Marine Biology Research

ISSN: 1745-1000 (Print) 1745-1019 (Online) Journal homepage: https://www.tandfonline.com/loi/smar20

Transplant bioassay induces different imposex responses in two species of the genus *Stramonita*

Ítalo Braga Castro , Cristina De Almeida Rocha-Barreira , Marcos Antônio Fernandez & Gregório Bigatti

To cite this article: Ítalo Braga Castro , Cristina De Almeida Rocha-Barreira , Marcos Antônio Fernandez & Gregório Bigatti (2012) Transplant bioassay induces different imposex responses in two species of the genus *Stramonita* , Marine Biology Research, 8:4, 397-404, DOI: 10.1080/17451000.2011.627923

To link to this article: https://doi.org/10.1080/17451000.2011.627923

Published online: 15 Feb 2012.

Marine Biology Research, 2012; 8: 397–404

ORIGINAL ARTICLE

Transplant bioassay induces different imposex responses in two species of the genus *Stramonita*

ÍTALO BRAGA CASTRO[1]*, CRISTINA DE ALMEIDA ROCHA-BARREIRA[2], MARCOS ANTÔNIO FERNANDEZ[3] & GREGÓRIO BIGATTI[4]

[1]*Instituto de Oceanografia – Universidade Federal do Rio Grande (IO – FURG), Rio Grande, RS, Brasil,* [2]*Instituto de Ciências do Mar (LABOMAR – UFC), Fortaleza-Ceará-Brazil,* [3]*Universidade Estadual do Rio de Janeiro (UERJ), Programa de Ecotoxicologia Marinha, Departamento de Oceanografia, Rio de Janeiro, and* [4]*LARBIM, Centro Nacional Patagonico (CENPAT), CONICET, Argentina*

Abstract

A bioassay to study differential specific responses in imposex development due to marine organotin pollution was done by transplanting specimens of the gastropods *Stramonita haemastoma* and *Stramonita rustica* from an imposex-free area to a marina with high marine traffic inside a ship repair yard, a place where local populations of *S. haemastoma* were known to show high indices of imposex. Three hundred sexually mature, imposex-free specimens of each species were kept in cages for 120 days, and samples of 30 individuals were periodically analysed for imposex development. Shell length, penis length and vas deferens development were recorded and imposex development indices (% imposex, RPLI and VDSI) were calculated. Our results indicated that imposex induction in *S. haemastoma* is faster and more sensitive than in *S. rustica*. Imposex incidence in *S. haemastoma* could be a useful tool for monitoring marine pollution by organotin compounds in harbours along the Brazilian coast.

Key words: *Imposex, sensitivity,* Stramonita haemastoma, Stramonita rustica, *organotin, bioassay*

Introduction

Imposex (Smith 1971) is probably the most studied biological effect of organotin (OT) pollution. This endocrine disruption causes masculinization in female prosobranch gastropods, with affected females developing a penis and/or a vas deferens, which in some species led to sterilization and even death (Bryan et al. 1986; Gibbs & Bryan 1986).

Nowadays, imposex is a widespread problem, affecting more than 200 gastropod species (Shi et al. 2005; Sternberg et al. 2010). The occurrence and intensity of imposex is widely accepted as proportional to environmental levels of OT compounds, with a clear cause and effect relationship demonstrated (Matthiessen & Gibbs 1998; Horiguchi 2009; Sternberg et al. 2010). Hence, imposex has been widely used as an effective biomarker of OT pollution (Garaventa et al. 2006; Gravel et al. 2006; Vasconcelos et al. 2006; Galante-Oliveira et al. 2010; Mohamat-Yusuff et al. 2010). However, sensitivity may be different even among species from the same genus (Stroben 1996; Tan 1999), and a calibration of the intensity of imposex development is desirable for monitoring studies, making results comparable when more than one species is used (Gibbs et al. 1997).

Matthews (1968) reported the occurrence of six species belonging to the genus *Stramonita* on rocky shores along the Brazilian coast, of which *Stramonita haemastoma* (Linnaeus, 1767) and *Stramonita rustica* (Lamarck, 1822) have the largest spatial distribution. Moreover, *S. haemastoma* is the most abundant muricid species in Brazilian coastal environments, inhabiting mainly hard substrates in intertidal zones and eventually subtidal areas. Furthermore, this species is a key predator in rocky beach environments,

*Correspondence: Ítalo Braga Castro, Instituto de Oceanografia – Universidade Federal do Rio Grande (IO – FURG), Av. Itália s/n, Campus Carreiros, C.P. 474, CEP 96201-900 Rio Grande, RS, Brasil. E-mail: italobraga@gmail.com

