Marine Pollution Bulletin xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Biomonitoring of TBT contamination and imposex incidence along 4700 km of Argentinean shoreline (SW Atlantic: From 38S to 54S)

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ARTICLE INFO

Keywords:
Imposex
Marine sediments
TBT pollution
Argentina
Biomonitoring
Caenogastropoda

ABSTRACT

The imposex incidence and TBT pollution were investigated along 4,700 km of Argentinean coast, including city harbors and proximal zones without marine traffic. We analyzed 1805 individuals from 12 gastropod species, including families Volutidae, Muricidae, Nassariidea, Calyptraidae, Marginellidae, and Buccinidae, and found the imposex phenomenon for the first time in six species. In high marine traffic zones, TBT pollution was registered and the percentage of imposex was high, while these occurrences were null in areas without boat traffic. The species that best reflect the degree of imposex were those inhabiting sandy/muddy or mixed bottoms. TBT determination and imposex incidence indicate that pollution was focused only in ports with high marine traffic or in areas where ship hulls are painted. This is the first report of an imposex-sediment approach to evaluate organotin contamination along the coast of a South American country.

1. Introduction

The introduction of TBT compounds into the international market of antifouling paints occurred during the 1960s and rapidly expanded due the reduced cost and efficiency of these materials. The undesirable effects in natural environments were discovered a few years later, including the imposition of masculine characters over female gastropods, termed imposex (Blaber, 1970; Smith, 1971). Numerous studies on such organismal effects have been published since then, which have indicated that more than 200 species of marine gastropods are affected by TBT (Gibbs, 1996; Gibbs and Bryan, 1994; Gooding et al., 1999, 2003; Huaquín et al., 2004; Oehlmann et al., 1998; Terlizzi et al., 2004, among others).

Imposex in caenogastropods is the most studied negative effect of TBT. Other effects of this contaminant in biota include malformations, mortality, and hormonal imbalance in dolphins, crabs, lobsters, oysters, invertebrate larvae, sea grasses, and algae (Evans, 1999 and references therein).

Marine shorelines occupy 4725 km of the coast of Argentina (Barragán et al., 2003). The imposex phenomenon was registered for the first time in the Buenos Aires province (off Mar del Plata city coast: 37°50′S) of Argentina during 2001, by Penchaszadeh et al. Several studies have been published since then on imposex and TBT presence on the coast of Mar del Plata and north-patagonic gulfs (Goldberg et al., 2004; Bigatti and Penchaszadeh, 2005; Bigatti and Carranza, 2007; Cledón et al., 2006). Although, the use of TBT has been banned worldwide since 2008 (Evans et al., 1995), and the use of paints containing TBT is forbidden in Argentina (government regulation 4/98, DPMA, 1998), organotin (OT) antifouling paints are still commercialized in this country.

Imposex is a reversible phenomenon at the individual or population level (Queiroz et al., 2007), but is probably a matter of major complexity on an ecosystem scale, as sediments may contain TBT and other organotin compound years after use is prohibited. According to some studies, the consequences of TBT are irreversible for most species of snails (Evans and Nicholson, 2000). In Australia, imposex has been shown to be evident ten years after tributyltin restrictions (Gibson and Wilson, 2003).

This research represents the first detailed monitoring work of snails as bioindicators linked to TBT dosage and utilizing

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doi:10.1016/j.marpolbul.2009.01.001

sedimentographic analysis along 4725 km of the Argentine marine coast, which includes the most important city harbors as well as areas without human impact.

The aim of this work was to study the TBT contamination along the coast of Argentina in order to suggest government management policies that diminish pollution levels and to identify snail species that are simple, fast biomonitoring tools for the detection of TBT pollution.

2. Material and methods

2.1. Sampling

The study area was comprised of almost the entire Argentine coast, from Southern Mar del Plata (38° 01′S 57° 32′W) to Lapataia Bay (54° 51′S, 68° 27′W) near Ushuaia (Fig. 1) during march 2006–april 2008, including city harbors and proximal zones without marine traffic. A sample of *Buccinanops cochlidium* collected by one of the authors (A.A.) in Mar del Plata during spring 2003 was also included. Caenogastropods were collected from the intertidal or subtidal zone to 20 m depths by SCUBA diving, dredges, snail baited traps, or manually during low tides. Specimens were collected live and carried to the lab for morphometric and imposex analyses. Since TBT deposited in marine sediments can be bioavailable for many years (Strand and Asmund, 2003), samples of sediments were collected with a dredge or manually at the points shown in Fig. 1. A 50 g sediment sample was placed in refrigerated black vials after collection. Sediments were stored at −20 °C until analysis in the Federal Maritime and Hydrographic Agency (BSH), Laboratory Suelldorf, Hamburg, Germany and the Instituto Argentino de Oceanografía (IADO-CONICET), Argentina.

