



Imposex Incidence in *Gastropod* Species from Santa Marta Coastal Zone, Colombian Caribbean Sea

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Abstract

Imposex is a phenomenon widely associated with environmental exposure to organotin compounds which were quite common components of antifouling paints applied on boats and ship hulls. Here we study the incidence of imposex in neogastropods and its relation with water quality and maritime traffic in the coastal strip of Santa Marta, Colombia. Imposex was determined via specialized indexes and related to the organisms' size, somatic conditions, variables of water quality and maritime traffic, in a space–time assessment. There was evidence of imposex in five species *Plicopurpura patula*, *Vasula deltoidea*, *Stramonita haemastoma*, *S. floridana*, and *Gemophos auritulus*. *Purpura patula* and *Vasula deltoidea* species were found in all sampling sites. The results have proved that imposex is highly influenced by the maritime traffic variable, with greater prevalence during the dry season, and with *P. patula* being more sensitive than *V. deltoidea*.

Keywords Imposex · Sea water contamination · Colombian Caribbean · Gastropoda · Maritime traffic

Several human activities generate hazardous substances that have been released in aquatic environments during the last 30–40 years. Among these, organotin compounds, synthetic chemical products such as Tributyltin (TBT) and Triphenyltin (TPT), are closely linked to maritime transport (Dobson and Cabridenc 1990). Since the 1960s, TBT-based antifouling paints were an effective solution used by the naval industry to prevent the settlement of biofouling in ship hulls. However, its deleterious effects on marine biota, specially imposex on gastropods, has led the International Maritime Organization (IMO) to implement a global Convention on

the Control of Harmful Antifouling Systems in Ships (AFS Convention) in 2001. Currently, the use of TBT-based antifouling paints is banned in IMO-register ships (Castro et al. 2012). Despite that, recent reports of TBT levels and its related biological effects are still seen in America (Castro et al. 2018), Asia (Wang et al. 2019), Africa (El Ayari and El Menif 2019), Oceania (Jones and Ross 2018) and Europe (Ruiz et al. 2018).

It is understood that marine-coastal ecosystems provide important environmental services through renewable and non-renewable resources, besides having a strong relationship with economic activities including 90% of world commerce. In this regard, assessments of environmental quality, mainly in zones under the influence of ship traffic, are important to guide coastal areas management, seeking to conserve the biodiversity and integrity of biological communities, especially within marine protected areas (Castro 2019). Considering specifically TBT contamination, which is closely related to ship traffic areas, the use of gastropods as tools in biomonitoring programs has been recommended to assess pollution in marine zones (Morabito et al. 2009). Imposex incidence was described as a superimposition of non-functional male sexual characteristics (penis and/or vas deferens) in females, which is irreversible and specifically induced in environmental conditions by TBT exposure (Horiguchi et al. 2006; Radke et al. 2008). Therefore,

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imposex incidence has become a suitable tool used to monitor TBT marine contamination around the world. In Latin America, despite prohibition issued by AFS convention, several recent studies demonstrated imposex occurrence in the coastal areas of Brazil (Otegui et al. 2019), Argentina (Brio et al. 2016), Perú (Castro et al. 2018), Panamá (Batista-Andrade et al. 2018), Chile (Mattos et al. 2017), Ecuador (Rodríguez-Grimón et al. 2016) and Venezuela (Paz-Villarraga et al. 2015), besides in Caribbean areas as British Virgin Islands (Tittley-O’Neal et al. 2011), and American Virgin Islands (Strand et al. 2009). In Santa Marta coast, maritime traffic can be more intense in some areas compared with others, with differences between the type of transport, highlighting tourism, fishing, commercial trade and of industrial uses (CIOH 2012). Therefore, the present study aimed to perform a time–space assessment of imposex incidence using different gastropod species from Santa Marta coastal zone, in Colombia.

