

# Beach filling and imposex in *Olivancillaria deshayesiana* (Mollusca: Gastropoda: Olividae) from the coast of Mar del Plata, Argentina

S. VALERIA TESO AND PABLO E. PENCHASZADEH

Museo Argentino de Ciencias Naturales-CONICET, Avenida Angel Gallardo 470, (1045) Buenos Aires, Argentina

*Beach filling activities were conducted along the Mar del Plata coastline, Argentina, with sediments dredged from the harbour mouth between November 1998 and April 1999. We determined the percentage of imposex of Olivancillaria deshayesiana before, during and immediately after beach filling. The current situation (2005–2007) of imposex incidence was compared between populations from Mar del Plata and Mar Chiquita, a less impacted area. In addition, we evaluated differences in shell shape and body weight between females with and without imposex. The percentage of imposex was significantly higher during (85.3%) and after (73.9%) beach filling than before it (31.0%). No differences in body weight were found in the female population but shell length was significantly lower in females with imposex. As expected, the Mar Chiquita population showed no signs of imposex. This is the first report of imposex in beaches nourished with sand dredged from the port. Our results highlight the importance of analysing more carefully the sediment before beach filling. On the basis of the high level of imposex recorded near the port of Mar del Plata, O. deshayesiana can be used as a biological indicator of marine contamination with butyltin compounds.*

**Keywords:** Argentina, beach filling, imposex, *Olivancillaria deshayesiana*, TBT

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## INTRODUCTION

Imposex leads to the development of additional male sex organs (penis and/or vas deferens) in female gastropods (Smith, 1981). It is induced by exposure to tributyltin (TBT), a commercial product present in antifouling paints for ships and boats (Gibbs & Bryan, 1994). TBT is also used as a biocide in wood preservatives, textiles, dispersion paints and agricultural pesticides, and as UV stabilizer in many plastics (Oehlmann *et al.*, 1998). In neogastropods, imposex can occur at TBT concentrations lower than 0.5 ng/l (Bryan *et al.*, 1986; Alzieu, 2000).

Beach filling, defined as the addition of sediments transported from other source locations, is seen as a solution against coastal erosion. It is increasingly being used in many parts of the world including Europe, North America and South America (Marcomini & López, 1999; Van Dalfsen *et al.*, 2000; Simonini *et al.*, 2005; Colosio *et al.*, 2007; Harriague & Albertelli, 2007).

In Argentina, beach filling activities were conducted along the Mar del Plata coastline (south-western Atlantic, 38°02'S), between November 1998 and April 1999 (López, personal communication). This resulted in the transport of almost 2.5 million m<sup>2</sup> of sediments dredged from the harbour entrance (Marcomini & López, 1999). Several studies have

evaluated the incidence of imposex after the nourishment operation in this area. Penchaszadeh *et al.* (2001) found a high degree of imposex in the gastropods *Adelomelon brasili-ana* (Lamarck, 1811) and *Buccinanops monilifer* (Kiener, 1834) in the last months of 2000, and Goldberg *et al.* (2004) reported tributyltin in water and sediments in Mar del Plata and Mar Chiquita (south-western Atlantic, 37°46'S), and in egg capsules of *A. brasili-ana* in Mar del Plata. Cledón *et al.* (2006) reported the occurrence of imposex in *A. brasili-ana* and the proportion and distribution of butyltin compounds (BTs) in sediments in Mar del Plata, from November 2000 to November 2002.

There are many studies of the effect of nourishment with sand extracted from offshore areas on different invertebrate communities (Sardá *et al.*, 2000; Van Dalfsen *et al.*, 2000; Colosio *et al.*, 2007). In contrast, information involving sediments from harbours and port areas is scarce. TBT is highly concentrated at these sites due to its low degradation rate, with a half-life ranging between 0.9 and 5.2 years (De Mora *et al.*, 1989, 1995; Stewart & De Mora, 1990). This fact highlights the importance of assessing TBT contamination in port sediments.