2.2. Imposex incidence

The sex of all collected gastropods was determined based on the presence or absence of sexual accessory glands (albumen, capsule, and pedal glands) as well as the color of the gonads (characteristic of each sex). The penis length and the presence of *vas deferens* in females and males were noted at the same time. The percentage of imposexed females was recorded, and the Relative Penis Length Index (RPLI) was calculated according to the following equation (Gibbs and Bryan, 1994):

$$\mathrm{RPLI} = [(\text{mean length of female penis size})/(\text{mean length of male penis size})] * 100$$

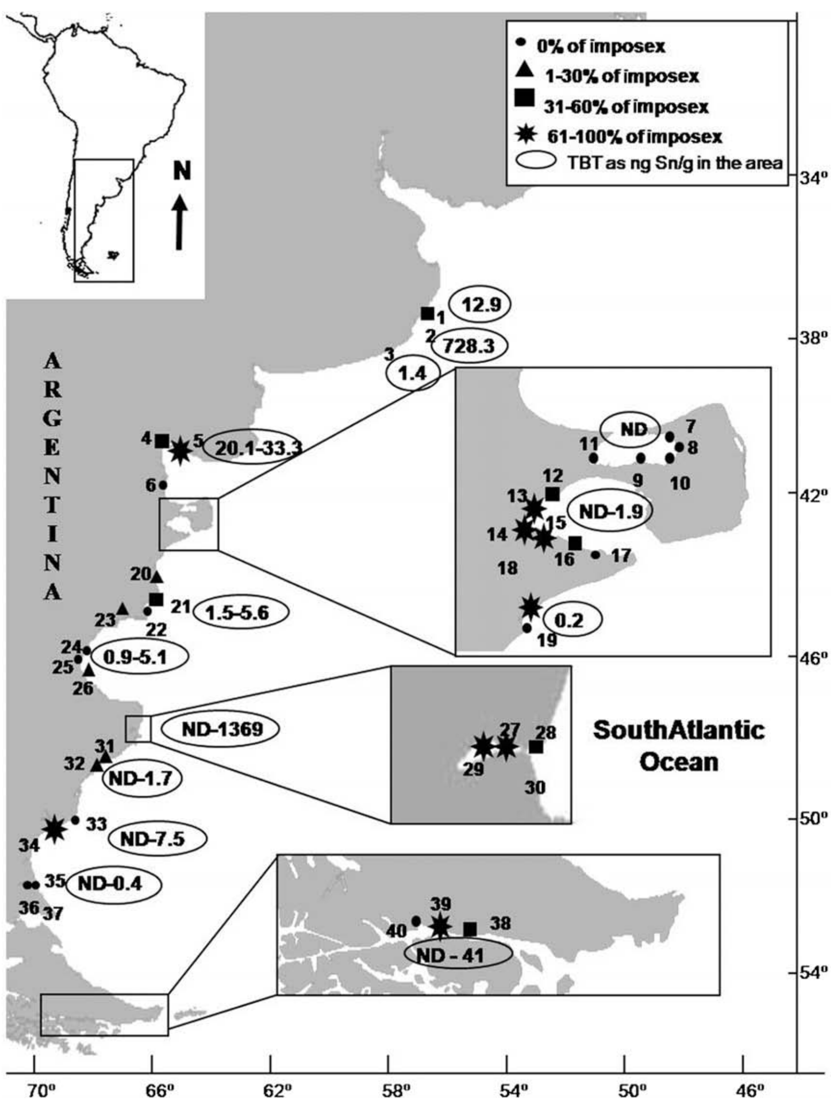

Fig. 1. TBT contamination and imposex incidence along the Argentinean coast. ND: not detected. Number corresponds to those in Table 1 and Fig. 4.

