



THE INCREASING INCIDENCE OF IMPOSEX IN STRAMONITA HAEMASTOMA (MOLLUSCA: GASTROPODA: MURICIDAE) AFTER THE ESTABLISHMENT OF THE PECÉM HARBOR, CEARÁ STATE, NORTHEAST BRAZIL

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ABSTRACT

Imposex is characterized by the development of masculine sexual organs in neogastropod females, caused by organotin contamination of the seawater. These compounds, as tributyltin (TBT), are used world-wide as biocidal agents in antifouling paints, commonly used to covering boats and off shore metallic structures. Five sampling sites were determined along the coast of Ceará State, Northeast Brazil in March 2001. In each site were collected 50 adults of neogastropod *Stramonita haemastoma* and indexes that quantify imposex (% imposex, RPLI, RPSI and VDSI) were observed. Other two collecting times were conducted (April 2003 and March 2004) at the same sites and imposex indexes were observed again. The indexes observed in the three collecting times showed an increase in the incidence of imposex at the sampling sites, probably caused by the establishment of Pecém Harbor and its increasing shipping activities

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INTRODUCTION

Imposex is characterized by the development of masculine sexual organs, like penis and vas deferens, in neogastropod females (Smith, 1971). This abnormality is a consequence of an endocrine disruption induced by marine environment contamination by tin organic compounds, as tributyltin (TBT) and triphenyltin (TPT), (Morcillo & Porte, 1999; Depledge & Billinghurst, 1999).

These compounds are used world-wide as biocidal agents in antifouling paints, commonly used to covering boats and off shore metallic structures. The use of TBT and/or TPT-based antifouling paints started during the 60's, when it was known that these compounds had biocidal action and we can find the first report of harmful effects of these paints during the 70's (Alzieu et al., 1982; Ruiz et al., 1996). Nowadays, more than 70% of world's fleet (Champ, 2000) is still using organotin copolymers, releasing approximately 4ug/cm² of tin compounds per day to the seawater (Horiguchi et al., 1994).

Morphological changes induced by imposex are very visible, so, they became an important way to detect marine ecosystems contamination by organotin compounds. This kind of detection was done in England, where the study of imposex in *Nucella lapillus* allowed a large monitoring of TBT

contamination (Gibbs & Bryan, 1987). Monitoring works that used neogastropods to detect this kind of contamination were made in the coast area of many countries: Portugal (Spence, 1990), Singapore (Ellis & Pattisina, 1990), New Zealand (Stewart et al., 1992;

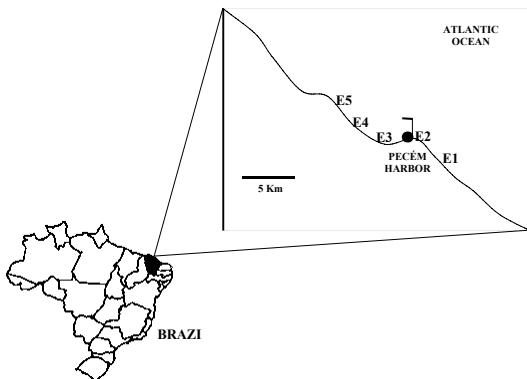


Fig. 1.
Sampling sites in the coast of Ceará State, Northeast Brazil.

Smith, 1996), Japan (Horiguchi et al., 1995; Horiguchi et al., 1997), Canada (Tester et al., 1996), Ireland (Minchin et al., 1997), Spain (Morcillo & Porte, 1998), Thailand (Bech, 1998) and Hong Kong (Blackmore, 2000). All these works showed clearly the influence of harbor areas on the results of indexes that quantify imposex.

The first report of imposex in the Atlantic coast of South America was in Brazil, where this abnormality was observed in the neogastropod *Stramonita haemastoma* in the coast of São Paulo City (Magalhães et al., 1997), Fortaleza City (Castro et al., 2000) and Rio de Janeiro City (Fernandez et al., 2002). Further monitoring works were made in Maceió City (Camillo et al., 2004) and Natal City (Castro et al., 2004), where it was studied imposex in *Stramonita rustica*.

To improve industry products transportation in Ceará State, the state government started to construct, in 1996, the Pecém Harbor. The location, before harbor establishment, was not developed and there was not ship activity. The purpose of the present paper is to verify the organotin contamination of the area near Pecém Harbour, using imposex in *Stramonita haemastoma* as bioindicator.

MATERIALS AND METHODS

It was determined 5 points as sampling sites along the coast of Ceará State, Northeast Brazil. The predominant maritime currents of the region as well as the proximity in relation to the Pecém Harbor were considered to the determination of these points: Cauípe's Beach (E1), Pecém Harbor (E2), Pecém's Beach (E3) and Taíba's Beach (E4 e E5) (Fig. 1).

Three collecting times were conducted: March 2001, April 2003 and March 2004. In each site were collected, manually, 50 adults specimen of neogastropod *Stramonita haemastoma*. The animals were kept plastic boxes containing water from the respective site and they were led to the laboratory. The mollusks were preliminary narcotized with magnesium chloride solution 3.5% (Huet et al., 1995). Subsequently length of each shell was measured from the apex to the distal end of the siphonal canal using calipers and they were removed to reach the soft parts.

