinae (*Voluta musica* from Jacksonville Shell Club), Adelomelon (*A. beckii* by M. Yamada), Alcithoe (*A. lutea* from Femorale), Arctomelon (*A. steansii* from Atlantic Shells), Cottonia (*C. nodoplicata* by P. Granja), Harpulina (*H. lapponica* by P. Granja), Provocator (*P. pulcher* by G. & Ph. Poppe), Zidona (*Z. dufresnei* by G. & Ph. Poppe).

They are mostly carnivorous, feeding on molluscs and other small marine invertebrate animals, which they envelop with their foot (Morton 1986).

The volutes vary from 9 to 500 mm in shell length. The shell is dextral, ovate to fusiform, sometimes involute, coloured or with elaborated patterns and often coated with a smooth, highly polished glaze, or more rarely having a periostracum. The protoconch is generally smooth, sometimes reaching three whorls.

The aperture (Fig. 2) is elongate and large, usually about the third of the shell length and protrudes into a short but well-defined anterior canal. Most have both, a short deep siphonal canal and a canal at the top of the aperture.

Columellar plaits (or folds) are usually present in variable numbers, generally between three and five.

The mantle, siphon, proboscis and foot are highly coloured, sometimes complementing the pattern of the shell.

The foot is broad and large and an operculum is present only in few small sized genera. The head is small, wide and flattened, usually with a large central lobe that in some groups is divided in two, on which are situated the tentacles. When eyes are present, they are small and situated at the tentacles' base. The inhalant siphon is large and overlies the head, with generally two appendages at its base.

The mantle in some genera is capable of externally enveloping the shell.

Spawn varies within the family from solitary horny or calcareous capsules to complex egg masses comprising many capsules. The egg masses are usually attached to hard substrate. All volutids produce large egg capsules containing few embryos (Darragh & Ponder 1998).

The embryos grow, sometimes over several months, developing into crawling juveniles (Penchaszadeh et al. 1999). These juveniles are sometimes very large (greater than 10 mm). In many volutes from South America extravitelline food consists of albumen, having no nurse eggs (Penchaszadeh et al. 1999). There are a few fossil species that have a multispiral protoconch, suggesting pelagic larval development. Direct development in recent taxa has resulted in many locally distinct populations, and often a hazy delineation of species (Darragh & Ponder 1998).

In Argentinean waters, the family Volutidae comprises 5 genera (Adelomelon, Minicymbiola, Odontocymbiola, Provocator and Zidona). There are some of the more appreciated molluscs by shell collectors. The fishery important volutes of Argentina are four species, *Zidona dufresnei, Odontocymbiola magellanica, Adelomelon beckii and A. brasiliana* (Lasta et al. 1998). *A. beckii* is the largest mollusc of these waters, reaching almost 50 cm, while *A. brasiliana* is a very interesting coastal species because of its unattached giant egg capsules.

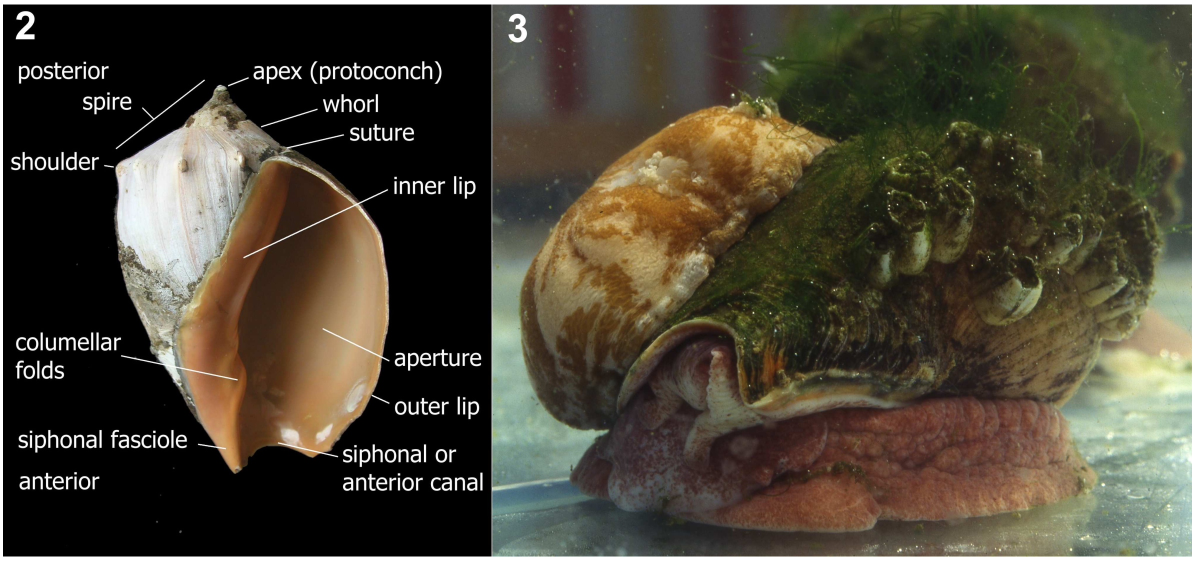

Figure 2-3: 2. Anatomical parts of a marine gastropod shell. 3. Living adult specimen of *Adelomelon brasiliana* with an anemone on its shell.

1.2. *Adelomelon brasiliana*

Family: Volutidae Rafinesque, 1815

Subfamily: Zidoninae A. & H. Adams, 1853

Genus: *Adelomelon* Dall, 1906

Species: *Adelomelon brasiliana* Lamarck, 1811

Common name: "caracol negro" (black snail)

Description: this species is registered in subtropical fossil deposits of the Pleistocene and Holocene eras from Brazil to Patagonia (Aguirre & Farinati 2000). *A. brasiliana* is characterized by a large solid and oval shell, measuring up to 200 mm in length. The shell spire is short and conic with the last whorl being very large and occupying almost the totality of the length. Usually epibiotized by anemones and barnacles (Fig.3), it possesses an external cover that consists of a thick dark brown periostracum, which is the cause of the species common name. When this periostracum is detached, the pale yellowish to pink surface presents strong and irregular growth grooves. Sometimes it has a sculpture composed by tubercles, which remain covered by subsequent shell growth.

The large oval opening occupies approximately 5/6 of the length of the shell, showing the orange-yellowish internal side. The subrect short columella presents from 2 to 4 folds (Fig. 2), being the basal greater and stronger. The shell siphonal channel is short and wide.

Figure 4: Map of zoogeographic provinces modified from Boschi (2000). *Adelomelon brasiliana* is distributed along the Argentinean province (yellow) from Río de Janeiro to Río Negro.

1.2.1. Biogeography

Distribution: This species lives in the southwestern Atlantic Ocean from Río de Janeiro (Brazil) to Río Negro (Argentina) (Ríos 1975) in the zoogeographic Argentinean Province (Fig.4). This province includes geographical features like the Río de la Plata, the dos Patos Lagoon and other estuaries, which influence the species distribution and abundance (Boschi 2000). Moreover, there is a permanent interaction between the cold Malvinas (Falkland) current and the warm Brazilian current (Boschi 2000). Many authors described this area as a transitional zone between the Brazilian and the Malvinas fronts (Fig. 5) (Dana 1853 and Palacio 1982). In this context, *A. brasiliana* inhabits shallow waters from 8 m to 70 m depth (Riestra & Fabiano 2000), appearing often in shallower waters or even on the beach when hundreds of live specimens are washed up by storms.

Bio-ecology: This volutid inhabits sandy - muddy coastal bottoms. It is an infaunal-epifaunal inhabitant, which slids on the substratum half burrowed in it. *A. brasiliana* is a dioic species in which males have a conspicuous penis that can reach over 20 mm in length. Females have

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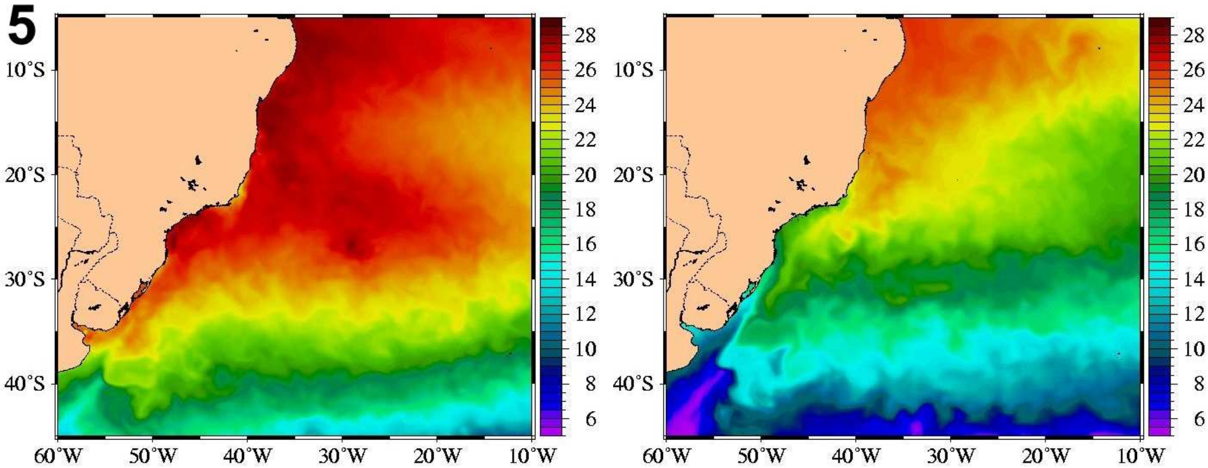


Figure 5: Summer and winter sea surface water temperature (°C) of the southwestern Atlantic –from Naval Research Lab. (NRL) Global Navy Coastal Ocean Model (NCOM)- reflecting the seasonal fluctuation of the Brazil-Malvinas confluence, which defines the limits of the Argentinean province and the distribution of *Adelomelon brasiliana*.

a genital pore used for copulation and spawning of the egg capsules. Individuals of this species can exceed 170 mm in shell length and their shell-free wet weight can be over 300g. *A. brasiliana* is of great scientific interest, because it is the only known volutid laying free egg capsules (Penchaszadeh & De Mahieu 1976, Penchaszadeh et al. 1999). D' Orbigny (1846) mentioned significant abundance of them in San Blas Bay (Province of Buenos Aires, Argentina). In 1889, Dall refered to the presence of egg capsules in outer waters of the Río de la Plata. Carcelles (1944) carried out observations on the egg capsules of the black snail, registering a presence from 5 to 15 embryos per capsule. Penchaszadeh et al. (1999) reported up to 33 embryos, contained in spherical egg capsules of 140 ml in volume, being one of the largest recorded gastropod egg capsules (Fig. 6).

Since all volutids are carnivorous (Darragh & Ponder 1998), it has been supposed that *A. brasiliana* feeds on mussels, but until the present study there have been no studies on its diet.

1.2.2. Fishery

The Chilean muricid "loco", *Concholepas concholepas*, was exported to Asiatic countries to supply the abalone, *Haliotis* spp, demand (Rabí & Maraví 1997). After years of declination of the stocks of this muricid, the fishery was closed during the 80's and early 90's in Chile (Geoghan & Castilla 1987, Bernal et al. 1999). A result of the gradual disappearance of the "loco" in the international market was an increasing interest in the extraction of *A. brasiliana* (Equipo Ecoplata 1996). Under these circumstances, the fishery of *A. brasiliana* reached 150 t per month in Uruguay in 1991 (Riestra & Fabiano, 2000).

Figure 6-7: 6. *A. brasiliana* egg capsule with embryos in advanced development. 7. Capture of a bottom trawl composed by many demersal fishes, *Adelomelon brasiliana*, *Amiantis purpuratus*, prawns and crabs.

This resource was exploited off the coast of José Ignacio (Department of Maldonado, Uruguay) and fundamentally off the coast of the Department of Rocha, Uruguay (close to La Pedrera and Valizas) in a 900 ha area (Equipo Ecoplata 1996). The operation depth range was between 8 m and 12 m. During the exploitation period 49 boats of 7 m length were registered operating on this Uruguayan population. Landings reached an average of 1 t per trip.

In Argentina the species is a by-catch (Fig. 7) of the coastal prawn fishery and usually is returned to the water, so the population is nearly unexploited.

There are studies about the distribution (Testud 1970), anatomy (Novelli & Novelli 1982) and egg capsules of *A. brasiliana* (De Mahieu et al. 1974; Penchaszadeh et al. 2000) but none about its reproductive ecology or environment.

In order to develop control rules for its exploitation and to ensure a long-term sustainable yield, it is necessary to improve knowledge about this species.

1.2.3. Contamination with Organotin compounds (OTs)

An important aspect to be considered in a commercial species is the fact that an external factor can produce a disturbance in the environment that can change the equilibrium of the population. In such a case the population could be reduced and even disappear, if the disturbance is not detected and its effect studied. Coastal species disturbances, which can alter the population ecological traits, are mainly of antropogenic origin. Since the 1960s

Tributyltin (TBT) compounds are used in paints to prevent organisms settling on ship hulls. The antifouling compounds were proven to cause many disturbances in the biota as they leach into the water. TBT has been shown to cause external masculinization of females, termed imposex, through the inhibition of sulphur conjugation of testosterone and its phase I metabolites, resulting in a build-up of androgens in the tissues (Ronis & Mason, 1996).

The application of OTs has been banned in many countries and its use on vessels of less than 25 m in length is forbidden. In Argentina, the law (ordenanza) 4/98 (DPMA) Tomo 6 Anexo 1 prohibits the application of TBT on sport boats.

Many species of molluscs are sensitive to TBT (Langston, 1996) and imposex was described in at least 120 neogastropod species (Schulte-Oehlmann et al, 1997).

Morcillo et al*.* (1997) and Stäb et al. (1996) pointed out that OTs are accumulated in the gastropod hepatopancreas, affecting their reproductive capacity.

On the other hand, the ingestion of TBT can produce an impaired immune response in lower doses (Snoeij et al. 1988), to neural and renal damage (Lin et al. 1998; Lin and Hsueh, 1993).

Many authors have studied the mechanisms through which the tissue damage is produced. Modifications were described on the cytoskeleton (Chow and Orrenius, 1994), disruption of protein synthesis, impairment of mitochondrial activity (Snoeij et al, 1986) and apoptosis (Stridh et al. 1999). Since *A. brasiliana* is a potential product for human consumption it is necessary to investigate the quality of the product. To date there have been no reports of imposex or OTs determination in these gastropods.

1.3. AIM OF THE STUDY

The problematic economical situation of the South American countries induced in the last decade increasing desemployment rates in these countries. There is a need to create new jobs places, but low capital availability to invest in the creation of productive infrastructures for sustainable jobs impedes this development. One viable way to start the reactivation of employment is to extend the use of the existing infrastructures to new production fields. There is no need of an important investment for exploiting *Adelomelon brasiliana*, since it is a bycatch of the Argentinean prawn fishery. There are studies on its quality as a fishery product in Uruguay (Fernández et al. 1999, Fernández 1999), where it is already exploited and on its red tide toxin content in Argentina (Carreto et al. 1996). Its good quality and international demand together with its availability make of *A. brasiliana* an interesting target for the Argentinean coastal fleet that operates in this area.

The aim of this study is to create a solid scientific basis for the management of the potentially exploitable volutid gastropod *Adelomelon brasiliana* in Argentina. For this purpose, their reproductive cycle, individual growth, and size and age at sexual maturity were studied. The pollution level of the environment was also studied in relation to the level of contaminant incorporated in these snails, and how this affects their reproductive capacity.

2. STUDY SITE

2.1. WATER MASSES AND CURRENTS ON THE ARGENTINEAN CONTINENTAL SHELF

The Atlantic Ocean off Argentina presents a great variety of fronts because of the diversity of its water masses. Among many fronts influencing the Argentinean shelf, the more relevant for the present study are the Polar Front (Gordon 1971), the Subtropical Front (Gordon 1989) and the coastal fronts as a confluence of continental water with platform water, e.g. the Río de la Plata Front between 34°S and 37°S (Guerrero et al. 1997). The Sub-Antarctic waters from the northern limit of the Drake Passage and the Malvinas Current provide the principal water volume to the platform. The tidal currents and the bottom influence cause a vertical homogeneity in coastal zones. These zones also present a different temperature and salinity than the rest of the platform because of the input of freshwater from the continent. These water masses were defined as coastal waters (Thomsen 1962), having salinity inferior to 33.7 UPS and a temperature between 6.5 and 21°C depending of the season.

Martos and Piccolo (1988) and Guerrero & Piola (1997) delimited the homogeneous coastal domain and an external domain with the isobath of 40 m. This separation, which usually has been associated to the fishery of the white croaker *Micropogonias furnieri* (Lasta & Acha, 1996), has important biological implications, since it concentrates particulate material and plankton, which increases the productivity of the area in comparison with the rest of the shelf (Podesta & Esaias 1988, Lutz & Carreto 1991 and Negri et al. 1992).

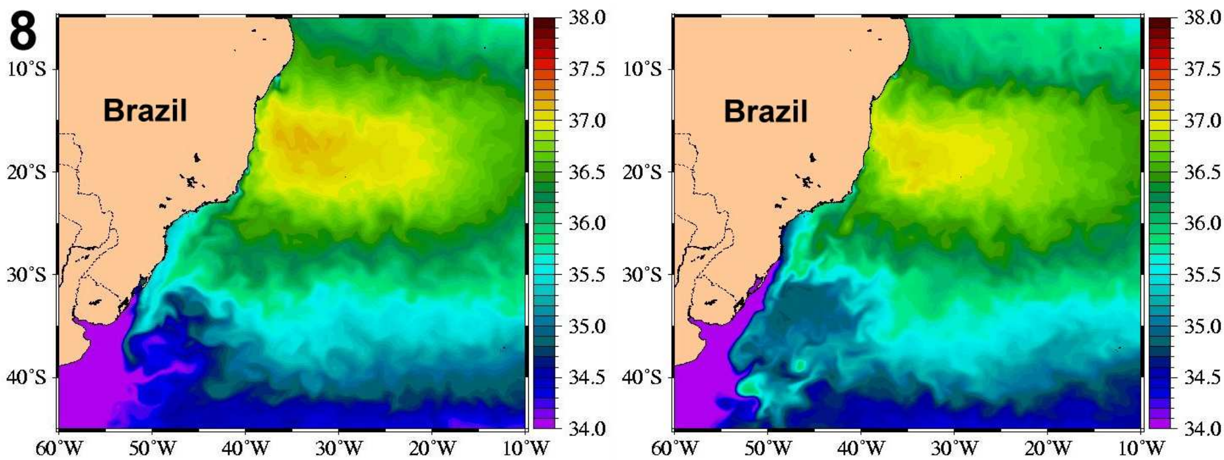


Figure 8: Sea surface salinity in UPS –from Naval Research Lab. (NRL) Global Navy Coastal Ocean Model (NCOM). Left: Summer 24/01/2004. Right: Winter 17/07/2004.

Piola et al. (in press) characterized the influence of the Río de la Plata. They reported variations due to the wind direction. In winter, wind is predominant from SE and diluted waters reach Florianopolis or even further north until Sao Paulo, Brazil (Fig. 8); whereas in summer, with wind direction from SW, the Río de la Plata waters are taken into the sea (Fig.8).

The Río de la Plata discharges between 15 and $20x10^3$ m^3/s and sometimes can duplicate this value (Guerrero et al 1997). At the same time, Lagoa dos Patos, in southern Brazil (32°S), discharges less than 10% compared with the Río de la Plata values (Möller et al 1991).

There are different opinions about the direction and magnitude of the regional currents. Models including the mean wind effect indicate a NNE flux (Fig. 9) with surface velocities of 0.10m/s (Semtner & Chervin, 1992). However the Río de la Plata influence reaches Mar del Plata southwards (Negri et al. 1992, Guerrero et al. 1997). This would be the result of oceanic wind, which forces this circulation up to the 37°S. Balech (1971) and Carreto et al (1995) reported a warm coastal drift SSW during the summer (Fig. 9), reaching the Buenos Aires Province, Severov (1990) called it "Argentinean flux". Antarctic water would be mixed with smaller volumes of subtropical coastal water, which would explain the presence of subtropical plankton in this area (Balech 1971, 1986). Moreover, Boltovskoy (1981) argues

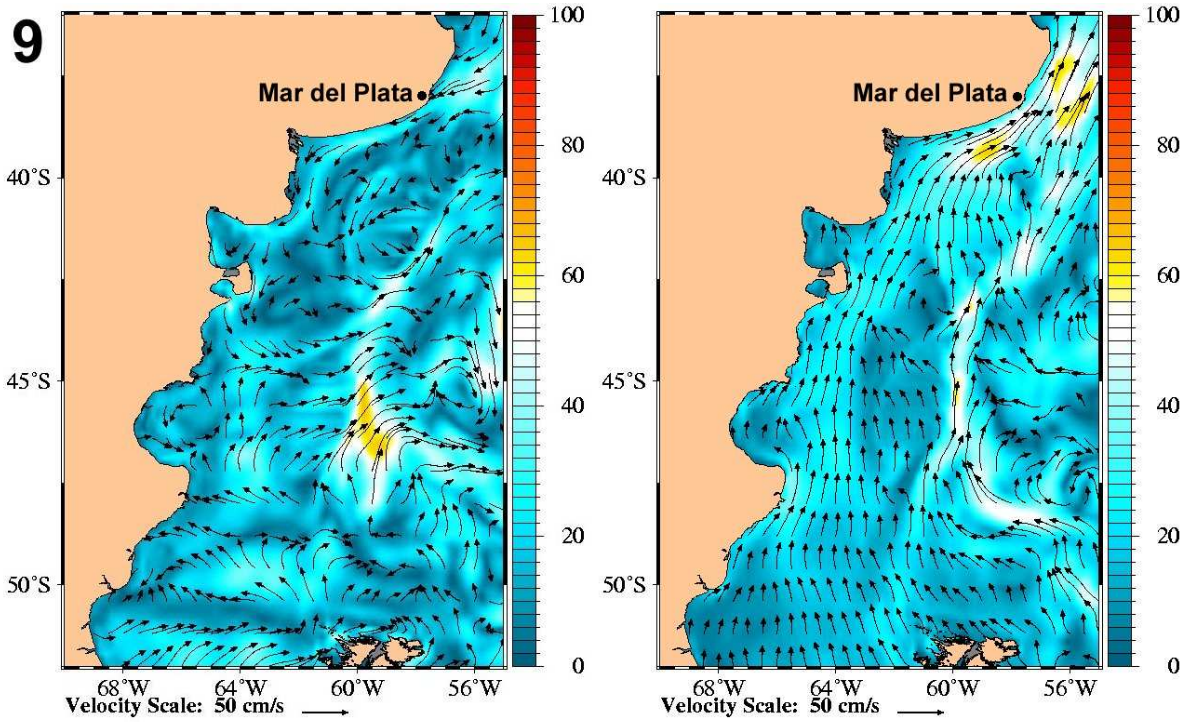


Figure 9: surface currents over speed in cm/s –from Naval Research Lab. (NRL) global Navy Coastal Ocean Model (NCOM). Left: Summer 01/02/2004. Rigth: Winter 15/07/2004.

that the Buenos Aires coastal warm water located above the 40°S originates with seasonal warming.

In summary, most of the biogeographic schemes agree with the presence of a discontinuity around 20-23°S at the eastern coast of South America, which is generally interpreted as a boundary between tropical and subtropical domains (Dadon & Boltovskoy, 1982, Bisbal 1995). This break was already suggested 150 years ago on the basis of SST data (Dana, 1853), and was subsequently confirmed on the basis of floral and faunal data. It is especially noticeable in benthic assemblages where a large proportion of the north-Brazilian species is shared with the Caribbean (Semenov 1978).

