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Imposex occurrence in *Nassarius vibex* from South America: a potential bioindicator in estuarine environments

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Research on organotin compounds (OTCs) contamination in South America is focused on sand/rocky environments; estuarine areas have not yet been investigated. Imposex is an important tool to evaluate OTC contamination. The aim of the present study was to assess the sensitivity of a typical estuarine species to OTC contamination and to biomonitor the spatial extent of such compounds in the estuary. Sampling was conducted along the Ceará River estuary (north-eastern Brazil) and imposex expression was quantified in Nassarius vibex *females. Imposexed females developed small papillae or penis, with smaller lengths compared to males. Passive monitoring was conducted on 10 sampling stations around 2 shipyards using imposex quantification indices. Imposex percentage in the population ranged from 0 to 33.3% and relative penis length index ranged from 0 to 26.4%. The present research is the first application of* N. vibex *as a bioindicator of organotin compounds in Brazil.*

Keywords: bioindicator, estuary, imposex, *Nassarius vibex*, Ceará River

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INTRODUCTION

The effect of organotin contamination is different in each affected group of organisms. Neogastropod molluscs respond to the organotin exposure by developing a syndrome called imposex, in which females develop masculine secondary structures as penis and/or vas deferens (Gibbs *et al.*, 1987).

Nassarius species have great potential for environmental impacts research, because of their wide distribution and resistance to pollution (Coelho *et al.*, 2006). Nassariids have been used in imposex studies in many different countries, such as *N. reticulatus* (Barreiro *et al.*, 2001; Barroso *et al.*, 2002a,b, 2005a,b; Sousa *et al.*, 2005) in Spain and Portugal, *N. kraussianus* in South Africa (Marshall & Rajkumar, 2003) and *N. festivus* in China (Chan & Morton, 2003).

Nassarius vibex (Say, 1822) is distributed from Cape Cod to Florida in the USA, West Indies, Venezuela and Brazil. On the Brazilian coast, this species is distributed from the state of Pará to Santa Catarina (Rios, 1994). It is commonly found in north-eastern Brazil in estuaries, over muddy sand flats (Matthews, 1968). Demaintenon (2001a,b) first reported the occurrence of imposex in *N. vibex* populations from the USA.

The first studies on the occurrence of imposex in South America were accomplished by Gooding *et al.* (1999) in Chile, Castro *et al.* (2000) in Brazil and Penchaszadeh *et al.* (2001) in Argentina.

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Species used as bioindicators of organotin compounds contamination in those countries are habitants of sandy or rocky shores, such as: *Stramonita haemastoma*, (Fernandez *et al.*, 2005; Castro *et al.*, 2007a,b; Limaverde *et al.*, 2007), *S. rustica* (Castro *et al.*, 2007b; Limaverde *et al.*, 2007) and *Odontocymbiola magellanica* (Bigatti & Penchaszadeh, 2005). Therefore, this study evaluates the potential of *Nassarius vibex* as an indicator of the presence of organotin compounds in estuarine environments and its application to monitor the spatial extent of OTC contamination along the Ceará River estuary.

MATERIALS AND METHODS

The Ceará River estuary is located in the metropolitan region of Fortaleza City, north-eastern Brazil. Two shipyards, whose activities are the maintenance of medium and small ships, are present in the estuary. To monitor the contamination extent along the estuary 10 sampling stations were selected, 5 on each river margin, in October 2006. Distance in metres from the shipyards area was obtained using GPS (Table 1).

In the laboratory, individuals were narcotized with saline solution of $MgCl_2$ (7%) for a minimum time of 1 hour 30 minutes. Shell length was measured with a vernier caliper of 0.05 mm precision and soft parts were removed using a lathe. Sex was determined based on the presence of capsule gland and/or bursa copulatrix in females and seminal vesicle in males. Penis length of males and imposexed females were measured using a graduated graph paper with 1.0 mm

Table 1. Imposex quantification in *Nassarius vibex* at the sampling stations along Ceará River estuary.

Sampling stations	Distance from shipyards (m)	Number of organisms	% imposex	RPLI
S1	510	41	10.5	7.6
S2	300	30	33.3	26.4
S3	250	29	0	0
S4	470	30	18.2	21.2
S5	690	5	0	0
S6	1010	36	6.7	6.6
S7	630	51	12	8.82
S8	250	45	18.2	12.1
S9	70	22	28.6	7.25
S10	704	4	0	0

RPLI, relative penis length index.

precision. Imposex development level in the population was quantified using the percentage of imposexed females and relative penis length index (RPLI) [(female mean penis length)/(male mean penis length) × 100]. RPLI is a more appropriate index for nassariids since this species presents dorso/ventral flattened penis (Bryan *et al.*, 1993; Barroso & Moreira, 1998).

The animals were partially deposited in the Professor Henry Ramos Matthews's Malacological Collection of the Universidade Federal do Ceará, Fortaleza.