*Olivancillaria deshayesiana* (Ducros de Saint Germain, 1857) (Mollusca: Gastropoda: Olividae) is commonly found from Rio de Janeiro, Brazil, to Mar del Plata at a depth of 6–12 m (Rios, 1994).

The aims of this work were: (1) to evaluate the presence of imposex in *O. deshayesiana* females before, during and after the nourishment operations in Mar del Plata mentioned

**Corresponding author:**  
S.V. Teso  
Email: valeteso@bg.fcen.uba.ar

above; (2) to compare the current incidence of imposex in *O. deshayesiana* in Mar de Plata and Mar Chiquita, with low marine traffic and no beach filling activities; and (3) in the case of imposex occurrence, to evaluate differences in shell shape and body weight between *O. deshayesiana* females with and without imposex.

## MATERIALS AND METHODS

### Sampling and study site

Mar del Plata (south-western Atlantic, 38°02'S; Figure 1) is an open coast characterized by rocky shores and sandy and sandy-silty bottoms and is the site of the most important coastal fishery fleet of Argentina (Olivier *et al.*, 1968; Penchaszadeh *et al.*, 2001).

Specimens of *Olivancillaria deshayesiana* were collected before, during and after the beach filling operation mentioned above (December 1995–February 1998, January–February 1999 and November 1999, respectively).

To determine the current incidence of imposex in *O. deshayesiana*, samples were collected on a monthly basis between October 2005 and August 2007 in Mar del Plata, Argentina. Specimens of these same species were collected in December 2000 and December 2006 in Mar Chiquita (south-western Atlantic, 37°46'S; Figure 1), Argentina.

All samples were collected between 5 and 12 m deep by bottom trawling with a net 15 mm of mesh size.

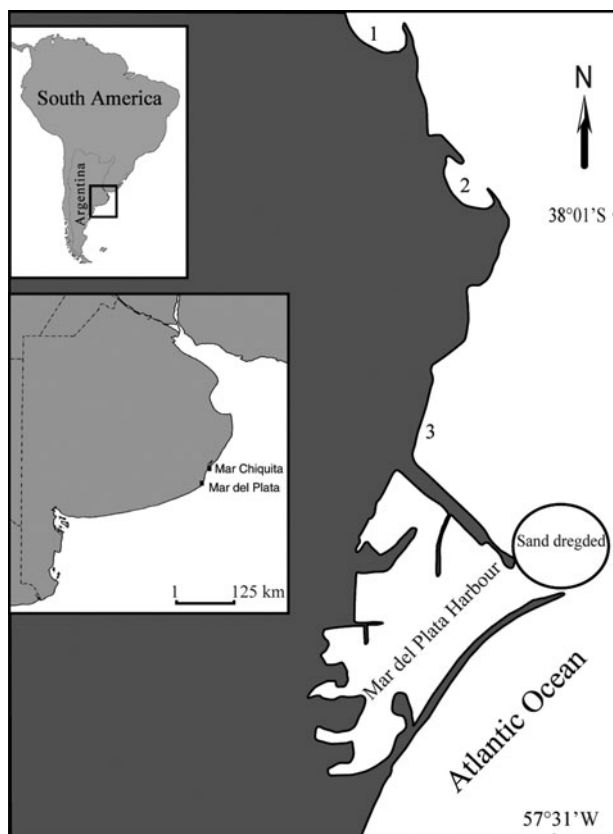


Fig. 1. Map showing the sampled area. 1, 2 and 3 sampled beaches.

