



IMPOSEX IN THREE PROSOBRANCH SPECIES FROM ILHA DO JAPONÊS, RIO DE JANEIRO, SOUTHEAST BRAZIL

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ABSTRACT:

Imposex in gastropods is used worldwide as a bioindicator of organotin compounds (OTs). Samples of three prosobranch mollusc species were collected (*Stramonita haemastoma*, *Leucozonia nassa* and *Cymatium parthenopeum*) in the Ilha do Japonês tidal flat, Rio de Janeiro, Southeast Brazil. The individuals were led to the laboratory and were examined for occurrence of imposex. Imposex was observed and described for each species: *S. haemastoma* showed the higher indexes (% imposex, RPSI and RPLI) followed by *L. nassa* and *C. parthenopeum*. The results suggest that *S. haemastoma* is the most indicate species to organotin pollution monitoring at Ilha do Japonês, Brazil.

INTRODUCTION

Imposex is characterized by the development of male characteristics in females prosobranch gastropods (Smith, 1971). It is a phenomenon caused by the exposition of those animals to the organotin compounds (OTs) of antifouling paints (Gibbs & Bryan, 1987; Matthiessen & Gibbs, 1998). Organotin compounds (OTs) are usually used in antifouling paints for boat hulls and other structures submitted to the seawater direct contact (Bryan *et al.*, 1986). Imposex anomaly usually has observed in areas with high boating activity.

In general, the study of imposex has been used at several places of the world as a tool to organotin compounds contamination monitoring, once the methods of chemical analysis are extremely onerous (Oehlmann *et al.*, 1996).

In Brazil, monitoring studies using the imposex in the species *Stramonita haemastoma* was accomplished in São Paulo State (Magalhães *et al.*, 1997), Rio de Janeiro State (Fernandez *et al.*, 2002) and Ceará State (Castro *et al.*, 2000; Lima, *et al.*, 2003). Similar studies using the species *Stramonita rustica* was also developed in Alagoas State (Camillo *et al.*, 2004) and Rio Grande do Norte State (Castro *et al.*, 2004).

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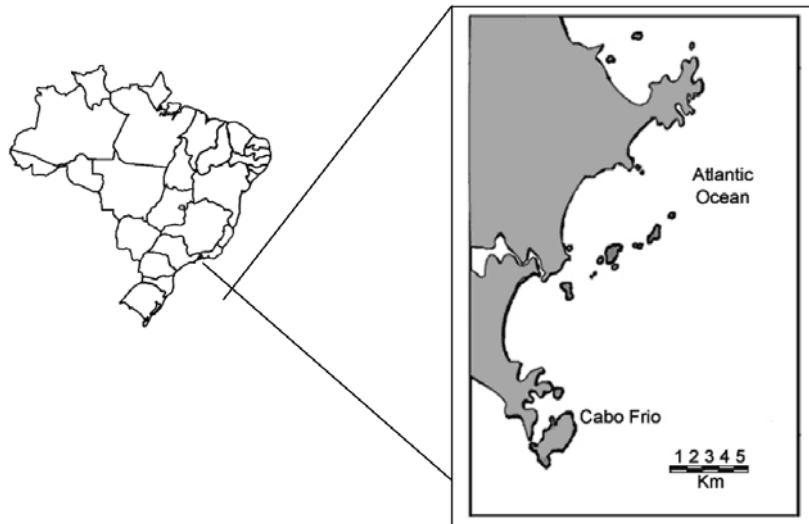


Figure 1:
Study area

The tidal flat of Ilha do Japonês shelters several species of gastropods, including the muricid *Stramonita haemastoma*, fasciolarid *Leucozonia nassa* and the cymatid *Cymatium parthenopeum*. That area is located in the entrance of the Itajuru Canal, where there is a great marina with intense flow of boats. Nowadays, imposex is described to 120 prosobranchs, but only some of those animals can be used in monitoring studies. According to Bech (1999), different species can present different imposex degrees, even when submitted at the same exhibition organotin compounds levels. The present investigation verifies the imposex occurrence in the species *Stramonita haemastoma*, *Leucozonia nassa* and *Cymatium parthenopeum* collected in the tidal flat of Ilha do Japonês and comparing the possible imposex indexes obtained for the three species in order to know the ideal indicator for organotin compound contamination in this area.

MATERIAL AND METHODS:

During the low tide, were collected manually 30 adults of *Stramonita haemastoma* and *Leucozonia nassa* and 12 adults of *Cymatium parthenopeum* in the Ilha do Japonês tidal flat (Figure 1). These animals were kept in plastic boxes containing seawater from the respective sites and they were led to the laboratory.