Data were analysed using software STATISTICA v.7.0. Non-parametric Kruskal–Wallis tests ($P < 0.05$) were used to determine significant statistical differences between sampling stations and imposex levels. Spearman tests ($P < 0.05$) measured the correlation between the shipyards distance and imposex expression in the population.

RESULTS

Penis is located, in *Nassarius vibex*, behind the right cephalic tentacle. This characteristic is common to other neogastropods. Imposexed females were characterized by the presence of strictly female structures such as glandular mass (capsule and albumen glands) and the presence of male structures such as penis or papillae, located in the equivalent region to males (Figure 1). The higher value of penis length observed in females was 4.5 mm. Percentage of imposexed females varied along the sampling stations (Figure 2; Table 1). The highest percentage occurred on Station S2, 300 m distant from the shipyards, with 33.3% of affected females. Sampling Station S9, 70 m distant from the shipyards, also presented high percentage of imposexed females (28.6%). Three sampling stations displayed an absence of any imposex level in the population (Stations 3, 5 and 10), however two stations did not achieve the required sampling number for analysis. The RPLI, which correlates male and female penis sizes, ranged from 0 to 26.4% and presented the higher value in sampling Station S2.

The non-parametric Kruskal–Wallis test ($P = 0.4373$) revealed no significant differences between imposex expression along the sampling stations for the imposex quantification indices used in the monitoring. Spearman correlation between the shipyards distance and the percentage of imposexed females along the sampling station was negative ($r_s = -0.58$, $P = 0.075$) and not significant, although it indicated a tendency to increased imposex levels near the shipyards.

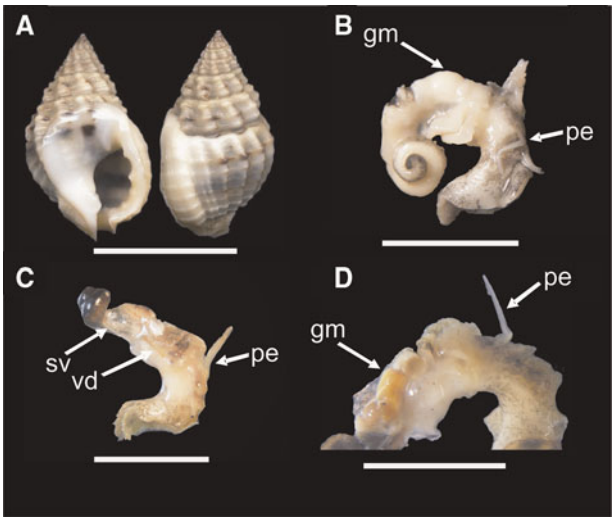

Fig. 1. *Nassarius vibex*. (A) Adult shell; (B and D) soft parts of an imposexed female; (C) soft part of a male. Abbreviations: gm, glandular mass; pe, penis; sv, seminal vesicle; vd, vas deferens.

DISCUSSION

The present research is the first application of *Nassarius vibex* as a bioindicator of organotin compounds in Brazil. *Nassarius vibex* is widely distributed along Brazilian coast estuaries and therefore has great potential to be a reliable sentinel organism to monitor estuaries. It is important to mention that the main OTC sources in the environment are harbours and marinas that are commonly located inside estuarine areas (Michaud & Pelletier, 2006). Although the International Maritime Organization (IMO) global banning of OTCs in antifouling paints started in 2003 and banning of ships painted with organotin compounds in 2008, the prohibition has not yet come into force in many developing countries (Shi *et al.*, 2005). Therefore, it is extremely important to monitor organotin contamination in order to inspect the enforcement of IMO rulings.

The assessment of imposex quantification indices along the Ceará River estuary demonstrated that they were low, with maximum percentage of imposexed females of 33.3% and RPLI of 26.4%.

Previous studies accomplished in the Ceará River estuary by Lima-Verde *et al.* (2006) showed imposex indices similar to those found in the present study. Lima-Verde *et al.* (2006) observed an imposex incidence of 23.5% and 33.3% and RPLI of 37.8 and 60.1%. In Rio de Janeiro, Cardoso *et al.* (2009) found 100% of imposexed females, with RPLI equal to 39.1%. In another study, *N. nitidus* presented imposex incidence of 88% up to 100% and RPLI between 6.1 and 46.7% in Lagoon of Venice, Italy (Pavoni *et al.*, 2007). *Nassarius reticulatus* presented imposex incidence of 0 up to 100% and RPLI ranged between 0 and 64% along the Portuguese coast (Rato *et al.*, 2008).