For controls, samples of all species were collected in areas of very low or null marine traffic. Percentages of imposex-affected females were calculated as the number of females with penis and/or *vas deferens* with respect to all females sampled of each species.

2.3. TBT determinations on sediments

Wet sediments, weighing 15 g, were analyzed following the procedure described by Cledón et al. (2006) and Delucchi et al. (2006). The reference material BCR 646 freshwater sediments (TBT-, DBT-, MBT-, TPhT-, DPhT-, and MPhT-certified) was used as a control for all chemical analyses (analytical data: BCR646 Certified TBT 480 ng/g sediment dry weight Recovered TBT 372). The detection limits of OTs were in the order of 0.2 ng/g dry weights. Concentrations are reported for each OT as mass per gram of sediments. Tributyltin was determined by gas chromatography (GC). The analytical procedure was performed under DIN-Norm 38407-F13 (modified) for water, sewage, and mud. Samples were mixed with 250 ng (62.5 ng of each compound) of an internal standard consisting of tetrapropyltin (TePrT), tripropyltin (TPrT), monoheptyltin (MHT), and diheptyltin (DHT) at 100 ng/ml in order to control the recovery effectiveness. Each sample was extracted during 20 min in an ultrasonic bath.

Each sample was extracted with 20 ml methanol, 40 g of a 10% NaCl solution, 40 ml sodium acetate/acetic buffer (pH approximately 4.6), and finally 100 ml hexane. Ethylation was performed by adding 3.2 ml of 10% Na tetraethylborate to each extract, with 30 min between each addition. From the hexane solution, 50 ml was collected with a 10 ml pipette. To this 50 ml hexane solution, 5 g of sodium sulfate was added and dried for 60 min. This dry solution was concentrated to 2–3 ml in a rotary evaporator. Purification of 15 ml of hexane:acetate 92:8 eluate was performed with a silica gel column. The resultant was concentrated to 1.5 ml.

The OT content in extracts was measured with the GC/AED-System. The GC used was a HP6890 gas chromatograph equipped with an atomic-emission detector HP G2350A with ICP plasma. The separation was performed on a GC-column 30 m HP-5MS 5% phenylmethyl-siloxane, 0.25 mm ID coated with 0.25 μm film phase. Injection was performed with a Gerstel Cold- System at 40 °C, and separation with a temperature program of 40–320 °C, within 35 min. Helium was used as the carrier gas at a low-rate of 2.7 ml/min. The ICP was maintained with H_2, O_2 and Ar/CH_4 and N_2 as the make-up gases (2000 °C). AED-signals were measured at 303 and 301 nm wavelengths.

2.4. Sedimentographic analysis

In each sample site of TBT detection, a sedimentographic analysis was performed using a series of consecutive Standard sieves at 1 interval Phi was defined as the negative logarithm of the grain dimension in millimeters of the base two (decreasing hole diameters of 2000 μm, 1000 μm, 500 μm, 250 μm, 125 μm, and 62 μm, following Gray (1981)). Samples of 50–100 g (wet weight) were dried at 60 °C for 48 h and separated following sedimentographic classification as gravel, sand (coarse, medium and fine), and silt/clay.

3. Results

3.1. Imposex incidence and TBT determinations on sediments

A total of 40 localities were sampled. As a general rule, all zones with marine traffic were polluted by TBT and the imposex phenomenon was observed in these areas. The percentage of imposex, RPLI, and TBT concentration for each species and sites are shown in Table 1 and Fig. 1.

We analyzed 1805 individuals from 12 gastropod species, including the families Volutidae, Muricidae, Nassariidea, Calyptraidae, Marginellidae, and Buccinidae. Species registered were: *Odontocymbiola magellanica, Crepidula aculeata*, *Trophon geversianus, Adelomelon ancilla*, *Adelomelon ferussacii*, *Adelomelon brasiliana*, *Prunum martini*, *Buccinanops globulosus, B. cochlidium*, *Buccinanops squalidum, Pareuthria plumbea,* and *Ximenopsis muriciformis*. The percentage of imposex was high in high marine traffic zones (Table 1), while imposex was null in pristine areas. The sampled zones with high TBT pollution and imposex incidence were the commercial port of Mar del Plata, commercial and fishery ports of San Antonio Oeste and San Antonio Este, commercial and tourist ports of Puerto Madryn, the fishery port of Puerto Deseado, the fishery port of Puerto San Julián, the fishery port of Puerto Santa Cruz, and the sport port of Ushuaia (AFASYN).