At 25-28°S the axis of the Brazil Current moves offshore because of the Malvinas Current (Brandini 1990).

Resgalla 1993 described the migration of the confluence of the Subantarctic and the Subtropical Fronts that reaches 31° S in winter and retracts to 39°S in summer (Fig. 5).

At 35°S, the mouth of the Río de la Plata defines a major hydrographic boundary, forming a dispersal barrier for many species.

The study site is located in the biogeographic province of Argentine (Boschi 2000) where the benthic assemblages show clear relationships with those in Brazilian shelf waters (Boltovskoy 1964). This is interpreted as the result of the summer intrusion of Brazil Current waters, which slip between the main axis of the Malvinas Current and the coast and move southward along the shelf (Boltovskoy 1999). A branch of the Brazil Current forms a coastal tongue with warm water organisms that reach 47°S creating a coastal system (Fig. 5). These two currents and the discharges of the Río de la Plata influence the environment where *A. brasiliana* lives.

The main sampling area was located off Mar del Plata city. After the occurrence of imposex was detected at the beginning of the sampled period, a second sampling place was established 50 km northwards near the Reserve of Mar Chiquita in order to detect possible alterations of the *A. brasiliana* reproductive biology in comparison with the first sampling area.

The Port of Mar del Plata is one of the biggest ports of Argentina, being a potential TBT pollution source. In order to study the pollution level in the area, the port entrance was established as the southern extreme of a coastal transect, whose northern end was located near the Reserve of Mar Chiquita. Five additional sampling places were established between both extremes of the transect (Fig. 10).

3. MATERIALS AND METHODS

3.1. SAMPLING

During a 24 months period between November 2000 and December 2002, about 25 *A. brasiliana* were captured monthly by a 30 min haul with the bottom trawl commonly used by the fishermen of the area (15 m mouth opening, 70 mm mesh size in the cod end) towed at approximatively 15 m depth by a 12 m fishing boat, at 1000 m off the coast from Punta Mogotes to Cabo Corrientes –southeast station-, off Mar del Plata (38°20'S ; 57°37'W), Argentina (Fig. 10). Seasonal samples were also taken during the same period near the Mar Chiquita area in order to detect possible reproductive differences over a short distance.

Surface water temperature was daily recorded at a single point by Dr. Marcelo Scelzo (Universidad Nacional de Mar del Plata) to the nearest 0.1°C with a mercury bulb thermometer.

3.2. MEASUREMENTS AND MATERIAL PROCESSING

Shell length (SL), width (SW), height (SH), and aperture length (AL) and width (AW) were measured (Fig. 11) with a vernier calliper to the nearest mm and soft parts of each individual were weighed as shell-free wet mass (SFWM) to the nearest g. The relationships between

Figure 10: Sampling area off the coast of the Buenos Aires province, Argentina. Stars indicate sampling stations.

the morphometric variables (SL, SW, SH, AL, AW, SFWM) were estimated by linear regression.
Penis length of all individuals (PL) was also registered to the nearest mm. Samples of the medial zone of the gonad were taken, fixed in Bouin solution for 48 hours and processed histologically.

3.2.1. Histological analysis of the reproductive cycle

Gonad samples of individuals with a SL over 130 mm were cut in 10 slides of 5 μm thick, which were stained with Hematoxilin-Eosine to study the monthly gametic development and to measure the oocyte diameters. In order to standardize the measurement for females, 5 oocytes with nucleoli were measured at their maximal diameter. Since neither the female nor the male gonad can be separated exactly from the visceral mass, it was not possible to calculate the Gonadosomatic Index.

The Condition Index (the ratio between soft parts weight and shell weight) was not the best proxy to evaluate the individuals' condition since shell weight oscillates too much among individuals of similar SL. This oscillation is caused by the presence of drilling polychaetes and *Lithophaga* mussels, so the SFWM was recorded throughout the year as an estimator of the animals' condition. The sex proportion and the reproductive condition were statistically analyzed with χ^2, while one-way ANOVA (Sokal & Rohlf 1969) was used to investigate the relationship between female SFWM and their gonadic stage. To minimize interference of somatic weight increase during growth, only females of 130-140 mm SL were used.

3.2.2. Growth and age

Individual growth and age were inferred from internal shell growth marks in 25 shells covering the whole SL range by (i) growth mark identification, (ii) validation of annual growth mark formation, and (iii) quantitative size-at-age determination. To check for internal growth marks, X-ray photographs of shells were done with a Hewlett Packard Faxitron 43855 mammograph with fixed anode, using AGFA-Strukturix D4 FW film and the parameter settings: focal film distance 45 cm, voltage 40 kV, 120- to 240-s exposure time. They revealed macroscopically visible bands perpendicular to the shell growth trajectory. Shells cuts were prepared to count and measure these marks.
Cuts were performed along the whorls following the suture from the apex to the posterior end of the aperture; this is, along the suture growth axis (SG) (Fig. 12). The cut surface was

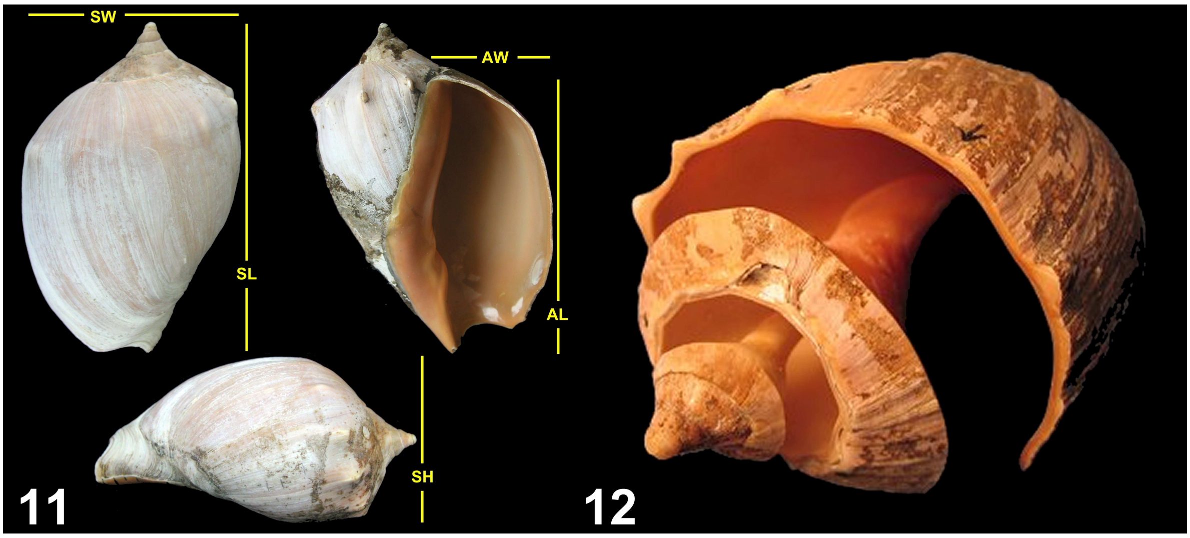


Figure 11 - 12: 11. Allometric measures of *A. brasiliana*. AL: aperture length, AW: aperture width, SH: shell height; SL: shell length, SW: shell width. 12. *A. brasiliana* shell cut through the whorls.

polished with fine-grained sandpaper and checked for growth marks in the three-layered shell by means of a stereomicroscope. The number of each mark along the cut and the corresponding distance from each mark to the shell apex following the SG were measured and interpreted as size-at-age data.

Stable oxygen isotope ratios ($\delta^{18}O$) were used to analyze whether these marks were formed annually, as described recently by Richardson (2001), Giménez et al. (2004) and others. The ratio of the stable oxygen isotopes ^{16}O and ^{18}O in biogenic $CaCO_3$ is inversely proportional to temperature during shell deposition. This relation is nearly linear between 5° and 30°C (Epstein et al. 1951, Epstein & Lowenstam 1953). Therefore, a mollusc shell from an environment with constant salinity and a distinct annual temperature cycle will show an oscillating pattern of $\delta^{18}O$ along the major growth axis (see examples in Santarelli & Gros 1985, Richardson 2001, Giménez et al. 2004). Six representative specimens (3 males of 137, 146 and 148 mm SL and 3 females of 136, 141 and 147 mm SL) of *A. brasiliana* were selected for isotope analysis. From each shell, about 120 samples (approx. 125 μg each) were drilled from the central shell layer at 1 to 4 mm distance along the cut from apex to shell aperture. Shell areas damaged by drilling polychaetes were not sampled.

Stable oxygen isotope composition was determined in the Stable Isotope Laboratory of the Alfred Wegener Institute with a Finnigan MAT251 mass spectrometer coupled to an automatic carbonate preparation device. Results were reported in δ-notation versus PDB (Vienna Pee Dee Belemnite) standard calibrated via NIST 19 (National Institute of Standards and Technology isotopic reference material 19). The precision of measurements was better than $\pm$0.08 ‰ for $\delta^{18}O$, based on repeated analysis of a laboratory-working standard over a one-year period. Spatial coincidence of subsequent $\delta^{18}O$ peaks and subsequent shell growth marks would indicate that one mark is formed each winter.

The iterative non-linear Newton algorithm was used to fit growth models to the resulting set of size-at-age data pairs. A logistic growth model

$SL_t = SL_\infty / (1 + e^{-k * (t-to)})$

was found to fit these data best.

3.2.3. Individual production

Individual annual somatic production P_i (SFWM) was calculated by the mass-specific growth rate method (see Brey 2001) using the size-growth function and the size-body mass relation:

$P_i = M_i * G_i$

where M_i represents mean individual body mass at size *i* and G_i the annual mass specific growth rate at size S_i given by:

$G_i = b * K * \ln (Sl_\infty / Sl_i)$

As samples were collected with a commercial bottom trawl with mesh size of 70 mm, smaller size classes are likely to be severely undersampled. Thus estimates of population production (P) and production to biomass ratio (P/B) based on the sample size distribution would strongly underestimate true values.

3.2.4. Mortality

Total mortality rate Z according to the single negative mortality model

$N_t = N_0 * e^{-Z*t}$

was estimated by a size-converted catch curve (Pauly 1984a,b)

$\ln(N_i/\Delta t) = a + b * t_i$

where N_i is number in size class i, Δt is the time required to grow through this size class, t_i is age at midsize of size class i, and $Z=-b$. This curve was based on the size-frequency distribution of the individuals sampled and the logistic growth function.

3.2.5. Length – frequency distribution

The sampled individuals of all months were plotted together in a size-frequency graphic in order to evaluate the size composition of the studied population.

3.2.6. Size and age at first maturity

The 452 analysed animals were collected during two summers, so all mature individuals presented mature gametes. Gonad samples were taken from the distal end of the visceral mass, fixed 48 hours in Bouin solution, dehydrated and imbibed in Paraplax. The 5 µm sections were stained with Haematoxilin-Eosine.

The percentages of mature females and males were plotted based on frequencies of mature individuals per size class. So the size class with half of the mature individuals is considered an estimate of the length at which 50% of the population becomes sexual mature.

Males penis length (PL) was measured to the nearest 0.1 mm with a vernier calliper, being interpreted as indicative of maturation (Hancock *et al* 1962, Gendron 1992 and Giménez & Penchaszadeh 2003). The size class with 50% of males with mature penis was also established.

Sexual maturation in snails produces a change in the relationship between SFWM and SL, since the reproductive system grows rapidly without a proportional change in size (Demaintenon 2001). According to this, SFWM of females and males was recorded as another criterion to analyse sexual maturity by plotting SFWM vs SL on a logarithmic scale to find the pair of straight lines, which fit the data best (Somerton 1980).

After size at maturation was determined, shells of 10 individuals belonging to this range were aged, based on internal growth marks as described above.

The number of marks and corresponding SL of each individual were recorded to relate them with it corresponding maturate stage.

3.2.7. Trophic relations

Volutids are reported to manipulate their prey with the foot (Morton 1986) so every time an *A. brasiliana* individual was caught while holding an animal in its foot, the species item was registered in order to establish a list of potential preferred prey.

Muscle tissue samples of sympatric species such as the bivalves *Amiantis purpurata, Solen tehuelchus*, the gastropods *Olivancillaria urseus, O. deshayesiana, Buccinanops monilifer,* the prawn *Pleoticus muelleri*, the anemone *Antholoba achaetes*, and *A. brasiliana* were dried at 60°C and ground using a mortar and pestle. Mass spectrometry analysis of nitrogen and carbon isotope content was carried out to determine which species is *A. brasiliana* prey and to compare with the previous observational list.

3.2.8. Imposex and TBT pollution

To study organotin (OT) pollution degree and its effect on the population, the relative penis size index (RPSI) and imposex percentage in *A. brasiliana* females was recorded along 30 months in Mar del Plata. As control, black snail seasonal samples were taken near the Reserve of Mar Chiquita.

Monthly percentage of imposex-affected females was calculated as the number of females with penis and/or vas deferens of all females sampled this month. The PL of living females and males was recorded to the nearest mm. Monthly RPSI was calculated according to the following equation (Gibbs & Bryan 1994)

$$\frac{\text{Female penis mean size}^3}{\text{Male penis mean size}^3} \; 100$$

TBT in *A. brasiliana* and sediment:

In January, July and December 2002, *A. brasiliana* samples were taken near the port. TBT and DBT content in the foot and hepatopancreas of the captured animals were determined by gas chromatography. The analytical procedure was carried out under DIN-Norm 38407-F13 (modified) for water, sewage and mud.

Five individuals per sample were analysed together in order to obtain mean BT values per month. For the 3 months, 1.5 g foot mix and 1.5 g hepatopancreas mix were separately extracted with 20ml methanol, 40 g of a NaCl 10% solution, 40 ml acetic buffer (pH approximately 4.6) and finally 100ml hexane. 250ng of an intern standard of Tetraropyltin, Tripropyltin, Monoheptyltin, and Diheptyltin 100ng/ml was added in order to control the effectiveness of the OTs recover.

The ethylation was carried out by adding 3 times 2ml Na-Tetraethylborate 10% with 30 minutes between each addition. 50ml of supernatant hexane were taken with a 10ml pipette. 5 g Na-sulphate were added to this 50 ml hexane to dry the solution during 60 min. This dry solution was concentrated to 2-3ml in a rotary evaporator.

A clean-up was made in a silica gel column with 15 ml of hexane/acetate 92/8 eluate. The resultant was concentrated again to 1.5ml.

The BT content in *A. brasiliana* egg capsules was also measured on 2 different occasions, one at the early phase of the sampling and one at the last sampling.

The measurement of extracts was done with GC/AED-System. GC: HP6890 gas chromatograph equipped with an Atomic-Emission-Detector HP G2350A with ICP-Plasma. The separation was carried out on a GC-Column 30m HP-5MS 5% Pheny-Methyl-Siloxan, 0.25mm ID coated with 0.25µm film phase. Injection was performed with a Gerstel Cold-

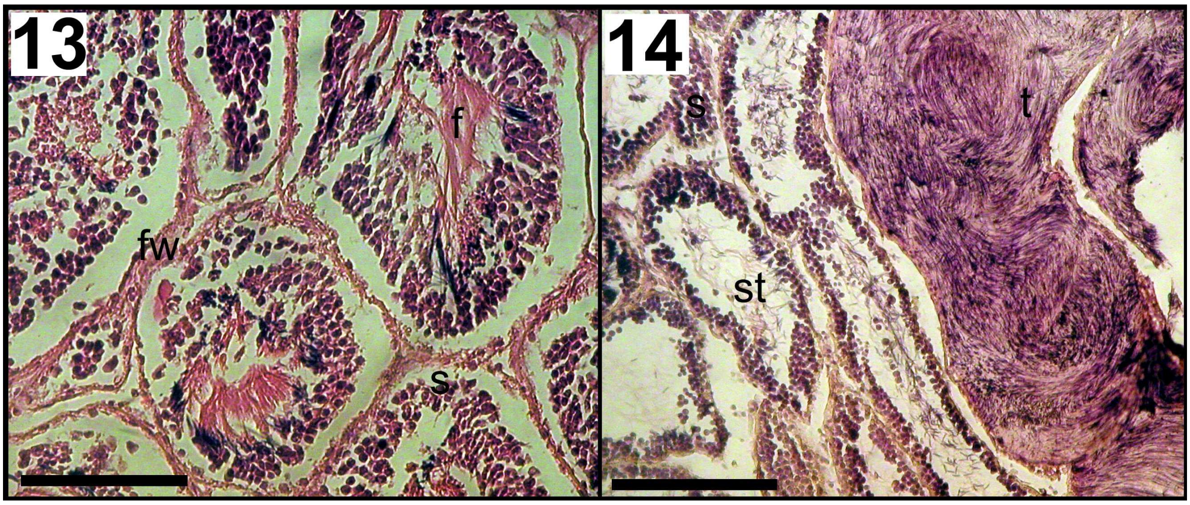
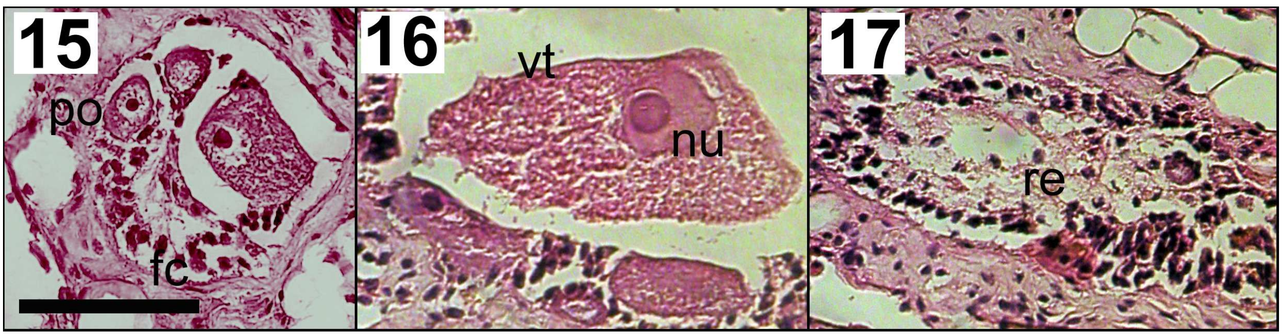


Figure 13 - 14: Male gonad of *Adelomelon brasiliana*. f: spermatozoid flagella, s: spermatids, fw: follicular walls. Scale bar= 100 µm. Figure 12: t: full spermatic tubules. Scale bar= 200 µm.

System at 40°C, separation with temperature program 40-320°C, within 35 min. Helium was used as the carrier gas at a flow-rate of 2.7ml*min^{-1}. The ICP was maintained with H_2, O_2 and Ar/CH_4 and N_2 as make-up gas (2000°C). AED-signals were measured at 303 and 301 nm wavelength.

Because TBT deposited in marine sediment can be bioavailable for years (Strand et al., 2003), samples of sediment were collected with a dredge in January 2001 and December 2002 in five places of a transect parallel to the coast (Fig. 8), limited by the port on the south and the Mar Chiquita coastal reserve on the north. 15 grams of wet sediment were analysed following the same procedure described above.

As a control for the chemical procedure the reference material BCR 646 freshwater sediment (TBT, DBT, MBT, TPhT, DPhT, MPhT certified) was used.



Figures 15 – 17: Female gonad of *Adelomelon brasiliana*. Scale bar= 100 µm. 15. Follicles with developing previtellogenic oocytes (po) and active follicular cells (fc). 16. Mature oocyte with conspicuous vitello (VT) and nucleus (nu). 17. Oocyte undergoing reabsorption (re).

The control for the snails was the reference material BCR 477 certified mussel tissue (Morabito et al., 1998). The detection limits of BTs and PTs were of the order of 0.2ng*g^{-1} dry weight.
The Bioconcentration factor was calculated as the TBT contain in gastropod tissue divided by the TBT contain in sediment (Takeuchi et al. 2001) as a form of comparison with other molluscan species affected by TBT.

4. RESULTS

4.1. REPRODUCTIVE CYCLE

The studied population of the black snail *A. brasiliana* presented a sex ratio not different from 1:1 (χ^2 = 0,79 df=1 p>0,05) during the sampled period. The males are easy to distinguish after shell removal because of their conspicuous penis. The gonads were situated in the distal part of the visceral mass. The ripe testis is dark violet, almost black, while the ovary is light brown and not distinguishable from the hepatopancreas.
Adult males had a fully active gonad all the year round. The colour ranged from beige to dark violet but in all cases the gonads were mature and active. The spermatic tubules contained all developing stages of masculine reproductive cells (Fig. 13). The spermatic ducts exhibited large amounts of spermatozoids throughout the year (Fig. 14). After gamete liberation the tubules remained almost empty. This liberation did not occur in synchrony for all individuals.

The female gametic cycle could be divided into five stages

- i- During the first growing stage, females developed oocytes along the germinal epithelium from the end of May until August (winter) (Fig. 15). The connective tissue was thick with many cell layers.
- ii- At the mature stage, cells were full of yolk measuring over 100 µm in diameter and the connective tissue remained well developed (Fig. 16).
- iii- During the first spawning stage, between September and October, many oocytes were released, the female gonad showed some empty follicles and new developing oocytes with diameters under 80 microns appeared.
- iv- In January a second spawning stage started. The presence of mature oocytes, however, remained low, the ovary appeared half empty, and some oocytes in previtellogenic and vitellogenic stages as well as reabsorption were observed.
- v- The reabsorption stage occurred from February to April, and depended on the water temperature. After releasing some more oocytes, many follicles were empty

and the few remaining oocytes underwent reabsorption (Fig. 17), so that the gonad presented fully empty follicles with thin connective tissue and some yellow residuals. Subsequently, new previtellogenic oocytes appeared, starting the active stage (i) of the following cycle.

From the histological analysis the female proportion in each developing stage was obtained (Fig. 18). It was found that the spawning presents a short peak in early spring followed by a sustained spawning period during summer.

No differences were observed between the populations of Mar del Plata and Mar Chiquita.

The SFWM for males showed variations during the study period reaching the maximum always at the end of winter, when the water temperature started to rise and in mid summer, when the water temperature reached its maximum. The SFWM minimum was reached at the beginning of summer and in autumn, when the water temperature was approximately 18°C (Fig. 19). The female SFWM showed a pattern similar to the males, maximum SFWM being reached at the end of winter. After a short recuperation in early summer, a new loss of weight occurred reaching a minimum in autumn (Fig.20).

