

## IMPOSEX IN *Odontocymbiola magellanica* (CAENOGASTROPODA: VOLUTIDAE) IN PATAGONIA.

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### ABSTRACT

*Odontocymbiola magellanica* (Gmelin, 1791) is a volutid snail from Patagonian shallow waters. Sampling by scuba diving in depths of 6-10 m was performed in Golfo Nuevo, Golfo San José and Golfo San Matías, Patagonia, in order to study the imposex occurrence. *O. magellanica* showed between 85% and 100% imposex near harbors with high marine traffic in Puerto Madryn, Golfo Nuevo, and a Relative Penis Size Index (RPSI) between 0.77 and 3.84; in scuba diving zones with sport boats traffic the imposex percentage decreased between 35% and 59% and the RPSI was between 0 and 0.15; in zones with low marine traffic the percentage of imposex was null. Although the species is under imposex occurrence in the area we observed females spawning in 2002. A posterior study in 2004 near the harbor zone showed the persistence of imposex in these marine gastropods. In Golfo San José the imposex was null and in San Antonio Este, Golfo San Matías the imposex was 100% and the RPSI 0.12. Imposex occurrence suggests the presence of TBT in the areas near harbors.

**KEY WORDS:** imposex, TBT, Volutidae, marine pollution.

### RESUMEN

**Imposex en *Odontocymbiola magellanica* (Caenogastropoda: Volutidae) en Patagonia.** *Odontocymbiola magellanica* (Gmelin, 1791) es un gasterópodo integrante de la familia Volutidae que habita aguas someras patagónicas. Se realizaron muestreos mediante buceo autónomo en profundidades entre 6 y 10 metros en localidades del Golfo nuevo, Golfo San José y Golfo San Matías con el fin de estudiar la existencia de imposex. El porcentaje de imposex observado en *O. magellanica* fue entre 85% y 100% en zonas cercanas a puertos con alto tráfico marítimo en Puerto Madryn, Golfo Nuevo, y un Índice Relativo del Largo del Pene (RPSI) entre 0.77 y 3.84; en zonas de buceo deportivo, con tráfico de embarcaciones pequeñas, el porcentaje de imposex fue entre 35% y 59% y el RPSI fue entre 0 y 0.15; en zonas con escaso tráfico marítimo el porcentaje de imposex fue nulo. Aunque esta especie presenta imposex en el área, observamos hembras oviponiendo en 2002. Un estudio posterior en 2004 cerca de la zona portuaria mostró la persistencia de imposex en estos gasterópodos marinos. En el Golfo San José el porcentaje de imposex fue nulo y en San Antonio Este, Golfo San Matías el porcentaje de imposex fue 100% y el RPSI 0.12. El fenómeno de imposex encontrado sugiere la presencia de TBT en las zonas cercanas a puertos.

**PALABRAS CLAVE:** imposex, TBT, Volutidae, contaminación marina.

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### INTRODUCTION

Species of the Volutidae family in the Southern Atlantic Ocean are about a dozen (Carcelles, 1944; Clench and Turner, 1964; Clench and Turner, 1970). They have been object of accidental extraction during trawling fishing, but in the last years a new market demand appeared, which involved specific fishing of few species. Up to date *Odontocymbiola magellanica* (Gmelin, 1791) is not a target species, it distributes from off Río de la Plata (35° S) to Chiloé Island in Southern Chile (Weaver and duPont, 1970; Osorio *et al.*, 1979). In depths between 100 and 110 meters off the Province of Buenos Aires, *O. magellanica* is part of the *Zygochlamys patagonica* (King & Broderip, 1832)

community. In Patagonian shallow waters it lives in mixed bottoms of gravel and sand. Volutids live buried into the substrate or upon it.