Materials and Methods

Santa Marta coastal zone presents two clearly defined climatic patterns: a dry season (December–April) and a rainy season (May–November), with an average yearly precipitation of 501 mm. (IDEAM 2020). The coastal fringe of Santa Marta is a highly dynamic zone, in which many different socioeconomic activities take place as tourism, fishing, commerce trade and carbon transport and storage, all of which impact sea environment conditions (INVEMAR-GEO 2015). Thus, were established two sampling sites within Santa Marta Bay (BSM): International Santa Marta Marine (SM) (11° 14' 31" N–74° 13' 4" W) and Punta de Betín (PdB) (11°15'01.8" N–74°13'14.9" W), and another

two sampling sites were established inside Tayrona National Natural Park (TNNP), which is a protected area: Concha Bay (BC) and Nenguange (Neg), located at the northeast of the main beaches of Concha (11° 18' 00" N–74° 8' 56" W) and Nenguange (11° 19' 27" N–74° 4' 42" W). In each site, manual collections of adult gastropod species (*P. patula*, *V. deltoidea*, *Stramonita haemastoma*, *Stramonita floridana*, and *Gemorphos auritulus*) were done in the intertidal zone and up to two meters deep on rocky substrates, between the years 2015 (April, July, and October), 2016 (March, May, August, and November) and 2017 (February) (Fig. 1). These sampling campaigns represented three and five months of dry and rainy seasons respectively.

Shell length (SL) was measured from the apex to the siphonal channel’s lip, using a digital caliper (± 0.01 mm). Soft tissues were removed from the shells and the shell’s weights were registered (± 0.001 g). Gender identification was based on the presence or absence of female sexual accessory glands and the penis length, which is normally longer in males than in females affected by imposex. Penis length (PL) and presence of vas deferens in females, were recorded at the same time. Imposex levels were assessed using the following indexes: % of imposex-affected females (I%), Female Penis Length Index (FPLI = mean penis length of all females in the sample including zero values), Relative Penis Length Index (RPLI = [FPLI/mean penis length of all males in the sample (MPLI)] $\times 100$) and Vas Deferens Sequence Index (VDSI), which is based on the development of male sexual characters (particularly vas deferens) additional to the female reproductive system, determined according to Gibbs et al. (1987) and Fernández et al. (2005).

Variables of water quality [total suspended solids (TSS), orthophosphates, nitrites, nitrates, ammonia, dispersed and dissolved petroleum hydrocarbons (DDPH), salinity, pH,

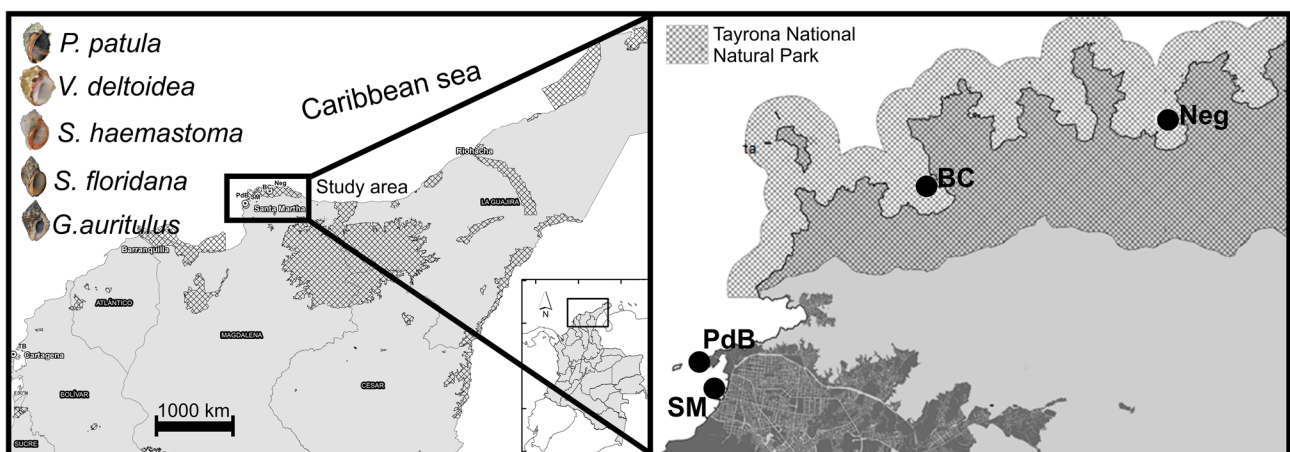


Fig. 1 Dependence between imposex effect and maritime traffic using BRT model for *P. patula* and *V. deltoidea*. Y axes are in centered logit scale, to have a zero average on the data distribution. The solid

line indicates the mean change in predictive deviation, and the shaded boundary is the standard error

dissolved oxygen (DO) and total coliforms (TC)], were obtained from the Vigilance Network for Conservation and Protection of Marine and Coastal Waters of Colombia (REDCAM, for its Spanish acronym), whose estimate follows standardized methodologies. Data from boats in each sampling station with information on the daily amount, length measure and permanence time of docking/anchorage reported in DIMAR (2018) were used. The length information for medium boats in sampling stations SM, BC and Neg, was complemented by the one given in the website www.marinetraffic.com. The monthly index for the sites: SM, PdB, BC, and Neg, was calculated by a direct relationship between the number of boats per month, average length and boat's permanence time, in each sampling station. Variable data were standardized to have values between 0 and 1, as shown in data treatment proposed by Guisande et al. (2013), and using Eqs. 1–4.