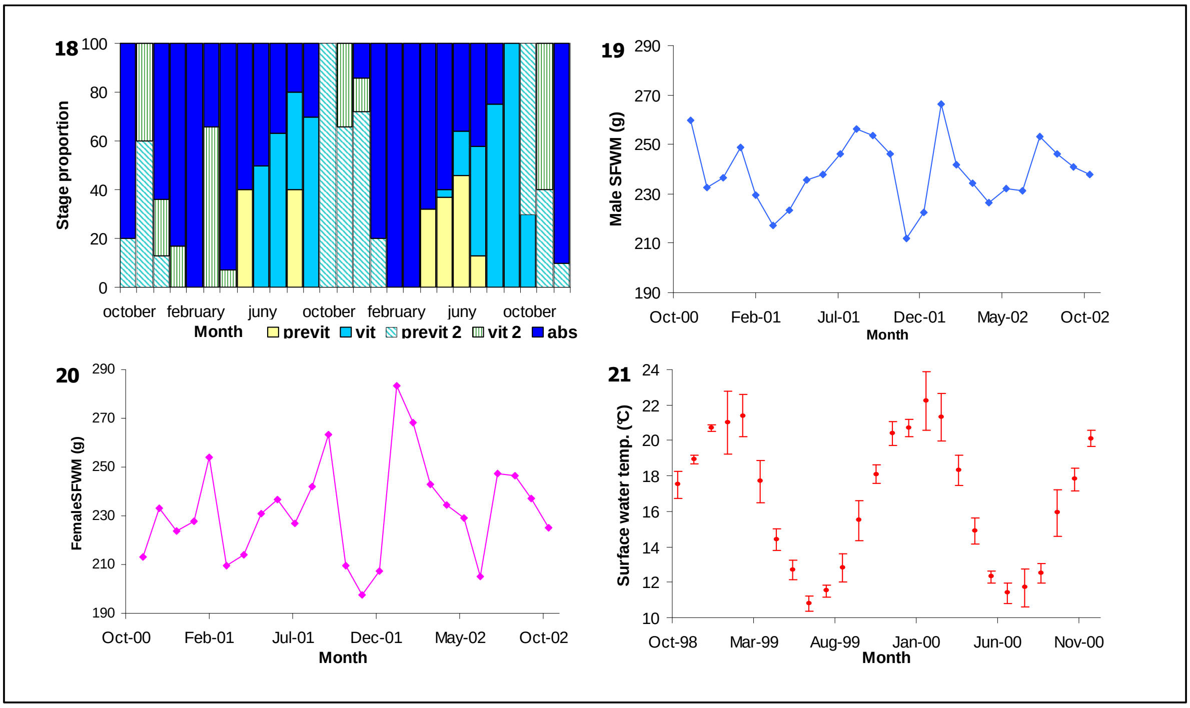


Figure 18-21: *Adelomelon brasiliana*, seasonality of reproductive cycle and surface water temperature 18. Female gonad stage. Each percentage obtained measuring 50 oocytes of each female of each sample. Previt: previtellogenic, Vit: vitellogenic, Previt2: second stage of oocyte production, Vit2: second spawning period, Abs: stage of reabsorption of sexual products. 19. Shell free wet mass (SFWM) of 130-140mm SL males. 20. SFWM of 130-140mm SL females. 21. Monthly water temperature (°C) during two years in Mar del Plata.

The one-way ANOVA indicates that the gametic cycle of females was related to the SFWM ($F=7.31$, $p<0.02$, $N=56$), which increased twice yearly (Fig. 18) in March and November 2001, and in June 2002. Both factors seem to be regulated by the water temperature cycle of the area (Fig. 21) with a maximum from December to March, the austral summer and a minimum from July to August, the austral winter.

4.2. INDIVIDUAL GROWTH AND PRODUCTION

4.2.1. Allometric morphometry

The 500 animals collected ranged from 34 mm to 174 mm shell length, modal length was about 140 mm SL.

Shell height (SH), width (SW), aperture width (AW) and aperture length (AL) were linearly related to shell length (SL):

$SW = 0.664 * SL - 3.618$; $r^2 = 0.979$; N= 500

$SH = 0.503 * SL – 1.304$; $r^2 = 0.979$; N=500

$AW = 0.4081 * SL – 7.2874$; $r^2 = 0.972$; N=500

$AL = 0.8468 * SL – 5.1959$; $r^2 = 0.976$; N=500

Shell free wet mass (SFWM) ranged from 1.4 g to 400.2 g, modal mass was about 255 g. SL and SFWM were exponentially related:

$Log(SFWM) = 3.5622 * log(SL) - 5.2008$; $r^2 = 0.971$; N= 500

Growth trajectory length SG, that is, the distance from apex to measurement point along the growth spiral was linearly related to SL:

$SG = 2.827 * SL + 2.728$; $r^2= 0.924$; N= 131

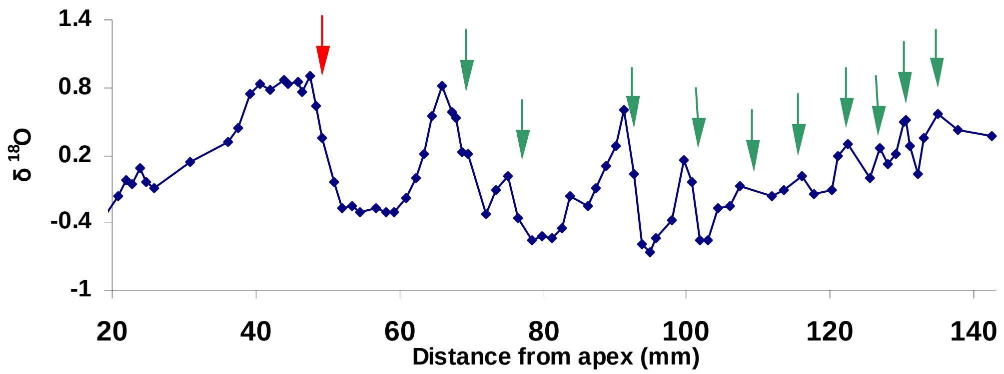


Figure 22: $\delta^{18}O$ profile related to shell length in an *A. brasiliana* of 144 mm SL, 12 years old. Peaks correspond to temperature minima (winters) and troughs to temperature maxima (summers). Green arrows indicate position of visible growth marks, while red arrow indicates first, non-visible internal growth mark.

4.2.2. Age and growth

The $\delta^{18}O$ profiles showed a distinct oscillating pattern in all six shells analysed (Fig 22). According to the palaeotemperature equation of Epstein & Lowenstam (1953), the amplitude of about 1 ‰ corresponds to a temperature range of >= 4 °C which is in the range of the annual cycle observed in the investigation area (Fig.21) (Guerrero et al. 1997). The distinct dark orange coloured shell growth marks visible in the shell cuts were situated closely to the maximum $\delta^{18}O$ values (Fig.22), that is, one mark is formed during each minimum temperature period in winter.

Apparently no visible growth band is formed during the first winter, which is, however, clearly identifiable by the first $\delta^{18}O$ peak (Fig.22). While getting older, *A. brasiliana* individual growth turns asymptotic and growth marks can be difficult to differentiate of each other (Fig. 23), so in old individuals age can be underestimated. The oldest individual found was at least 20 years old.

The logistic growth model fitted best the 131 size-at-age data pairs obtained from the 25 specimens analyzed (Fig. 24):

$S_t = 186.28 / (1 + e^{-0.185 * (t - 4.601)})$; $r^2 = 0.924$; N = 131

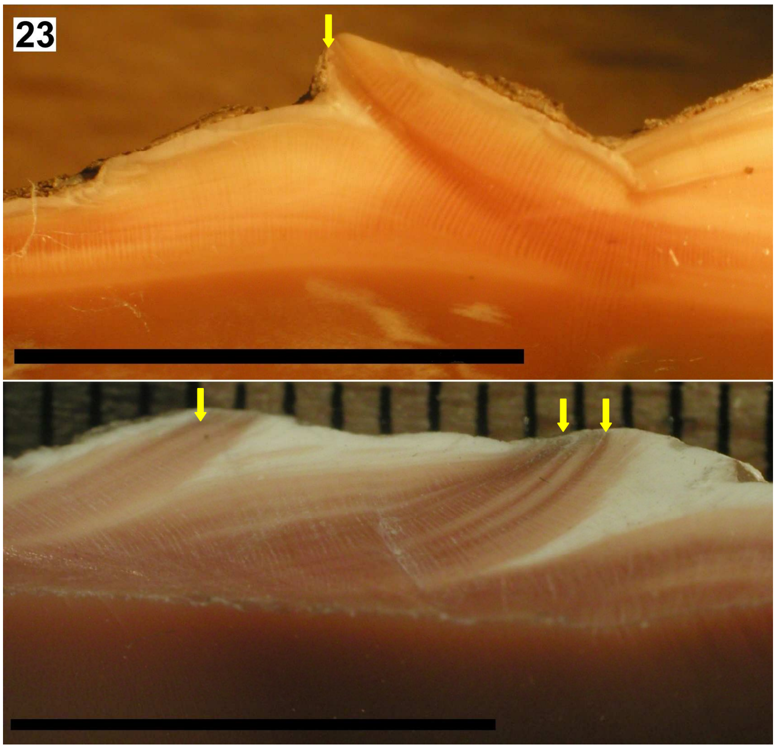


Figure 23: *A. brasiliana* growth marks (arrows) of young adult (12 years) and old adult (19 years). Scale bar = 10 mm.

4.2.3. Individual production

Maximum individual somatic production (P_i) amounts to 46.1 g SFWM year^{-1} at 145 mm SL (Fig. 25).

The size converted catch curve (Fig. 26) does not show a straight descending right arm, i.e. the single negative mortality model does not fit the data and hence a mortality rate Z cannot be calculated.

4.3. FIRST MATURITY SIZE AND AGE

No significant difference between years was found for the gonadic size at first sexual maturation of the population (χ^2 = 24,127), so all data were pooled together.

Females start the maturation process at 107 mm SL and 90 g SFWM. Ovaries of specimens under this SL presented no oocytes or oogenic stages.

Plotted data show that 50% of females are mature at 115 mm SL (Fig. 27). After this size, the proportion of mature females increases so rapidly that all of them were sexually mature at 120 mm SL and around 160 g SFWM.

Male gonads did not show any signs of maturation until the animals reached 102 mm SL and 96 g SFWM. A specimen of this SL presented gonad cells with different spermatogenic stages but no spermatozoids were observed. From the plotted data is possible to observe, that 50% of all males were mature at

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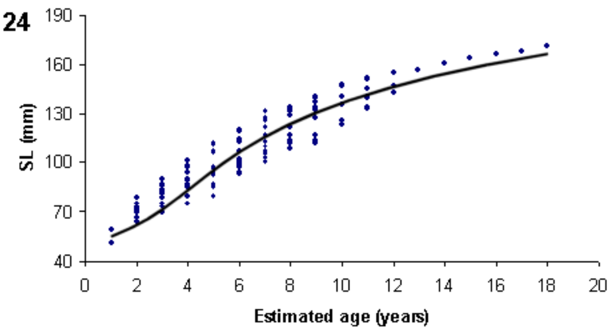

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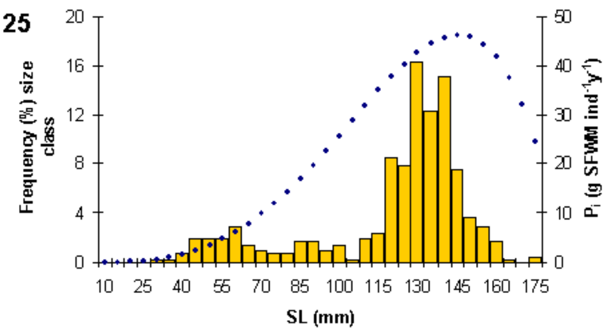
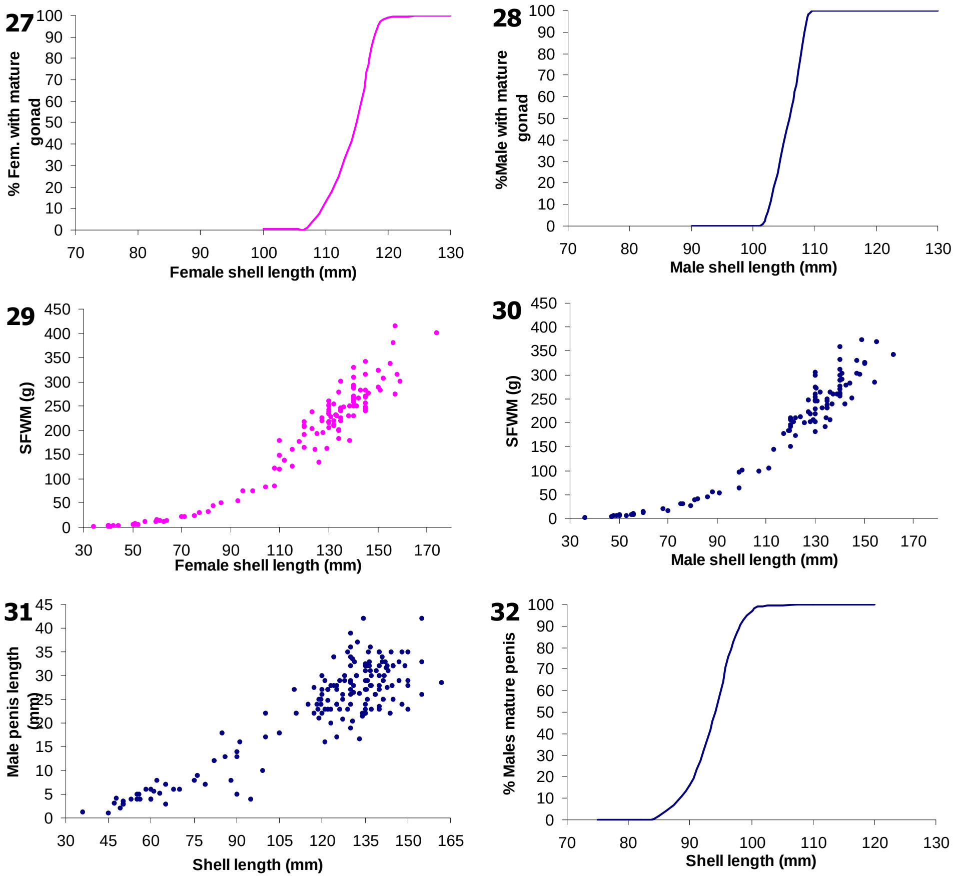


Figure 24 - 26: 24. Logistic growth curve fitted to 131 size-at-age data pairs obtained from 25 cut shells of *A. brasiliana*. r^2=0,92 SL=186,26mm/(1+*e$^{-0,185*(t-4,601)}$). 25. Size-frequency distribution of *A. brasiliana* (N= 960) in Mar del Plata during 2000-2002, using class intervals of 5 mm. Samples caught with a net of 70 mm mesh size. Dots: superimposed curve of individual production P_i (grams shell-free wet mass year^{-1}) versus size in *A. brasiliana*. 26. Size-converted catch curve based on the size-frequency sample (Fig. 25) and the growth function (Fig 24) of *A. brasiliana* solid circles: data excluded from regression.



Figures 27-32: *A. brasiliana*. 27. Percentages of females with mature gonads according to histology criteria plotted against shell length. 28. Same relation for males. 29. Shell free wet mass (SFWM) versus shell length for females. 30. Same relation for males. 31. Male penis length vs shell length. 32. Percentage of males' adult penis per shell length.

106 mm SL (Fig. 28). The totality of the male population was sexually mature at 110 mm SL and around 117 g SFWM.

These data indicate a wider maturation range for females, 13 mm, than males, 8 mm.

From the relation log SL – log SFWM of females, it is possible to divide the function in 2, with the split in 82 mm SL (Fig. 29), corresponding to the minimum SL of a female with mature reproductive system.

When the same criterion was applied to males, the split point was at 99 mm (Fig. 30) SL. The smallest male with mature PL was 91 mm SL (Fig. 31). The PL rises rapidly and 50% of mature individuals were recorded at 94.1 mm SL. All the sampled males showed mature penis at 101 mm in SL (Fig. 32).

4.3.1. Growth marks

The analyzed shells ranged from 80 to 130 mm in length and presented between 3 and 8 visible internal marks. Individuals of 110 mm in SL presented 6 marks while the analysis of stable isotopes showed 1 peak of $\delta^{18}O$ that was not optically observable (Fig. 33).

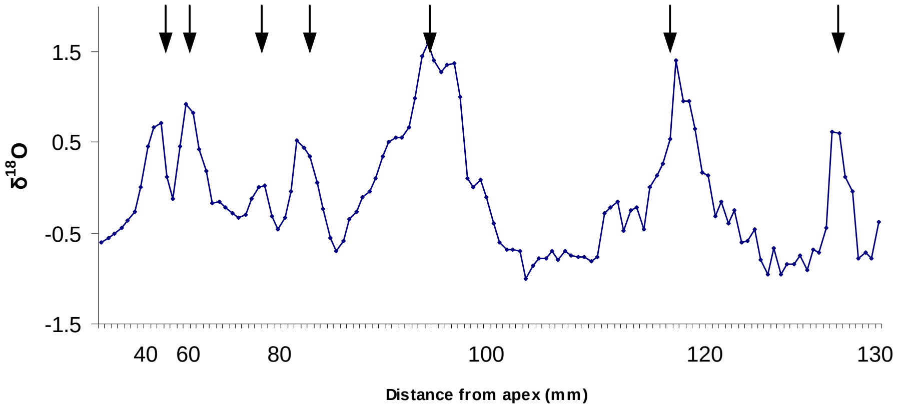


Figure 33: $\delta^{18}O$ profile related to shell length in an *A. brasiliana* of 136 mm SL, 7 years old. Main peaks (arrows) correspond to temperature minima (winters) and troughs to temperature maxima (summers).

4.4. TROPHIC RELATIONS

About 40 *A. brasiliana* individuals were found holding specimens of the clams *Amiantis purpurata* and *Solen tehuelchus* in different degrees of digestion, which were the only two prey items recorded.

The isotopic measurements confirmed that the studied population is feeding mainly on these two species (Fig.34).

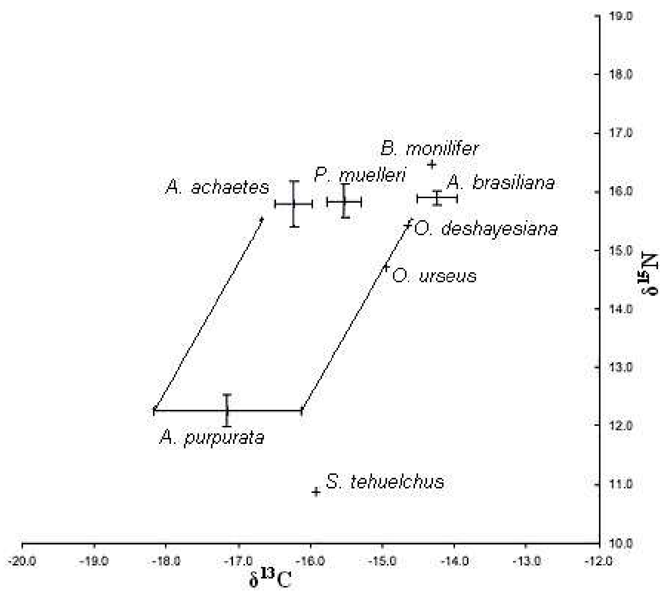


Figure 34: Carbon and nitrogen stable isotope relation of the most common invertebrate species –*Amiantis purpurata* and *Solen tehuelchus* (clams), *Antholoba achaetes* (anemone), *Pleoticus muelleri* (prawn), *A. brasiliana*, *Buccinanops molinifer*, *Olivancillaria deshayesiana* and *O. urseus* (gastropods)- off the coast of Mar del Plata. Lines indicate theoretical enrichment in a trophic level in species feeding exclusively on *A. purpurata*.

4.5. IMPOSEX AND TBT

Imposex affected *A. brasiliana* females were found in the area of Mar del Plata at the begin of the study (Fig. 35). The RPSI fluctuated between 0.18 and 1 while the imposex % was between 38.9 and 50%. The subsequent RPSI and imposex percentages monthly measured for *A. brasiliana* of this area are shown in the Fig. 36.

Figure 35: Example of normal *A. brasiliana* male penis (left) and imposex affected female penis (right).

The hepatopancreas of the analysed animals presented always a higher TBT and DBT contain than the foot (Fig. 37-38). Comparing the TBT and DBT content in the hepatopancreas a gradual increment of the second compound is observed in the successive months in the hepatopancreas, whereas the foot presents an oscillatory pattern.

BTs in egg capsules: The analysed egg capsules contained early and late stage embryos and the TBT content in embryos increased more than in intracapsular liquid (Table 1).

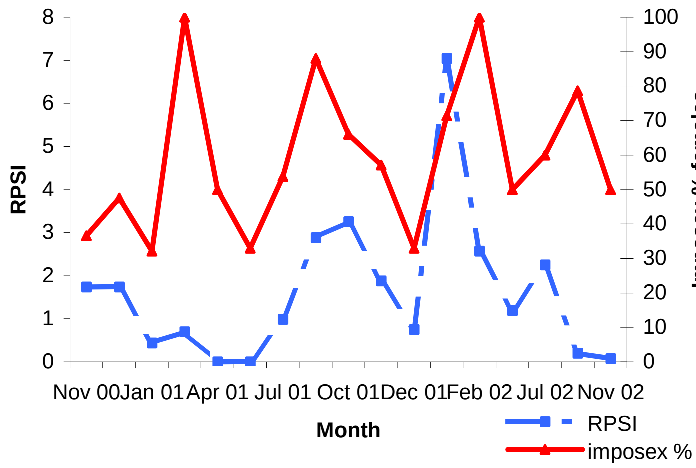


Figure 36: *A. brasiliana* Relative penis size index (RPSI) (stippled line) and percentage of imposex affected females (continuous line) during 2 consecutive years.

BTs in sediment: Sediment samples of 2001 contained 240 ng/g TBT in Mar del Plata, while no TBT was detected in Mar Chiquita's sediment. The analysis of the 2002 sediment samples

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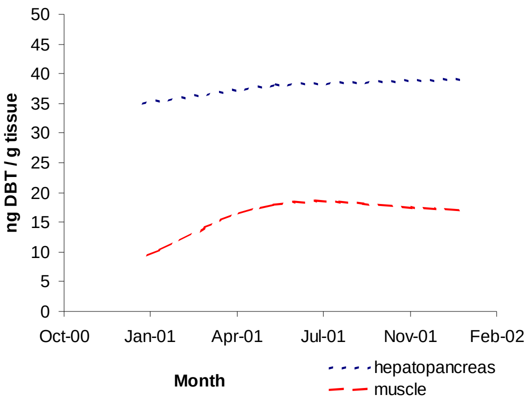

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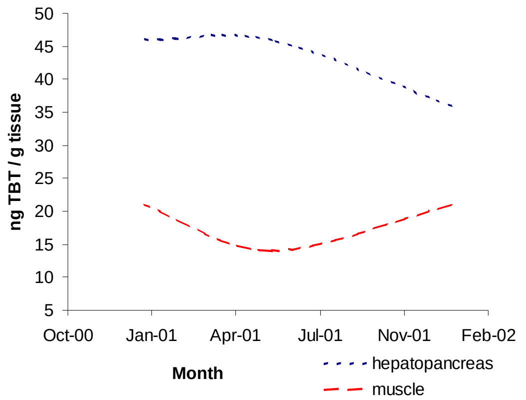

Figures 37-38: DBT and TBT content in hepatopancreas and muscle tissue of *A. brasiliana*.

confirms the gradient of BTs in the environment with a maximum of 2.6 ng/g of TBT in sediment at the port entrance decreasing to 1.2 ng/ at 50 km northwards, in the Mar Chiquita area. DBT presents the same pattern (Fig. 39).

Table 1: *A. brasiliana* egg capsules collected in different months. Development stage and TBT concentration in solid and liquid fractions.

Month	Development stage	Embryos and dispersed solids (TBT ng/ egg capsule)	Intracapsular liquid (ng/ egg capsule)
January 2001	0	14.2	250
January 2001	0	41.8	437
January 2001	1	390	618
January 2001	3	1180	683
January 2001	3	800	548
December 2002	0	-	25
December 2002	3	225	19

39

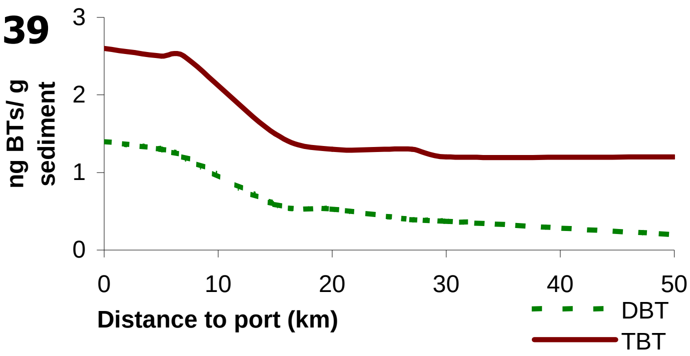

Figure 39: DBT (dots) and TBT concentration in sediment related to the distance to port.