The imposex phenomenon was detected for the first time in the following species: *P. martini*, *X. muriciformis*, *P. plumbea*, *A. ferussacii*, *T. geversianus*, and *B. cochlidium* (Fig. 2).

Species inhabiting soft or mixed bottoms, such as *B. globulosus, B. cochlidium, P. plumbea, X. muriciformis, P. martini, A. brasiliana, A. ferussacii, A. ancilla*, and *O. magellanica*, are the species that best reflected the imposex phenomenon in low or medium TBT pollution zones (Table 1). In comparison, *T. geversianus* and *C. aculeata* inhabiting intertidal hard bottoms with medium or low contamination did not develop sexual secondary characteristics. In the case of *T. geversianus*, the development of a small penis was registered only in zones with high TBT pollution and mud bottoms (Puerto Madryn, Puerto Deseado, Puerto San Julián and Ushuaia).

As a general rule, volutids exhibited high sensitivity to TBT contamination. *A. ancilla* displayed greater sensitivity than *O. magellanica* in a zone with low marine traffic (point 23, Table 1). In Puerto Madryn, these species are sympatric and the neoformation of multiple penises in both species was detected (Fig. 3), but the RPLI was higher for *A. ancilla*.

Within the Muricidae, *X. muriciformis* was more sensitive to TBT pollution than *T. geversianus*, as well as in comparison to volutid species. This occurred in Ushuaia (site 40, Table 1), where females of *X. muriciformis* presented a developed penis, while other species did not (Table 1).

3.2. Sedimentographic analysis

Sediment granulometry was variable between sites, with a predominance of sand fractions of especially fine and very fine sand. Only a few of those sites included fractions corresponding to a Phi of 5 (<62 μm). A few samples demonstrated the inverse pattern with predominantly coarse sand and gravel fractions (27, 38, and 39, intertidal samples). Sediment granulometry of the samples corresponding to each site (with TBT determinations) are shown in Fig. 4.

4. Discussion

This is the first report investigating the situation of imposex and TBT contamination along 4700 km of the Argentinean coast, covering two biogeographically distinct provinces. A survey of imposex-sediment to evaluate organotin contamination in marine environments in a restricted area within Rio de Janeiro (Brazil) was performed by Fernandez et al. (2005), which demonstrated imposex associated with TBT pollution.

Imposex incidence was determined in six species for the first time in this study, and only five other species with imposex were previously recorded in Argentina: *A. brasiliana*, *Buccinanops monilifer* (Penchaszadeh et al., 2001), *O. magellanica* (Bigatti and Penchaszadeh, 2005), *B. globulosus* (Narvarte et al., 2008), and *Olivancilaria deshayesiana* (Teso and Penchaszadeh, 2008).

Table 1
Boating activities, species, imposex percentage, RPLI, TBT concentrations, and the number of each species sampled from Buenos Aires to Chubut.

