

THAIS (STRAMONITA) RUSTICA (LAMARCK, 1822)
(MOLLUSCA: GASTROPODA:THAIDIDAE), A POTENTIAL
BIOINDICATOR OF CONTAMINATION BY ORGANOTIN NORTHEAST BRAZIL

Ítalo Braga de Castro^{1,2}, Carlos A. O. Meirelles¹, Helena Matthews-Cascon^{1,2}
& Marcos Antonio Fernandez³

¹Universidade Federal do Ceará, Instituto de Ciências do Mar (LABOMAR)
Laboratório de Zoobentos
(Av. da Abolição, 3207 – Bairro Meireles, 60165-081 – Fortaleza, CE, Brasil)
italo_braga@hotmail.com

²Universidade Federal do Ceará, Laboratório de Invertebrados Marinhos
Departamento de Biologia
(Bloco 909, Campus do Pici, 60455-970, Fortaleza, CE, Brasil)

³Universidade Estadual do Rio de Janeiro
Departamento de Oceanografia Química
(Rua São Francisco Xavier, 524, sala 4018E, Maracanã 20550-013, Rio de Janeiro, RJ, Brasil)

ABSTRACT

The use of antifouling paints containing the biocide compound tributyltin (TBT) has been shown as an inductor of imposex in neogastropods mollusks. Imposex is characterized by the development of male features in females, mainly the appearance of a non functional vas deferens and penis. Samples of *Thais rustica* were collected in eight sites in the metropolitan area of Natal city, Rio Grande do Norte state, northeast Brazil, and examined for occurrence of imposex, which was found in many females. The higher imposex levels were presented by samples from sites near city harbor.

RESUMO

A utilização de tintas antiincrustantes contendo o composto biocida tributilestanho (TBT) tem induzido moluscos neogastrópodes ao imposex. O imposex é caracterizado pelo surgimento de caracteres sexuais masculinos, sobretudo, pênis e vaso deferente não funcionais em fêmeas desses moluscos. Foram coletadas amostras de *Thais rústica* em oito estações ao longo da costa da cidade de Natal no Rio Grande do Norte. Esses animais foram analisados quanto a presença e o grau de imposex que apresentavam. Os níveis mais elevados de imposex foram observados na estação mais próxima ao porto.

Descriptors: Imposex, TBT, *Thais rustica*.

Descritores: Imposex, TBT, *Thais rustica*.

INTRODUCTION

Antifouling paints are used world-wide as covering of boats and other metallic structures in intention to prevent incrustations and corrosion which are processes that drastically reduce the income of boats and the durability of these structures.

To solve these problems, during the decades of 60 and 70, highly efficient paints were developed using tin organic compounds as tributyltin (TBT) and triphenyltin (TPT) as biocide agent. Since the 70's, it was known that these compounds were harmful to the marine environment, been detected an increase in the mortality rate and serious deformities in shells of

Crassostrea gigas cultivated, in the Arcachon bay in France (Alzieu *et al.*, 1982; Alzieu *et al.*, 1986; Alzieu *et al.*, 1989).

Imposex was verified in gastropods (Smith, 1971) and is characterized by the development of non functional masculine sexual organs, like penis and *vas deferens*, in females. This phenomenon is mainly caused by organotin contamination of the seawater and/or food cases. The problem was initially observed in *Nucella lapilus* from the southwest coast of England, where some populations of this prosobranch mollusk had severely declined (Bryan *et al.*, 1987b).

Many countries around the world had already evidenced imposex in gastropods. As a result some have implemented severe legislations that

banned the use of TBT based paints in boats smaller than 25 meters long (Minchin *et al.*, 1995).

In Japan, high levels of TBT contamination were verified in *Thais clavigera* (Horiguchi *et al.*, 1994 and Horiguchi *et al.*, 1998), *Thais bronni* (Horiguchi *et al.*, 1994) and in mesogastropods (Horiguchi *et al.*, 1995). Bioassays were made with some species of neogastropods to verify their sensitivity to TBT (Horiguchi *et al.*, 1997b).