5. DISCUSSION

5.1 REPRODUCTIVE TRAITS

Temperature is an important environmental factor in the regulation of gastropod gonadic development (Giese 1959, Kinne 1963, Fretter & Graham 1964, Giese & Pearse 1974, Kikuchi & Uki 1974, Martel et al. 1986). This seems also to be the case with *A. brasiliana* where the gametic cycle shows a clear seasonality related to the water temperature, coinciding with the observations on the closely related *Zidona dufresnei* (Giménez & Penchaszadeh 2001). Both volutids species are represented in the whole Argentinean Province. This could be related to the seasonal change of the Brazil – Malvinas confluence, which has the preferred reproductive water temperature of 20 °C (Fig. 4 and 5).

Oocytes grow and mature when the water temperature increases from the winter minimum, and spawning takes place when the mean water temperature rises to 17.5°C. The decrease of the mean water temperature terminates this process, and triggers the reabsorption of the remaining oocytes. So gametic activity was observed all year long, as in *Z. dufresnei* (Giménez & Penchaszadeh 2001).

In the population of *A. brasiliana* studied in the Mar del Plata area, no seasonal gonadic maturation cycle was observed in the males. Mature spermatozoids occurred in the spermatic follicles as well as in spermatic tubules throughout the year. This fact contrasts with the reports on other caenogastropods like *Buccinum undatum* having undeveloped testes at the end of winter (Martel et al. 1986) and *Neptunea arthritica* presenting a decrease in testis size prior to spring copulation (Takamaru & Fuji 1981).
In most caenogastropods, gametogenic activity throughout the reproductive cycle of a population is synchronous and can be divided into maturation stages (Giese & Pearse 1977).
In *A. brasiliana* females, five gonadic phases could be defined. Maturation among females occurs synchronously.
Reproduction takes place in spring and summer, when females are ready to spawn. Coinciding with the present results, Luzzatto & Penchaszadeh (2002) reported abundance of *A. brasiliana* egg capsules in nature during the whole year except for the coldest months (June-August) and a massive presence of them in every early spring.

Studies on *Buccinum undatum* from eastern Canada also indicate spawning in spring and summer (Martel et al. 1986, Kusnetsov 1963), however, other studies report spawning of this species in the winter months (Cunningham 1899, Sykes 1905, Havinga 1922, Lebour

1937, Moore 1937, Kristensen 1959, Hancock 1960, 1967, Fretter & Graham 1962, Bruce et al. 1963, Kideys et al. 1993). This duality of the reproductive season has also been reported for the genus Neptunea, e.g. *N. despecta* reproduces in summer (Martel et al. 1986b), while *N. antiqua* reproduces in winter (Pearse & Thorson 1967). Given the wide latitudinal distribution of *A. brasiliana*, it seems possible that populations at lower latitudes also reproduce over winter.

The SFWM seasonal variation is related to the gonadic cycle, but it is not possible to conclude that this SFWM variation is attributable largely to changes in gonad weight. The *A. brasiliana* female gonad is only a thin layer around the hepatopancreas tip. It is more likely that these changes in weight are due to activation of sexual glands, e.g. the albumen or capsule glands in *B. undatum* as mentioned by Martel et al. (1986).

Although I did not directly observe copulation, *A. brasiliana* males are capable of reproduction throughout the year. It is still to be studied if copulation is induced with increasing temperature as reported for *B. undatum* (Martel et al. 1986).

The male SFWM showed a similar, but less defined, pattern as female SFWM. This similarity contrasts with the fact that they do not show a clear seasonal gonadic cycle. Hence the SFWM seems to reflect also a glandular activity change, if so, this population would mate during spring and summer before egg capsules are released.

Concerning the validity of the ageing approach, traditional methods of ageing molluscs such as counting growth marks on/in the shells are not reliable by themselves as they lack a validation of the time intervals at which those marks are formed. In environments with an annual temperature amplitude of several °C stable oxygen isotope analysis can provide such validation, because $\delta^{18}O$ of carbonate shell deposits vary with temperature (Richardson 2001). Changes in salinity can affect $\delta^{18}O$, too (Epstein & Lowenstam 1953), but are negligible in the investigation area (constantly around 33.8 UPS, Guerrero & Piola 1997). Therefore the $\delta^{18}O$ profile in the shell of *A. brasiliana* reflects the water temperature cycle, i.e. each $\delta^{18}O$ peak corresponds to one winter water temperature minimum, thus providing a valid shell age scale.

Data on prosobranch growth are few. To compare them we use an auximetric grid of the index P (Fig. 40). Several authors (e.g. Moreau et al. 1986; Munro & Pauly, 1983; Pauly, 1979) demonstrated the suitability of composite indices for overall growth performance (OGP) for inter- and intraspecific comparisons. The index P is proportional to the maximum rate of body mass increase during lifetime, i.e. the mass increase at the inflexion point of the

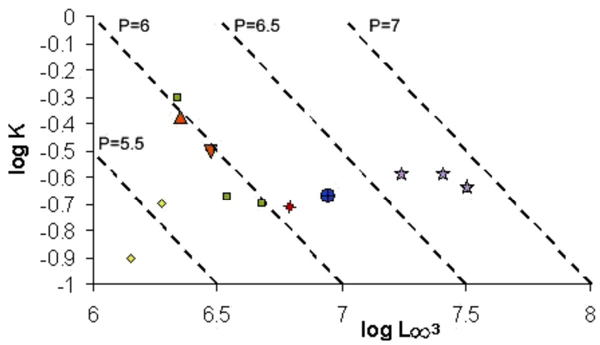

Figure 40: Auximetric grid comparing commercial marine gastropod species. ☆ : *Strombus gigas*, ⊕ : *Zidona dufresnei*, ✦ : *Adelomelon brasiliana*, □: *Concholepas concholepas*, ▽ : *Haliotis laevigata*, △ : *Haliotis rubra*, ◇ : *Buccinum undatum.*

VBGF, since few values of maximum body mass can be found in the literature and maximal mass is proportional to L∞. I calculated P using the obtained K and L∞ of *A. brasiliana* and literature data of another species by P = log ($K{*}L\infty^3$). OGP of Argentinean *A. brasiliana* (P = 6.077) locates near *Z. dufresnei* based on data from Gimenez et al. (2004) for the population living on mussel banks in the same area (P=6.284) and those of *C. concholepas* (P=5.983) based on Geaghan & Castilla (1987) (Fig. 40). The present value for *A. brasiliana* is also in line with P values of Australian populations of *Haliotis rubra* (P=5.967), *H laevigata* (P=5.958) based on Shepherd & Hearn (1983), the muricid *C. concholepas* of the Peruvian population (P=6.041) computed from Rabí & Maraví (1997) and from the Chilean population (P=5.87) from data of Rodriguez et al. (2001). This compilation of commercial species OGP data confirmed that P is species-specific and environmental differences can modify it. Species inhabiting tropical/subtropical regions such as *Strombus gigas* (from de Jesús-Navarrete 2000) show high OGP (P over 6.5). Temperate species have intermediate OGP (5.9 – 6.3), while *B. undatum* show the lowest OGP (Kideys 1996, Santarelli & Gros 1985) (under 5.6), which is a comparatively low performance.

Maximum biomass increase per year of *A. brasiliana* is higher than the maximum *Z. dufresnei* production reported by Giménez et al. (2004) surpassing it at 105 mm SL when *A. brasiliana* reaches 28.7 g SFWM year^{-1}. Rabí & Maraví (1997) reported a specific growth rate of *C. concholepas* of 39.8 g year^{-1} for the range between 30 and 80 mm SL, relating this important increase in biomass to prey consumption and water temperature, but at this size the "loco" is under the minimum catchable size. *A. brasiliana* increase individual production after maturation at a lower rate than this commercial muricid.

Table 2 shows that *A. brasiliana* is a gastropod with a long life span.

Concerning the size at sexual maturity, females appear to mature somewhat slower than males as indicated by both minimum size at sexual maturity and size at which 50% of the population is mature. Other gastropods also show differences between sexes, e.g. *Buccinum* *cyaneum* (Miloslavich & Dufresne 1994) or the closely related volutid *Zidona dufresnei* (Gimenez & Penchaszadeh 2004).

Size at first sexual maturity of *A. brasiliana* is similar to that reported for *Z. dufresnei*. However, it was observed in other gastropods, e.g. *B. undatum*, that size at first maturity can differ among separated areas (Hancock 1967, Martel et al. 1986, Kideys et al. 1993), so the present results on *A. brasiliana* are only applicable to the studied population.

The age at maturation of *A. brasiliana* is also very similar to commercial snails, e.g. *Haliotis rubra,* around 7 years (Prince et al. 1988), and *Z. dufresnei* (Table 2). Other commercial species reach maturity in shorter time, such as *S. gigas* (Table 2) and *C. concholepas* at 30 mm SL (Rodriguez et al. 2001). The two South Atlantic volutids reach sexual maturity very late, which could endanger the stocks if the size of captured animals begin to decrease as reported for *A. brasiliana* in Uruguay (Fernández 1999b) and for *Z. dufresnei* in Argentina (Giménez et al. 2004).

Together with their comparatively slow growth, this makes them quite vulnerable to overexploitation.

Table 2: Size and age at sexual maturity and life span of some commercial marine gastropods from different areas.

Gastropod species	Location	Size at first maturity (mm)	Age at first maturity (years)	Life span (years)	Author
Adelomelon brasiliana	Mar del Plata, Argentina	115	7	20	Present study
Buccinum undatum	Gulf of St. Lawrence, Canada	76	6	12	Gendron 1992
Concholepas concholepas	I, IV, X region Chile[1] Quintay Bay, Chile[2]	30[2]	-	10[1]	Stotz 2000[1] Rodriguez et al. 2001[2]
Strombus gigas	Bermuda[1] Caribbean Sea[2]	220[2]	3.5[2]	7[1]	Wefer & Killingley 1980[1] Orr & Berg 1987[2]
Zidona dufresnei	Buenos Aires, Argentina	120[1]	8[2]	17[2]	Giménez & Penchaszadeh 2003[1] Giménez et al. 2004[2]

5.2. IMPOSEX AND TBT POLLUTION

Regarding the influence of TBT in the reproductive success of the population, the amount of the contaminant in sediments of Mar del Plata in 2002 was already lower than in other port areas of the world such as in Osaka 10 ng/g (Harino et al. 1998), 6 - 520 ng/g in Westeinder Lake, Netherlands (Stäb et al. 1996) and 2.4-18.8 ng/g in Øresund and Kattegat/Skagerrak between Denmark and Sweden (Strand et al 2003).

TBT in sediment produces imposex at concentrations of 1ng Sn/l in *Nucella lapillus* (Gibbs et al 1987) and in *Hinia reticulata* (Storben et al 1992) but only at 7 ng/g in *B. undatum* (Mensink et al 2002). In the studied area, BTs content in the sediment changed from 240 ng_*g^{-1} in 2001 to 1.4 ng_*g^{-1} at the end of 2002 in Mar del Plata and from 0 ng_*g^{-1} in 2001 to 0.2 ng_*g^{-1} in 2002 in Mar Chiquita, indicating degradation and a current transport of the pollutant at the same time.

These BT concentrations induced the development of small penises in females captured near the port, but did not further affect the reproduction since the functional reproductive organs were not modified and gonads of these animals were histologically similar to those of specimens captured in Mar Chiquita, which did not present any signs of imposex.

The DBT contained in the hepatopancreas of the analysed gastropods increased during the last year of sampling reflecting the continuous incorporation and accumulation of the pollutant, which was over 30 times higher than the values for sediments. Such a high concentration in only few trophic levels was already reported in other organisms. Bioconcentration factors (BCF) are one way to compare this phenomenon among different organisms and can be as high as 5×10^4 (Takeuchi et al. 2001) depending on the BT content in the sediment and the species incorporation rate. Comparing all these facts with the reports on other molluscan species (Table 3) the studied population of *A. brasiliana* showed a low BCF.

The adult females of *B. undatum* are less sensitive to TBT than juveniles because they already have a developed genital system (Mensink et al 2002). In the present study, no indication of such an effect was registered since polluted and unpolluted animals reached maturity at the same length and age.

It still not clear how BT's are incorporated. Strand et al (2003) suggested that deposit feeders are ingesting the TBT rich fraction. *A. brasiliana* mainly feeds on *A. purpurata*, a bivalve living in close relation with the sediment. On the other hand Stäb et al. (1996)

Table 3: Bioconcentration factors reported for different molluscan species of different areas.

Species	Location	BCF	Reference
Gastropoda			
Adelomelon brasiliana	Argentina	7.7 – 32.8	Present study
Thais clavigera	Japan	5000 - 10000	Horiguchi et al. 1995
Nucella lapillus	England	29000	Bryan & Gibbs 1991
Bivalvia			
Dreissena polymorpha	Netherlands	20-45	Stäb et al. 1996
Nuculana pernula	Denmark/Sweden	138-404	Strand et al. 2003
Mytilus edulis	Denmark	7400-19000	Zoulian & Jensen 1989
Crenomytilus grayanus	Japan	11000	Suzuki et al. 1998
Mytilus galloprovincialis	Japan	11000	Takahashi et al. 1999

proposed that BTs are passed on primarily via the water. It seems possible that *A. brasiliana* incorporates BT's through both pathways and it should be studied, which one produces a stronger effect.

Mensink et al. (2002) suggested a relatively "clean" egg capsule environment for capsules of *Buccinum undatum*, since embryos developed normally in the capsule but died after hatching in heavily contaminated water. They propose that the female passes the TBT while producing the egg capsule.

The results of this study indicate that the liquid phase of the egg capsules of *A. brasiliana* depends on the TBT content of the environment.

The mechanism of TBT incorporation in the egg capsules still needs to be investigated. Nevertheless the increase in TBT content during the development of the embryos suggests that it is a dynamic process, which occurs while egg capsules roll on the sediment.

TBT-based antifoulants are still used on large vessels and consequently are found in ports. Due to the slow degradation of TBT in sediments, it is continuously accumulating. This may cause a problem if sediments are remobilized through events such as storms or dredging operations (Sarradin et al. 1991, Dowson et al. 1993, de Mora et al. 1995). During 1998 an enlargement of Mar del Plata beaches (Fig. 41) implied the mobilization of almost 2.5 million cubic metres of sand (Marcomini & López 1999) causing possibly the occurrence of imposex in the area.

The present findings are opposite to many studies reporting virtually no imposex at open coasts (Bright & Ellis 1989; Smith & Mc Veagh 1991; Foale 1993; Evans et al. 1995). Bryan et al. (1986), Evans et al. (1996, 1998) Minchin et al. (1995), Nyarko & Evans (1997) and Evans & Nicholson (2000) proposed that open oceanic coasts are free of TBT pollution at biologically significant levels because of the dilution effect of the sea. TBT pollution is localized in ports (Evans & Nicholson 2000) and disappears rapidly when distance from the port increases. Even after the 30 years in which it has been used as the major biocide in antifoulants, there are still healthy gastropod populations within a few kilometres from harbours all around the world (Evans et al. 1996, Smith & McVeagh 1991; Foale 1993; Nyarko & Evans 1997). Taking this into account, it seems that this phenomenon will no longer affect snail populations in Mar del Plata, due the incidence of the Brazil and Malvinas currents as well as the coastal front that wash away the pollutants to the NE and SW. However, the authorities should prevent new episodes based on the acquired experience.

Figure 41 - 42: 41. The central beach of Mar del Plata was enlarged together with other beaches of the city in 1998 at the same time that the port was dredged. (picture: http://www.todomardelplata.com). 42. Fishing boats of the artisanal coastal fleet of Mar del Plata (picture: http://www.todomardelplata.com).

5.3. FISHERY

5.3.1. Fishing fleet

The Argentinean coastal fleet of 347 boats represents almost the 50% of the fishing boast of Argentina. Twenty percent of the Argentinean fishermen are working for this fleet producing 5 % of the total captures (Lasta et al. 2001). The coastal fleet (Fig. 42) is composed by the short-range fleet (8 to 18 m length vessels) and the large-range fleet (18 to 28 m length vessels). 72% of its catches are landed in Mar del Plata (Lasta et al. 2001). Errazti et al. (2001) reported over 100 vessels of the short-range fleet operating from Mar del Plata. In the province of Buenos Aires, the fleet uses bottom trawls to catch all benthic and demersal species. In southern areas other kinds of net are used. In Patagonia some fishermen are diving to collect scallops (Ciocco et al. 1998) and octopus (Ré 1998). This technique is also applied in Peru and Chile to collect other benthic organisms such as echinoderms, clams and gastropods. This method is more species and size selective and friendlier for the environment since the bottom is not disturbed.

It is likely that the operative boats will use *A. brasiliana* as an alternative target as they already operate with other species, depending on resource availability and market conditions.

The prices of similar commercial species, such as the Chilean loco and the Australian abalone fluctuate between about 10 $/kg (Infopesca 2004) and 30 $/kg (Cook 2004), respectively. *A. brasiliana* is marketable in the same price class and thus represents an economically interesting target for the coastal fleet, which Lasta et al. (2001) define as a socially active fishery, very dynamic and producing employment.

5.3.2. Fishery regulations

A. brasiliana populations are patchily distributed and in a single haul of 20 min 200 kg can be captured (Fernandez 1995). Its large size and frequent occurrence present it as a valuable and exploitable resource, but on the other hand its slow growth and late maturity make it extremely vulnerable to overexploitation, which already happened with other commercial gastropods such as the abalone in Australia (Rogers-Bennett et al. 2002), the queen conch in the Caribbean Sea (Stoner & Ray-Culp 2000), the Chilean "loco" (Rabí & Maraví 1997), *Z. dufresnei* in Argentina (Giménez et al. 2004) and even the black snail in Uruguay (Fernández 1999b).

The only measures that have been proposed for the management of *A. brasiliana* in Uruguay are to give a special license only to those fishermen who already operate on this resource, limit fishing areas and introduce a minimum mesh size of 120 mm (Riestra & Fabiano 2000). However, to my knowledge, none of these ideas have been implemented yet. The proposed minimum mesh size overlaps with the size at first maturity reported here. Therefore the minimum mesh size should be at least 130 mm if bottom trawling is used. Furthermore it is necessary to investigate the selectivity of the net to be implemented.

Since the Argentinean population of *A. brasiliana* is located in shallow waters, a more adequate system would be the use of divers that collect the mature individuals.

Manríquez and Castilla (2001) analysed the effectiveness of protected and managed areas for the fishery of loco in Chile. It involves the participation of cooperatives of fishermen controlling the areas they exploit. This method requires a prudent exploitation of these areas to ensure the permanence of the stock living in it, but it allows growth to an optimal size. This could be an efficient system for the fishery of *A. brasiliana*. It is clear that the studied population of *A. brasiliana* can be sustainably exploited only with strong regulations. If trawling is maintained, it is necessary to get use scientific net with narrow meshes in order to get representative catches with respect to the size structure of the population.

6. PUBLICATIONS

This thesis includes six publications listed below. Additionally, my contribution to each study is explained.

Publication 1: Cledón, M., W. Arntz & P.E. Penchaszadeh. Accepted. Gonadal cycle in an *Adelomelon brasiliana* (Neogastropoda: Volutidae) population of Buenos Aires province, Argentina. Marine Biology.

I established the first contact with the fishermen for getting a boat to carry out the sampling as well as participated in the sampling excursions. The laboratory work, processing of the data, analyses and interpretation were done by myself and discussed with the last author. After writing the manuscript, it was improved with the co-authors.

Publication 2: Cledón M., T. Brey, P. E. Penchaszadeh, W. Arntz. Submitted. Individual growth and somatic production in *Adelomelon brasiliana* (Gastropoda; Volutidae) off Argentina. Marine Biology.

The concept and initial idea of this article was elaborated with the third author. I conducted all the practical work and the data processing. Thereafter the data analysis for the growth estimates and improvement of my first version of the manuscript was achieved in cooperation with the second author. Then it was improved with all co-authors.

Publication 3: Cledón M., T. Brey, W. Arntz, P. E. Penchaszadeh. Size and age of first sexual maturation in *Adelomelon brasiliana* (Neogastropoda; Volutidae) off Argentina.

The idea of sampling and material processing for this paper was elaborated by myself. My first version of the manuscript was improved by the comments of the three co-authors.

Publication 4: Penchaszadeh PE, Averbuj A, Cledón M. 2001. Imposex in gastropods from Argentina (South-Western Atlantic). Mar. Pollut. Bull.42: 790.

The original idea of this work belongs to the first author after incidental observations of the second author. I did the sampling, material processing and data collection of *Adelomelon brasiliana*. My first manuscript was reworked, improved and finished by the first author.

Publication 5: Raquel N. Goldberg, A. Averbuj, M. Cledón, D. Luzzatto and N. Sbarbati Nudelman. 2004. Search for triorganotins along the Mar del Plata (Argentina) marine coast: finding of tributyltin in egg capsules of a snail *Adelomelon brasiliana* (Lamarck, 1822) population showing imposex effects. Applied Organometallic Chemistry 18: 117–123

The original idea of this work belongs to me. I made sampling, material processing and data collection of *Adelomelon brasiliana* as well as collection of egg capsules. I wrote part of the manuscript. It was completed by the co-authors.

Publication 6: Cledón, M., Gerwinski, W., Hammermeister, E., Penchaszadeh, P. E. & Arntz, W. Organotin Compounds (OTs) in sediments and commercial gastropods off Mar del Plata, Argentina.

The original idea of monitoring the temporal and spatial OTs pollution pattern in sediment and black snails was developed by myself. I carried out the sampling and material reprocessing. Extraction of OTs and gas chromatography were performed in cooperation with the second and third co-authors. I did also the manuscript writing and the final version was discussed with all co-authors.

Publication 1:

Gonadal cycle in an *Adelomelon brasiliana* (Neogastropoda: Volutidae) population of Buenos Aires province, Argentina.

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Summary

The reproductive season of the population extends from September to April (austral spring and summer), showing synchronization with water temperature. Yolk content of the oocytes increases until they reach 200 µm in diameter before spawning. In autumn, a resting phase begins, in which no new oocytes develop, while in the unspawned reabsorption occurs. Gonadic development begins during the early winter when new previtellogenic oocytes appear. *Adelomelon brasiliana* has a yearly gonadal cycle with an activity peak in early spring and a prolongated spawning season in summer.

Introduction

Adelomelon brasiliana (Lamarck 1811) inhabits shallow waters between five and 20 metres depth on sandy bottoms of the southwestern Atlantic Ocean. According to Ríos (1975), its distribution covers the coastal area between Río de Janeiro, Brazil in the north and Río Negro, Argentina in the south.

A. brasiliana males have a conspicuous penis that measures over 20 mm in length. Females have a genital pore used for copulation and spawning of the egg capsules. It is a large sized species exceeding 170 mm of shell length and weighing more than 400g. *A. brasiliana* is the only one volutid known to have free egg capsules (Penchaszadeh, et al. 1999). These are approximately spherical, reaching 140 ml volume, being the biggest known among gastropod egg capsules and containing between 9 and 33 embryos (Penchaszadeh & De Mahieu 1976, Penchaszadeh et al. 1999).

The commercial importance of the black snail, *A. brasiliana*, emerged because of the closure of the fishery of "loco", *Concholepas concholepas*, during the 1980's and early 1990's in Chile after years of decline (Geoghan & Castilla 1987, Bernal et al. 1999). This gastropod was exported to Asiatic countries to supply the abalone, *Haliotis* sp, demand (Rabí & Maraví 1997)

The gradual disappearance of the Chilean loco in the international market was the cause of the increasing interest in the harvesting of *A. brasiliana* (Equipo Ecoplata 1996) and initiated a change of the fishery in La Paloma, Uruguay, where the principal target changed to the black snail (Nion 1999). In Argentina *A. brasiliana* is a very abundant species being captured by bottom trawling near the coast, but it has never been exploited.