Published in collaboration with the University of Bergen and the Institute of Marine Research, Norway, and the Marine Biological Laboratory, University of Copenhagen, Denmark

(Accepted 31 August 2011; Published online 14 February 2012; Printed 29 February 2012)

ISSN 1745-1000 print/ISSN 1745-1019 online
http://dx.doi.org/10.1080/17451000.2011.627923

feeding on barnacles, oysters and mussels (Gunter 1979). Additionally, some studies have reported that *S. haemastoma* is caught for human consumption by people along the Brazilian coast (Cavalcante-Braga et al. 2006). Both species showed imposex development and are good potential indicators of OT pollution in previous studies in Brazil (Fernandez et al. 2002; Castro et al. 2004), particularly since *S. haemastoma* is very common in tropical and subtropical areas, with populations spreading from southeastern Brazil to Florida, the Azores, eastern Atlantic and the Mediterranean (Spence et al. 1990; Rilov et al. 2000).

Imposex is widespread along the Brazilian coast (Castro et al. 2011b), indicating that antifouling pollution is probably continuing in many important coastal areas. Organotins have also been detected in sediments (Fernandez et al. 2005a; Santos et al. 2009; Oliveira et al. 2010) and animal tissues (Limaverde et al. 2007), mainly close to zones with high marine traffic zones such as the ports of Mucuripe, Rio de Janeiro, Paranaguá and Rio Grande (Castro et al. 2007a, 2007b). High imposex incidence have been observed in areas with high concentrations of OTs in sediments (Fernandez et al. 2005a), and a preliminary evaluation of possible human health effects of OTs ingestion through seafood was made in Brazil (Fernandez et al. 2005b). In Brazil, Resolution 357 of the Brazilian National Council of Environment (CONAMA 2005) recommends the maximum allowed concentrations of tributyltin (TBT) as 10 and 370 ng l^{-1}, according to the classes of saline waters (classes 1 and 2, respectively). In November 2007, the use of organotin-based antifouling paints was definitively banned in Brazil (NORMAM-23/DPC). However, the inspection and registration standards of all antifouling systems, as well as management of residues of these compounds, are not well controlled in Brazil. On the other hand, few OT monitoring studies were accomplished and the effectiveness of the restriction is poorly understood (Castro et al. 2011b). Even after the ban, some instances organotin pollution have been shown to continue (Toste et al. 2011).

The use of transplanted gastropods is useful for biomonitoring TBT pollution, as demonstrated with several species such as *Nucella lapillus* (Quintela et al. 2000; Smith et al. 2006), *Thais distinguenda* (Bech et al. 2002), *T. orbita* (Gibson & Wilson 2003) and *Hexaplex trunculus* (Lahbib et al. 2008). The present transplant experiment aimed to compare imposex responses between *S. haemastoma* and *S. rustica*, which are currently used as biomarkers for organotin pollution, along the coasts of Brazil.

Materials and methods

Stramonita haemastoma and *Stramonita rustica* with no signs of imposex were collected in September 2005 at Caponga Beach, Ceará state Brazil (Figure 1), prior to the TBT global ban in 2008. For each species, 300 adult specimens (shell length 20–40 mm) were collected, taken to the laboratory and kept in aerated aquaria. After a week of acclimation at the same salinity and temperature as the areas of origin, the animals were transferred to cages in the field. Cages were made of nylon netting, with five vertically arranged square wooden platforms measuring 45×45 cm. Animals were randomly distributed in two cages, separated by species. The cages, suspended from buoys, were settled in a small marina inside 'Industria Naval do Ceará' repair shipyard (see Figure 1). Animals were fed weekly with prey they would normally consume in the natural environment: *Crassostrea rhizophora* oysters (for *S. haemastoma*) and *Brachidontes* spp. mytilids (for *S. rustica*). These bivalves were collected from areas where the gastropods showed no imposex development. The imposex levels in the native *S. haemastoma* from the marina were checked every 30 days (positive control). Similarly, organisms of both species were collected at Caponga Beach every 60 days of the experiment to verify the imposex occurrence (negative control). Initially, 30 individuals of each species were sampled as $T = 0$, then similar samples were collected at $T = 15, 30, 45, 60, 75, 90, 105$ and 120 days. Animals collected at each sampling time were transported alive in aerated seawater to the laboratory.