The sexual determination was made based on the presence of the sperm-ingesting gland, observed only in females, the presence of prostate, exclusive to males, and the coloration of the gonads. All males as well as the females that presented imposex had their penises measured with a millimetric slide and the formation of the vas deferens in the females was also observed.

For the quantification of imposex, three index initially developed for the neogastropod *Nucella lapillus* (Gibbs and 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), the RPSI (relative penis size index) and the VDSI (vas deferens sequence index), scale that was originally developed by Gibbs et al. (1987) and later adapted for the use in *Stramonita haemastoma* in the Brazilian coast (Fernandez et al., 2002; Fernandez et al., 2005).

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. 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

Table 1.

Imposex indexes observed in the three collecting times (March 2001, April 2003 and March 2004).

| Collecting Time | Sample size | n female | % imposex | VDSI | RPLI | RPSI |
|-----------------|-------------|----------|-----------|------|-------|------|
| Site E1 | | | | | | |
| 03/2001 | 50 | 30 | 68,42 | 0,20 | 0,10 | 0,01 |
| 04/2003 | 50 | 26 | 69,2 | 0,85 | 1,33 | 0,01 |
| 03/2004 | 50 | 29 | 100,0 | 1,70 | 1,40 | 0,24 |
| Site E2 | | | | | | |
| 03/2001 | 50 | 24 | 66,6 | 0,50 | 1,20 | 0,01 |
| 04/2003 | 50 | 24 | 100,0 | 1,30 | 4,90 | 0,01 |
| 03/2004 | 50 | 27 | 100,0 | 2,00 | 28,37 | 2,33 |
| Site E3 | | | | | | |
| 03/2001 | 50 | 32 | 81,81 | 0,63 | 3,76 | 0,01 |
| 04/2003 | 50 | 30 | 100 | 1,45 | 11,27 | 0,14 |
| 03/2004 | 50 | 34 | 100 | 1,86 | 14,05 | 0,27 |
| Site E4 | | | | | | |
| 03/2001 | 50 | 28 | 7,4 | 0,01 | 0,53 | 0,01 |
| 04/2003 | 50 | 30 | 13,0 | 0,13 | 2,55 | 0,01 |
| 03/2004 | 50 | 30 | 20,0 | 0,25 | 2,63 | 0,01 |
| Site E5 | | | | | | |
| 03/2001 | 50 | 26 | 0,0 | 0,00 | 0,00 | 0,00 |
| 04/2003 | 50 | 24 | 6,6 | 0,08 | 1,41 | 0,01 |
| 03/2004 | 50 | 29 | 0,0 | 0,00 | 0,00 | 0,00 |

male penis.

The VDSI quantifies the degree of imposex in the animal through the sequence of the penis and vas deferens formation. This scale is composed of 7 levels: stage 0, a healthy female without a sign of penis or vas deferens; stage I, the beginning of the penis formation, characterized by a small wart; stage II, the penis appears formed with approximately 1mm in length and is observed the beginning of the vas deferens formation; stage III, the penis is already developed, with size superior to 1mm and it is also observed an incomplete vas deferens in the bottom of the palial cavity; stage IV, the penis is developed, with size superior to 1mm and a complete vas deferens can be observed; stage V, characterized by the blockage of the vulva and stage VI where the presence of aborted capsules in the capsule gland is observed.

RESULTS

All sites presented imposex in the conducted samplings (except site E5, in 2001 and 2004). The most elevated indexes were observed in March 2004, at site E2, where it was found VDSI = 2.00, RPLI = 28.37 and RPSI = 2.33. The index variations were higher at Pecém Harbor (E2) and Pecém (E3), where they increased along the collecting time. It was observed at these sites that all animals presented imposex (Table 1).

In all samples, the % imposex, RPLI, RPSI and

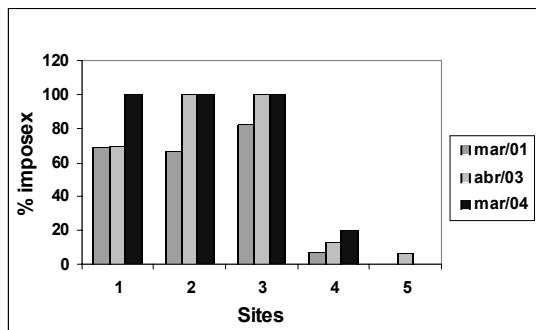


Fig. 2.
% imposex observed in *S. haemastoma* from 2001 to 2004.

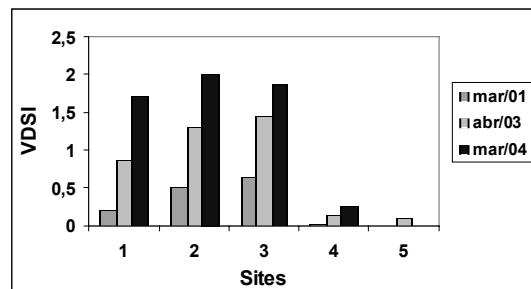


Fig. 3.
VDSI (vas deferent sequence index) values observed in *S. haemastoma* from 2001 to 2004.