There are studies about the distribution (Testud 1970), anatomy (Novelli & Novelli 1982), general taxonomic characteristics (INIDEP 2000) and egg capsules of *A. brasiliana* (De Mahieu et al. 1974; Penchaszadeh et al. 2000) but none about population traits or reproduction. It is necessary to improve knowledge of this species in order to develop control rules for its exploitation to ensure a long-term sustainable yield. The present study provides the first data on the reproductive dynamics of *A. brasiliana*.

Material and Methods

During a 24 months period between November 2000 and December 2002, about 25 adult *A. brasiliana* were captured monthly by bottom trawling, at 15 meters depth in the Mar del Plata area (38°20'S; 57°37'W), Argentina (Figure 1). Seasonal samples were also taken during the same period near the Mar Chiquita area in order to detect possible reproductive differences over a short distance.

Shell length (SL) was measured with a 1 mm precision using a Vernier calliper and shell free wet mass (SFWM) was determined with 0.1 g precision and surface water temperature was recorded daily at a single point (Dr. Marcelo Scelzo, Universidad Nacional de Mar del Plata) to the nearest 0.1°C with a mercury bulb thermometer in order to compare gonadic change during maturation with the annual temperature variation in the water column.

Samples of the medial zone of the gonad were taken, fixed in Bouin solution for 48 hours and processed histologically. Ten slides per individual, 5 µm thick, were stained with Hematoxilin-Eosine to study the monthly gametic development, to measure the oocyte diameters in females, and in order to standardize the measurement 5 oocytes per slide, presenting a nucleus with nucleoli were measured at their maximal diameter. Male gonads were easily distinguishable from the adjacent hepatopancreas because of their dark colour when mature, whereas females showed a gonad with similar coloration as the hepatopancreas. However neither the female nor the male gonad can be separated exactly

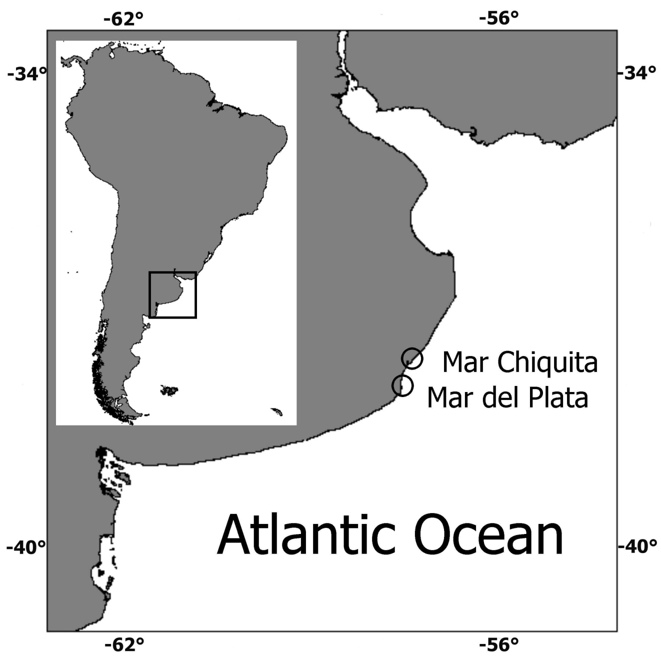


Figure 1: Map of the Buenos Aires Province shore, Argentina, showing the sample zones.

from the visceral mass, so the gonadosomatic index could not be determined. The Condition Index (the relation between soft part weight and shell weight) was not the best proxy to evaluate the individuals' condition since shell weight oscillates too much among individuals of similar SL. This oscillation is caused by the presence of drilling polichaetes and *Lithophaga* mussels, so the SFWM was recorded throughout the year as an estimator of the animals' condition. The sex proportion and the reproductive condition was statistically analysed with χ^2, one-way Anova (Sokal & Rohlf 1969) was used to investigate the relationship between female SFWM and their gonadic stage. To minimize interference of somatic weight increase during growth, only females of 130-140 mm SL were used.

Results

The studied population of the black snail *A. brasiliana* presented a sex ratio not different from 1:1 (χ^2 = 0,79 df=1 p>0,05 N= 500) during the sampled period. The males are easy to distinguish after shell removal because of their conspicuous penis. The gonads were situated in the distal part of the visceral mass. The ripe testis is dark violet, almost black, while the ovary is light brown and not distinguishable from the hepatopancreas.

Males had a fully active gonad all year round. Its colour ranged from beige to dark violet but in all cases the gonads were mature and active. The spermatic tubules contained all developing stages of masculine reproductive cells (Figure 2) and the spermatic ducts exhibited large amounts of spermatozoids throughout the year (Figure 3). After gamete liberation the tubules remained almost empty. This did not occur in synchrony for all individuals.

The female gametic cycle could be divided into five stages. (i) During the first growing stage, females developed oocytes along the germinal epithelium from the end of May until August

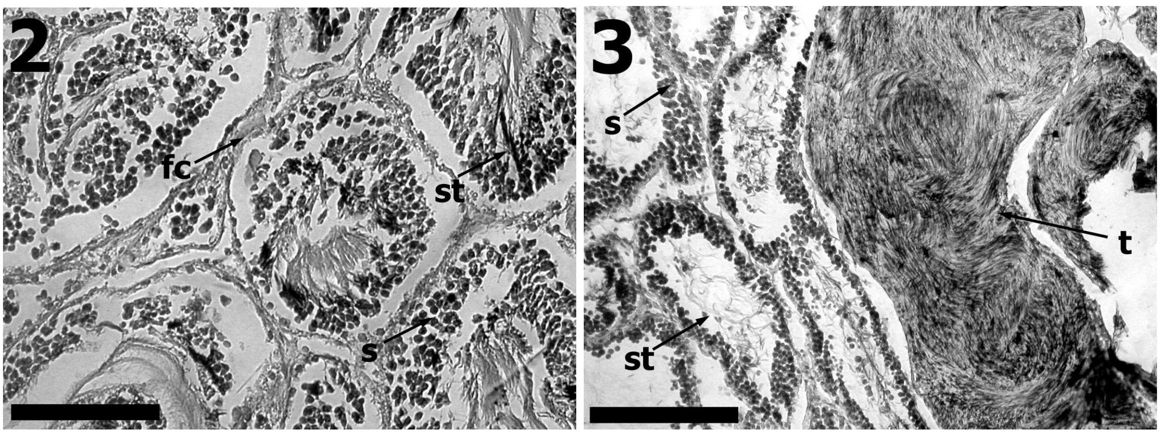
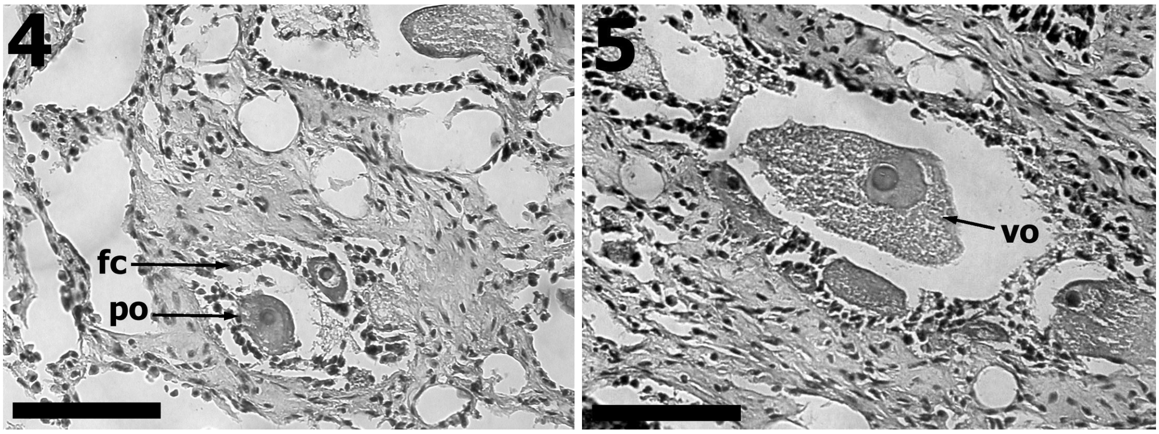
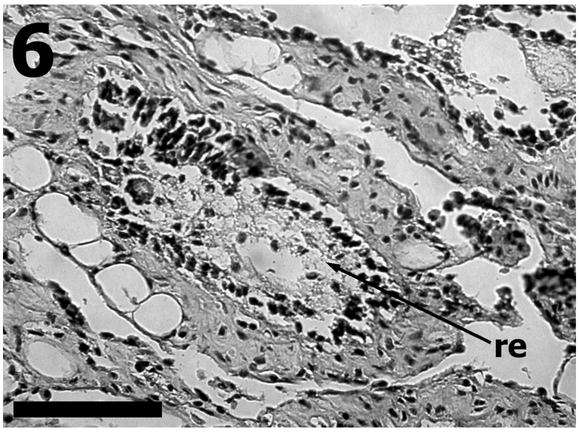


Figure 2: Male gonad of *Adelomelon brasiliana*. St: spermatozoids, s: spermatids, fc: follicular walls. Scale bar = 100 µm. Figure 3: Male gonad of *Adelomelon brasiliana* showing full spermatic tubules (t). Scale bar = 100 µm.





Figures 4-6: Female gonad of *Adelomelon brasiliana*. Scale bar = 100 µm. 4. Follicles with developing previtellogenic oocytes (po) and vitellogenic oocytes (vo). 5. Mature oocyte. 6. Oocyte undergoing reabsorption (re).

(winter) (Figure 4). The connective tissue was thick with many cell layers. (ii) At the mature stage, cells were full of yolk, and measured over 100 µm in diameter and the connective tissue remained well developed (Figure 5). (iii) During the first spawning stage, between September and October, many oocytes were released, and the female gonad showed some empty follicles as well as new developing oocytes with diameters under 80 microns.

(iv) In January a second spawning stage started. The presence of mature oocytes, however remained low, the ovary appeared half empty, and some oocytes in previtellogenic and vitellogenic stages as well as reabsorption were observed. (v) The reabsorption stage occurred from February to April, also depending on the water temperature. After releasing some more oocytes, many follicles were empty and the few remaining oocytes underwent reabsorption (Figure 6), so that the gonad presented fully empty follicles with thin connective tissue and some yellow residuals. Subsequently new previtellogenic oocytes appeared, starting the active stage (i) of the following cycle.

During anatomical dissections spermatozoids were found in the bursa copulatrix of females caught each month without a clear seasonal pattern, and from the histological analysis the female proportion in each developing stage was obtained (Figure 7). It was found that the spawning presents a short peak in early spring followed by a sustained spawning period during summer.

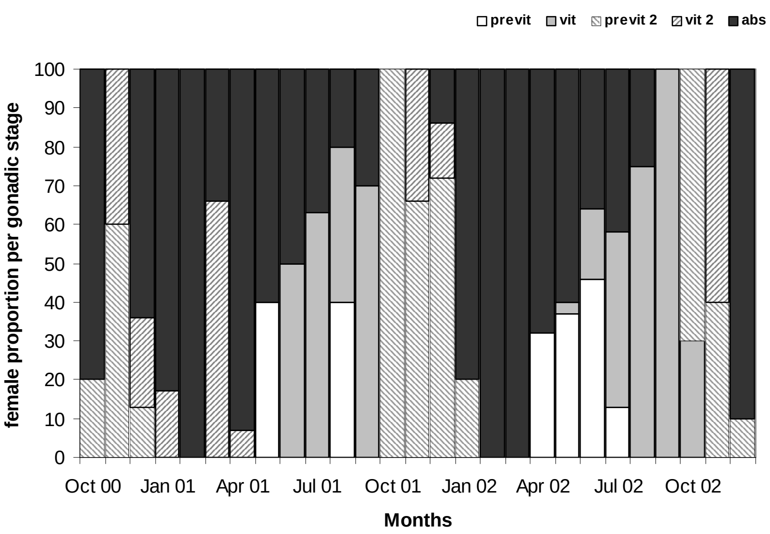


Figure 7: *Adelomelon brasiliana,* female gonadal stage. Each percentage was obtained by measuring 50 oocytes of each females of each sample. Previt: previtellogenic, Vit: vitellogenic, Previt2: second stage of oocyte production, Vit2: second spawning period, Abs: stage of reabsorption of sexual products.

No differences were observed between the populations of Mar del Plata and Mar Chiquita.

The SFWM for males showed variations during the studied period reaching the maximum always at the end of winter, when water temperature starts to rise and in mid summer, when water temperature reaches its maximum. The SFWM minimum was reached at the beginning of summer and in autumn, when the water temperature was approximately 18°C (Figure 8). The female SFWM showed a pattern similar to the males, maximum SFWM being reached at the end of winter. After a short recuperation in early summer, a new loss of weight occurred with minimum in autumn.

The one-way ANOVA indicates that the gametic cycle of females was related to the mean SFWM (Table 1), which decreased twice yearly (Fig. 8A); in March and November 2001, and in June 2002. Both factors seem to be regulated by the water temperature cycle of the area, which showed an annual pattern (Figure 8 C) with a maximum of about 20°C from December to March (austral summer) and a minimum of 8°C from July to August, the austral winter.

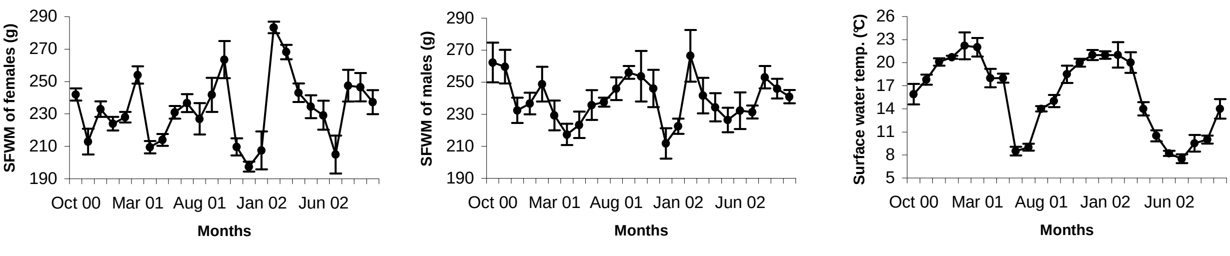


Figure 8: Shell free wet mass (SFWM) of females and males of *Adelomelon brasiliana* of the 130 mm shell length size class and monthly water temperature variation during two years in Mar del Plata, Argentina.

Discussion

Temperature is an important environmental factor in the regulation of gastropod gonadic development (Giese 1959, Kinne 1963, Fretter & Graham 1964, Giese & Pearse 1977, Kikuchi & Uki 1974, Martel et al. 1986). This seems also to be the case with *A. brasiliana* where the gametic cycle shows a clear seasonality related to the water temperature, coinciding with the observations on *Zidona dufresnei* (Giménez & Penchaszadeh 2001). Oocytes grow and mature when the water temperature increases from the winter minimum, and spawning takes place when the mean water temperature rises to 17.5°C. The decrease of the mean water temperature terminates this process, and triggers the reabsorption of the remaining oocytes. Gametic activity was observed all year long, as in *Z. dufresnei* (Giménez & Penchaszadeh 2001).

In the population of *A. brasiliana* studied in the Mar del Plata area, we did not observed a gonadic maturation cycle in the males, mature spermatozoids occurring in the spermatic follicles as well as in spermatic tubules throughout the year. This fact contrasts with the reports on other caenogastropods e.g. *Buccinum undatum* having undeveloped testes at the end of winter (Martel et al. 1986) and *Neptunea arthritica* presenting a decrease in testes size prior to spring copulation (Takamaru & Fuji 1981).

In most caenogastropods, gametogenic activity throughout the reproductive cycle of a population is synchronous and can be divided into maturation stages (Giese & Pearse 1977).

In the females of *A. brasiliana*, five gonadic phases could be defined, see results.

Maturation among females occurs synchronously.

Reproduction takes place in spring and summer, when females are ready to spawn. Coinciding with our results, Luzzatto & Penchaszadeh (2002) reported *A. brasiliana* egg capsules presence during the whole year except for the coldest months (June- August) with a massive abundance every early spring.

Studies on *Buccinum undatum* from eastern Canada also indicate spawning in spring and summer (Martel et al. 1986, Kusnetsov 1963), however, other studies reported spawning of this species in the winter months (Cunningham 1899, Sykes 1905, Havinga 1922, Lebour 1937, Moore 1937, Kristensen 1959, Hancock 1960, 1967, Fretter & Graham 1962, Bruce et al. 1963, Kideys et al. 1993). This duality of the reproductive season has also been observed for the genus *Neptunea*, e.g. *N. despecta* reproduces in summer (Martel et al. 1986b), *N. antiqua* reproduces in winter (Pearse & Thorson 1967). Given the wide latitudinal distribution of *A. brasiliana*, it seems possible that populations at lower latitudes also reproduce over winter.

The SFWM seasonal variation is related to the gonadic cycle, but it is not possible to conclude that this SFWM variation is attributable largely to changes in gonad weight, because the *A. brasiliana* female gonad is only a thin layer around the hepatopancreas tip. It is more likely that these changes in weight are due to activation of sexual glands, e.g. the albumen or capsule glands in *B. undatum* as mentioned by Martel et al. (1986).

Although we have not observed copulation, *A. brasiliana* males are capable of reproduction throughout the year, and living spermatozoids have been found in the female reproductive system all the year round. It is still to be studied if copulation is induced with increasing temperature as reported for *B. undatum* (Martel et al. 1986).

The male SFWM showed a similar but no so well defined pattern as female SFWM. This similarity contrasts with the fact that they do not show a clear seasonal gonadic cycle. Hence the SFWM seems to reflect a glandular activity change, if so, the population could mate during spring and summer before the egg-capsules are released.

Argentinian volutid stocks seem to depend on relatively closed and isolated populations been highly vulnerable to the localized fishing (Orensanz et al. 1996), but *A. brasiliana* is the only South American volitud with free egg capsules (Penchaszadeh et al. 1999) and since embryos develop in 90 days (Luzzatto & Penchaszadeh 2002) the rate of dispersion of this species must be very high compared with other american volutids, e.g. currents near the study area varied between 0.1 $m{*}s^{-1}$ northwards (Lanfredi & Capurro 1971) and 0.3 $m{*}s^{-1}$ southwards (Lanfredi 1972), so that an egg capsule can be transported over 700 km during its development, implying that *A. brasiliana* populations are not so isolated.

In summary, the long reproductive season of the species, together with its free egg capsules with a long development are advantages that make it more resistant to fishing than other volutids. On the other hand, this species reaches its maximum individual production rate very late (Cledón et al. submitted) and its size at sexual maturity is greater than other commercial gastropods (Cledón et al. in prep.), so that there is the need for accurate fishing legislation for this species.

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Publication 2:

Individual growth and somatic production in *Adelomelon brasiliana* (Gastropoda; Volutidae) off Argentina.

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Summary

Growth, age and somatic production of the benthic top predator *Adelomelon brasiliana* were studied at its Argentinean distribution on the South American Atlantic shelf. Stable oxygen isotope ratios confirmed annual formation of internal shell growth marks. Modal shell length of the population was 140 mm, while modal shell free wet mass was 255 g. A logistic growth function (SL_∞ = 186.28 mm, K = 0.185, t_0 = 4.601) fitted 131 pairs of size-at-age data (25 shells) best. *A. brasiliana* is a very long-lived species, reaching up to 20 years of age. The maximum individual somatic production of 46 g_* shell free wet mass year^{-1} is attained at 145 mm shell length which corresponds to about 12 years of age.

Introduction

The neogastropod *Adelomelon brasiliana* (Volutidae) occurs along the South American Atlantic coast between 23°S and 41°S. High Asian demand for gastropod meat induced the commercial exploitation of this species in the early 1990's in Uruguay, mainly as a response to the depletion of the "loco" *Concholepas concholepas* stock in Chile (Equipo EcoPlata. Eds. 1996, Rabí & Maraví 1997). The Argentinean stock is not commercially exploited but *A. brasiliana* is captured in high numbers as bycatch of the shallow water shrimp fishery along the coast of the Buenos Aires Province. As in most Argentinean Volutidae, biological and ecological information on *A. brasiliana* is scare (Lasta et al. 1998). There are some studies on its egg capsules (Penchaszadeh, et al. 2000, Penchaszadeh, et al. 2000b, Penchaszadeh & G. De Mahieu. 1976) and a recent description of its reproductive cycle (Cledón et al. 2004).

The aim of this study is to analyse individual growth, age and somatic production in order to provide some basic parameters of population dynamics, which will be required for evaluation and future successful management of this potentially valuable resource in Argentinan waters.

Material and Methods

The study was carried out along the coast of the Buenos Aires province, Argentina, off Mar del Plata (38° 20'S; 57° 37'W). About 20 individuals of *A. brasiliana* were captured monthly between November 2000 and December 2002 by one 30 min haul with a bottom trawl (15 m mouth opening, 70 mm mesh size in the cod end) towed by a 12 m fishing boat at approximatively 15 m depth, 1000 m off the coast. Soft parts were weighed (shell free wet mass, SFWM) to the nearest g and shell length (SL), width (SW), high (SH), aperture length (AL), width (AW) and spiral growth trajectory (SG) (Fig. 1) were measured with a vernier calliper to the nearest mm. The relationships between these morphometric parameters were described by linear regression.

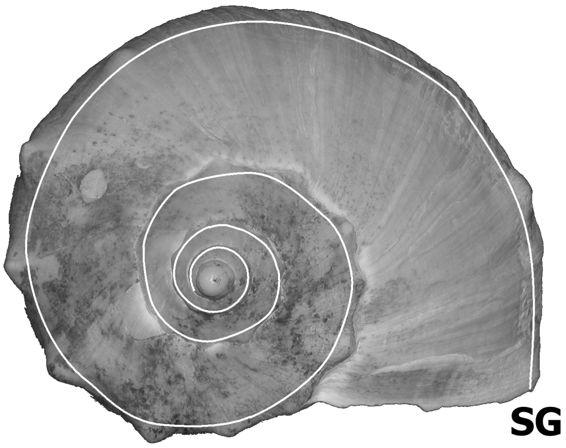

Figure 1: Distal view of *A. brasiliana* shell. Samples of carbonate for isotopes analysis were taken along spiral growth trajectory (SG).

Growth and age

Individual growth and age were inferred from internal shell growth marks in 25 shells covering the whole SL range by (i) growth mark identification, (ii) validation of annual growth mark formation, and (iii) quantitative size-at-age determination. To check for internal growth marks, X-ray photographs of shells were taken with a Hewlett Packard Faxitron 43855 mammograph with fixed anode, using AGFA-Strukturix D4 FW film and the parameter settings: focal film distance 45 cm, voltage 40 kV, 120- to 240-s exposure time. They revealed macroscopically visible bands perpendicular to the shell growth trajectory. Shell cuts were prepared to count and measure these marks. Cuts were performed along the whorls following the spiral growth trajectory from the apex to the posterior end of the aperture (Fig. 1). The cut surface was polished with fine-grained sandpaper and checked for growth marks in the three-layered shell by means of a stereomicroscope. Growth marks were numbered subsequently and the distance from the shell apex to each mark, i.e. growth trajectory length SG was measured. Mark number and corrsponding SG were interpreted as size-at-age data.