$$\text{Traffic} = \text{VEc} + \text{VEe} + \text{VEt} \quad (1)$$

$$\text{VEc} = \frac{C - \text{Min}}{\text{Max} - \text{Min}} \quad (2)$$

$$\text{VEe} = \frac{E - \text{Min}}{\text{Max} - \text{Min}} \quad (3)$$

$$\text{VEt} = \frac{T - \text{Min}}{\text{Max} - \text{Min}} \quad (4)$$

where *VEc* is the number of ships; *VEe* is the ships' length, and *VEt* represents the ship's permanence time in docking zones. The variables *C*, *E*, and *T* represent the observations of number of ships, ships' length and permanence time respectively, employing the maximum (Max) and minimum (Min) values of all the data available for each variable.

The comparison among averages was performed using ANOVA and Welch's t-test. If a significant difference between three or more means was detected, Tukey's multiple comparison test was applied. If one of the assumptions was not fulfilled, the Kruskal–Wallis non-parametrical analysis was performed. Similarly, Mann–Whitney U tests were used to compare two medians presenting non-normal distribution. Contingency tables were done (χ^2 Pearson test/G-test of independence with Williams' correction) for spatial–temporal comparisons of imposex levels, and to assess in this phenomenon in each species. In addition, linear regression analysis was used to evaluate the correlation between shell length and penis length of males and of imposex affected females, by species. A Spearman correlation analysis was made in order to extract the variables with the highest significant correlation coefficient with direct association to the prevalence of imposex (I%); using these variables, a model of Boosted Regression Trees (BRT) was executed using the

“gbm” package in the “R” statistical program (Elith and Leathwick 2017), to obtain the relative contributions or importance of each independent variable on imposex. To evaluate the sensitivity of *P. patula* and *V. deltoidea* species in manifesting imposex according to the effect of the predictor variable, maritime traffic, breakpoint regressions were performed (Davies 1987). All the data handling was done with the “R” statistical program, via the interface RWizard (Guisande et al. 2013). All the tests were made assuming a value of probability below 5% ($p < 0.05$), to reject the null hypothesis.

Results and Discussion

Organisms of five gastropod species were sampled. *Plicopurpura patula* and *Vasula deltoidea* were found in all sampling sites, while *Stramonita haemastoma*, *Stramonita floridana* and *Gemorphos auritulus*, were only present in SM. *P. patula* (N = 351) and *G. auritulus* (N = 76) were collected between the mesolittoral and supralittoral zones; while *S. haemastoma* (N = 72), *S. floridana* (N = 14) and *V. deltoidea* (N = 177), were limited to the infralittoral zone and up to a depth range of 2 m (Table 1). The specimens of *P. patula* and *V. deltoidea* collected at the SM station presented smaller absolute shell size, probable due to effects related to environmental stress to which they have been constantly subjected due to the proximity of anthropic contamination sources; this behavior has been described previously for prosobranch gastropods (McClanahan 1992; Crowe et al. 2000).

In BSM imposex was observed in all sampled species and over the studied years. The highest values of imposex prevalence were reported in SM (Table 1). In the TNNP protected zone, *P. patula* females were only affected during dry season (Pearson's Chi-square test, $p < 0.001$). The first case of imposex in the Caribbean Sea was detected 20 years ago in a reproductive biology study of *Melongena melongena* (Hernández and Stotz 2004). So far, imposex had been recorded for 12 species, belonging to the families Muricidae, Melongenidae, Fasciolaridae, Strombidae, and Volutidae (Hernández and Stotz 2004; Miloslavich et al. 2007; Strand et al. 2009; Titley-O'Neal et al. 2011; Peralta et al. 2014; Paz-Villarraga et al. 2015; Batista-Andrade et al. 2018; Sierra-Marquez et al. 2018;). The present study, in addition to recording the impact on four of these muricides, reports imposex phenomenon for the species *Gemophos auritulus* belonging to Pisaniidae family. Imposex impact on *Gemophos auritulus* constitutes the second world report for the species in 40 years (Jenner 1979), and our work constitutes also the first report in Caribbean Sea waters. Imposex in Muricidae Family has been reported in more than 100 species, with *S. haemastoma* being the most frequent reported species in Latin America studies (Titley-O'Neal et al. 2011);