Many gastropod species have demonstrated TBT sensitivity and could be used as TBT monitors in the marine environment (Gibbs and Bryan, 1994; Bright and Ellis, 1990; Bettin *et al.*, 1996). Imposex occurrence is explained as a result of an anti-fouling paint compound, TBT (tributyltin) (Bryan *et al.*, 1986), whose presence is related to high marine traffic (Smith, 1981; Gibbs *et al.*, 1987). The grade of TBT presence in the water or sediment causes a directly related response on gastropods, consisting in the appearance of a *vas deferens* and sometimes a penis in females. In

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some cases the *vas deferens* develops in such a way that causes the obstruction of the female's ovipore (Gibbs, 1996); other effect could be *bursa copulatrix* splitting (Gibbs *et al.*, 1990).

In Latin America, imposex occurrence was first observed in Chile by Gooding *et al.*, (1999) and in Brazil by Braga de Castro *et al.* (2000). In Argentina imposex occurrence was detected for the first time in Mar del Plata harbor; Buenos Aires province (Penchaszadeh *et al.*, 2001). Later studies were performed in the South Atlantic and Pacific coasts (Caetano and Absalão, 2002; Fernandez *et al.*, 2002; Goldberg *et al.*, 2004), confirming the relation between imposex occurrence and TBT concentration in water, sediments and egg capsules.

#### MATERIAL AND METHODS

In order to determine the imposex occurrence, specimens of *O. magellanica* were collected from 10 stations in Golfo Nuevo, one station in Golfo San José and one station from Golfo San Matías during 2000 – 2004 (Table 1). Golfo Nuevo localities 1 to 4 (Table 1) are situated in the same area with high marine traffic; localities 5 to 8 are in a near by area with lower marine traffic, with presence of sport and diving ships; localities 9 and 10 are situated in different zones in the gulf, in areas with very low marine traffic. Station 11 and 12 are situated in different gulfs, with no marine traffic in the former and high marine traffic in the latter. The low number of specimens collected in some stations is due random distribution of this species with low densities (1-

members of the Volutidae in the SW Atlantic (Ayçaguer, 2002). Internally sex was determined by the presence of the egg capsule gland. Penis length was measured with calipers. The per centage of females with imposex, average female penis length and Relative Penis Size Index (RPSI) (see Gibbs and Bryan, 1994) was calculated for each species in each location. Gonad pieces of 3 mm thick were fixed in Bouin's solution for 3 hours and then preserved in ethanol 70%. Later dehydration and inclusion in paraffin were performed. Histological cuts of 5 micrometers were made for each individual and stained with hematoxiline-eosine as to control the gonadic sex. Special attention was taken in reproductive seasons, and with females in oviposition. Slides were observed under an Axiostar Zeiss optic microscope; *vas deferens* was observed in a Zeiss stereoscopic microscope; digital photographs were taken with a Sound Vision digital microscope camera version 2.0 and figures were prepared by using a Photoshop 5.5 program.

#### RESULTS

A total of 235 specimens of *O. magellanica* were sampled between September 2000 and July 2004. In the samplings performed during 2002 – 2004 the species showed an imposex occurrence between 85% and 100% near harbors with high marine traffic and inside gulfs (Puerto Madryn and San Antonio Este), and a RPSI between 0.77 and 3.84; in scuba diving zones, into the gulf and with sport ships traffic the imposex percentage was between 35% and 59% and the RPSI was between

<b>Sampling site</b>	<b>% imposex</b>	<b>RPSI</b>	<b>Total N</b>
1-Parque Piedras	71-100	1.3-2.2	24
2-Salmonera Ventura	100	0.91	4
3-Alpesca	100	0.69-1.3	30
4-Aluar	87.5-100	0.77-3.84	35
5-Tamar I	0-100	0-0.044	20
6-Tamar II	50	0.00023	10
7-Punta Este	18.2-100	0.011-0.157	30
8-Playa Paraná	0-100	0-0.118	43
9-Cerro Avanzado	0	0	4
10-Punta Pardelas	0	0	11
11-Playa Villarino (Golfo San José)	0	0	13
12-Playa Villarino (Golfo San Matías)	100	0.123	11

Table 1. Sampling sites, imposex occurrence percentage and RPSI for *Odontocymbiola magellanica* from Golfo Nuevo except when noted.