Stable oxygen isotope ratios ($\delta^{18}O$) were used to analyse whether these marks were formed annually, as described recently by Giménez et al. (2004) and others. The ratio of the stable oxygen isotopes ^{16}O and ^{18}O in biogenic $CaCO_3$ is inversely proportional to temperature during shell deposition. This relation is nearly linear between 5° and 30°C (Epstein et al. 1951, Epstein & Lowenstam 1953). Therefore, a mollusc shell from an environment with rather constant salinity and a distinct annual temperature cycle will show an oscillating pattern of $\delta^{18}O$ along the major growth axis (see examples in Santarelli & Gros 1985, Richardson 2001, Giménez et al. 2004). Six representative specimens (3 males of 137, 146 and 148 mm SL and 3 females of 136, 141 and 147 mm SL) of *A. brasiliana* were selected for isotope analysis. From each shell, about 120 samples (approx. 125 µg each) were drilled from the central shell layer at 1 to 4 mm distance along the cut from apex to shell aperture. Shell areas damaged by drilling polychaetes were not sampled.

Stable oxygen isotope composition was determined in the Stable Isotope Laboratory of the Alfred Wegener Institute with a Finnigan MAT251 mass spectrometer coupled to an automatic carbonate preparation device. Results were reported in δ-notation versus PDB (Vienna Pee Dee Belemnite) standard calibrated via NIST 19 (National Institute of Standards and Technology isotopic reference material 19). The precision of measurements was better than ±0.08 ‰ for $\delta^{18}O$, based on repeated analysis of a laboratory-working standard over a one-year period. Spatial coincidence of subsequent $\delta^{18}O$ peaks and subsequent shell growth marks would indicate that one mark is formed each winter.

The iterative non-linear Newton algorithm was used to fit growth models to the resulting set of size-at-age data pairs. A logistic growth model

$SL_t = SL_\infty / (1 + e^{-k * (t-to)})$

was found to fit these data best.

Individual production

Individual annual somatic production P_i (SFWM) of *A. brasiliana* was calculated by the mass-specific growth rate method (see Brey 2001) using the size-growth function and the size-body mass relation:

$P_i = M_i * G_i$

where M_i represents mean individual body mass at size i and G_i the annual mass specific growth rate at size S_i given by:

$G_i = b * K * \ln (Sl_\infty / Sl_i)$

As samples were collected with a commercial bottom trawl with mesh size of 70 mm, smaller size classes are likely to be severely undersampled. Thus we restrained from computing population production (P) and production to biomass ratio (P/B) based on the sample size distribution, as these estimates would strongly underestimate true values.

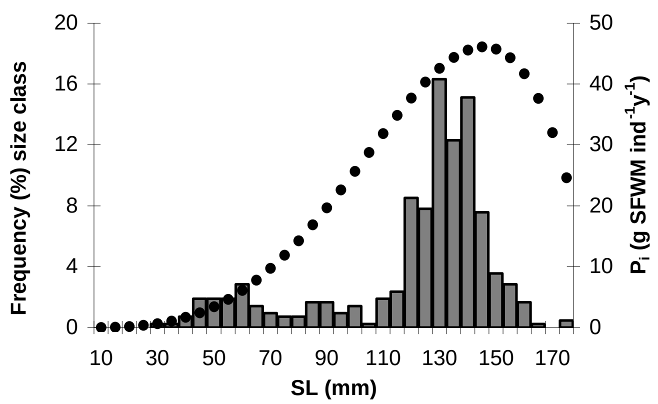

Figure 2: Size-frequency distribution of *A. brasiliana* (N= 500) from Mar del Plata in the years 2000-2002 using class intervals of 5 mm. Dots: superimposed curve of individual production P_i (grams shell-free wet mass*years^{-1}) versus size in *A. brasiliana*.

Mortality

Total mortality rate Z according to the single negative mortality model

$N_t = N_0 * e^{-Z*t}$

was estimated by a size-converted catch curve (Pauly 1984a, b)

$\ln(N_i/\Delta t) = a + b * t_i$

where N_i is number in size class i, Δt is the time required to grow through this size class, t_i is age at midsize of size class i, and $Z=-b$. This curve was based on the size-frequency distribution of the individuals sampled and the logistic growth function.

Results

Morphometrics

The 500 animals collected ranged from 34 mm to 174 mm shell length, modal length was about 140 mm in SL (Fig. 2).

Shell height (SH), width (SW), aperture width (AW) and aperture length (AL) were linearly related to shell length (SL):

$SW = 0.664 * SL - 3.618$; $r^2 = 0.979$; N= 500

$SH = 0.503 * SL - 1.304$; $r^2 = 0.979$; N=500

$AW = 0.4081 * SL - 7.2874$; $r^2 = 0.972$; N=500

$AL = 0.8468 * SL - 5.1959$; $r^2 = 0.976$; N=500

Shell free wet mass (SFWM) ranged from 1.4 g to 400.2 g, modal mass was about 255 g. SL and SFWM were exponentially related:

$\log(SFWM) = 3.5622 * \log(SL) - 5.2008$; $r^2 = 0.971$; N= 500

Growth trajectory length SG, that is, the distance from apex to measurement point along the growth spiral (Fig.1) was linearly related to SL:

$SG = 2.827 * SL + 2.728$; $r^2 = 0.924$; N= 131

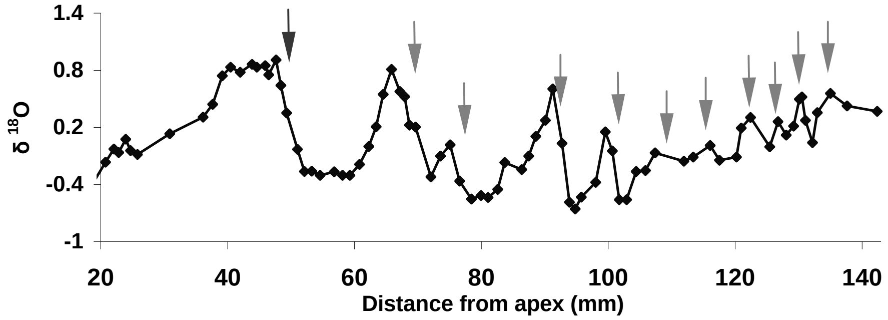

Figure 3: $\delta^{18}O$ profile along a SG transect from apex to shell edge plotted against correspondent shell length in an *A. brasiliana* individual of 147 mm SL, 12 years old.

Age and growth

The $\delta^{18}O$ profiles showed a distinct oscillating pattern in all six shells analysed (Fig 3). According to the paleotemperature equation of Epstein & Lowenstam (1953), the amplitude of about 1 ‰ corresponds to a temperature range of >= 4 °C which is in the range of the annual cycle observed in the investigation area (Guerrero et al. 1997, Cledón et al. in press). The distinct dark orange coloured shell growth marks visible in the shell cuts were situated closely to the maximum $\delta^{18}O$ values (Fig.3), that is, one mark is formed during each temperature minimum period in winter. Apparently no growth band is formed during the first winter, which is, however, clearly indicated by the first $\delta^{18}O$ peak (Fig.3). The oldest animal found has 20 years of age.

The 131 size-at-age data pairs obtained from the 25 specimens analysed were fitted best by the logistic growth model (Fig. 4):

$S_t = 186.28 / (1 + e^{-0.185 * (t - 4.601)})$; $r^2 = 0.924$; N = 131

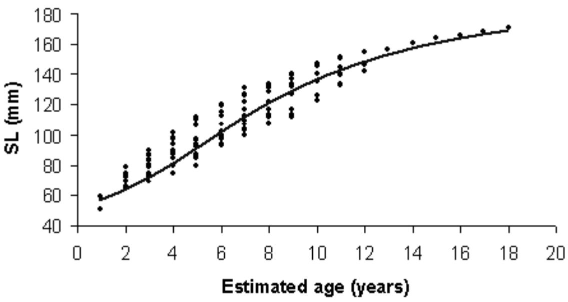

Figure 4: Logistic growth curve fitted to 131 size-at.age data pairs obtained from 25 cut shells of *A brasiliana* $SL=186,26mm/(1+*e^{-0,185*(t-4,601)})$ N=131, $r^2=0,92$

Individual production:

Individual somatic production (P_i) increased steadily with size to a maximum of 46.1 g SFWM year^{-1} at 145 mm SL and decreased again thereafter (Fig. 2).

The size converted catch curve (Fig. 5) does not show a straight descending right arm, i.e. the single negative mortality model does not fit the data and hence a mortality rate Z cannot be calculated.

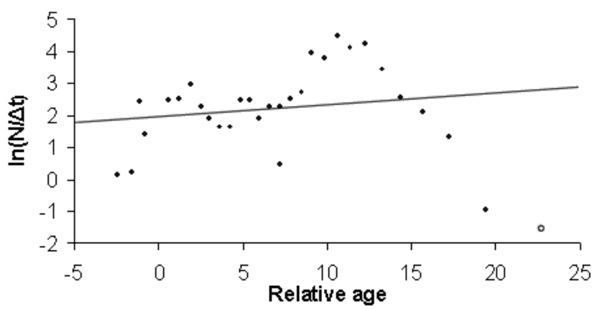

Figure 5: Size-converted catch curve based on the size-frequency sample (Fig.2) and the growth function (Fig.4) of *A. brasiliana* Solid circles: data excluded from regression.

Discussion

Validity of ageing approach

Traditional methods of ageing molluscs such as counting growth marks on/in the shells are not reliable by themselves as they lack a validation of the time intervals at which those marks are formed. In environments with an annual temperature amplitude of several °C stable oxygen isotope analysis can provide such validation, because $\delta^{18}O$ of carbonate shell deposits vary with temperature (Richardson 2001). Changes in salinity can affect $\delta^{18}O$, too (Epstein & Lowenstam 1953), but are negligible in the investigation area (constantly around 33.8 UPS, Guerrero & Piola 1997). Therefore the $\delta^{18}O$ profile in the shell of *A. brasiliana* reflects the water temperature cycle, i.e. each $\delta^{18}O$ peak corresponds to one winter water temperature minimum, thus providing a valid shell age scale.

A. brasiliana from the Mar del Plata area (maximum age = 20 years) appears to be rather long lived compared to other large gastropods such as *Buccinum undatum* (12 years, Gendron 1992), *Concholepas concholepas* (10 years, Stotz 2000), *Gazameda gunii* (7 years, Carrick 1980). *Strombus costatus* and *S. gigas* (5 and 7 years, respectively, Wefer & Killingley 1980), and *Zidona dufresnei*, (17 years, Gimenez et al. 2004).

We used the index of overall growth performance (OGP) $P = \log(K*L_\infty^3)$ (e.g. Moreau et al. 1986; Munro & Pauly, 1983; Pauly, 1979) to compare growth between various large gastropod species. OGP of *A. brasiliana* (P = 6.077) is well in the range of values refering to other large gatropod species from temperate regions (Fig. 6).

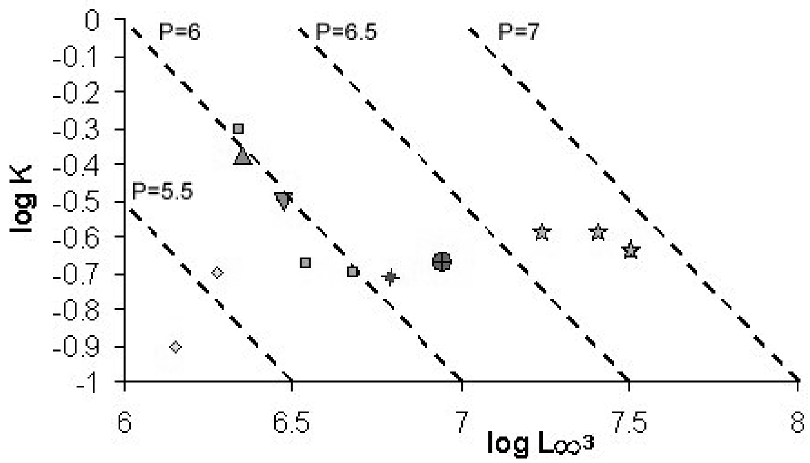

Figure 6: auximetric grid comparing growth performance in commercially exploited marine gastropod species. ☆: *Strombus gigas* (de Jesús-Navarrete 2001), ⊕ : *Zidona dufresnei* (Gimenez et al. 2004), ✧ : *Adelomelon brasiliana* (this study), □: *Concholepas concholepas* (Rabí &Maraví 1997, Rodriguez et al. 2001, Geaghan & Castilla 1987), △: *Haliotis laevigata* (Shepherd & Hearn 1983), ▽: *Haliotis rubra* (Shepherd & Hearn 1983), ◇: *Buccinum undatum* (Kideys 1996, Santarelli & Gros 1985). Stippled lines indicate lines of equal growth performance.

Its large size and frequent occurrence presents *A. brasiliana* as valuable and exploitable resource, but on the other hand its slow growth and late maturity (Cledón et al. submitted) make it extremely vulnerable to overexploitation, which already happened with other commercial gastropods such as the Australian abalone (Rogers-Bennett et al. 2002), the queen conch (Stoner & Ray-Culp 2000), the chilean "loco" (Rabí & Maraví 1997) and now is observable in the Argentinean *Z. dufresnei* fishery (Giménez et al. 2004).

Only precautionary measures have been proposed so far for the management of *A. brasiliana* in Uruguay (area limitation, creation of a special license, introduction of a minimum mesh size of 120 mm) (Riestra & Fabiano 2000) but to our knowledge they have not been implemented yet. Under this panorama, the studied population of *A. brasiliana* can be sustainably exploited only with strong regulations.

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Publication 3:

Size and age at sexual maturity in *Adelomelon brasiliana* (Neogastropoda; Volutidae) off Argentina.

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Summary

Size at first maturity of *Adelomelon brasiliana* near Mar del Plata, Argentina, was established analyzing gonadal tissue samples and the secondary sexual characteristics. Age was determined through growth marks. Minimum shell length (SL) at sexual maturity is 107 mm for females and 102 mm for males, whereas 50% of the females are mature at 115 mm and the males at 107 mm SL (approx. 7 years of age). All females were mature at 125 mm and males at 115 mm SL (up to 8 years of age). The comparative late sexual maturation in this species makes the population quite sensitive to overfishing.

Introduction

The southamerican Neogastropod *Adelomelon brasiliana* (Lamarck, 1811) is distributed from 23°S to 41°S along the Atlantic coast (Novelli & Novelli 1982) on sandy bottoms between 10 and 70 m water depth (Riestra & Fabiano 2000). High Asian demand for gastropod meat induced the commercial exploitation of this species in the early 1990's in Uruguay, mainly as a response to the depletion of the *Concholepas concholepas* stock in Chile (Equipo EcoPlata. Eds. 1996, Rabí & Maraví 1997). The Argentinean stock is not yet a fishery target, albeit *A. brasiliana* is captured in high numbers as bycatch of the shallow water prawn fishery along the coast of the Buenos Aires Province. Despite its commercial potential, biological and ecological information on *A. brasiliana* is scare. There are some studies on the ecological role of its egg capsules (Penchaszadeh, et al. 2000), which are among the biggest gastropod egg capsules (Penchaszadeh, et al. 2000b, Penchaszadeh & De Mahieu 1976). The recent description of its reproductive cycle (Cledón et al. accepted) is the first study dealing with reporting some important population traits for the comprehension of the ecology of this volutid. The aim of this study is to analyse the size and age at first sexual maturity of *A. brasiliana* off Mar del Plata in order to provide some basic parameters of population dynamics, which will be required for evaluation and future successful management of this valuable resource in Argentinean waters.

Material and Methods

The study was carried out along the coast of the Buenos Aires province, Argentina, off Mar del Plata (38° 20'S; 57° 37'W). The 452 analysed animals were collected during two summers, so all mature individuals presented mature gametes. Gonad samples were taken from the distal end of the visceral mass, fixed 48 hours in Bouin solution, dehydrated and imbibed in Paraplax. The 5 µm sections were stained with Haematoxilin-Eosine.

The percentages of mature females and males were plotted based on frequencies of mature individuals per size class. So the size class with half of the mature individuals is considered an estimate of the length at which 50% of the population becomes sexual mature.

Male's penis length (PL) was measured to the nearest 0.1 mm with a vernier calliper, being interpreted as indicative of maturation (Hancock et al. 1962, Gendron 1992 and Giménez & Penchaszadeh 2003). The size class with 50% of males with mature penis was also established.

Sexual maturation in snails produces a change in the relationship between SFWM and SL, since the reproductive system grows rapidly without a proportional change in size (Demaintenon 2000). According to this, SFWM of females and males was recorded as another criterion to analyse sexual maturity by plotting SFWM vs SL on a logarithmic scale to find the pair of straight lines, which fit the data best (Somerton 1980).

After size at maturation was determined, shells of 10 individuals belonging to this range were aged, based on internal growth marks as described by Cledón et al. (in press). The number of marks and corresponding SL of each individual were recorded to relate them with it corresponding maturate stage.

Results

No significant difference between years was found for the gonadic size at first sexual maturation of the population (χ^2 = 24,127), so all data were pooled together.

Females can start the maturation process at 107 mm SL and 90 g SFWM. Ovaries of specimens under this SL presented no oocytes or oogenic stages.

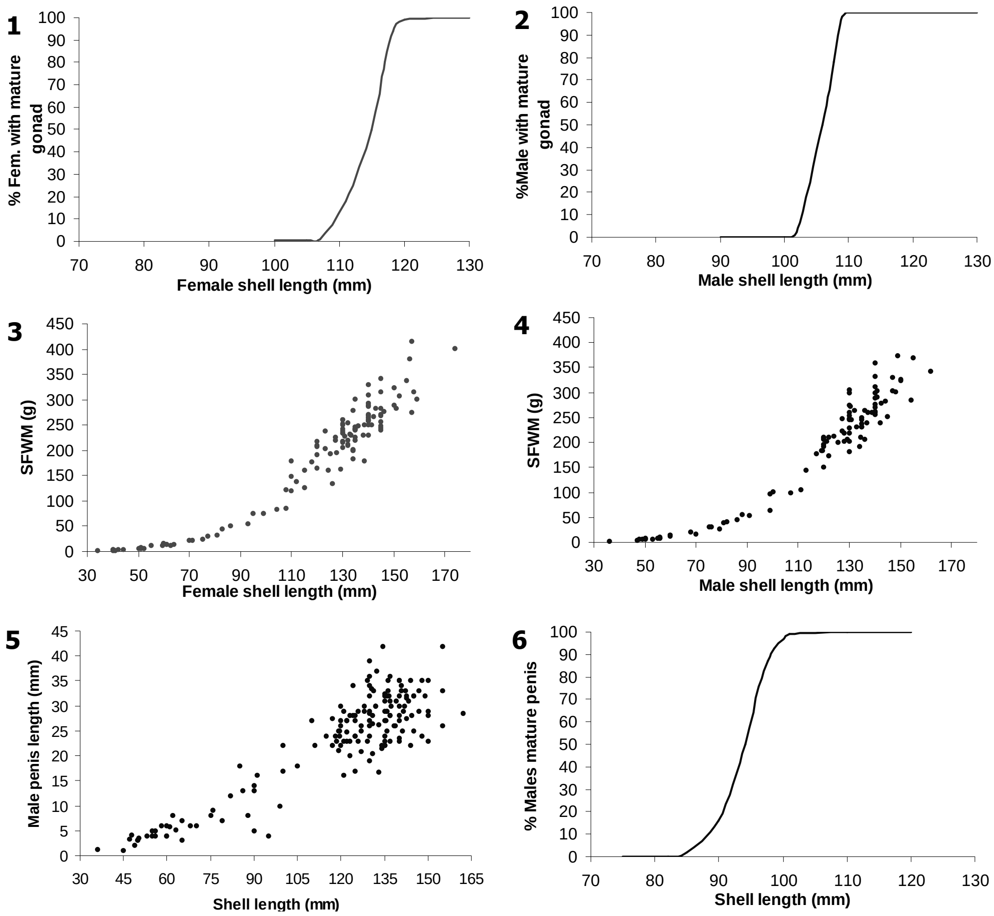
Plotted data show the 50% of females are mature at 115 mm SL (Fig. 1). After this size, the proportion of mature females increases so rapidly that all of them were sexually mature at 120 mm SL and around 160 g SFWM.

Male gonads did not show any signs of maturation until the animals reached 102 mm SL and 96 g SFWM. A specimen of this SL presented gonad cells with different spermatogenic stages but no spermatozoids were observed. From the plotted data is possible to observe, that 50% of all males were mature at 106 mm SL (Fig. 2). The totality of the male population was sexually mature at 110 mm SL and around 117 g SFWM.

This data indicate a wider maturation range for females, 13 mm, than males, 8 mm.

From the relation log SL – log SFWM of females, it is possible to divide the function in 2, with the split in 82 mm SL (Fig. 3), corresponding to the minimum SL of a female with mature reproductive system.

When the same criterion was applied to males, the split point was at 99 mm (Fig. 4) SL.



Figures 1-6: *A. brasiliana*. 1 Female percentage of mature gonads according to histology criteria plotted against shell length. 2. Same relation for males. 3. Shell free wet mass (SFWM) versus shell length for females. 4. Same relation for males. 5. Male penis length vs shell length. 6. Percentage of male's adult penis per shell length.

The smallest male with mature PL was 91 mm in SL (Fig. 5).

The PL rises rapidly and the 50% of mature individuals were recorded at 94.1 mm in SL

The totally of the sampled males showed mature penis at 101 mm in SL (Fig. 6).

The analyzed shells ranged from 80 to 130 mm in length and presented between 3 and 8 visible internal marks. Individuals of 110 mm in SL presented 6 visible marks.

Discussion

Females appear to mature somewhat slower than males as indicated by both minimum size at sexual maturity and size at which 50% of the population is mature. Other gastropods also show differences between sexes, e.g. *Buccinum cyaneum* (Miloslavich & Dufresne 1994) or the closely related volutid *Zidona dufresnei* (Gimenez & Penchaszadeh 2004).

Size at first sexual maturity of *A. brasiliana* is similar to the reported for *Z. dufresnei* living in an adjacent area, which is another commercial volutid inhabiting the Argentinean Province.

Nevertheless it was observed in other gastropods, e.g. *B. undatum*, that size at first maturity can differ among separated areas (Hancock 1967, Martel et al. 1986, Kideys et al. 1993) so the present results on *A. brasiliana* are only applicable to the studied population.

Table 1: size and age at sexual maturity and life span of some commercial marine gastropods.

Gastropod species	Size at first maturity (mm)	Age at first maturity (years)	Life span (years)	Author
Adelomelon brasiliana	115	7	20	Present study
Buccinum undatum	76	6	12	Gendron 1992
Concholepas concholepas	30*	-	10	Stotz 2000 *Rodriguez et al. 2001
Strombus gigas	220	3.5	7*	*Wefer & Killingley 1980 Orr & Berg 1987
Zidona dufresnei	120*	8	17	Giménez et al. 2004 *Giménez & Penchaszadeh 2003

A. brasiliana matures in 7 years, because it does not produce a visible first year mark (Cledón et al. submitted).

The age at maturation of *A. brasiliana* is also very similar to commercial snails, e.g. *Haliotis rubra,* around 7 years, (Prince et al. 1988) and *Z. dufresnei* (Table 1). Other commercial species reach maturity in shorter time, such as *S. gigas* (Table 1) and *C. concholepas* at 30 mm SL (Rodriguez et al. 2001).

The two South Atlantic volutids reach sexual maturity very late, which could endanger the stocks if the abundance or size of captured animals begin to decrease as reported for *A. brasiliana* in Uruguay (Fernández 1999) and for *Z. dufresnei* in Argentina (Giménez et al. 2004).

Together with their comparatively slow growth (Cledón et al. submitted), this makes them quite vulnerable to overexploitation.

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Publication 4:

Imposex in gastropods from Argentina (South-western Atlantic).