### Laboratory procedures

To determine the presence of abnormalities in the sexual apparatus, the specimens were dissected and the mantle cavity was opened under a stereomicroscope by a longitudinal cut through the hypobranchial gland. Snails were sexed based on the presence of the capsule gland. The lengths of the shell and penis were measured with a Vernier caliper. The gonads of each individual were fixed in Bouin's solution, dehydrated in a graded ethanol series (70%, 80%, 96% and 100%), embedded in paraffin and resin, cut at 5 µm thickness and stained with Harris haematoxylin and eosin. Sex was confirmed by the histological examination of gonad sections under a Zeiss Axio Imager Z1 microscope. The body weight (without shell) of fresh female individuals was also measured. The following values were calculated for each species in each sampling site: mean female penis length, mean male penis length, mean shell length and relative penis size index (RPSI), defined as  $((\text{mean length of female penis})^3 / (\text{mean length of male penis})^3) * 100$  (Gibbs & Bryan, 1994). The imposex frequency was calculated as the proportion of females with imposex compared to the total number of females in the sample.

### Statistical analysis

Differences in the frequency of imposex between periods were tested with the Chi-square test. Differences in the sex-ratio between periods were determined with the Chi-Square test for trend. The correlation between total shell length and penis length per sex per period was determined using the Spearman's correlation coefficient. Differences in the size-distribution between males and females were assessed with the one-way ANOVA test. The distribution of sizes between females with and without imposex was compared with the Kolmogorov–Smirnov test, and their respective body weights (without shells) were compared with the one-way ANOVA test. The data were pooled for these analyses because the sample size of females with and without imposex in each period was statistically insufficient (Bech, 2002). Differences between the penis length of males and females exhibiting imposex were tested with the one-way ANOVA test.

## RESULTS

The imposex phenomenon, detected as the neoformation of a penis and a vas deferens in females, occurred throughout the study period in the Mar del Plata area (Tables 1 & 2). No individuals with obstructed vagina were observed.

Table 1. Mean percentage of imposex and mean RPSI (relative penis size index) in *Olivancillaria deshayesiana* from Mar del Plata area before (December 1995–February 1998), during (January–February 1999) and immediately after beach filling (November 1999).

Dates	N	% Males/females	Imposex (%)	RPSI (%)
Before	91	57.1/42.9	31.0	1.8
During	92	64.1/35.9	84.8	2.0
After	56	58.9/41.1	73.9	3.4

**Table 2.** Sampling sites, mean percentage of imposex and mean RPSI (relative penis size index) in *Olivancillaria deshayesiana* during October 2005–August 2007.

Sampling site	Imposex (%)	RPSI (%)	N (total)
Mar del Plata	81.7	2.6	284
Mar Chiquita	0	0	59

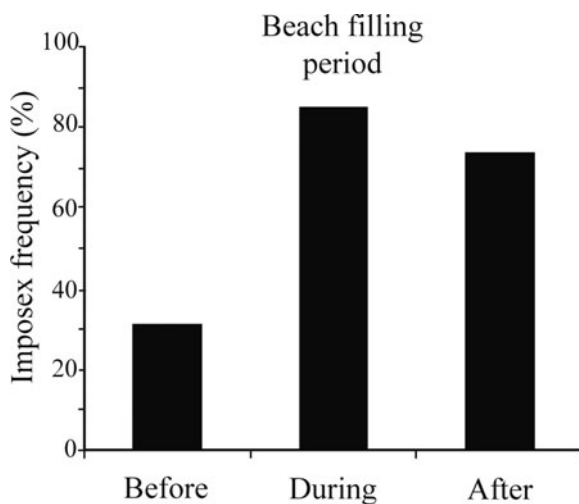
The frequency of imposex in *O. deshayesiana* was significantly higher during (84.8%) and after (73.9%) beach filling than before it (31.0%) ( $\chi^2=23.8$  and  $18.2$ , respectively;  $P < 0.01$  in both cases) (Table 1; Figure 2).

The sex-ratio was significantly higher during beach filling, with males outnumbering females ( $\chi^2 = 7.3$ ,  $P < 0.01$ ), and no significant differences were found before and after beach filling ( $\chi^2 = 1.9$  and  $1.8$ , respectively;  $P > 0.05$  in both cases) (Table 1).