Despite the reduced percentage, imposex occurrence is directly related to organotin contamination in that site, since imposex only develops in gastropods exposed to OTCs

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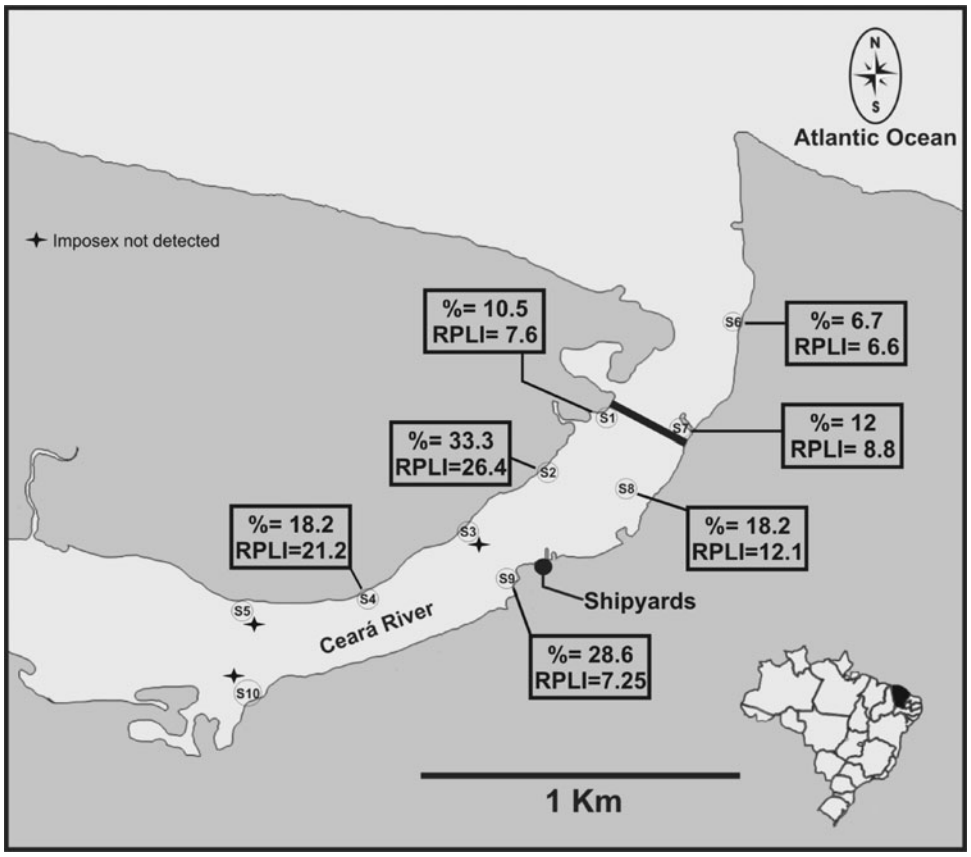

Fig. 2. Map of the Ceará River estuary with sampling stations locations. Imposex percentage and relative penis length index (RPLI) can be observed near each sampling station.

(Smith, 1981; Matthiessen & Gibbs, 1998). It is irrefutable that the *N. vibex* population in the present study is sensitive to OTCs and responded to its presence in the environment by developing this syndrome.

Pavoni *et al.* (2007) accomplished imposex determinations and OTC chemical analyses in *N. nitidus* and observed a positive correlation between both results. Similarly other studies proved the relationship between OTC concentration and imposex alterations (Barroso *et al.*, 2002a; Rato *et al.*, 2008). Additional experiments of imposex induction under TBT exposure were accomplished by Chan & Morton (2003) in *N. festivus*. Those results showed a strong cause–effect relationship between imposex and the presence of OTC in the environment.

Low imposex indices in *N. vibex* can be explained by the following hypotheses: (1) low local organotin contamination due to a small influence of the shipyards; (2) variable residency time of water due to tidal fluctuations; and (3) low sensitivity of the chosen species. More detailed studies are needed to test these hypotheses and to determine the minimum OTC concentration capable of causing imposex in females.

Bigatti *et al.* (2009) in a study on TBT biomonitoring contamination and imposex incidence along the Argentinean coast showed that species inhabiting soft or mixed bottoms are the ones that best reflect the imposex phenomenon in low and medium TBT polluted zones.

The present research focused on sampling adult specimens, however, according to Barroso *et al.* (2005a), to implement systematic monitoring programmes, the age of the sampled specimens has to be considered. Considering that the concentrations of OTCs in the environment would remain stable or reduced due to the IMO ban and that imposex is irreversible, if only adult individuals were used in research, there would be no need to repeat sampling frequently (annually or every 2 years) because animals with equal ages would be sampled again and results would not reflect time-dependent changes in TBT pollution. If sampling intervals were shorter, younger specimens would have to be sampled (Barroso *et al.*, 2005a). Imposex levels observed in *N. vibex* sampled in the Ceará River estuary indicated that this species represents a potential bioindicator of organotin compounds because of its wide distribution, abundance, easy field identification and easy sampling.

In conclusion, *Nassarius vibex* females in the Ceará River estuary developed imposex, constituting an alternative tool for the detection of OTCs in estuarine environments. Monitoring results suggest that shipyards in the Ceará River are the probable source of OTC contamination demonstrated by a tendency of increased imposex levels in sampling points near the shipyards.

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