2 spec/100 m<sup>2</sup>). Samples were taken by scuba diving in depth ranging from 6 to 10 m. Externally the sex was determined by the presence of the pedal gland in the anterior portion of the female foot, as seen in other

0.00023 and 0.15; in zones with low marine traffic and near the gulf's opening the per centage of imposex was null (Fig. 1, Table 1). Females developed first a *vas deferens* and then a small penis (Fig. 2), which is notably

smaller than the male penis (Fig. 3). Histological sections confirmed the sex of the females with imposex. In two cases we could observe females in oviposition in the field with a small penis developed, but the vagina was not blocked. The penis size for females with imposex was always less than 9 mm.

In 2004 we took samples again in the harbor zone in Golfo Nuevo in order to monitor the persistence of the phenomenon and observed that the imposex percentage was 100% with a RPSI of 1.67 (one female with a penis of 11 mm length). In this sampling we found a female with an overdeveloped *vas deferens* near the vagina, but not obtruding it.

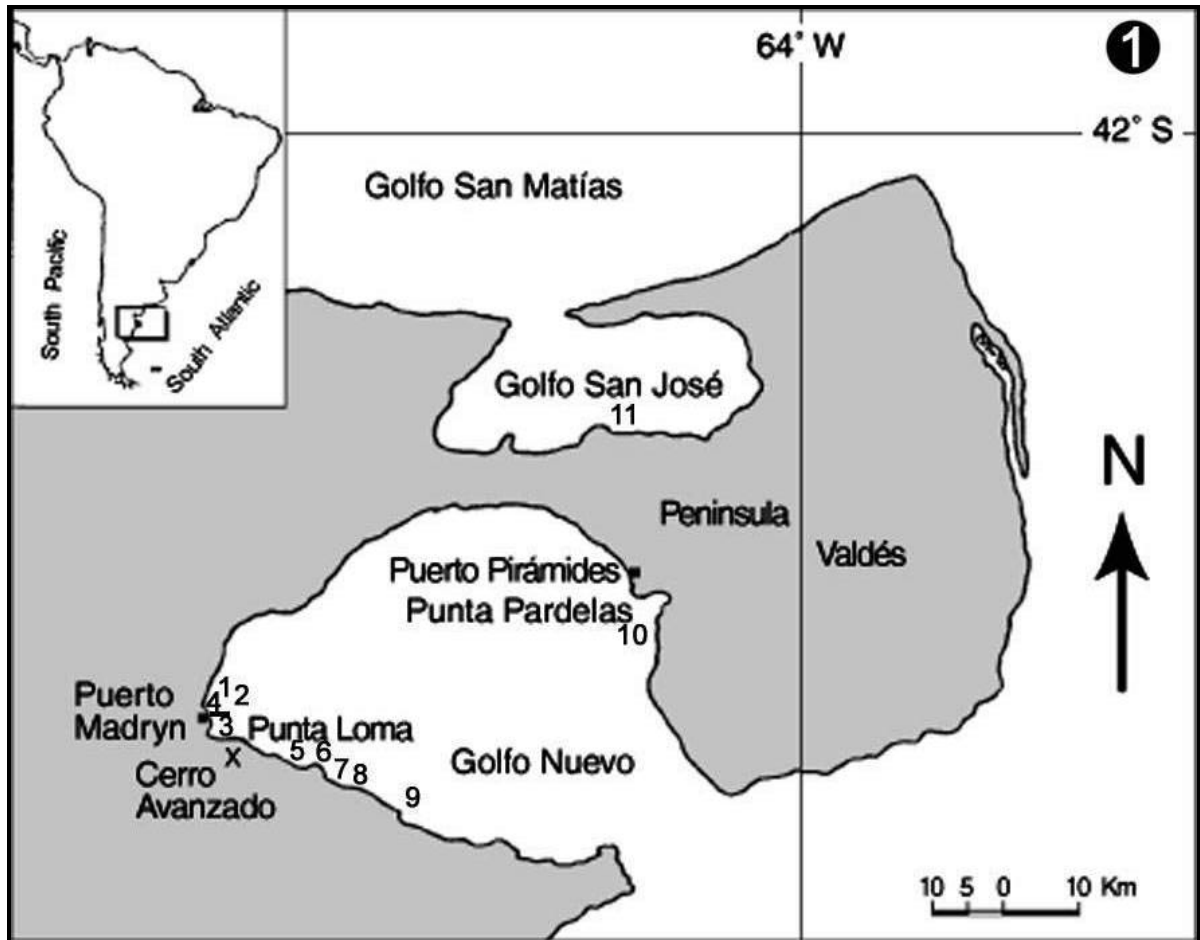


Figure 1. Map showing sampling locations for Golfo Nuevo and Golfo San José (1 to 11). The number corresponds to the localities shown in Table 1 (location 12 from Golfo San Matías not shown in the map).

### DISCUSSION

The high imposex percentage in Patagonian species could be related to low speed marine currents and high marine traffic. Geographically, Golfo Nuevo is an environment with high depths and a tight mouth, with low speed water currents and very pronounced tides with maximums of 5 meters (Pastorino, 1994). Large vessels and harbors are painted with antifouling paints, being potentially the main source of TBT contamination in this area.

Results for *O. magellanica* indicates that in high marine traffic zones the imposex percentage was bigger than in zones with low marine traffic or scuba diving areas; in zones with low marine traffic the imposex was