In the laboratory, snail shell length was measured from the apex to the extremity of the siphonal canal with vernier calipers. Animals were then narcotized in 3.5% $MgCl_2$ (Huet et al. 1996) and soft parts extracted from the shells. Sexual identification was done by the presence of sperm ingesting gland, albumen and capsule glands in females and prostate in males. Penis length was measured to the nearest millimetre using calipers in both males and imposex-affected females. The imposex incidence and severity were assessed using the following indices:% of imposex-affected females (I%), Female Penis Length Index (FPLI = mean penis length of all females in the sample, including the zero values of aphalic females), Relative Penis Length Index (RPLI = [mean penis length in females/mean penis length in males] $\times$ 100)(Gibbs et al. 1987). Although animals were relaxed with $MgCl_2$, it can cause an increase penis measurements (Vasconcelos et al. 2011); all the measurements were done identically for all the individuals, so comparisons could be made in this experiment. Future works using the same species as in this work must take into account

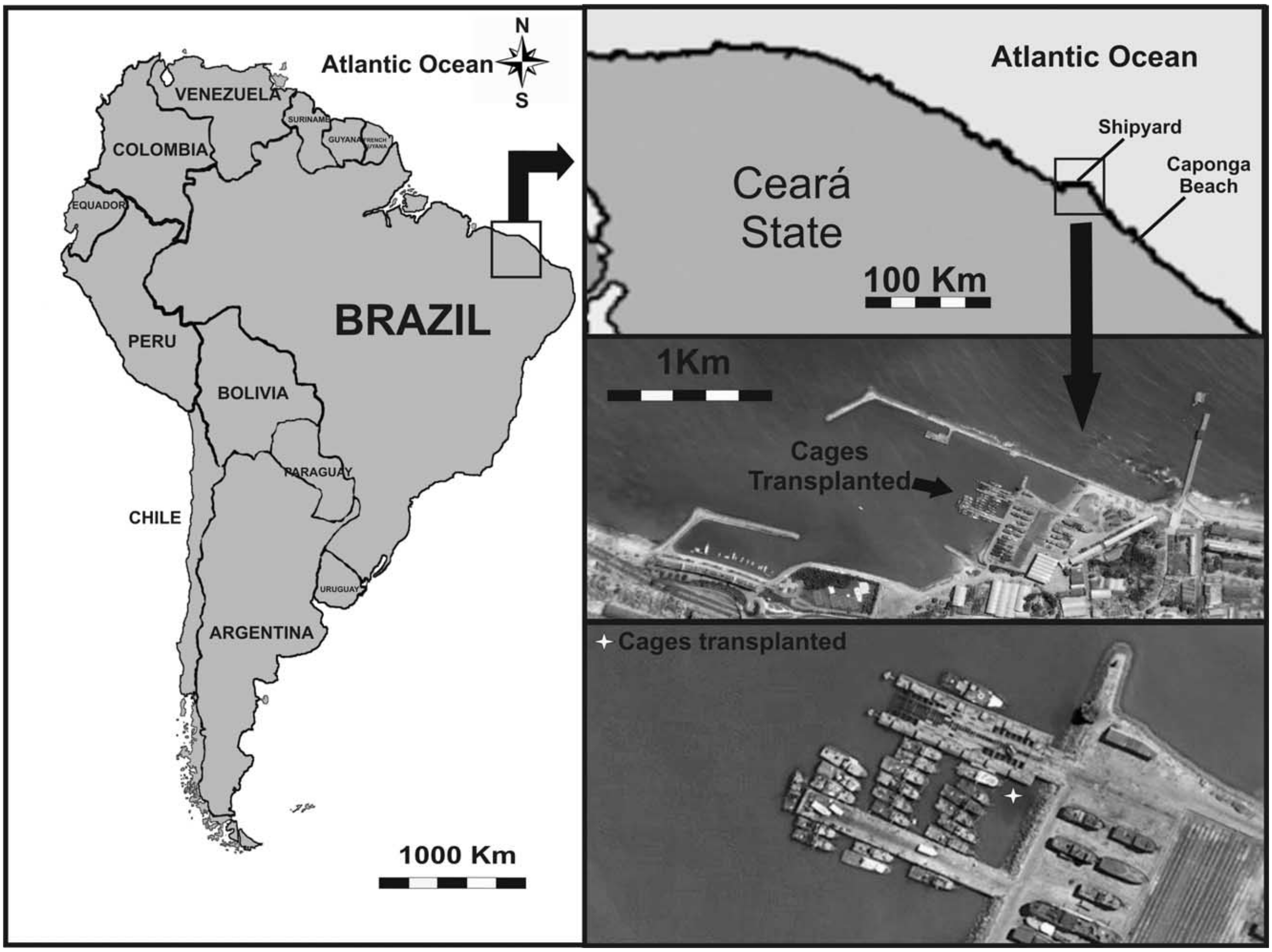

Figure 1. Sampling site (imposex-free) at Caponga beach and transplant site in a crowded shipyard.

the procedures made here for comparisons with our results. The Vas Deferens Sequence Index (VDSI), based on the development of male sexual characters (penis and/or vas deferens) in females, was calculated using a six-stage scale similar to the classic Gibbs scale for *Nucella lapillus* (Gibbs et al. 1987), adapted for *S. haemastoma* according to Fernandez et al. (2005a). In brief, the VDSI stages were determined as follows: (0) normal female, (I) beginning of penis or vas deferens formation, (II) penis developed [length < 2 mm], (III) penis developed [length > 2 mm], (IV) completely developed vas deferens, (V) vulva blocked by vas deferens growth, and (VI) dark mass of aborted eggs in the capsule gland.