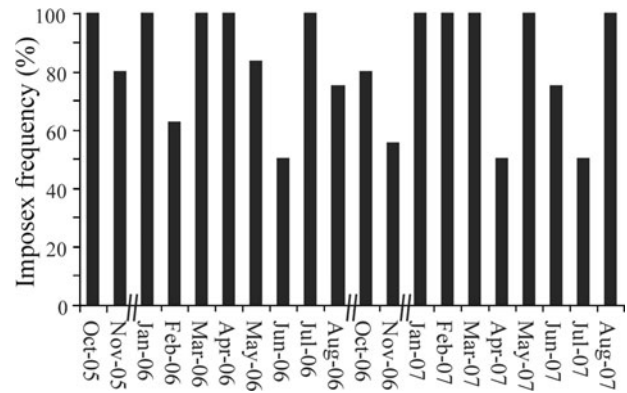
There was a significant positive correlation between the shell length and penis length of males during and after beach filling (Spearman  $R = 0.03$  and  $0.04$ , respectively;  $P < 0.05$  in both cases), while no significant correlation was found before it (Spearman  $R = 0.04$ ,  $P = 0.2$ ).

Between 2005 and 2007, the imposex frequency of *O. deshayesiana* females collected in Mar del Plata showed monthly variations, with a minimum value of 50% (Figure 3). The sex ratio was male-biased ( $\chi^2 = 3.61$ ,  $P < 0.01$ ). A significant positive correlation between shell length and penis length was found in males (Spearman  $R = 0.03$ ,  $P < 0.05$ ).

The mean shell length was significantly higher in males than in females when considering the entire study period (Table 3). Mean shell length was significantly higher in females without imposex (Table 4; Figure 4A). No significant differences in mean body weight were found between females with and without imposex ( $F = 1.92$ ,  $P = 0.2$ ). No correlation was found between shell length and penis length of females when considering the entire study period. The mean penis length of males was significantly greater than that of females exhibiting imposex when considering the entire study period (Table 3).



**Fig. 2.** Mean imposex frequency in *Olivancillaria deshayesiana* females in Mar del Plata, Argentina, before, during and after beach filling.



**Fig. 3.** Mean imposex frequency in *Olivancillaria deshayesiana* females from Mar del Plata, Argentina, during the study period.

No imposex was detected in *O. deshayesiana* samples from Mar Chiquita (Table 2); the sex ratio was 1:1 ( $\chi^2 = 0.9$ ,  $P = 0.3$ ), and there were no significant differences in the shell length between males and females ( $F = 3.4$ ,  $P = 0.07$ ), with mean shell lengths of  $21.3 \pm 2.3$  and  $21.0 \pm 2.5$  mm, respectively (Figure 4B).

## DISCUSSION

The presence of imposex in *Olivancillaria deshayesiana* throughout the study period confirms the negative influence of the port of Mar del Plata on the local gastropod population, although imposex appears to be mild as sterilization was not found. Currently, the frequency of imposex is as high as 81.7% and the RPSI ranges from 1.8 to 3.4. In the same study area, Penchaszadeh *et al.* (2001) found an imposex frequency between 33.3 and 85.7% and a RPSI between 0.14 and 0.34 for a declining population of *B. monilifer*.

The increase in imposex frequency from 31.0 to 73.9% before and after beach filling, respectively, was probably due to a high concentration of TBT in the sediment dredged from the port of Mar del Plata. This may confirm the suggestion made by Stewart & De Mora (1990) that TBT shows a tendency to accumulate in sediments, which may cause a problem if they are remobilized through events such as storms or dredging operations. TBT concentration decreases rapidly in the water column (2.5 days–20 weeks), while it is considerably more persistent in the sediments (0.9–5.2

**Table 3.** Results of ANOVA test for (1) shell length between males and females of *Olivancillaria deshayesiana* and (2) penis length between males and females with imposex of *O. deshayesiana* during the study period (October 2005–August 2007).