Penchaszadeh P.E.(1), Averbuj A. (2) & Cledón M. (2). Marine Pollution Bulletin. 42(9): 790-791.

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NOTE

Imposex in Gastropods from Argentina (South-Western Atlantic)

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Imposex is a widespread phenomenon in marine molluscs, documented in more than 120 gastropod species. Its occurrence is explained as a result of an anti-fouling paint compound called TBT (tributyltin) (Bryan *et al.*, 1986), whose presence is related to high marine traffic. The amount of TBT in the water or sediment is directly related to the masculinization of female gastropod molluscs of some species. This masculinization appears as a penis and sometimes vas deferens in females. Concentrations lower than 0.5 ng l^{-1} can induce imposex in meso and neogastropods (Bryan *et al.*, 1986).

In order to study if imposex was occurring in the SW Atlantic, specimens of two common gastropods *Buccinanops monilifer* and *Adelomelon brasiliana* were collected during the five last months of 2000 from Mar del Plata harbor (Lat. 37°50′ S) in a high boating activity area. The snails were collected by bottom trawling. Sex was determined on the basis of the presence or absence of the egg capsule gland. Juveniles and parasited individuals were excluded from the analysis. We considered as adult snails with a shell length greater than 32 mm for *B. monilifer* and greater than 100 mm for *A. brasiliana*. The presence or absence of a penis was recorded and the penis length was measured with 0.1 mm precision callipers. Penis was measured from its base where it joins with the body behind the right tentacle to the tip. Measurements were conducted on unnarcotized snails relaxed by cold exposure in a 4°C refrigerator. The percentage of females with imposex, average female penis length and relative penis size index (RPSI) (Gibbs and Bryan, 1994) was calculated for each species. Histological sections of each individual were stained with Hematoxilin-eosin to confirm its gonadic sex.

Imposex was clearly identified in *B. monilifer*. The percentage of imposex varied among the monthly samples from 33.3% to 85.7%. RPSI ranged from 0.14 to 0.34. Histological examination confirmed that all *B. monilifer* specimens with a penis of more than 10 mm were males and those with no penis or a penis of less than 10 mm were females. The specimens of *A. brasiliana* also showed imposex, with the percentage varying from 38.9 to 50%. RPSI ranged from 0.18 and 1.0. Histological sections analysis demonstrated that all *A. brasiliana* individuals with penises larger than 15 mm were males and all with no penis or a penis shorter than 10 mm were females.

Mar del Plata harbors the most important coastal fishery fleet in Argentina, with a coastal fleet of about 200 boats and an offshore fleet of about 100 vessels (Errazti and Bertolotti, 1998).

The sedimentary reservoir of TBT seems to contribute to its persistence here, in contrast with the rapid rates of degradation observed in the water column and in laboratory studies of suspended material. TBT has been observed to persist in sediments from 0.9–5.2 years (Gibbs and Bryan, 1994). Sediment collected near the harbor mouth at Mar del Plata has been added to its tourist beaches, during which 2 480 000 m^3 of sediments was moved during 1998 (Marcomini and López, 1999). The transfer of sediments may have increased exposure to pollutants. TBT concentrations as low as 0.5 ng l^{-1} can result in changes in morphology of some gastropods (Gibbs *et al.*, 1987).

A high degree of imposex in *B. monilifer* near Mar del Plata may be related to the observed decline of the population in the coastal waters in front of the city of Mar del Plata, the very low breeding activity observed for the very few females carrying egg capsules (which are attached to the callous region of the mother shell), the lack of juveniles and male predominance in the adults.

From these results we conclude that the Mar del Plata locality shows a high imposex occurrence, but the relatively low RPSI could reflect a contaminated area that is far from being in an extreme state. This less than extreme state could be related to the sampling zone being in open ocean and not in an enclosed system such as occurs with inlets or semi-closed gulfs or bays. Studies carried out in the harbor or near the beaches could provide more extreme figures for imposex.

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This is the first report of imposex from the large coastline of Argentina. Imposex has been previously recorded for South America, in Chile by Gooding *et al.* (1999) and in Brazil by Braga de Castro *et al.* (2000).

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Publication 5:

Search for triorganotins along the Mar del Plata (Argentina) marine coast: finding the tribultyltin in egg capsules of a snail *Adelomelon brasiliana* (Lamarck, 1822) population showing imposex effects.

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Search for triorganotins along the Mar del Plata (Argentina) marine coast: finding of tributyltin in egg capsules of a snail *Adelomelon brasiliana* (Lamarck, 1822) population showing imposex effects

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Cases of imposex were clearly identified in *Adelomelon brasiliana* living in the Mar del Plata (Argentina) coastal area; percentages as high as 50.0% were determined among the samples studied. These were the first reported cases of ocurrence of imposex in this type of gastropod. Since this is one of the known tributyltin (TBTs) effects, and no previous reports of determination of TBTs in gastropods eggs were found, methods were developed for the speciation and quantitative determination of organotins in *A. brasiliana* egg capsules. Determination of organotins in samples collected in the Mar del Plata area showed contents of tributyltin chloride (TBT) as high as 400 ng l^{-1} in water and 6.50 µg g^{-1} in sediments of areas of intensive boat traffic. The results showed the presence of TBT in the egg capsules of *A. brasiliana* at three different instars (range 0.264–1.86 µg per egg). As far as we know, this is the first report of the finding of TBT in gastropod egg capsules. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: antifouling additives; tributyltin (TBT); biological effects; imposex; Argentine gastropods; organotins; gastropods eggs; *Adelomelon brasiliana*

INTRODUCTION

Since the commercialization in the early 1960s of triorganotin (TOT) compounds as antifouling paints, and especially after the introduction of the self-polishing copolymer (SCP) formulation in the 1970s, organotins have been heavily used; by the mid-1980s they were used on over 80% of the world's commercial fleet. However, severe damage to some marine aquatic organisms has been reported, and the use of TOT as an antifouling additive in boat paints is being limited, and is even banned in several countries. A recent review[1] thoroughly covers most of the more important features reported in the literature. Some organic biocidal compounds, termed organic boosters, were proposed as alternative antifoulants after the ban on tributyltins (TBTs).[2,3] Nevertheless, the long-term potential risks of these compounds are mostly unknown yet, and contaminations by organic boosters in the coastal waters of Greece,[4] the UK[3,5] and the USA[6] have recently been reported.

Some of the most frequent and acute effects of TBTs were observed in gastropods: chronic toxicity is shown by endocrine disruption, leading to effects such as imposex, intersex and masculinization of females.[7,8] Recent reviews report more than 140 different species of gastropod that have been observed to be affected by imposex.[1,9,10] (Argentine species are not among the cited species.) The bioaccumulation potential of TBTs by top trophic-level organisms had

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been considered to be low until a recent report on the contamination by TOTs of cetaceans and pinnipeds in various regions of the world;[11] TBTs has also been found in the liver of Beluga whales (*Delphinapterus leucas*) from Canada.[12] On the other hand, we have previously reported the deleterious effect of tributyltin chloride (TBT) on *Euglena gracilis* and *Chlorella* sp., two freshwater microorganisms.[13] The observed effects (measured by growth rate and chlorophyll content) were concentration dependent. Further research using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) confirmed a TBT concentration dependence of the cell damage (A. Nudelman *et al.*, unpublished results). More recent studies carried out with TBT-dosed *E. gracilis* showed some restoration-promoting effects of iron-encaging–zeolite-processed water.[14] The deleterious effects of triphenyltin chloride (TPTCl) on the alga *Spirulina subsalsa* were recently shown by SEM and TEM.[15]

Novel organotin compounds have recently been synthesized and fully characterized.[16–18] Some of these are proposed to be used as insecticides,[17] especially those effective for the control of insects in their larval instar.[18] Some of these recommendations[17,18] are based on the early determinations of TOT stabilities that established that toxic TOT easily degraded in the environment to non-toxic tin species.[19] The presumed biodegradability should made them advantageous alternatives to the organophosphorus insecticides.[18] Nevertheless, more recent determinations of the half-lives of TBT have shown them to be as high as 8.7 years in sediments[20] and 4–17 years in the bivalve *Venerupis decussata*.[21] The stabilities of TOTs in natural media being surprisingly higher than the data previously reported strongly argues against their introduction into the environment as insecticides.

All the chemical studies on the toxicity effects of TOTs and on TBT determinations in the environment recorded in the literature refer to developed countries in the Northern Hemisphere, where there is usually legislative control. To the best of our knowledge, only one recent study monitoring butyltin contamination in green mussels collected from various Asian developing countries has been reported.[22] The study shows that polluted areas, such as Hong Kong, Malaysia, India, the Philippines and Thailand, revealed levels comparable to those in developed nations. Only one study of environmental butyltin determinations in the Latin American region has been reported. Recent determinations in surface sediments from the São Paulo State coast (Brazil) showed high concentration levels of TBT (360–670 ng g^{-1}) in zones of intensive boat traffic.[23] The first cases of imposex in marine species living along Argentine marine coasts, namely *Adelomelon brasiliana* and *Buccinanops monilifer*, were recently reported by Penchaszadeh *et al*. As this is one of the known TBT effects, it was of interest to carry out a search for TBT in water and sediments of this coastal area and in biological samples (egg capsules) of *A. brasiliana.* Methods for the quantitative determination of TBT in gastropod egg capsules have to be developed, since no methods were found in the literature. The present paper reports the finding of TBT in *A. brasiliana* egg capsules in different stages of development, as well as in water and sediments from the Mar del Plata area.

EXPERIMENTAL

Materials

Tributyltin chloride (TBT) was purchased from Aldrich Chem. Co. (USA) and used as received. Triphenyltin (TPT) was freshly synthesized by an adaptation of a previously reported procedure.[25] Samples of butyl tin trichloride 95% (MBT, Aldrich) and dibutyltin dichloride (DBT, Aldrich) were generously provided by Professor L. Ebdon (University of Plymouth, UK). Certified samples of TBT and of TPT, (standard stock solution in toluene), both Cica reagents from KANTO Chem. Co., Inc. (Japan), were generously provided by Dr Kazuko Mizuishi (Ministry of Public Health, Tokyo, Japan). Silicagel Merck, grade 60, 230–400 Mesh, 60A° was obtained from Aldrich Chem. Co., Inc. High-performance liquid chromatography (HPLC)-grade hexane was bi-distilled. Since this solvent is used for the sample treatment, the purification was repeated until no impurities were detected by gas chromatography (GC) under the splitless system.

A HP 5890 Series II Plus gas chromatograph (Agilent Technologies, Avondale, PA) equipped with a flame ionization detector (FID) system and a 30 m × 0.25 mm HP-5 (phenyl-methylsilicone 5%) capillary column (coated with a 0.25 μm thickness film) and an FID was used. High-purity nitrogen was the carrier gas; the column head pressure was controlled at 4 psi (i.e. 0.273 84 atm = 206.867 mmHg). The temperature program used was: 60 °C for 2 min, 10 °C min^{-1} to 250 °C, hold for 15 min. The injector and detector temperatures were 200 °C. To obtain maximum sensitivity, the chromatographic analyses were made under *splitless* conditions.

In some cases, GC–mass spectrometry (MS) was also carried out under conditions similar to the GC analysis and the typical clusters of tin-containing ions were observed. GC–MS spectra were recorded on a BG-Trio-2 spectrometer. In the TBT mass spectrum listed below (only the most abundant peaks are given), clusters corresponding to the molecular ion, and to the loss of 57, (2 × 57) and (3 × 57) mass units are shown between brackets. MS m/z (relative intensity) [292, (1.59), 289, (1.2) (M^+)]; [235 (67), 234 (37.5), 233 (56.7), 232 (32.1); 231 (35.4) (M^+-57)]; [183 (29) 181 (29.6), 180 (8.3), 179 (100), 178 (57.1), 177 (100), 176 (59.3); 175, (88.5) (M^+-(2 × 57)) (M^+-1-(2 × 57)]; 121 (42.0), 120 (25.5), 119 (35.8), 118 (19.3), 117 (18.7) (M^+-(3 × 57)].

Sampling

Living specimens of adult *A. brasiliana* were collected by bottom trawling from two sampling stations: one located at 1 km north from Mar del Plata harbour in a high boating-activity area, and the other in front of the restricted Marine

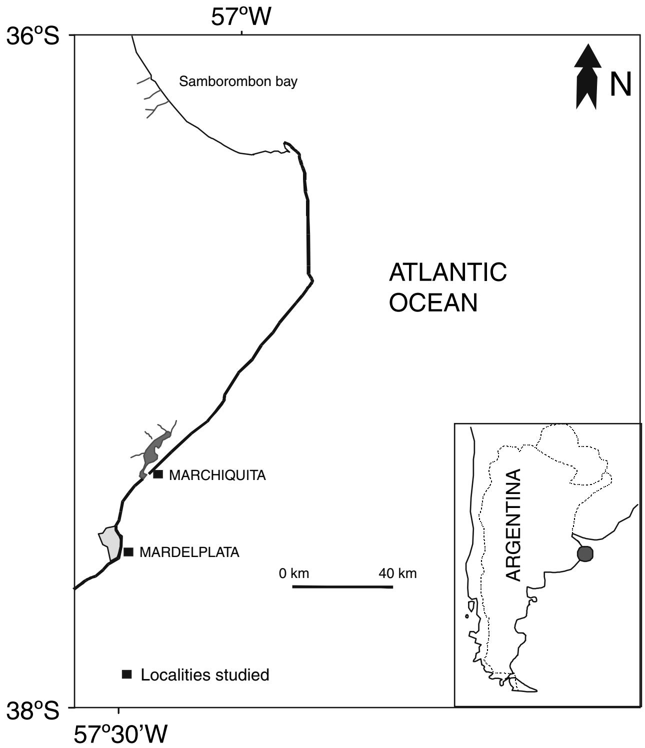

Figure 1. Sampling location. Shaded zones indicate the areas where samples of water, sediments and specimen were collected.

Reserve of Mar Chiquita, a very low boating-activity area (see the map in Fig. 1). Samples of water and sediments were collected from the beach, the coast, the North Pier and the harbour of Mar del Plata, including a very crowded boating area. Additional samples were also collected in Mar Chiquita and in Valeria del Mar, a resort area with low boat traffic, located about 100 km north from Mar del Plata. The egg capsules of *A. brasiliana* were collected near the Mar del Plata coast.

Methods for the imposex determinations were those previously reported.[24] The percentage of females with imposex, average female penis length and relative penis size index calculated for each species in each location.

Chemical analysis

Determinations of TBTs were carried out by GC after derivatization with sodium borohydride. Water and sediment samples were processed following methodologies reported elsewhere.[26] To determine the detection limit of the whole procedure, standards solutions containing known amounts of TBT, TPT, MBT and DBT were processed as the environmental samples. The minimum quantitatively detectable concentration (defined as the signal equal to three times the standard deviation of the baseline noise value) was of $0.08 \pm 0.01\ \mu g\ l^{-1}$ for TBT.

Derivatization with sodium borohydride

To processed standard solution of TBT in hexane (5 ml) or to the sample solution (5 ml) in a 50 ml round-bottomed flask, 5 ml of $NaBH_4$ solution (0.08 g in 5 ml ethanol) were added; the mixture was allowed to react for 1 h. The reaction mixture was washed with 10 ml of 10% aqueous NaCl solution; the organic layer was dried with anhydrous sodium sulfate and then purified through a silicagel column. The eluate was gently concentrated under vacuum at room temperature up to 0.2 ml; it is very important to avoid heating and also to complete solvent distillation, since losses of TBT hydride (TBTH) can occur. It was checked that under the controlled conditions described complete TBTH is recovered. 0.5 µl of the solution was injected for the CG analysis. Decaline (*cis*,*trans*-decahydronaphthalene) was used as subrogate. The chromatogram of the subrogate showed two signals, one at 7.4 min and the other at 8.1 min retention time. The latter exhibits the larger area, and this was taken as the reference.

Determination of TBT in *Adelomelon brasiliana* egg capsules

The giant egg capsules of *A. brasiliana* (with *ca* 100 ml of intracapsular liquid) were classified according to the embryos' development stage.[27] The eggs were cut with a knife; the liquid was separated from the solid phase by a syringe and treated separately.

For the liquid phase 40 ml of the phase were dispersed with 5 ml of 10% aqueous NaCl solution, 20 ml of hexane were added and stirred for 15 min. The material was centrifuged until net phase separation was achieved. The organic phase was transferred to a dry Erlenmeyer flask fitted with a Nalgene cap; this phase was dried with anhydrous sodium sulfate, then filtered and concentrated to nearly 5 ml at room temperature under reduced pressure. A similar procedure as described before was followed for the derivatization and chemical analysis of TBT.

The solid phase was treated consecutively with 5, 5, and 2.5 ml of hexane. The organic phase was worked up as described above and TBT was determined as described previously.

For both phases, the efficiency of the extraction of TBT was controlled in the preliminary runs by using five hexane aliquots and processing separately both the 1–3 and the 4–5 aliquots. No signals for TBT were found in the analysis of the second extraction batch.

RESULTS AND DISCUSSION

Imposex is clearly identified in *Buccinanops monilifer* from the Mar del Plata area; the percentage of imposex varies from 33.3 to 85.7% among the studied samples collected at different times in several areas of Mar del Plata.[24] The *A. brasiliana* specimens collected from the same area also showed imposex occurrence with a percentage variation from 38.9 to 50%.[24] *B.*

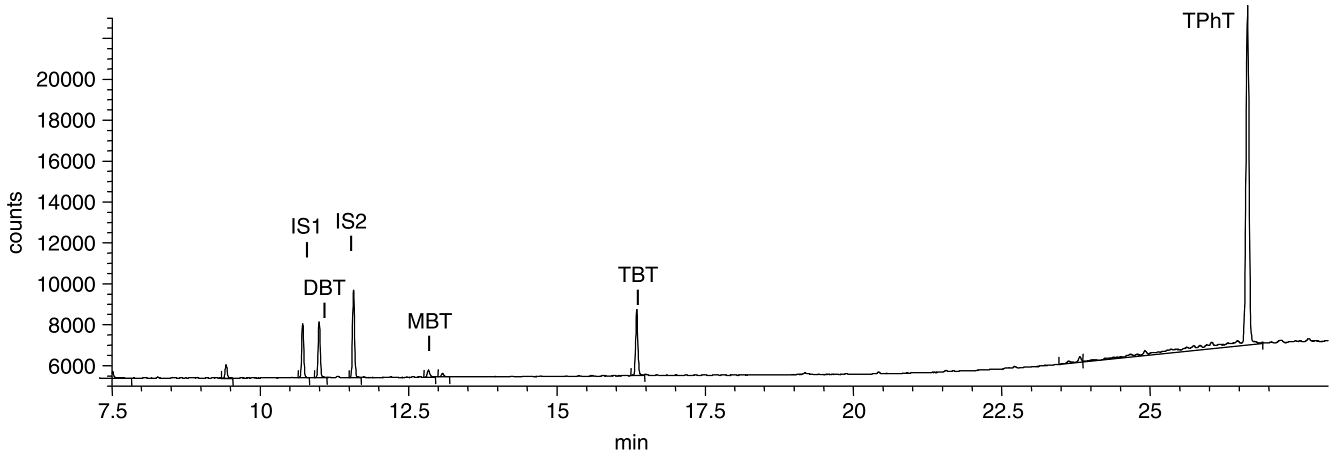

Figure 2. Chromatogram of a typical mixture of standards processed as described in the Experimental section. Amounts of each TOT in this mixture: DBT: 17 μg; TBT: 22.5 μg; MBT: 28 μg; TPT: 250 μg.

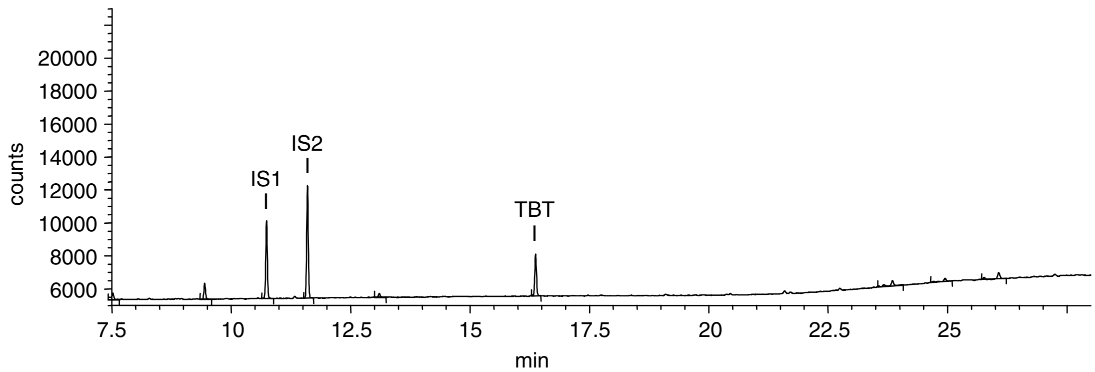

Figure 3. Chromatogram of a sample of water from the Mar del Plata coast (sample 4 of Table 1) processed as described in the Experimental section.

monilifer and *A. brasiliana* from the Mar Chiquita area showed no sighs of imposex and no TBTs were detected in water or sediments from that area. Mar del Plata harbour contains the most important coastal fishery fleet, constituting about 200 boats; it concentrates 82.5% of the coastal catch landings from the Province of Buenos Aires; the harbour also has about 100 boats in the offshore fleet.[28]

TBT determination in water and sediments

Various analytical techniques for organotins and their degradation products in environmental matrices have been reported.[29] GC after chemical derivatization is one of the most popular techniques for butyltin[30–34] and methyltin species[35] analysis. GC analysis is currently being performed utilizing one of many suitable detection methods.[36–39] Several HPLC determinations of TOTs have been published;[40,41] nevertheless, since in HPLC separations for organotin applications the peaks are generally broader than those encountered with GC, this technique was chosen as the most suitable for the purposes of the present work.

The methods currently used for the determination of environmental TOTs involve various analytical steps, such as extraction, derivatization, separation, and final detection, and this multiplies the risks of analytical errors.[42,43] In order to improve and ensure good quality control of tin speciation analysis, a series of interlaboratory studies have been organized in the past few years;[44] we have tried to follow most of the recommendations given. The analysis of each sample was made at least by individual triplicate, especially in the cases of samples with minor contents, to ensure that the products detected were present in the sample and not just artefacts of the analytical procedure.

Figure 2 shows a typical chromatogram of a standard solution containing TBT, DBT, MBT, TPT and the subrogate (called IS1 and IS2 in Figs 2 and 3). MBT is highly hygroscopic and the actual concentration in the mixture could not be defined with the same precision as the rest of the TOTs. Figure 3 shows the chromatogram obtained for a sample of water from the Mar del Plata coast (sample 4), working under splitless conditions. For some cases, the identity of the peak

Table 1. Concentrations of TBT determined in water and sediment samples

	Sample	Concentration of TBT[a]		Total tin[b]	
		Water ($ng\ l^{-1}$)	Sediment ($ng\ g^{-1}$)	Water ($ng\ l^{-1}$)	Sediment ($ng\ g^{-1}$)
1	Mar del Plata beach	180	160		
2		190	240		
3	Mar del Plata coast	200	Not determined		
4		400	Not determined	405	Not determined
5		300	Not determined		
6	Harbour[c]	4800[d]	4300		
7		8000[d]	6500	7900	6400
8		7500[d]	5000		
9	Mar del Plata North Pier	4800[d]	1300	4820	1250
10		4000[d]	1100		
11		4500[d]	1400		
12	Mar Chiquita	0	0		
13		0	0		
14	Valeria del Mar[e]	0	2.5		
15		0	0		

[a] Concentrations are reported as Sn, according to Ref. 12. Detection limit for quantification: 80 $ng\ l^{-1}$.
[b] Total tin determined by HGAAS. Error ±5%.
[c] Very crowded boating area of Mar del Plata harbour.
[d] Supernatant liquid of the deep sediment.
[e] Samples were collected after storm with winds from the south, named 'Sudestada'.

at the retention time of TBT was confirmed by GC–MS of the derivatized samples as giving TBTH. In all cases, the clusters corresponding to the molecular weight of TBTH (292 for ^{120}Sn), as well as the ions of m/e (M^+-1), (M^+-1-57), [M^+-1-(2 × 57)] and [M^+-1-(3 × 57)], were clearly shown.