Table 1 Biometric and imposex parameters obtained for *Plicopurpura patula*, *Vasula deltoidea*, *Stramonita haemastoma*, *Stramonita floridana* and *Gemorphos auritulus* in climatic seasons, Santa Marta coastal zone

Site	N	M/F	MSL ± sd (mm)	MPL ± sd (mm)	FSL ± sd (mm)	FPL ± sd (mm)			RPLI			VDSI ± sd (mm)			Imposex %		
						Dry	Rainy	Global	Dry	Rainy	Global	Dry	Rainy	Global	Dry	Rainy	Global
<i>Plicopurpura patula</i>																	
SM	81	33/48	21.9 ± 5.2	6.8 ± 1.1	20.0 ± 7.8	1.2 ± 1.1	0.4 ± 0.4	0.8 ± 0.9	13.1	7.5	12.7	2.0 ± 0.7	1.3 ± 0.9	1.6 ± 0.9	100	86	92
PdB	93	28/65	32.6 ± 8.1	11.3 ± 3.4	32.0 ± 12.1	3.5 ± 4.5	0.4 ± 1.9	2.5 ± 4.1	32.6	3.7	23.0	2.2 ± 1.6	1.0 ± 1.3	1.8 ± 1.6	70	38	60
BC	85	21/64	23.4 ± 3.5	7.0 ± 1.2	23.4 ± 3.1	1.5 ± 2.7	0.4 ± 0.9	1.3 ± 2.5	26.7	0.0	10.9	0.8 ± 1.3	0.4 ± 0.9	0.7 ± 1.2	36	10	28
Neg	92	36/56	29.8 ± 4.8	10.0 ± 2.6	28.5 ± 9.5	1.9 ± 3.1	0.0 ± 0.0	1.0 ± 2.5	40.0	0.0	12.4	0.9 ± 1.3	0.0 ± 0.0	0.5 ± 1.0	34	0	18
<i>Vasula deltoidea</i>																	
SM	36	5/31	28.4 ± 5.7	7.4 ± 1.7	37.3 ± 6.1	-	3.9 ± 3.4	3.9 ± 3.4	52.4	8.6	25.6	2.3 ± 0.8	-	2.3 ± 0.8	100	100	100
PdB	30	14/16	33.8 ± 3.3	9.8 ± 2.5	35.0 ± 4.1	-	0.3 ± 1.0	0.3 ± 1.0	-	2.6	2.6	-	1.9 ± 2.8	1.9 ± 2.8	-	38	38
BC	66	29/37	37.8 ± 3.5	7.5 ± 2.4	40.5 ± 4.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0	0.0	0.0 ± 0.0	0.5 ± 1.1	0.5 ± 1.1	0	19	11
Neg	45	27/18	36.4 ± 2.7	6.4 ± 1.6	39.3 ± 2.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0	0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0	0	0
<i>Stramonita haemastoma</i>																	
SM	72	39/33	41.9 ± 8.6	9.4 ± 4.3	41.2 ± 8.3	0.2 ± 0.4	0.4 ± 0.9	0.4 ± 0.8	1.7	4.6	3.9	1.0 ± 0.6	0.7 ± 0.8	0.8 ± 0.8	67	48	52
<i>Stramonita floridana</i>																	
SM	14	7/7	41.4 ± 5.6	11.3 ± 3.0	41.9 ± 5.2	-	0.1 ± 0.0	0.1 ± 0.0	-	0.2	0.2	-	0.3 ± 0.4	0.3 ± 0.4	-	25	25
<i>Gemorphos auritulus</i>																	
SM	76	44/32	28.0 ± 2.7	13.0 ± 2.6	24.7 ± 2.2	4.3 ± 2.5	0.1 ± 0.8	0.8 ± 1.9	28.7	1.2	6.1	2.4 ± 1.2	0.1 ± 0.6	0.5 ± 1.1	80	4	16

Male/Female (M/F), Male shell length (MSL), Female shell length (FSL), Male penis length (MPL), Female penis length (FPL), Relative penis length index (RPLI), Vas deferens sequence index (VDSI), Imposex percentage (I%)

SM Santa Marta International Marine, PdB Punta de Betín, BC Concha Bay, Neg Nenguange

while, *S. floridana* has only presented one report during a study of imposex induction at laboratory level (Lima et al. 2006), making our report the first one of imposex impact for this species in the Caribbean Sea.