null. Although the number of studied specimens in some areas was low, the fact that a penis is developed in females could be related to imposex occurrence in the studied areas. This suggests the presence of TBT compounds in the harbor area, nevertheless further studies of water, sediments and egg capsules concentrations of TBT must be done in order to confirm this hypothesis. In Mar del Plata imposex occurrence was observed in *Adelomelon brasiliense* (Lamarck, 1811), a volutid, and *Buccinanops monilifer* (Valenciennes, 1834), a nassariid (Penchaszadeh *et al.*, 2001). In posterior studies (Goldberg *et al.*, 2004), TBT was found in *A. brasiliense* egg capsules and water samplings in the Mar del Plata harbor area, confirming the

incidence of the compound in the developing of secondary male characteristics in volutid females.

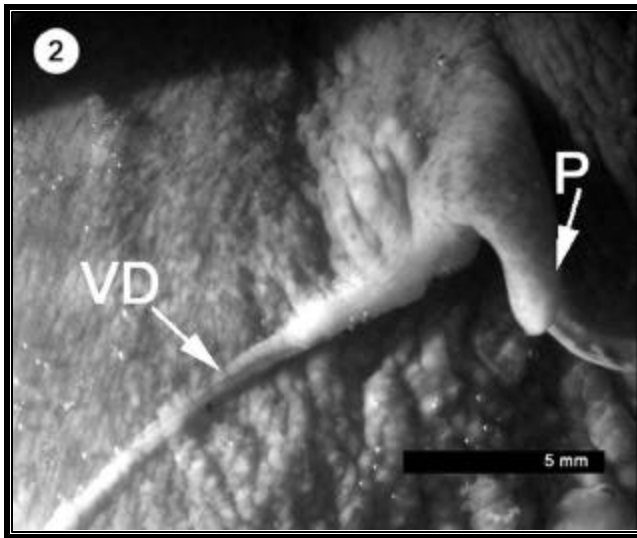


Figure 2. Female of *Odontocymbiola magellanica* with imposex, showing the vas deferens (VD) and penis (P).

Evidences indicate that imposex occurrence continue in the area at least until December 2004. We suggest that the RPSI in marine gastropods could be used as a first indicator of TBT pollution in the Argentinean coasts. This was used and confirmed in other species in the country (Penchaszadeh *et al.*, 2001;

Goldberg *et al.*, 2004) and in other species around the world (Bryan *et al.*, 1987; Evans *et al.*, 1995; Bech, 1998; Tan, 1997, among others). Studies of water and



Figure 3. Male of *Odontocymbiola magellanica* without the shell. (P): penis.

bottom samplings are needed to confirm the existence of TBT in high marine traffic zones in Patagonia. Imposex occurrence in marine Caenogastropods could be a potential tool to monitor pollution along the Argentine coasts.

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