The results obtained in the analysis of water and sediment samples for TBT and total tin are summarized in Table 1. As can be observed, TBT was not detected in samples of water from the Mar Chiquita area (samples 12 and 13 in Table 1), nor in Valeria del Mar (samples 14 and 15), a neighbouring open beach. In samples of water collected near the Mar del Plata (MdP) beach, (samples 1 and 2) and also in the MdP coastal area (samples 3–5), TBT was found in significant amounts. Small leisure boats arrive at the MdP coasts, and these might be the cause of the increased [TBT] with regard to the beach area. Interestingly, determination of the total tin concentration in the water and sediment samples by hydride generation atomic absorption spectrometry (HGAAS) indicated that the TBT made up mostly 100% of the tin, suggesting that anthropogenic organotins represent the major source of tin in these areas (data are given in the last column of Table 1). The observed results for [TBT] in the range 180–400 $ng\ l^{-1}$ in the waters of these two zones are higher than those reported, for example, by Suzuki *et al.*[45] i.e. 242 ± 30 $ng\ l^{-1}$ (72.6 $ng\ l^{-1}$ expressed as tin), for depuration experiments in Moroiso Bay (Japan), but they are comparable to the higher value of 480 $ng\ l^{-1}$ determined in December 1994 in the Aburatsbo Bay (Japan).[46] Nevertheless, more recent determinations at more than 100 sampling points in Japan showed important decreases of organotins in seawater: the maximum observed value was 9.8 $ng\ l^{-1}$,[1,47] and the concentrations of TBT in the open sea and in deep-sea locations are lower (a maximum of 0.1 $ng\ l^{-1}$).[48]

The sedimentary reservoir of TBT is important, given its apparent persistence. In contrast to the more rapid rates of degradation observed in the water column in laboratory studies (about 6–10 days) and in seawater (several weeks to several months according to recent determinations),[49] TBT has been proved to have a half-life in sediments in the range 0.9–5.2 years;[50] and a value as high as 8.7 years has recently been reported.[20] Therefore, the observed concentration ranges of TBT in sediments are usually higher than those in water. In the present study, the [TBT] in sediments of the MdP beach are not much higher than those observed in the seawater, suggesting that the inputs of TBT in this area could be relatively recent (probably arising from TBT coming from the more contaminated, crowded areas). On the other hand, at the MdP North Pier (where the boat traffic is intense and the area was restricted water exchange) and in samples coming from the MdP harbour (where big commercial vessels operate), the TBT concentrations determined in sediments are very large. The values found in the supernatant liquids of the sediment samples were also very high; we have found no reports on such high [TBT] in the recent literature. These values show the accumulation of TBT since probably more than 15 years ago. The highest reported values we have found were those of Amouroux *et al.*,[51] who found concentrations of TBT of 3340 ng g^{-1} in sand and 600 ng g^{-1} in silt collected in the vicinity of the shipyard in Arcachon Harbour (France). A recently published range of measured TBT in different aquatic environments shows values within the range 1–1000 ng g^{-1} for harbour sediments.[52] The highest concentration levels observed in recent determinations of TBT in São Paulo State (Brazil) show 360 ng g^{-1} in Santos harbour and 670 ng g^{-1} in Guaruja marina, which seem to be related to intensive boat traffic.[23]

TBT determination in egg capsules of *Adelomelon brasiliana*

Interest in the effects of TBT on eggs of different species is currently being shown. Toxicity studies of TBT in fertilized eggs of gilthead seabream *Sparus aurata*[53] and also in eggs of *Ascidia malaca*[20] have recently been published, and the results confirm the acute toxicity of TBT; they showed that the fertilization process is affected greatly and the reproduction of ascidians under unfavourable environmental conditions is prevented. These studies were carried out by incubation of the eggs in solutions of TBT of variable concentration, and the different degrees of damage were evaluated. No determinations of TBT in the eggs were reported.

In the present study, egg capsules of *A. brasiliana* at different stages of development were collected near the more contaminated areas and methods were developed for the determination of TBT concentration in the liquid phase and in the solid phase. Table 2 shows the results obtained for egg capsules at different stages. It can be observed that, even in early stages of embryogenesis, TBT is present in the liquid, as in the dispersed phases; in fact, the total amount of TBT/egg capsule present in the liquid phase is much greater than that in the dispersed phase. In early stages of development, the amount of TBT is increasing, although it is still relatively low; the content/egg capsule of the liquid phase is also relatively higher than in the solid phase. Contrastingly, at stage 3, the TBT content in the solid phase is very high and the content/egg is higher than in the liquid phase.

It is probable that, if the egg matures, the new born gastropod will show a high degree of imposex, masculinization of the females and other known disorders caused by organotins. Although the egg capsules are permeable to TBT, the concentration observed even in the stage 0 of *A. brasiliana* eggs, which is much higher than that determined in the water of the more contaminated areas, suggests that TBT could be transferred from the gastropod females to the eggs.

To the best of our knowledge, this is the first report of the finding of TBT in the egg capsules of gastropods. TBT has been found in a neonate Beluga whale; since the necropsy revealed that the neonate had no milk in its stomach, the only explanation for the presence or butyltin compounds in the liver is via *in utero* transfer from maternal blood to the foetus.[12]

Table 2. Concentrations of TBT determined in eggs[a]

Development stage	Solid phase (ng/ovicapsule)	Liquid phase ng ml^{-1}	Liquid phase ng/ovicapsule
0	14.2[b]	3.2	250
		2.9	240
0	41.8[b]	5.6	437
		4.2	328
1	390[c]	7.1	618
		2.4	213
		5.3	460
3	1180[d]	6.8	683
		5.3	484
3	800	6.0	548
		5.0	456

[a] Concentrations are reported as tin, according to Ref. 12.
[b] Dispersed solid.
[c] The solid phase was not abundant.
[d] Both phases had sharp limits.

CONCLUSIONS

Our results indicate that high concentrations of TBT in water and sediments in the Mar del Plata area, especially in the harbour and North Pier, are responsible for the high level of imposex observed in *B. monilifer* and also in *A. brasiliana*. The results also show that the TBT content made up mostly 100% of the tin present in the area, confirming that anthropogenic organotins represent the major source of tin there. The observed decrease in the population of *B. monilifer* and *A. brasiliana* in the Mar del Plata area make clear the need for controlling the environmental risks associated with the release of TBT, and the urgent ban of tin-based antifouling paints, at least for leisure and fishing boats. In view of the present results, fishermen's practices of cleaning and re-painting their boats along the Mar del Plata coasts should also be controlled.

The first reported finding of TBT in *A. brasiliana* egg capsules showed the accumulation of TBT. The observed concentrations, which are much higher than the environmental TBT concentration (surprisingly high in the liquid phase of early states of embryogenesis), likely suggest that it could also partially arise by transferability of TBT from the contaminated mother.

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Publication 6:

Imposex and Organotin compounds in marine gastropods and sediments from the Mar del Plata coast, Argentina.

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Abstract

The occurrence of imposex in *A. brasiliana* during two consecutive years near the principal fishery port of Argentina and its relation with TBT and DBT contain in muscle tissue and hepatopancreas was investigated using gas chromatography. Percentage of imposex affected females increased over 80 % at summers end and spring of both years. But its intensity, given by the RPSI, fluctuated usually around low values*. A. brasiliana* hepatopancreas samples presented a very low concentration of TBT (36-46 ng/g dry weight). Egg capsules developed in this environment presented normal viable embryos, which had similar TBT concentration than the capsule wall (15 ng/g) while the intracapsular liquid was only 2 ng/g, similar to the surrounding environment. The proportion and distribution of BTs in sediment indicate a major contamination event and the posterior transport of the polluted sediment mediated by currents.

Introduction

Since the 1960s Tributyltin (TBT) compounds are used in paints to prevent organisms settling on ship hulls. The antifouling compounds were proven to cause many disturbances in the biota as they leach into the water. TBT has been shown to cause external masculinization of females, termed imposex, through the inhibition of sulphur conjugation of testosterone and its phase I metabolites, resulting in a build-up of androgens in the tissues (Ronis & Mason, 1996).

The application of OTs has been banned in many countries and its use on vessels of less than 25 m in length is forbidden. In Argentina, the law (ordenanza) 4/98 (DPMA) Tomo 6 Anexo 1 prohibits the application of TBT on sports boats.

Many species of molluscs are sensitive to TBT (Langston, 1996) and imposex was described in at least 120 neogastropod species (Schulte-Oehlmann et al, 1997). Penchazsadeh et al*.* (2001) reported the occurrence of imposex in the coast of Buenos Aires province, Argentina for two gastropods species, *Adelomelon brasiliana* and *Buccinanops monilifer* but until this date there have been no reports of OTs determination in this gastropods.

Morcillo et al*.* (1997) and Stäb et al. (1996) pointed out that OTs are accumulated in the gastropod hepatopancreas affecting their reproductive capacity.

On the other hand, the ingestion of TBT can produce an impaired immune response in lower doses (Snoeij et al. 1988), to neural and renal damage (Lin et al. 1998; Lin and Hsueh, 1993).

Its is very important to control the TBT level in some marine invertebrates because its economic importance as sea food as well as the reproductive impairment TBT can produce, which can endanger the affected population.

Here were investigated the OTs levels in sediment, commercial gastropods and their egg capsules along 50 km of coast.

Materials and Methods

Sampling

To study OTs pollution degree and its effect on the population, the relative penis size index (RPSI) and imposex percentage in *A. brasiliana* females were recorded along 24 months in Mar del Plata (Fig. 1). As control, black snail seasonal samples were taken near the Mar Chiquita coastal reserve, where no TBT was recorded before (Goldberg et al. 2004).

Monthly percentage of imposex-affected females was calculated as the number of females with penis and/or vas deferens of all females sampled this month.

Monthly RPSI was calculated according to the following equation (Gibbs & Bryan 1994)

$$\frac{\text{Female penis mean size}^3}{\text{Male penis mean size}^3} 100$$

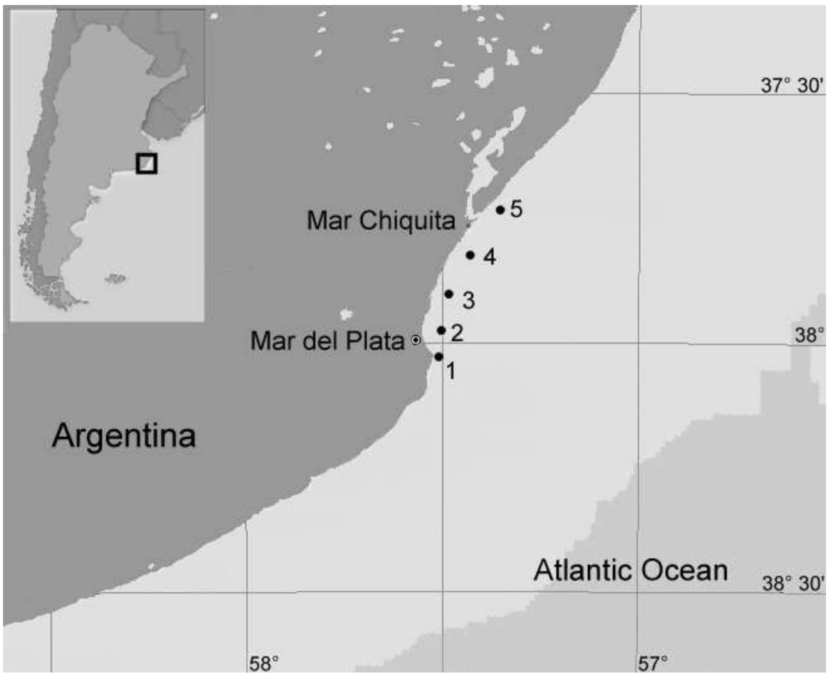

Figure 1: Sampling sations for sediment along the coast of Buenos Aires, Argentina. Samples of *A. brasiliana* were taken from the southern location.

TBT in *A. brasiliana* and sediment:

In January, July and December 2002, *A. brasiliana* samples were taken near the port. TBT and DBT content in the foot and hepatopancreas of the captured animals were determined by Gas chromatography. The analytical procedure was carried out under DIN-Norm 38407-F13 (modified) for water, sewage and mud.

Five individuals per sample were analysed together in order to obtain mean BT values per month. For the 3 months, 1.5 g foot mix and 1.5 g hepatopancreas mix were separately extracted with 20ml methanol, 40 g of a NaCl 10% solution, 40 ml acetic buffer (pH approximately 4.6) and finally 100ml hexane. 250ng of an intern standard of Tetraropyltin, Tripropyltin, Monoheptyltin, and Diheptyltin 100ng/ml was added in order to control the effectiveness of the OTs recover.

The ethylation was carried out by adding 3 times 2ml Na-Tetraethylborate 10% with 30 minutes between each addition. 50ml of supernatant hexane were taken with a 10ml pipette. 5 g Na-sulphate were added to this 50 ml hexane to dry the solution during 60 min. This dry solution was concentrated to 2-3ml in a rotary evaporator.

A clean-up was made in a silica gel column with 15 ml of hexane/acetate 92/8 eluate. The resultant was concentrated again to 1.5ml.

The BTs' content in *A. brasiliana* egg capsules was also measured.

The measurement of extracts was done with GC/AED-System. GC: HP6890 gas chromatograph equipped with an Atomic-Emission-Detector HP G2350A with ICP-Plasma. The separation was carried out on a GC-Column 30m HP-5MS 5% Pheny-Methyl-Siloxan, 0.25mm ID coated with 0.25µm film phase. Injection was performed with a Gerstel Cold-System at 40°C, separation with temperature program 40-320°C, within 35min. Helium was used as the carrier gas at a flow-rate of $2.7 ml*min^{-1}$. The ICP was maintained with H_2, O_2 and Ar/CH_4 and N_2 as make-up gas (2000°C). AED-signals were measured at 303 and 301 nm wavelength.

Because TBT deposited in marine sediment can be bioavailable for years (Strand et al., 2003), samples of sediment were collected with a dredge in January 2001 and December 2002 in five places of a transect parallel to the coast (Figure 8), limited by the port on the south and the Mar Chiquita coastal reserve on the north. 15 grams of wet sediment were analysed following the same procedure described above.

As a control for the chemical procedure the reference material BCR 646 freshwater sediment (TBT, DBT, MBT, TPhT, DPhT, MPhT certified) was used.

The control for the snails was the reference material BCR 477 certified mussel tissue (Morabito et al., 1998). The detection limits of BTs and PTs were of the order of $0.2 ng*g^{-1}$ dry weight.

The Bioconcentration Factor (BCF) was calculated as the TBT content in gastropod tissue divided by the TBT content in sediment (Takeuchi et al. 2001) as a form of comparison with other molluscan species affected by TBT in the world.

Results

The analysis of sediment samples indicate the presence of a gradient of BTs in the environment with a maximum at the port entrance decreasing towards Mar Chiquita. DBT presents the same pattern (Table 1). There is a clear dominance of MBT and a low presence of TBT in all sediment samples.

Table 1: Organotin content in sediment samples from Mar del Plata to Mar Chiquita, Argentina

Location	1 [ng/g]	2 [ng/g]	2′ [ng/g]	3 [ng/g]	3′ [ng/g]	4 [ng/g]	4′ [ng/g]	5 [ng/g]
MBT	14,39	12,13	12,27	16,11	16,89	15,21	15,70	13,78
TePrT	50,00	50,00	50,00	50,00	50,00	50,00	50,00	50,00
DBT	8,25	7,85	7,51	6,12	6,94	5,56	5,74	5,44
TBT	2,45	2,29	2,13	1,41	1,60	1,00	1,05	0,50
TeBT	0,19	0,22	0,22	0,29	0,24	0,29	0,24	0,22

The hepatopancreas of the analysed animals presented always a higher TBT and DBT contain than the foot (Table 2). DBT content in hepatopancreas gradually increases in the hepatopancreas, whereas the foot presents an oscillatory pattern.

Table 2: Organotin content in tissue samples ($ng*g^{-1}$) of A brasiliana females from Mar del Plata, Argentina, along a year.

Date	January 2002		June 2002		December 2002	
SAMPLE	Muscle	Hepatopancreas	Muscle	Hepatopancreas	Muscle	Hepatopancreas
TBT	72	42	92	28	92	42
DBT	39	17	38	18	35	9.1
MBT	133	26	29	35	31	10
TPhT	8.9	10	19	4.3	33	30
DPhT	0	0	0	0	0	0
MPhT	0	0	0	0	1.1	1

BTs in egg capsules: The analysed egg capsules contained early and late stage embryos and the TBT content in embryos increased more than in intracapsular liquid (Table 3).
The percentage of imposex affected females decreased and the RPSI fluctuated during the sample period (Table 4). Almost half of the population showed no sings of imposex at the end of the sampling.

Table 3: OTs concentration in ng g^{-1} in the 3 parts of an egg capsule in late development stage and in intracapsular liquid of an egg capsule in early stage of development.

Capsule stage	Capsule part	TBT	DBT	MBT	TPhT	MPhT
late	Intracapsular liquid	2	1.3	3.0	0.4	0
late	Capsule wall	18	10	21	82	2.3
late	Embryos in late stage	15	4.4	10	8.9	0
early	Intracapsular liquid	2.8	2.2	2.0	0.9	0

Table 4: Degree of imposex in *A brasiliana* females of Mar del Plata, Argentina

Month	RPSI	% of imposex affected females
Nov 00	1.74	36
Dec00	1.74	47
Jan 01	0.44	32
Feb 01	0.55	53
Mar01	0.70	100
Apr 01	0.00	50
May 01	0.00	40
Jun01	0.00	33
Jul 01	0.99	53
Aug01	2.88	88
Sep 01	3.00	70
Oct 01	3.25	66
Nov01	1.88	57
Dec 01	0.75	33
Jan02	7.04	71
Feb 02	2.57	100
Mar 02	2.10	80
Apr 02	1.80	70
May 02	1.30	60
Jun02	1.19	50
Jul 02	2.25	60
Aug02	0.20	78
Sep 02	0.15	70
Oct 02	0.10	60
Nov 02	0.08	50

Discussion

TBT low concentration in sediment of the study area is the result of the natural degradation process of this compound and a lack of new mayor inputs, while the high level of MBT indicates an old contamination event reported by Penchaszadeh et al. (2001).

The amount of the contaminant in sediments of Mar del Plata in 2002 was already lower than in other port areas of the world such as in Osaka 10 ng/g (Harino et al1998), 6 - 520 ng/g in Westeinder Lake, Netherlands (Stäb et al. 1996) and 2.4-18.8 ng/g in Øresund and Kattegat/Skagerrak (Strand et al 2003).

TBT in sediment produces imposex at concentrations of 1ng Sn/l in *Nucella lapillus* (Gibbs et al 1987) and in *Hinia reticulata* (Storben et al 1992) but only at 7 ng/g in *B. undatum* (Mensink et al 2002). In the studied area, BTs content in the sediment changed from 240 $ng{*}g^{-1}$ in 2001 (Goldberg et al. 2004) to 1.4 $ng{*}g^{-1}$ at the end of 2002 in Mar del Plata and from 0 $ng{*}g^{-1}$ in 2001 (Goldberg et al. 2004) to 0.2 $ng{*}g^{-1}$ in 2002 in Mar Chiquita, indicating a reduction and a current transport of the pollutant at the same time.
These BTs concentrations induced the development of small penises in females captured near the port, but did not further affect the reproduction since the functional reproductive organs were not modified and gonads of these animals were histologically similar to those of specimens captured in Mar Chiquita (Cledón et al. accepted), which did not presented any signs of imposex.

The DBT contained in the hepatopancreas of the analysed gastropods increased during the last year of sampling reflecting the continuous incorporation and accumulation of the pollutant, which was over 30 times higher than the values for sediments. Such a high concentration in only few trophic levels was already reported in other organisms. Bioconcentration factors (BCF) are one way to compare this phenomenon among different organisms and can be as high as 5×10^4 (Takeuchi et al. 2001) depending on the BT content in the sediment and the species incorporation rate. Comparing all these facts with the reports on other molluscan species (Table 5) the studied population of *A. brasiliana* showed a low BCF.
The adult females of *B. undatum* are less sensitive to TBT than juveniles because they already have a developed genital system (Mensink et al 2002). In the present study, now indication of such an effect was registered since polluted and unpolluted animals reached maturity at the same length and age (Cledón et al.submitted).
It still not clear how BT's are incorporated, Strand et al (2003) suggested that deposit feeders are ingesting the TBT rich fraction. *A. brasiliana* mainly feeds on *A. purpurata*, a bivalve living in close relation with the sediment. On the other hand Stäb et al. (1996) proposed that BTs are passed on primarily via the water. It seems possible that *A. brasiliana* incorporates BT's through both pathways and it should be studied which one produces a stronger effect.

Table 5: Bioconcentration factors reported for different molluscan species.

Species	BCF	Reference
Gastropoda		
Adelomelon brasiliana	7.7 – 32.8	Present study
Thais clavigera	5000 - 10000	Horiguchi et al. 1995
Nucella lapillus	29000	Bryan & Gibbs 1991
Bivalvia		
Dreissena polimorpha	20-45	Stäb et al. 1996
Nuculana pernula	138-404	Strand et al. 2003
Mytilus edulis	7400-19000	Zoulian & Jensen 1989
Crenomytilus grayanus	11000	Suzuki et al. 1998
Mytilus galloprovincialis	11000	Takahashi et al. 1999

Mensink et al. (2002) suggested those egg capsules are a relatively clean environment for *Buccinum undatum* embryos, since embryos developed normally in the capsule but died after hatching in heavily-contaminated water.

The results of this study indicate that the liquid phase of the egg capsules of *A. brasiliana* depends on the TBT content of the environment.

The mechanism of TBT incorporation in the egg capsules still needs to be investigated. Nevertheless the increase in TBT content during the development of the embryos suggests that it is a dynamic process, which occurs while egg capsules role on the sediment.

TBT-based antifoulants are still used on large vessels and consequently are found in ports. Due to the slow degradation of TBT in sediments, it is continuously accumulating. This may cause a problem if sediments are remobilized through events such as storms or dredging operations (Sarradin et al. 1991, Dowson et al. 1993, de Mora et al. 1995). During 1998 an enlargement of Mar del Plata beaches implied the mobilization of almost 2.5 million cubic metres of sand (Marcomini & López 1999) causing possibly the occurrence of imposex in the area. The present findings are opposite to many studies reporting virtually no imposex at open coasts (Bright & Ellis 1989; Smith & Mc Veagh 1991; Foale 1993; Evans et al. 1995).

Bryan et al. (1986), Evans et al. (1996, 1998) Minchin et al. (1995), Nyarko & Evans (1997), and Evans & Nicholson (2000) proposed that open oceanic coasts are free of TBT pollution at biologically significant levels probably because of the dilution effect of the sea. TBT pollution is localized in ports (Evans & Nicholson 2000) and disappears rapidly when distance from the port increases. Even after the 30 years in which it has been used as the major biocide in antifoulants, there are still healthy gastropods populations within a few kilometres from harbours all around the world (Evans et al. 1996, Smith & McVeagh 1991; Foale 1993; Nyarko & Evans 1997).

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