The sensitivity of *P. patula* to imposex was influenced by size, with small (< 25 mm) and medium-sized females ($\geq 25 \leq 40$ mm) being more affected than large-sized ones (> 40 mm) (Pearson's Chi-square test, $p=0.016$; Williams' corrected independence G-test, $p=0.017$). The manifestation of imposex with highest prevalence and intensity in small and medium-sized specimens of *Stramonita brasiliensis*, was described at the experimental level by Rossato et al. (2019), in contrast to El Ayari et al. (2015) and Otegui et al. (2019), who demonstrated in a field research on the same species a higher level of impact on larger females. The fact that in BSM, the affected females presented the lowest relative shell weights compared to healthy females, is an indicator that TBT contamination in addition to endocrine disruption related to reproduction, is affecting other physiological processes such as shell structural formation and growth Rico (2017) reported that imposex affected *P. patula* specimens had the thinnest shells and decreased shell density in the same area of study. Similarly, Márquez et al. (2011) described the same behavior between the appearance of there is a pattern in the behavior of physiological responses of gastropods species sensitive to TBT contamination. The climatic season was an important factor that influenced the prevalence of imposex in *P. patula* and *V. deltoidea*. A recent study using *S. brasiliensis* indicates that imposex is influenced by climatic season and the gastropod's age (Rossato et al. 2019). During dry season, the appearance of imposex malformations became more visible, which was more evident in the sites with high maritime traffic. Responses observed in *P. patula*, presenting a different seasonal sensitivity, are related to changes in population structure with climatic seasons in Caribbean Sea, as described by Susan (2004). The highest CI in *P. patula* and the tendency to present increased penis length in males, evidence that the reproductive period occurs primarily during dry season (Ramón and Amor 2002; Vasconcelos et al. 2011), and during this reproductive stage this species is more sensitive to exposure to the pollutant TBT. An experimental study by Rossato et al. (2019), showed how *S. brasiliensis* females were more susceptible to TBT when exposed during their reproductive period. The highest prevalence of imposex occurred at the SM sampling station, despite that the calculated maritime traffic was lower than in the port (PdB); this is explained by the fact that in SM there is permanent presence of small boats performing tourist and fishing activities that are not controlled under IMO regulation. In fact, recent studies have reported higher imposex incidence associated to TBT contamination in marinas and moorings used by small vessels since they are poorly regulated in comparison to commercial

ports (Castro and Fillmann 2012; Paz-Villarraga et al. 2015; Laranjeiro et al. 2018). Considering the presence of tourist boats in all monitored sites and that the detection of imposex phenomenon was widespread, the high relation between imposex and maritime traffic is clearly evident. In the two sampling stations corresponding to TNNP (BC and Neg), the detection of imposex is explained as due to the proximity to navigation routes of tourist, fishing and merchant vessels; in fact, bays and inlets in TNNP are reference points for the anchoring of tourist boats on official navigation charts for Colombian Caribbean waters (CIOH 2012), and there is the possibility that this phenomenon will continue being detected for a long time. This is supported by the findings of Schøyen et al. (2019), who evaluated butyltin concentrations between 1991 and 2017, and found significantly higher levels of TBT and imposex in coastal areas near to shipping routes and with shipping repair facilities.

Comparative analysis of variables in sampling stations showed differences in pH, DDPH, and TC, with a trend of more deteriorated conditions of water quality in SM sampling site. The influence of climatic seasonality was evident during dry season, in which higher levels of salinity, pH, nitrates, and ammonium were reported, while TC concentration showed higher values during rainy season, being significant only in SM with an average of 438 NMP/100 mL ($p<0.05$). Maritime traffic was the most important variable influencing the groups of healthy and affected females. The BRT analysis showed it as the variable with the highest contribution to the model, obtaining relative contributions of 63.24% and 69.09% for *P. patula* and *V. deltoidea*, respectively. In our work, it was estimated that the imposex phenomenon occurs even at very low levels of maritime traffic (< 0.10), this may indicate that the affected species are very sensitive to the polluting effect inherent to this traffic (e.g. tin butyls and particularly TBT). The breakpoint represents the minimum estimated level of maritime traffic to detect a prevalence of imposex of 100%. Thus, *P. patula* reached the breaking point with a 0.362 [95% confidence intervals (CI) 0.342–0.381] of maritime traffic, compared to the value obtained in *V. deltoidea*: 0.484 (95% CI 0.459–0.509). Figure 2, shows that there are differences in species sensitivity to the exposure to the pollutant, indicating that *P. patula* is the most sensitive species in the group of studied species in this work. Although it has been reported that *P. patula* and *V. deltoidea* species show a similar sensitivity to development of imposex after exposure to TBT, a previous comparative study between both species detected differences in the potential for TBT accumulation in tissues, with *V. deltoidea* being higher than *P. patula*, and that the difference in imposex intensity measured by VDSI, begins to be slightly higher in *V. deltoidea* at concentration levels above 40 (ng Sn/g dw) in animal tissue (Strand et al. 2009). This result suggests the existence of a differential genotypic capacity in channeling