Raw data were checked for normality using the Lilliefors test. Interspecific tests for FPLI and VDSI variation at Day 15 and Day 120 were done using the Mann–Whitney U-test. Correlation of the mean values of the imposex indices was inferred by the Pearson coefficient. In all statistical analyses, significance level was $p < 0.05$.

Results

Snails sampled at the control site did not show imposex incidence at 0, 60 and 120 days, while females of both transplanted species developed penises during the experiment (Figure 2). After 15 days of transplantation, *Stramonita haemastoma* was the only species presenting signs of imposex in 90% of the females (Figure 3). At this time, development of the vas deferens was registered in 78.6% of the females (Figure 4), while a penis could be measured in only one snail (3.5%). No females of *S. rustica* showed imposex at $T = 15$ days. However, at $T = 30$ days, 38.5% of females started to show imposex development, albeit lower than *S. haemastoma* with $FPLI = 0.4$ and VDS stage I. At this time, imposex development in *S. haemastoma* was much higher, with all females, presenting imposex (Figure 3). Faster imposex development in *S. haemastoma* was confirmed throughout the study period. At Day 90, imposex was induced in all females of both species. Female penises were much more developed in *S. haemastoma*, reaching values of RPLI comparable to

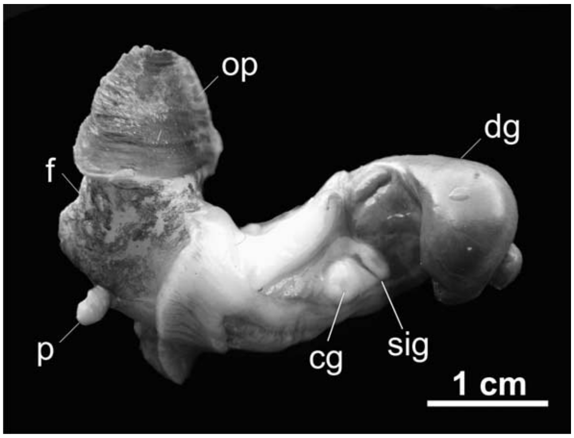

Figure 2. Imposex-affected *Stramonita haemastoma* female showing penis (p) and sperm ingesting gland (sig), operculum (op), digestive gland (dg), foot (f) and capsule gland (cg).

those found in snails sampled in the polluted area at the end of the 120 days (Figure 3). Vagina blocked by vas deferens tissues (VDSI ≥ V) was observed in native organisms (9 – 17% of the samples). In transplanted *S. haemastoma*, genital blocking was detected after 75 days of transplantation, with percentage ranging between 11.1 and 28.6%, while in *S. rustica*, imposex development was much less pronounced than *S. haemasoma* at the end of the experiment (Figure 4).

All imposex indices showed a strong Pearson correlation (r^2) for the duration of the experiment, for *S. haemastoma* (FPLI = 0.96, VDSI = 0.91, RPLI = 0.96) and for *S. rustica* (FPLI = 0.91, VDSI = 0.93, RPLI = 0.94). Due to the initial imposex-free condition of females, comparisons were made from Day 15 to Day 120. As shown in Table I, females of both species showed significant penis development in this period. Imposex development, as indicated by FPLI and VDSI, was significantly different between species.

Discussion

Our results demonstrated that imposex is induced very quickly in both species, probably due to high sensitivity to TBT pollution as registered worldwide (Fent 2006; Bigatti et al. 2009; Santos et al. 2009; Castro et al. 2011a). The maximum time used in this experiment was only 120 days, but the rapid responses of the individuals treated (100% imposex at 30 days in *Stramonita haemastoma* and 90 days in *S. rustica*) allowed us to confirm the different