Sampling sites	Boating Activity	Species	% Imposex	RPLI	TBT (ng Sn/g)	N
1. Mar del Plata-Playa Grande	High	*A. brasiliana*	43	39	12.9	24
2. Mar del Plata (harbor)	High	*Not found*			728.3	
3. Quequen	High	–	–	–	1.4	–
4. San Antonio Oeste (Port)	High	*B. globulosus*	31.42	6.39	20.1–33.3	43
5. San Antonio Este (Port)	High	*P. martini*	100	26.66	ND-0.9	4
		C. aculeata	0	0		8
6. Puerto Lobos	Low	*B. cochlidium*	0	0	NA	13
7. Playa Bengoa	Low	*O. magellanica*	0	0	NA	2
8. Punta Conos	Low	*O. magellanica*	0	0	NA	4
9. Playa Villarino	Low	*O. magellanica*	0	0	ND	23
		B. cochlidium	0	0		128
		T. geversianus	0	0		3
10. Playa Fracaso	Low	*O. magellanica*	0	0		4
11. Riacho San José	Low	*O. magellanica*	0	0	NA	27
12. Playa Casino	medium	*O. magellanica*	50	0.78	NA	4
13. Parque Piedras	High	*A. ancilla*	100	36.64	1.7	56
		O. magellanica	100	26.31		30
		T. geversianus	7.69	2.93		24
14. Puerto Madryn (Port)	High	*B. globulosus*	100	31.96	ND-0.4	57
		T. geversianus	0	0		21
15. Punta Cuevas	Medium	*B. globulosus*	100	10.53	1.9	69
16. Playa Paraná	Medium/low	*A. ancilla*	42.85	11.53	NA	12
		O. magellanica	33.33	2.53		10
17. Cerro Avanzado (Control)	Low	*O. magellanica*	0	0	NA	19
18.Rawson(Port)	Medium/high	*B. globulosus*	62.5	6.82	NA	14
19. Playa Magaña	Medium	*T. geversianus*	0	0	0.2	11
		C. aculeata	0	0		27
20. Puerto Camarones (Port)	Medium	*P. plumbea*	41.17	2.72	1.5	40
		T. geversianus	0	0		39
		C. aculeata	0	0		5
21. Caleta Sara-intertidal	Medium	*P. plumbea*	8.33	0.58	5.6	55
22. Caleta Sara-6 m depth	Medium	*P. plumbea*	0	0	NA	28
		T. geversianus	0	0		3
		C. aculeata	0	0		23
23. Bahía San Gregorio	Low	*O. magellanica*	0	0	NA	6
		A. ancilla	25	0.23		10
24. Caleta Cordova	High	*P. plumbea*	0	0	5.1	45
		T. geversianus	0	0		23
25. Cro. Rivadavia (Port)	High	*T. geversianus*	0	0	0.9	62
		C. aculeata	0	0		37
		P. plumbea	0	0		23
26. Rada Tilly	Low	*P. plumbea*	5	0.58	ND	38
		C. aculeata	0	0		53
		T. geversianus	0	*		6
27. Puerto Deseado-Shipyard	High	*P. plumbea*	0	*	2.2	2
28. Puerto Deseado-20 m depth	High	*P. plumbea*	100	30.43	1369.58	4
29. Puerto Deseado-Fish factory	High	*T. geversianus*	36	2.89	NA	15
30. Puerto Deseado-Intertidal	High	*P. plumbea*	88.37	16.04	21.2	88
31. Puerto Deseado (control)	Low	*Not found*			ND	
32. Puerto San Julián (harbor)	Low	*P. plumbea*	2.56	0.41	ND-1.65	87
		T. geversianus	0	0		151
33. Puerto San Julián-Cascada	Low	*T. geversianus*	14.28	3.12	ND	14
		B. squalidum	0	0		12
		P. plumbea	0	0		15
34. Punta Quilla (control)	Low	*T. geversianus*	0	0		7
35. Punta Quilla (harbor)	Medium/high	*P. ferusacci*	66.66	28.66	ND-7.5	6
		T. geversianus	4.54	0.03		61
36. Rio Gallegos-Punta Loyola (harbor)	Medium	*T. geversianus*	0	0	ND-0.3	2
37. Rio Gallegos-Puerto YCF	Low	*T. geversianus*	0	0	ND	1
38. Río Gallegos (control)	Low	*Not found*			0.4	
39. Ushuaia (harbor)	High	*P. plumbea*	100	84.77	41	5
		X. muriciformis	100	44.57		83
		T. geversianus	70.58	7.91		29
40. Ushuaia-Península	Medium	*X. muriciformis*	53.84	17.42	NA	41
		P. plumbea	0	0		8
		O. magellanica	0	*		1
		A. ancilla	0	0		2
41. Ushuaia-Bahía Lapataia (control)	Low	*P. plumbea*	0	0	ND	20
		X. muriciformis	0	0		15
		T. geversianus	0	0		3
Total						1805

* **N**o male sampled NA: no analyzed ND: no detectable.