VDSI showed a gradual decrease when the distance of the sites from the harbor was increased (Table 1).

RPSI oscillations were low (almost all of sites oscillated between 0.00 and 0.27, except E2), however, % imposex, RPLI and VDSI showed important variations, as we can see in figures 2, 3, 4 and 5. While this work was being conducted, the shipping activities in Pecém Harbor increased, been detected almost 290 ships and boats in 2003 (Fig. 6).

DISCUSSION

The results showed that the increasing shipping activities in Pecém Harbor induced imposex in *Stramonita haemastoma*, caused by the increasing using of antifouling organotin-based paints. This situation was observed in other *Stramonita* species in Japan (Horigushi et al., 1997), Singapore (Tan, 1997) and Thailand (Bech, 1999).

Pecém Harbor construction (in an area that had

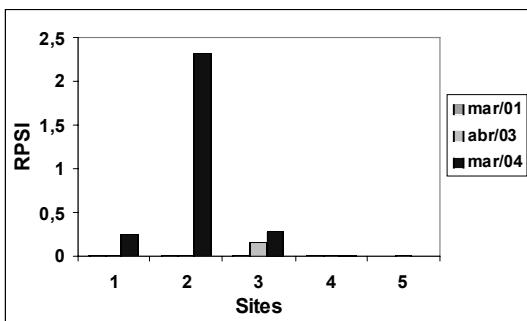


Fig. 4.

RPSI (relative penis size index) values observed in *S. haemastoma* from 2001 to 2004.

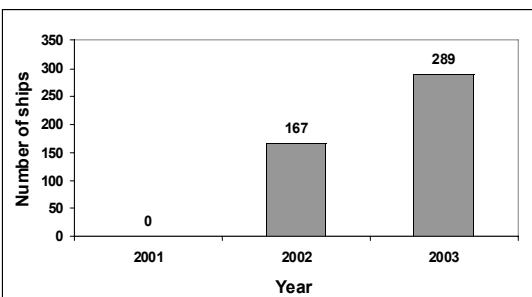


Fig. 6.

Number of ships that moored in Pecém Harbor from 2001 to 2003.

indexes at sites located far from harbor mooring area. This situation also occurred in Phuket Island, Thailand, i. e., indexes decreased when the distance of sampling sites from a marina was increased (Bech, 2002).

The graphs (Figs. 2, 3, 4 and 5) showed increasing marked oscillations on % imposex, RPLI e VDSI at sampling sites in four years, probably caused by the increasing activities of Pecém Harbor, however, RPSI oscillations were not so marked if they were compared with others indexes. These oscillations could be due to the RPSI characteristics, i. e., it shows better results when the highest imposex degrees are detected, when size of female penises has almost the same length of male penises (Fernandez et al., 2002).

The Pecém Harbor opened for business in September 26, 2003, so, the last collecting time of this work was 7 months after the establishment of the harbor. Since that, it's still working with low shipping activities. To try to avoid any possibility of pollution impacts at the marine coast ecosystem, the harbor was constructed as an off shore structure (Deusdadara, 1996). These facts probably justify the low imposex degree observed in the study area. However, indexes showed a marked increase in the occurrence and incidence of imposex. Probably, shipping activities at Pecém Harbor will increase, consequently, imposex indexes and degree in *S. haemastoma* and other neogastropods will too. This fact was observed at large harbor areas, for example, in Japan, where there are regulations prohibiting the use of TBT-based antifouling (Champ, 2000).

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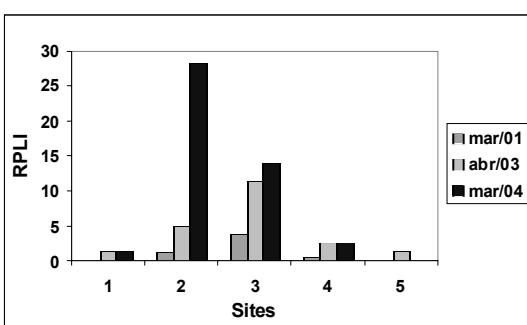


Fig. 5.

RPLI relative penis length index values observed in *S. haemastoma* from 2001 to 2004.

insignificant shipping activity) was probably responsible for the occurrence and the increasing incidence of imposex in harbor and nearest areas. A similar work made in Thailand showed that after a marina construction, the number of yachts increased and it was linked to the increasing incidence of imposex in *Chicoreus capucinus* and *Thais distingua* (Bech, 2002).

In March 2001, when Pecém Harbor was still being constructed, the lower imposex indexes and degree observed at sites could be due to the presence of some boats that were working on harbor construction. In 2002 and 2003, 167 and 289 (Fig. 6) ships moored in Pecém Harbor, respectively. The consequence of this increasing number of shipping activity can be observed in the increasing imposex indexes, and probably, in seawater organotin concentration.

The predominant maritime current direction of the study area is from east to west (Maia, 1998), so, we observed there was a gradual decrease of imposex

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