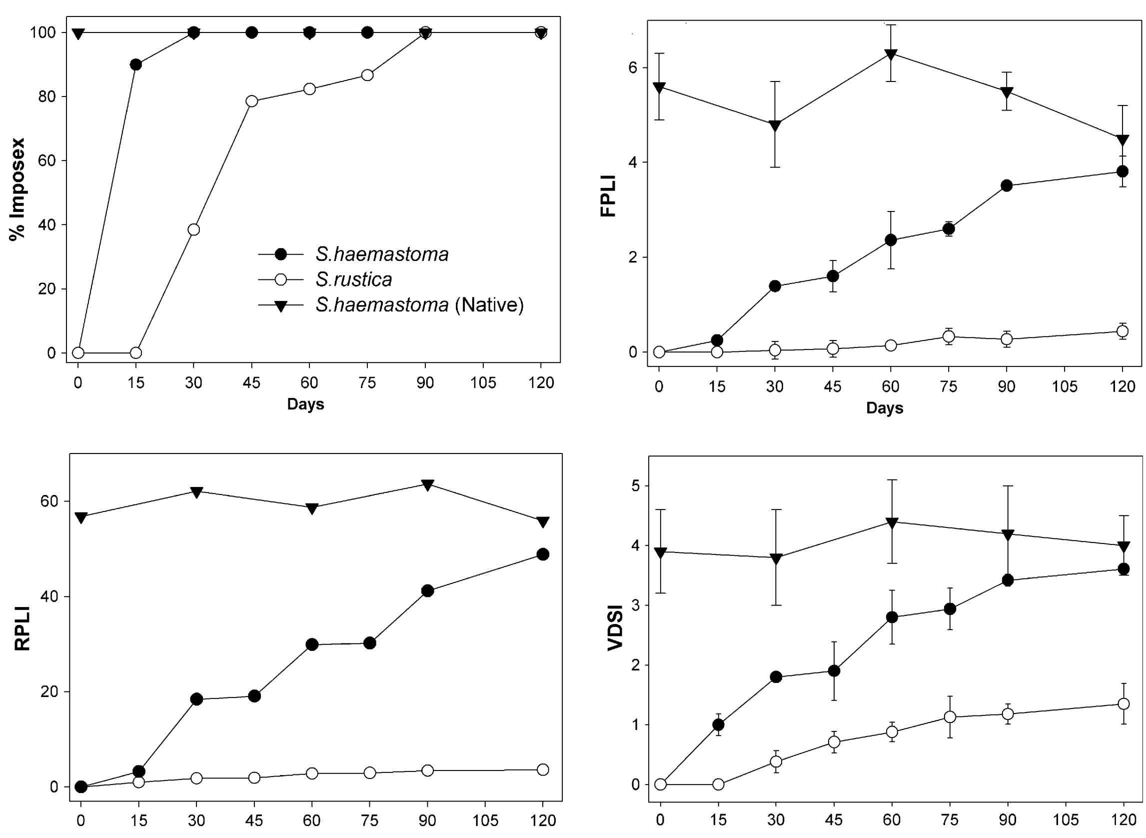

Figure 3. Variations in the imposex indices (I%, RPLI, FPLI and VDSI) of *Stramonita haemastoma* and *S. rustica* transplanted to a repair shipyard during 120 days. Bars indicate standard deviation.

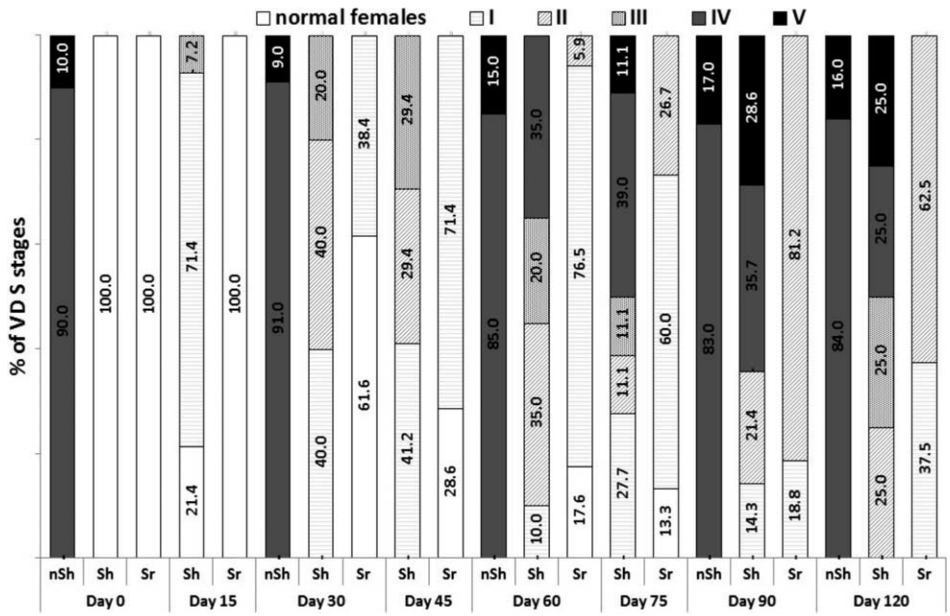

Figure 4. Frequency of VDS stages registered throughout the transplant experiment (native *Stramonita haemastoma* (nSh), *S. haemastoma* (Sh) and *S. rustica* (Sr) transplanted).