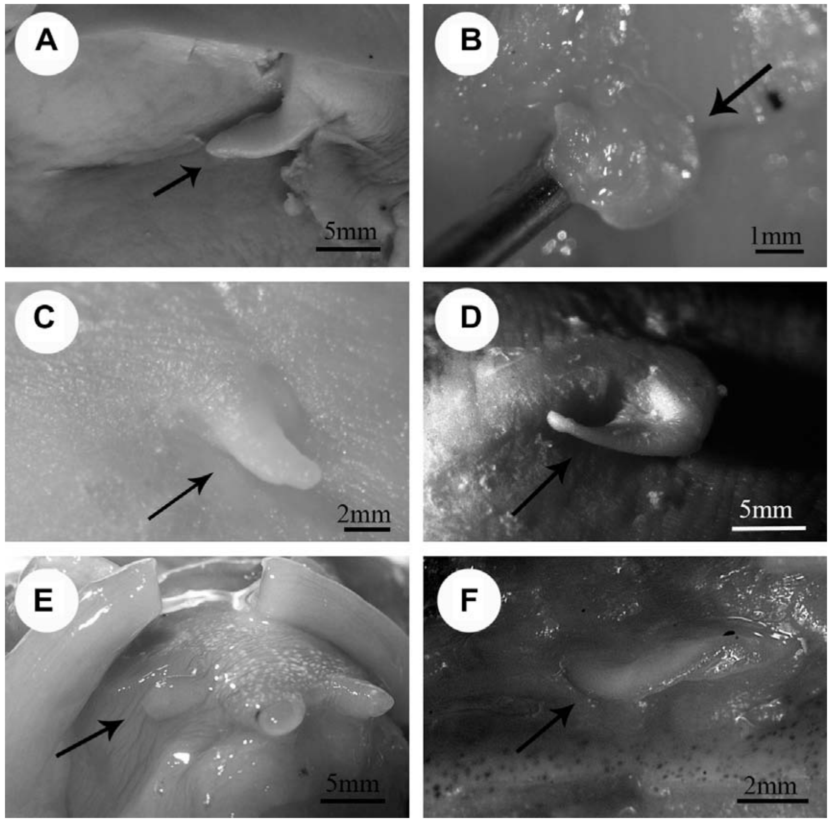

Fig. 2. Species that were determined to display imposex for the first time. Female penis (arrows) of *Pachycymbiola ferusaccii* from Punta Quilla (A); *Trophon geversianus* from Ushuaia port (B); *Xymenopsis muriciformis* from Ushuaia (Peninsula) (C); *Buccinanops cochlidium* from Mar del Plata port (D); *Paraeuthria plumbea* from Puerto Deseado harbor (E); and *Prunum martini* from San Antonio Este.

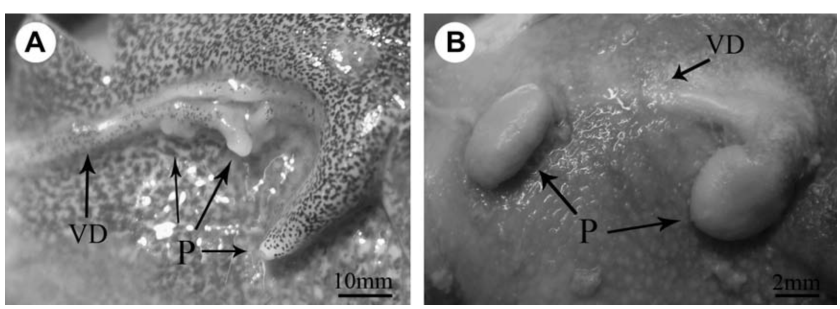

Fig. 3. Imposexed females with multiple penises (P) recorded in volutids from Puerto Madryn harbor. A: *Adelomelon ancilla* with multiple small penises and an engrossed *vas deferens* (VD). B: *Odontocymbiola magellanica* with two developed penises.

All the ports in the country were surveyed in this study, excluding the port of Bahía Blanca (38°45′S; 61°45′W). Data regarding TBT contamination in the Bahía Blanca estuary was previously reported by Delucchi et al. (2006). Although, no biological indices were measured in caenogastropods for that area, TBT concentrations ranging from non-detectable levels to 170.3 ng Sn/g were measured in the inner region of the estuary, and higher levels of 3,288 ng Sn/g were detected near the dry dock at Puerto Belgrano. DBT values ranging between non-detectable and 75.2 ng Sn/g were obtained along the principal channel, but the extreme concentration of 1645 ng Sn/g was measured at Puerto Belgrano (military port), which indicated organotin pollution. Another study in Mar del Plata (Site 1 of this work) of *O. deshayesiana* from 1995–2007 found that the percentage of imposex was significantly higher during (85.3%) and after (73.9%) beach filling (with sand taken from the port) than before the procedure (31.0%) (Teso and Penchaszadeh, 2008). These results and the concentration of TBT found in this zone in this study may explain the imposex incidence in *O. deshayesiana* and in *A. brasiliana* (43% of imposex, determined in this study).