The molluscs were preliminary narcotized with magnesium chloride solution 3,5% (Huet *et al.*, 1995). Subsequently, length of each snail was measured from the apex to the distal end of the siphonal canal using callipers. The shell of the animals was crushed with a hammer, soft parts were placed in a petri dish, and examined using a binocular microscope.

The sexual determination in *Stramonita haemastoma* was made based on the presence of the sperm-ingesting gland, only in females, and prostate in males. The oviducts of *Leucozonia nassa* and *Cymatium parthenopeum* presence was examined.

The individuals of *Stramonita haemastoma* which presented both sperm ingesting gland and penis were considered females with some imposex level. Individuals of *Leucozonia nassa* and *Cymatium parthenopeum* with oviducts and penis were also considered imposexed females. All males as well as the females that presented imposex, had their penis measured with a millimetric slide and the vas deferens formation in the females was also observed. The imposexed females percentage was calculated.

The imposex quantification was usually made by two indexes initially developed for the neogastropod *Nucella lapillus* (Gibbs & Bryan, 1987; Gibbs *et al.*,

1987) and successfully used for others neogastropods (Minchin *et al.*, 1996; Gooding *et al.*, 1999) were used: the RPLI (relative penis length index) and RPSI (relative penis size index). The RPLI is an index that quantifies the degree of imposex in the population and is obtained from the equation: (Mean length of female penis) / (Mean length of male penis) X 100. This index is better applied in low contaminated areas (Fernandez *et al.*, 2002). The RPSI quantifies the degree of imposex in the population by the equation: (Mean length of female penis)³ / (Mean length of male penis)³ X 100. This cubical index is better applied in highly contaminated areas, when the length of the female penis approaches the length of the male penis.

RESULTS

The imposex was observed in the three analyzed species. The largest imposex index was observed in *Stramonita haemastoma* that presented 100% of females with morphologic alterations, RPSI 7.2 and RPLI 41.72. In this species, it was observed females with penis length average of 30 mm (Figure 02) (approximately 41% of the penis length of the males).

The imposex indexes observed in *Leucozonia nassa* were: 98,15% of imposexed females, RPSI 1,9 and RPLI 26,7. The average penis length of the imposexed females was 26,7% of the average length penis of the males. Most of the animals presented penis with a robust base and a long scourge in the edge. The vas deferens was visualized in most of the organisms. Only one female was observed with two penis (Figure 03).

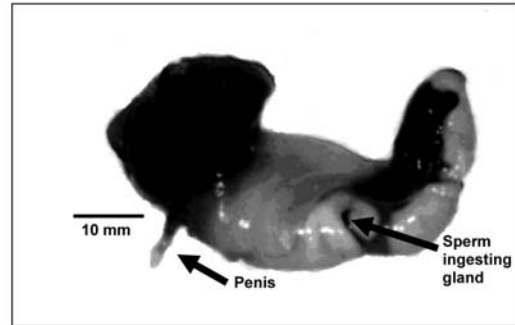


Figure 2:
Stramonita haemastoma female with imposex.

The cymatid *Cymatium parthenopeum* presented lower imposex indexes than *Stramonita haemastoma* and *Leucozonia nassa*, been observed 87,5% of imposexed females, RPSI 0,35 and RPLI 15,22. The average penis length of females was 15,22% of penis length of the males. It was also observed the formation of a groove-shaped vas deferens in males and imposexed females (Figure 04).

The figures 05, 06 and 07 show comparatively imposex indexes obtained for the three studied species.

DISCUSSION:

This study constituted the first report of imposex in the faciolarid *Leucozonia nassa*. Studies accomplished in Japan had already determined previously the phenomenon in species of the same family, as *Fusinus perplexus perplexus* (Horiguchi *et al.*, 1997).

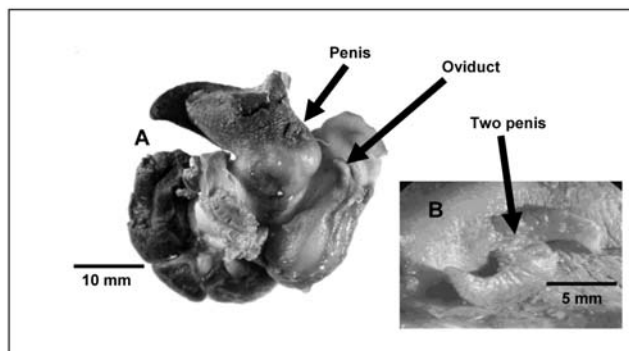


Figure 3:
Leucozonia nassa female with imposex (A) Detail of two penis in *L. nassa* (B).