physiological responses of both species. Nevertheless, future experiments regarding specifically VDSI should be done over longer periods of time to reach the same condition as in native species. As biomonitoring studies are frequently hampered by the spatial distribution of bioindicator species (Liu et al. 1997), monitoring studies could provide relevant information about the imposex response of the bioindicator species (Bech et al. 2002; Lahbib et al. 2008). Several studies compared the sensitivity for imposex responses in different gastropod species (Bech 1999; Barroso et al. 2000; Tewari et al. 2002; Bigatti et al. 2009). However, even when species of the same genus are compared, specific differences in sensitivity may be apparent (see Stroben et al. 1995). These differences may be explained by differential physiological responses, bioaccumulation factors or excretion rates for xenobiotics, or even specific feeding habits (Gibbs et al. 1997). The results obtained by Huet et al. (1995) comparing VDSI and RPSI (Relative Penis Size index) development in *Ocenebra erinacea*, *Nucella lapillus* and *Hinia* (-*Nassarius*) *reticulata* support this observation.

In Brazil, *S. haemastoma* has been employed as a bioindicator species in many studies (Fernandez et al. 2005), as has *S. rustica* (Camillo et al. 2004; Castro et al. 2004), but both species seldom occur together. Near sites with high marine traffic as marinas, harbours and shipyards, sterile *S. haemastoma* were frequently detected in Brazilian coastal waters (Fernandez et al. 2005a). On the other hand, sterility was not observed in *S. rustica* populations in similar conditions. Another study with *S. haemastoma* showed that imposex development could be fast (in the order of 15–20 days), particularly when juvenile animals were exposed (Mensink et al. 2002). Those results are in accordance with our study that detected first signs of imposex at Day 30 and 100% imposex at Day 90 in both species. On the other hand, transplanted *S. haemastoma* showed an extremely fast response with 100% imposex at Day 30. In transplant

Table I. Mann–Whitney tests for intraspecific and interspecific comparisons of FPLI and VDSI in *Stramonita haemastoma* and *S. rustica*.

Tests			*p*-value
Intraspecific	*S. haemastoma*	FPLI (15 days) × (120 days)	0.000143
		VDSI (15 days) × (120 days)	0.000137
	S. rustica	FPLI (15 days) × (120 days)	0.000001
		VDSI (15 days) × (120 days)	0.000001
Interspecific		Initial FPLI (15 days) *S. haemastoma* × *S. rustica*	0.000254
		Final FPLI (120 days) *S. haemastoma* × *S. rustica*	0.000143
		Initial VDSI (15 days) *S. haemastoma* × *S. rustica*	0.000002
		Final VDSI (120 days) *S. haemastoma* × *S. rustica*	0.000235

experiments using other species, similar but slower responses were registered: Lahbib et al. (2008) reported 100% imposex in *Hexaplex trunculus* from Tunisia approximately 5 months after their release into a zone with high marine traffic. In the Australian *Thais orbita*, imposex incidence reached 73% after 9 weeks of transplantation (Gibson & Wilson 2003), while in *Thais distinguenda* imposex incidence reached 86.4% after 12 months of transplantation (Bech et al. 2002).

The present study clearly demonstrated that *S. haemastoma* is more sensitive to imposex induction than is *S. rustica*. The first species showed a faster imposex development and faster penis growth in females. It is remarkable that *S. haemastoma* starts to develop a vas deferens 15 days after transplantation. A field study performed in Bizerta channel (Tunisia) by Lahbib et al. (2010) demonstrated that *S. haemastoma* is less sensitive than *H. trunculus* to the same exposition to contaminated waters. Differences in species responses were also recorded by Bech (1998) for *T. distinguenda* from Phuket Island (Thailand) and by Bech (1999), that compared imposex development in the muricids *T. distinguenda*, *Thais bitubercularis* and *Morula musiva*. The last study indicated that *T. bitubercularis* was more sensitive, thus being a preferable bioindicator. Bigatti et al. (2009) also registered different imposex responses in two species of muricids from Patagonia exposed to the same TBT contamination in zones with high marine traffic. It is worth noting that while biomonitoring studies should always prefer the most sensitive species, these should also be abundant and broadly distributed to make the study viable; when these last conditions cannot be satisfied, it would be preferable to use a less sensitive but more abundant species (Forbes et al. 2006). In any case, comparative studies may allow verification of the differences among imposex responses when different species are sampled from the same areas (Stewart et al. 1992; Tan 1999; Tan & Liu 2001; Birchenough et al. 2002). In the case of *S. haemastoma*, it is distributed along almost all the Brazilian coast, and its abundance, high sensitivity and rapid imposex response make it a very good tool for monitoring marine pollution by OTs. Although TBT determinations were not performed in the studied area, the imposex incidence registered in this study could only be explained from contamination by antifouling paints. Although other harbour pollutants, such as hydrocarbons or heavy metals, are expected to be present (Commendatore et al. 2000; Bigatti et al. 2009), according to the current knowledge these classes of compounds do not induce the imposex response. In this sense, the degree of contamination of the soft tissues of *S. haemastoma* should be analysed as this species is consumed as food along the Brazilian coast.