Period	Sex	N	(1) Mean $\pm$ SD (mm)		P	(2) Mean $\pm$ SD (mm)		P
			Mean	SD		Mean	SD	
Before	Male	52	27.1	$\pm 2.8$	<0.01	11.1	$\pm 3.1$	<0.01
	Female	39	25.7	$\pm 2.2$		2.9	$\pm 1.1$	
During	Male	59	26.7	$\pm 2.5$	=0.02	14.8	$\pm 3.7$	<0.01
	Female	33	25.5	$\pm 2.1$		4.0	$\pm 1.9$	
After	Male	33	27.3	$\pm 2.0$	=0.01	10	$\pm 1.5$	<0.01
	Female	23	25.9	$\pm 2.2$		3.1	$\pm 1.7$	
Present	Male	169	28.1	$\pm 2.4$	<0.01	9.4	$\pm 3.0$	<0.01
	Female	115	26.6	$\pm 2.6$		2.8	$\pm 1.7$	

**Table 4.** Results of Kolmogorov–Smirnov test for shell length between females without and with imposex of *Olivancillaria deshayesiana* during the study period.

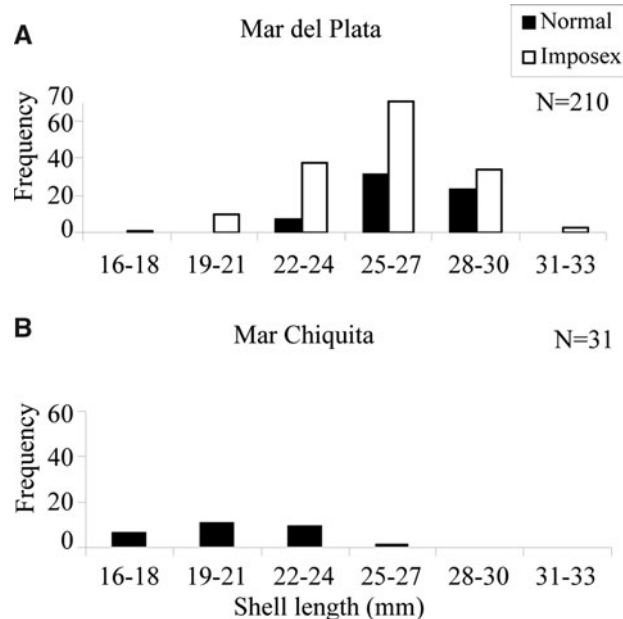
Sex	N	Mean $\pm$ SD (mm)	P
Normal females	60	27.1 $\pm$ 1.7	<0.05
Females with imposex	150	25.8 $\pm$ 2.6	

years, with a maximum of 8.7 years) (De Mora *et al.*, 1989; Stewart & De Mora, 1990; Stewart & Thompson, 1997). The fact that the frequency of imposex in *O. deshayesiana* was greater during beach filling (85.3%) than after it (73.9%) could be explained by the direct effect of high concentrations of TBT in the water column and sediments in this period (Gibbs *et al.*, 1988; Ide *et al.*, 1997). Moreover, the higher mean penis length ( $4.0 \pm 1.9$  mm) recorded during this period may indicate that the species shows a rapid response to TBT.

Evans & Nicholson (2000) pointed out that biological measurements of imposex should be confirmed by chemical measurements of TBT in tissue, sediment and/or water samples. In Mar del Plata there are no data available on the presence of TBT for the study period, but Goldberg *et al.* (2004) found this chemical in sediment and water in 2001.

The penis length of males was greater than that of females with imposex throughout the study period. This pattern has also been reported for *Olivancillaria vesica vesica* (Gmelin, 1791) from Brazil (Caetano & Absalão, 2002) and for other gastropod species (Bech, 2002; Huaquín *et al.*, 2004).