Imposex in *Thais rustica* was reported for the first time in the coast of the state of Alagoas, where monitoring works were made using this species as a possible indicator of organotin contamination (Quadros, personal communication)*.

The purpose of the present paper is to provide a preliminary biomonitoring of imposex in *Thais rustica* from Natal City coast, Northeast Brazil.

MATERIALS AND METHODS

It was determined 8 points as sampling sites along the coast of the city of Natal-RN, Brazil. The predominant maritime currents of the region as well as the proximity in relation to the Natal Harbor, situated in the estuary of the Potengi River were considered to the determination of these points (Fig.1).

Field Procedures

In each site were collected, manually, 25 adults of *Thais rustica*, except in the pier of Redinha's Beach where, despite the effort, only 8 individuals were collected. These animals were kept plastic boxes containing water from the respective sites and they were led to the laboratory.

Laboratory Procedures

The mollusks 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 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. The percentage of females presenting imposex was calculated.

For the quantification of imposex, three index 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), 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 *Thais haemastoma* in the Brazilian coast (Fernandez *et al.*, 2002).

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 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

Four of the eight sampling sites presented animals with imposex. The most elevated indexes observed were in the area of the port of Natal (site 6) where it was found RPLI = 42.1, RPSI = 7.7 and the animals were all in stage IV of VDSI. The indexes in the estuary of the Potengi River (site 5) were: RPLI = 27.3 RPSI = 2.03 and VDSI oscillating between I and III. In the Forte's beach (site 4) observed RPLI corresponded to 7.2, RPSI to 0.03 and VDSI oscillated between 0 and III. The site that presented the lowest indexes was the beach of Redinha (site 7) where they were RPLI < 0.1, RPSI < 0.01 and VDSI = I. The others sites (1,2,3 and 8) didn't present imposex (Table 1).

(*) Quadros, J. 2002. Universidade Estadual do Rio de Janeiro.

DISCUSSION

The occurrence of imposex is a consequence of contamination by anti-fouling paints and, in particular, by their main biotoxic ingredient, the tributyltin that acts on the endocrine control of the reproductive system of some gastropods (Gibbs *et al.*, 1997; Mathiessen & Gibbs, 1998). For this reason, the occurrence of imposex in Gastropoda has been used to monitor the contamination by tributyltin in several countries, as England (Bryan *et al.*, 1987a, b, c), Japan (Horigushi *et al.*, 1994, 1995, 1998; Tan, 1997) and Thailand (Evans *et al.*, 1995; Bech, 1998, 1999).

The occurrence of imposex in *Thais rustica* populations was observed in this study in the area around Natal, Northeast Brazil. Nevertheless, the indices were very low even in the sites close to the city harbor. The same fact was observed for *Thais rustica* populations from Maceió city (Quadros, personal

communication)*. The low indices of imposex in these populations could be due to low levels of contamination in these areas or to a low sensibility for the organotin compounds by this species.

Among the over 120 mollusc species where imposex was registered, 38 belong to the Thaididae family (Ellis & Pattisina, 1990; Stewart *et al.*, 1992; Stewart & Mora, 1992; Evans *et al.*, 1995; Tester *et al.*, 1996; Horigushi *et al.*, 1997a, b) and 18 of them to the genus *Thais* Roding, 1798 (Tan, 1997; Swennen *et al.*, 1997; Evans, 1999; Castro *et al.*, 2000). This fact suggests that Thaididae species are good indicators of contamination by organotin, and the use of *Thais rustica* as a organotin indicator in Northeast Brazil should be object for further studies.

(*) Quadros, J. 2002. Universidade Estadual do Rio de Janeiro.