Imposex development in *S. haemastoma* was extremely fast when compared to similar assays done in Thailand with *T. distinguenda* (Bech 2002a; Bech 2002b; Bech et al. 2002). Therefore, *S. haemastoma* is likely to be a useful bioindicator of OT pollution on rocky shores along approximately 9000 km of Brazilian coastline. However, the current study was performed before the global TBT ban issued in September 2008 by the International Maritime Organization. In this context, it would be interesting to continue sampling analyses in order to verify the effectiveness of the global TBT ban along the Brazilian coast. Knowing the differences in imposex induction between *S. haemastoma* and *S. rustica* is a useful tool for future monitoring programmes of TBT pollution in Brazilian coastal areas. As stated before (Fernandez & Pinheiro 2007), the very slow transition from TBT-based to TBT-free antifoulings in developing countries continues to make imposex a very useful tool to evaluate the extent and intensity of impact from antifoulants in coastal areas.

References

Barroso CM, Moreira MH, Gibbs PE. 2000. Comparison of imposex and intersex development in four prosobranch species for TBT monitoring of a southern European estuarine system (Ria de Aveiro, NW Portugal). Marine Ecology Progress Series 201:221–32.

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 18:129–38.

Bech M. 1999. Sensitivity of different gastropod to tributyltin contamination. Phuket Marine Biological Center Special Publication 19:1–6.

Bech M. 2002a. A survey of imposex in muricids from 1996 to 2000 and identification of optimal indicators of tributyltin contamination along the east coast of Phuket Island, Thailand. Marine Pollution Bulletin 44:887–96.

Bech M. 2002b. Imposex and tributyltin contamination as a consequence of the establishment of a marina, and increasing yachting activities at Phuket Island, Thailand. Environmental Pollution 117:421–29.

Bech M, Strand J, Jacobsen JA. 2002. Development of imposex and accumulation of butyltin in the tropical muricid *Thais distinguenda* transplanted to a TBT contaminated site. Environmental Pollution 119:253–60.

Bigatti G, Primost MA, Cledón M, Averbuj A, Theobald N, Gerwinski W, et al. 2009. Biomonitoring of TBT contamination and imposex incidence along 4700 km of Argentinean shoreline (SW Atlantic: From 38S to 54S). Marine Pollution Bulletin 58:695–701.

Birchenough AC, Barnes N, Evans SM, Hinz H, Kronke I, Moss C. 2002. A review and assessment of tributyltin contamination in the North Sea, based on surveys of butyltin tissue burdens and imposex/intersex in four species of neogastropods. Marine Pollution Bulletin 44:534–43.

Bryan GW, Gibbs PE, Hummerstone LG, Burt GR. 1986. The decline of the gastropod *Nucella lapillus* around southwest England – Evidence for the effect of tributyltin from antifouling paints. Journal of the Marine Biological Association of the United Kingdom 66:611–40.

Camillo E, Quadros J, Castro IB, Fernandez MAS. 2004. Imposex in *Thais rustica* (Mollusca:Neogastropoda) (Lamarck, 1822) as an indicator of organotin compounds pollution at Maceió coast (Northeastern Brazil). Brazilian Journal of Oceanography 52:101–105.

Castro IB, Bemvenuti CE, Fillmann G. 2007a. Preliminary appraisal of imposex in areas under the influence of southern Brazilian harbors. Journal of the Brazilian Society of Ecotoxicology 2:73–79.

Castro IB, Lima AFA, Braga ARC, Rocha-Barreira CA. 2007b. Imposex in two muricid species (Mollusca: Gastropoda) from the northeastern Brazilian coast. Journal of the Brazilian Society of Ecotoxicology 2:81–91.

Castro IB, Meirelles CAO, Matthews-Cascon H, Fernandez MAS. 2004. *Thais (Stramonita) rustica* (Lamarck, 1822) (Mollusca: Gastropoda: Thaididae), a potential bioindicator of contamination by organotin in northeast Brazil. Brazilian Journal of Oceanography 52:135–39.