Despite the close proximity and similar environmental conditions of Mar del Plata and Mar Chiquita, the *O. deshayesiana* populations showed different patterns. In contrast to males from Mar Chiquita, those from Mar del Plata had a longer shell and were more numerous than females (during beach filling and at present). In addition, the mean



**Fig. 4.** *Olivancillaria deshayesiana*. Size–frequency distribution of (A) females with and without imposex from Mar del Plata currently and (B) females without imposex from Mar Chiquita.

shell length of the population in Mar Chiquita was lower than that of the population in Mar del Plata. In highly TBT contaminated areas along the Northumbrian coast, England, Evans *et al.* (1991) found a higher proportion of *Nucella lapillus* (Linnaeus, 1758) males compared with females. Normally, gonochoric molluscs show a general sex-ratio pattern of 1:1 (Fretter & Graham, 1994), as in *A. brasiliensis* and *Zidona dufresnei* (Donovan, 1823), which are found near the study area (Gimenez & Penchaszadeh, 2002; Cledón *et al.*, 2005). These considerations, together with a report by Goldberg *et al.* (2004) indicating high TBT concentrations in Mar del Plata, suggest that the differences between the studied populations could be due to higher levels of TBT contamination in Mar del Plata.

There are several studies on the effect of TBT on shell size. In a study of the genetic and morphological diversity of *N. lapillus*, Plejdrup *et al.* (2006) recorded the largest phenotypic variation in shell size in a population with imposex from a polluted area. Bigatti & Carranza (2007) reported that females of *Odontocymbiola magellanica* (Gmelin, 1791) with imposex were larger than those without imposex using multivariate analysis, and they obtained no significant differences in shell length with univariate analysis. We found that *O. deshayesiana* females without imposex were significantly longer than females with imposex, while Son & Hughes (2000) reported that the shell size of *N. lapillus* from North Wales increased significantly with increasing degree of imposex. This lack of consistency among results suggests that the effect of TBT on shell size depends on the gastropod species.

In Argentina, the first discovery of imposex was reported for Mar del Plata at the end of 2000 by Penchaszadeh *et al.* (2001), and Cledón *et al.* (2006) suggested that imposex resulted from the beach filling operation carried out in November 1998–April 1999. However, our results indicated that this phenomenon was present at least since December 1995.

Based on the fact that the degree of imposex decreases with decreasing levels of TBT contamination in the environment (Evans & Nicholson, 2000), the ordinance 4/98 of the Environment Protection Secretary of Argentina (DPMA, 1998) prohibits the application of TBT on boats in the study area (Cledón *et al.*, 2006). The present study reveals an increase in the intensity of imposex between 1995 and 2007 with evidence of the use of painting containing TBT (Goldberg *et al.*, 2004).

The vast majority of studies on imposex have been conducted near marinas, harbours or likely sources of TBT contamination, probably because TBT pollution decreases as distance from the port increases (Evans & Nicholson, 2000). We found no signs of imposex in the *O. deshayesiana* population from Mar Chiquita. A negative result was also reported by Goldberg *et al.* (2004) in *A. brasiliensis* and *B. monilifer* collected in the same area. This could be explained by the distance between Mar Chiquita and the port (30 km), but Goldberg *et al.* (2004) detected an increase in TBT sediments from  $0 \text{ ng} \times \text{g}^{-1}$  in 2001 to  $0.2 \text{ ng} \times \text{g}^{-1}$  in 2002, and Cledón *et al.* (2006) suggested a current transport of the pollutant from Mar del Plata to the Mar Chiquita coast.

According to the literature, beach filling may have no effect on macrofaunal assemblages (Guillén & Hoeksatra, 1997; Harriague & Albertelli, 2007) or may be highly deleterious (Van Dalssen *et al.*, 2000). To our knowledge, this is the first report on imposex in an area nourished with sand



dredged from a port. Our results highlight the importance of analysing more carefully the sediment before beach filling. *Olivancillaria deshayesiana* can be used as a biological indicator of marine contamination with organotin compounds on the basis of the higher values of imposex recorded near the port of Mar del Plata.

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**Correspondence should be addressed to:**

S.V. Teso  
Museo Argentino de Ciencias  
Naturales-CONICET  
Avenida Angel Gallardo 470  
(1045) Buenos Aires  
Argentina  
email: valeteso@bg.fcen.uba.ar