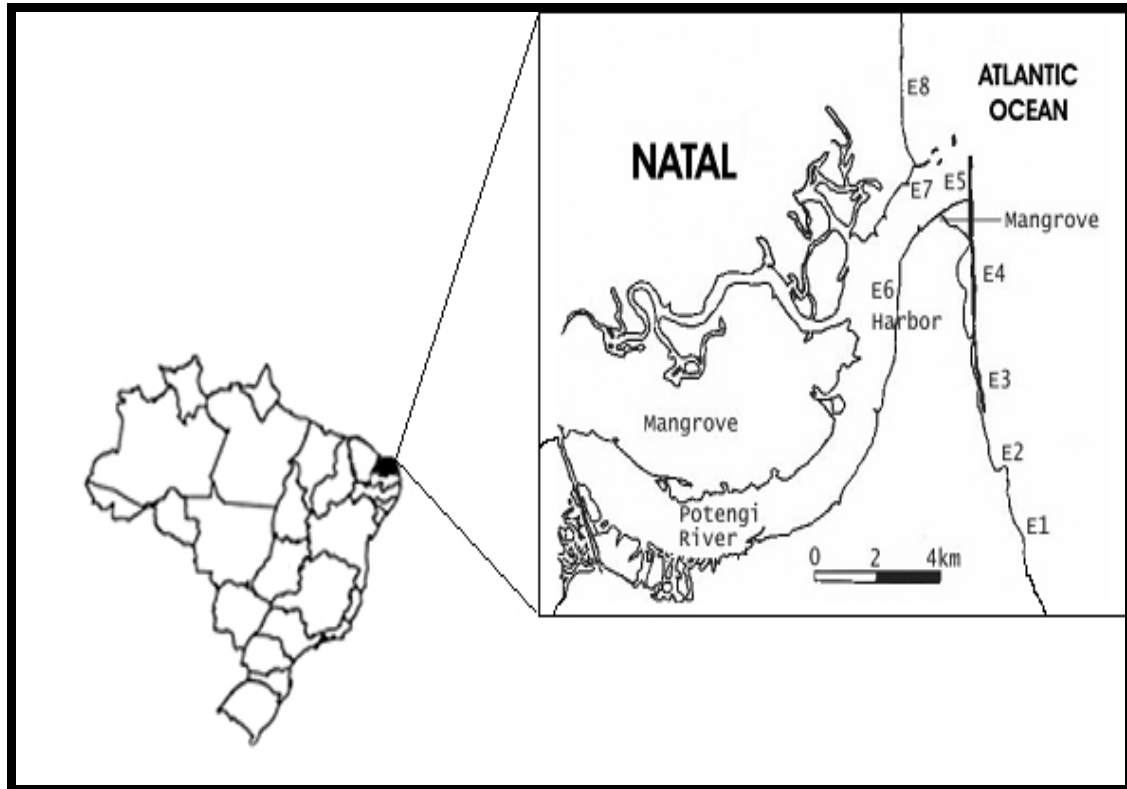


Fig. 1. Sampling sites in the coast of Natal – RN, Brazil.

Table 1. Results of the monitoring of Natal - RN

Sites	Period	Location	n(Males/ Females)	% imposex	RPLI	RPSI	VDSI
(E1)	July 2002	Barreira D'Água	7/18	0	0	0	0
(E2)	July 2002	Artista's Beach	9/16	0	0	0	0
(E3)	July 2002	Meio's Beach	13/12	0	0	0	0
(E4)	July 2002	Forte's Beach	8/17	66.7	7.2	0.03	0-I - II - III
(E5)	July 2002	Estuary of the Potengi River	10/15	100	27.3	2.03	I-II-III
(E6)	July 2002	Porto – Balsa	13/12	100	42.1	7.70	IV
(E7)	July 2002	Pier of Redinha	5/3	100	< 0.1	< 0.01	I
(E8)	July 2002	Santa Rita's Beach	8/17	0	0	0	0