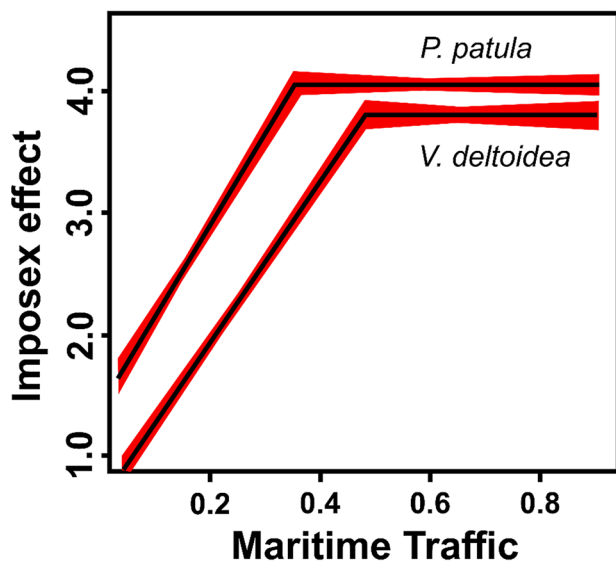


Fig. 2 Sites and gastropod species sampled in Santa Marta coastal zone

TBT contamination and its effects on animal tissue, giving *P. patula* an adaptive advantage over *V. deltoidea*, in showing greater sensitivity and at the same time, a better channeling capacity to the effects of contamination by TBT.

TNNP is a protected area in the northern Colombian region, belonging to the Magdalena department and located 34 km away from Santa Marta city. This park, included in category II, according to the International Union for Conservation of Nature (IUCN), is a hotspot of coral reef biodiversity in the Caribbean region (Bayraktarov et al. 2014). Besides, TNNP has been declared an integral part of UNESCO's international network of Biosphere Reserves, due to its strategic importance for the conservation of coral reef features including 110 species of corals, 202 species of sponges, 700 species of mollusks, and over 400 species of sea and river fish (GANP 2020). Low to moderate imposex incidence (10% to 34%) was detected in two gastropod species (*P. patula* and *V. deltoidea*) collected inside TNNP (Table 1). Considering that imposex occurrence denotes clear TBT (or triphenyltin) contamination, it is very probable that many other inhabitant organisms of the contaminated zone, are being exposed to this hazardous compound. Therefore, our findings regarding imposex incidence indicate that conservation goals of TNNP may be under threat. Indeed, a similar situation was already demonstrated in another Biosphere Reserve as Galapagos islands—Ecuador (Rodríguez-Grimón et al. 2016). National parks aim to protect natural biodiversity including the ecological structure and environmental processes. Moreover, such areas should promote education and recreation (Dudley 2008). In this regard, TNNP is the second most visited national park in

Colombia, receiving several small and medium touristic boats that may be related to TBT inputs. It should be considered that in the port areas inside Santa Marta Bay (SM and PdB), surface water currents have values that do not exceed 0.2 m/s, being slightly higher in rainy months and that the prevailing directions of marine currents have indicated a flow out of the bay (INVEMAR-GEO 2015); therefore, TBT releases from Santa Marta port area might be mobilized through coastal currents. Imposex incidence within TNNP, after TBT global ban, indicates that specific regulations and conservation plans should be addressed mainly in strategic coastal areas playing an essential role in Caribbean biodiversity conservation. It is highly probable that many other inhabitant organisms of these sites, are being exposed to this hazardous compound, therefore, the conservation goals of TNNP may be under threat.

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