As a general rule, a high degree of imposex was observed in zones with high TBT concentrations (high marine traffic) and fine grain sediments. Sampling sites with a high prevalence of very fine sand and silt/clay fractions presented high percentages of imposex, even when TBT levels were low. TBT is extremely surface-active and the most important sink process of TBT is adsorption onto particulate material with subsequent sedimentation (de Mora et al., 1995). The percentage of organic matter was not registered in this study and should be considered in future works, since this is an important parameter that affects the sorption of organotin compounds onto sediments.

TBT determination indicates that pollution is focused only in ports were ship hulls are painted or marine traffic is high. Species inhabiting these areas are more greatly exposed to tributyltins,

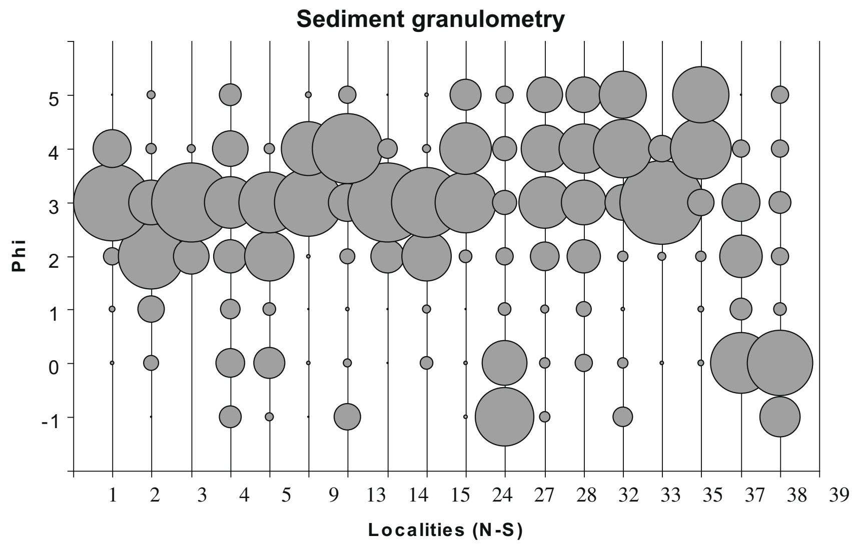

Fig. 4. Percent granulometry for different sample sites (the number in the *x* axis corresponds to those in Table 1 and Fig. 1).

and, therefore, the incidence of imposex in those species is generally high. The determined physiological variations in this study that result in different responses for each of the studied species must be studied in further detail. Patagonian studies regarding hydrocarbon pollution have demonstrated that this pollutant is primarily concentrated at ports with and without crude oil exploitation activity, and there is variability in hydrocarbon levels among analyzed harbors. These areas constitute sources of anthropogenic hydrocarbons or "hot spots" that can affect not only the point source area, but also adjacent or more distant coastal sectors (Commendatore and Esteves, 2007), and are also likely to be polluted with TBT from vessels (the current study). Ingestion of hydrocarbons resulted in mortality of embryos of the marine gastropod *Ilyanassa obsoleta* (Pechenik and Miller, 1983), which is a factor that must be investigated in the populations studied herein to further assess the conservation status of these species.

The species that best reflected the degree of imposex were those inhabiting sandy/muddy or mixed bottoms, which are environments where organotin retention is found at higher concentrations (retention up to five years). In comparison, species inhabiting intertidal hard bottoms, which are environments with a reduced time of exposure to organotins (retention of TBT is up to nine months in the water column), are not good indicators of TBT contamination. In these sites, contamination is not retained *in situ*, and further studies on contaminants dispersion and dilution are needed.