REFERENCES

- Alzieu, C.; Heral, M.; Thibaud, Y.; Dardignac, M. & Feuillet, M. 1982. Influence des peintures antisalissures a base d'organostanniques sur la calcification de la coquille de l'huitre *Crassostrea gigas*. *Revue Trav. Inst. Pêches marit.*, 45(2):101-116.
- Alzieu, C.; Sanjuan, J.; Delteil, J. P. & Borel, M. 1986. Tin contamination in Arcachon Bay: effects on oyster shell anomalies. *Mar. Pollut. Bull.*, 17(11): 494-498.
- Alzieu, C.; Sanjuan, J.; Michel, P.; Borel, M.; Dreno, J. P. 1989. Monitoring and assesment of butyltins in Atlantic Coastal Waters. *Mar. Pollut. Bull.*, 17(11): 494-498.
- Bech, M. 1998. Imposex and population characteristics of *Thais distinguenda* as an indicator of organotin contamination along the south east coast of Phuket Island, Thailand. *Phuket Marine Biological Center Special Publication*, 18(1):129-138.
- Bech, M. 1999. Sensitivity of different gastropod to tributyltin contamination. *Phuket Marine Biological Center Special Publication*, 19(1):1-6.
- Bryan, G. M.; Gibbs, P. E.; Burt, G. R. & Humerstone, L. G. 1987a. The affects of tributyltin (TBT) accumulation on adult dog-whelks, *Nucella lapillus*: Long-term field and laboratory experiments. *J. mar. biol. Ass. U.K.*, 67:525-544.
- Bryan, G. M.; Gibbs, P. E.; Humerstone, L. G. & Burt, G. R. 1987b. The decline of the gastropod *Nucella lapillus* around Southwest England: Evidence for tributyltin from antifouling paints. *J. mar. biol. Ass. U.K.*, 66:611-640.
- Bryan, G. M.; Gibbs, P. E.; Pascoe, P. L. & Burt, G. R. 1987c. The use of the dog-whelk *Nucella lapillus*, as na indicator of tributyltin(TBT) contaminacion. *J. Mar. biol. Ass. U.K.*, 67:507-523.
- Castro, I. B.; Cascon, H. M. & Fernandez, M. A. 2000. Imposex em *Thais haemastoma* (Linnaeus, 1767) (Mollusca:Gastropoda), uma indicação da contaminação por organoestânicos na costa do Município de Fortaleza - Ceará - Brasil. *Arg. Ciênc. Mar., Bras.*, 33:51-56.
- Ellis, D. V. & Pattisina, L. A. 1990. Widespread neogastropod imposex: a biological indicator of global TBT contamination? *Mar. Pollut. Bull.*, 24(5):248-253.
- Evans, S. M.; Dawson, M.; Day, J.; Frid, C. L. J.; Gill, M. C.; Pattisina, L. A. & Porter, J. 1995. Domestic waste and TBT pollution in coastal areas of Ambom Island. *Mar. Pollut. Bull.*, 30(2):109-115.
- Evans, S. M.; Evans, P. M. & Leksono, T. 1999. Widespread recovery of dogwelks, *Nucella lapillus* (L.), from tributyltin contamination in the North Sea and Clyde Sea. *Mar. Pollut. Bull.*, 32(3):263-269.
- Fernandez, M. A.; Limaverde, A. M.; Castro, I. B.; Almeida, A. C. M. & Wargener, A. L. M. 2002. Occurrence of imposex in the *Thais haemastoma*: possible evidence of environmental contamination derived from organotin compounds in Rio de Janeiro and Fortaleza, Brazil. *Publ. Hlth. Rept.*, 18(2):463-476.
- Gibbs, P. E. & Bryan, G. W. 1987. TBT paints and demise of the dog-whelk *Nucella lapillus* (Gastropoda). *J. mar. biol. Ass. U.K.*, 68:1482-1487.
- Gibbs, P. E.; Bryan, G. W.; Pascoe, P. L. & Burt, G. R. 1987. The use of dog-whelk *Nucella lapillus*, as na indicator of tributyltin (TBT) contamination. *J. mar. biol. Ass. U.K.*, 67:507-523.
- Gibbs, P.; Bebianno, M. J. & Coelho, M. R. 1997. Evidence of the differential sensitivity of neogastropods to tributyltin (TBT) pollution with notes on a species (*Columbella rustica*) lacking the imposex response. *Environ. Pollut.*, 18:1219-1224.
- Gooding, M.; Gallardo, C. & Leblanc, G. 1999. Imposex in three marine gastropod species in Chile and potential impact on muriciculture. *Mar. Pollut. Bull.*, 38(12):1227-1231.
- Horiguchi, T.; Shiraishi, H.; Shimizu, M. & Morita, M. 1994. Imposex and organotin compounds in *Thais clavigera* and *T. bronni* in Japan. *J. mar. biol. Ass. U.K.*, 74:651-669.
- Horiguchi, T.; Shiraishi, H.; Shimizu, M.; Yamazaki, S. & Morita, M. 1995. Imposex in japanese gastropods (Neogastropoda and Mesogastropoda): effects of tributyltin and triphenyltin from antifouling paints. *Mar. Pollut. Bull.*, 4(12):402-405.
- Horiguchi, T.; Shiraishi, H.; Shimizu, M. & Morita, M. 1997a. Effects of triphenyltin chloride and five other organotin compounds on the development of imposex in the rock shell, *Thais clavigera*. *Environ. Pollut.*, 95(1):85-91.