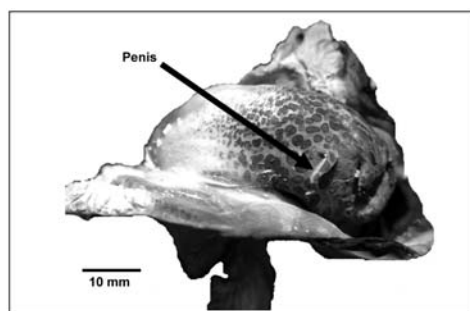


Figure 4:
Cymatium parthenoepum female with imposex.

Similarly, there was not a previously imposex report to the mesogastropod *Cymatium parthenoepum*, although other mesogastropods had already been mentioned as indicators of the contamination for organotin compounds (Horiguchi *et al.*, 1997 and Horiguchi *et al.*, 1995). Considering this aspect, as reported by Horiguchi *et al.* (1995), as mesogastropods are fewer sensitive to the organotin compounds than the neogastropods, the concentrations sufficiently high to induce imposex in neogastropods don't affect mesogastropods.

Although VDSI is an index usually used to quantify imposex in gastropods, it was not used in this study. This index needs to be standardized before the application in the species studied, once alternative routes to the imposex development are known (Stroben *et al.*, 1995).

Considering that the organotin exposition degree of the three species was the same, and once all of them were collected at the same place, it was possible to compare "*in situ*" the observed imposex levels for the each species. Different species can present several levels of organotin compounds sensibility. In this study, *Stramonita haemastoma* was, among the analyzed species, the most sensitive. In a similar way, Bech (1999) studied the sensibility difference among *Thais distinguenda*, *Thais bitubercularis* and *Morula musiva*. *Thais distinguenda* was respectively the most sensitive species followed by *Thais bitubercularis* and *Morula musiva*.

Among the 120 mollusc species known that are sensitive to organotin contamination, 38 species

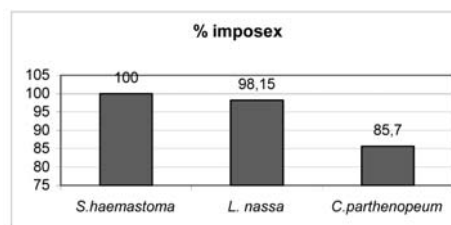


Figure 5:
% imposex in *S. haemastoma*, *L. nassa* e *C. parthenoepum*

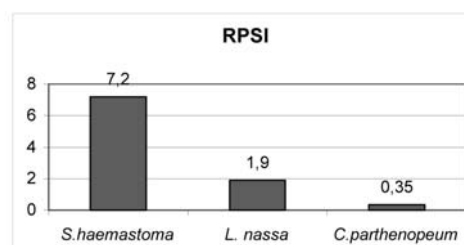


Figure 6:
RPSI in *S. haemastoma*, *L. nassa* e *C. parthenoepum*

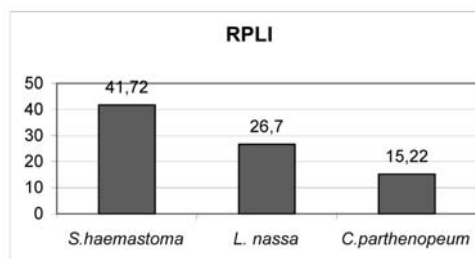


Figure 7:
RPLI in *S. haemastoma*, *L. nassa* e *C. parthenoepum*

belong to the family Muricidae (Horigushi *et al.*, 1997a and 1997b; Ellis & Pattisina, 1990; Stewart *et al.*, 1992; Evans, 1995; Stewart & Mora, 1992; Tester *et al.*, 1996) and 18 belong to the genus *Thais* Roding, 1798 (Tan, 1997; Liu, 1997; Swennen *et al.*, 1997; Evans, 1999; Castro *et al.*, 2000). This sensibility makes species from this family and this genus the most used organisms as bioindicators of organotin contamination in the world.

According to Stewart *et al.* (1992), the choice of the ideal bioindicator, besides its sensibility to the

pollutant, should be due to its abundance, limited mobility, intertidal occurrence and widespread distribution. *Stramonita haemastoma* from Japones Island tidal flat had all of the above-mentioned requirements.

It is dissuaded the use of *Cymatium parthenopeum* as organotin bioindicator due its low imposex indexes (that suggests the low sensitive to OTs) and also its low abundance at Ilha do Japonês tidal flat. Similarly, the use of *Leucozonia nassa* should also be avoided due to the presence of a flagellate penis that not allow accurate measures for RPSI and RPLI analisis. Similar difficulty was found by Tan (1999) that worked on *Thais gradata* in the Strait of Johor (Singapore). The use of *Leucozonia nassa* in environmental monitoring becomes still more difficult, once the occurrence of a individual with two penis can indicate the existence of different routes of imposex development.

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