Volutid species exhibited imposex incidence with low TBT concentrations (1.7 ng Sn/g) in Golfo Nuevo, denoting that these organisms are very sensitive to the contaminant. *A. ancilla* demonstrated a higher imposex percentage and RPLI than *O. magellanica* within the same zone. Another volutid from Punta Quilla, *A. ferussacii*, displayed imposex with higher TBT concentrations than the previously mentioned species (up to 33.3 ng Sn/g), and at similar numbers in *P. plumbea, B. globulosus, B. cochlidium,* and *A. brasiliana*. In a site with medium to low marine traffic in the Ushuaia Peninsula, *Xymenopsis muriciformis* developed penises and *vas deferens*, while volutids did not. Although TBT concentrations could not be measured in this zone, the sensitivity of this species seems to be higher than *A. ancilla*. In San Antonio Este, *P. martini* exhibited 100% imposex and a RPLI of 26.66 with very low concentrations of TBT (up to 0.9 ng Sn/g). The species *T. geversianus* is distributed along the entire Argentinean coast (Pastorino, 2005) and exhibited the development of a penis, 70.6% imposex, and a RPLI of 7.9 at TBT concentrations greater than 41 ng Sn/g. In the same site, *X. muriciformis* and *P. plumbea* presented 100% imposex and RPLI up to 84.77. Several studies have utilized cenogastropods as biological indicators to monitor TBT contamination. These studies have been performed in Scotland (Bailey and Davies, 1988), England (Gibbs et al., 1987, 1991), Ireland (Schulte-Oehlmann et al., 1993; Minchin et al., 1995, 1996), France (Oehlmann et al., 1993), and outside of Europe in the United States (Short et al., 1989; Saavedra Alvarez and Ellis, 1990), Canada (Bright and Ellis, 1990), southeast Asia (Ellis and Pattisina, 1990), New Zealand (Stewart et al., 1992), and Australia (Kohn and Almasi, 1993). We propose the use of the species reported in this study to biomonitor TBT pollution along Argentinean coasts.

The case of multiple penis development in volutid females from the port of Puerto Madryn could be attributed to the exposure of these populations to TBT (and perhaps other contaminants) for more than 30 years while the port was modified (Bigatti and Carranza, 2007) and to higher sensitivity to TBT contamination. In the species *O. magellanica*, previous studies have indicated shell malformations and weight loss of 10% in imposexed females, while *O. deshayesiana* from Mar del Plata Teso and Penchaszadeh (2008) registered no differences in body weight in the female population, but shell length was significantly lower in females with imposex.

Studies with natural antifouling compounds that do not affect the biota are encouraged in Argentina, such as those utilizing the Australian algae *Delisea pulchra* and *Dilophus marginatus*, the former of which contains compounds that are currently being commercially developed as antifoulants (Gribben et al., 2006). These algae produce metabolites that affect the settlement response of larvae to two non-polar extracts, which are naturally derived settlement inhibitors.

5. Conclusions

The situation of TBT contamination and imposex incidence along the Argentinean coast reported in this study must be taken into account by local and regional government authorities to create policies that avoid the use of these kind of pollutants and encourage the use of antifouling compounds that are free of TBT in this country and in South America.

Please cite this article in press as: Bigatti, G., et al. Biomonitoring of TBT contamination and imposex incidence along 4700 km ... Mar. Pollut. Bull. (2009), doi:10.1016/j.marpolbul.2009.01.001

Our results demonstrate that all the ports of the Argentinean coast are affected by TBT pollution, which is associated with imposex incidence in caenogastropods.

Taking into account the imposex incidence and TBT concentrations determined in this study, we concluded that the species indicating low TBT pollution (high sensitivity to the pollutant) are: *X. muriciformis*, *A. ancilla*, and *P. martini*. The species indicating medium TBT contamination are: *P. plumbea, B. globulosus, B. cochlidium, O. magellanica*, *A. brasiliana*, and *A. ferussacii.* A good indicator of high TBT concentrations (less sensitive to the contaminant) is *T. geversianus*. All of these species are commonly found and easy to collect in the studied sites, and these results can be utilized for future biomonitoring in order to infer TBT contamination prior to performing expensive biochemical analysis to determine the compound.

Acknowledgements

We are grateful to Daniel Aureliano, Fabian Quiroga, Ricardo Vera, Julio Rúa, and Miguel A. Díaz for sampling support. Carlos Sanchez Antelo and Federico del Brío assisted with laboratory analyses. Guido Pastorino assisted with photographs. This work was supported by GEF-PNUD ARG/02/018 Subproyecto AC-56 and PICT Redes-01869.

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