- Horiguchi, T.; Shiraishi, H.; Shimizu, M. & Morita, M. 1997b. Imposex in sea snails, caused by organotin (tributyltin and triphenyltin) pollution in Japan: a survey. *Appl Org. Chem.*, 11:451–455.
- Horiguchi, T.; Hyeon-Seo, C.; Shiraishi, H.; Shibata, Y.; Soma, M.; Morita, M. & Shimizu, M. 1998. Field studies on imposex and organotin accumulation in the rock shell, *Thais clavigera*, from the Seto Inland Sea and the Sanriku region, Japan. *Sci. total Environment*, 214:65–70.
- Huet, M.; Fiorini, P.; Oehlmann, J. & Stroben, E. 1995. Comparison of imposex response in three prosobanch species. *Hydrobiologia*, 309:29–35.
- Matthiessen, P. & Gibbs, P. E. 1998. Critical appraisal of the evidence for tributyltin – mediated endocrine disruption in mollusks. *Environ. Toxicol. Chem.*, 17(1):37–43.
- Minchin, D.; Oehlmann, J.; Duggan, C. B. & Stroben, E. 1995. Marine TBT antifouling contamination in Ireland, following legislation in 1987. *Mar. Pollut. Bull.*, 30(10):633–639.
- Minchin, D.; Stroben, E.; Oehlmann, J.; Bauer, B.; Duggan, C. & Keatinge, M. 1996. Biological indicators used to map organotin contamination in Cork Harbour, Ireland. *Mar. Pollut. Bull.*, 32(2):188–195.
- Smith, B. S. 1971. Sexuality in the American mud-snail *Nassarius obsoletus* Say. *Proc. malac. Soc. Lond.*, 39:377–378.
- Stewart, C. & Mora, S. J. 1992. Elevated tri(n-butyl)tin concentrations in shellfish and sediments from Suva Harbour, Fiji. *Appl. Organ. Chem.*, 6: 507-512.
- Stewart, C.; Mora, S. J.; Jones, M. R. L. & Miller M. C. 1992. Imposex in New Zealand neogastropods. *Mar. Pollut. Bull.*, 24(4):204–209.
- Swennen, C.; Ruttanadukul, N.; Ardseungnern, S.; Singh, H. R.; Mesinck, B. P. & Hallers-Tiabbes, C. C. 1997. Imposex in sublittoral and littoral gastropods from the Gulf of Thailand and Strait of Malacca in relation to shipping. *Environ. Technol.*, 18:1245-1254.
- Tan, K. S. 1997. Imposex in three Species of *Thais* from Singapore, with additional observations on *T. clavigera* (Kuster) from Japan. *Mar. Pollut. Bull.*, 34(7):577-581.
- Tester, M.; Ellis, D. V. & Thompson, J. A. J. 1996. Neogastropods imposex for monitoring recovery from marine TBT contamination. *Environ. Toxicol. Chem.*, 15(4):560–567.

(Manuscript received 21 May 2003; revised 03 February 2004; accepted 15 May 2004)