Castro ÍB, Arroyo MF, Costa PG, Fillmann G. 2011a. Butyltin compounds and imposex levels in Ecuador. Archives of Environmental Contamination and Toxicology, in press (1-10. doi:10.1007/s00244-011-9670-2).

Castro ÍB, Perina F, Fillmann G. 2011b. Organotin contamination in South American coastal areas. Environmental Monitoring and Assessment, in press (1-10. doi:10.1007/s10661-011-2078-7).

Cavalcante-Braga AR, Castro IB, Rocha-Barreira CA. 2006. Compostos organoestânicos: Um risco potencial para contaminação do pescado marinho. Boletim Técnico Científico do Cepene 14:103–07.

Commendatore MG, Esteves JL, Colombo JC. 2000. Hydrocarbons in coastal sediments of Patagonia, Argentina: Levels and probable sources. Marine Pollution Bulletin 40:989–98.

CONAMA. 2005. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes, e dá outras providências. Brazil, Diário Oficial da União 357:58–63.

Fent K. 2006. Worldwide occurrence of organotins from antifouling paints and effects in the aquatic environment. In IK Konstantinou editor. Handbook of Environmental Chemistry: Antifouling Paint Biocides. Berlin: Springer-Verlag, p 71–100.

Fernandez MA, Pinheiro F. 2007. New approaches for monitoring the marine environment: The case of antifouling paints. International Journal of Environment and Health 1:427–48.

Fernandez MA, Wagener AdLR, Limaverde AM, Scofield AL, Pinheiro FM, Rodrigues E. 2005a. Imposex and surface sediment speciation: A combined approach to evaluate organotin contamination in Guanabara Bay, Rio de Janeiro, Brazil. Marine Environmental Research 59:435–52.

Fernandez MAS, Limaverde AC, Castro IB, Wagener AdLR, Almeida ACO. 2002. Occurrence of imposex in *Thais haemastoma*: Possible evidence of environmental contamination derived from organotin compounds in Rio de Janeiro and Fortaleza, Brazil. Reports in Public Health 18:463–76.

Fernandez MA, Limaverde AM, Scofield AL, Wagener AdLR. 2005b. Preliminary evaluation of human health risks from ingestion of organotin contaminated seafood in Brazil. Brazilian Journal of Oceanography 53:75–77.

Forbes VE, Palmqvist A, Bach L. 2006. The use and misuse of biomarkers in ecotoxicology. Environmental Toxicology and Chemistry 25:272–80.

Galante-Oliveira S, Oliveira I, Pacheco M, Barroso CM. 2010. *Hydrobia ulvae* imposex levels at Ria de Aveiro (NW Portugal) between 1998 and 2007: A counter-current bioindicator? Journal of Environmental Monitoring 12:500–07.

Garaventa F, Pellizzato F, Faimali M, Terlizzi A, Medakovic D, Geraci S, et al. 2006. Imposex in *Hexaplex trunculus* at some sites on the North Mediterranean Coast as a base-line for future evaluation of the effectiveness of the total ban on organotin based antifouling paints. Hydrobiologia 555:281–87.

Gibbs PE, Bebianno MJ, Coelho MR. 1997. Evidence of the differential sensitivity of neogastropods to tributyltin (TBT) pollution, with notes on a species (*Columbella rustica*) lacking the imposex response. Environmental Technology 18:1219–24.

Gibbs PE, Bryan GM, Pascoe PL, Burt GR. 1987. The use of dog-whelk *Nucella lapillus*, as an indicator of tributyltin (TBT) contamination. Journal of the Marine Biological Association of the United Kingdom 67:507–23.

Gibbs PE, Bryan GW. 1986. Reproductive failure in populations of the dog-whelk, *Nucella lapillus*, caused by imposex induced by tributyltin from antifouling paints. Journal of the Marine Biological Association of the United Kingdom 66:767–75.

Gibson CP, Wilson SP. 2003. Imposex still evident in eastern Australia 10 years after tributyltin restrictions. Marine Environmental Research 55:101–12.

Gravel P, Johanning K, McLachlan J, Vargas JA, Oberdorster E. 2006. Imposex in the intertidal snail *Thais brevidentata* (Gastropoda: Muricidae) from the Pacific coast of Costa Rica. Revista de Biologia Tropical 54:21–26.

Gunter G. 1979. Studies of the southern oyster borer, *Thais haemastoma*. Gulf Research Reports 6:249–60.

Horiguchi T. 2009. Mechanism of imposex induced by organotins in gastropods. In Arai T, Harino H, Ohji M, Langston WJ, editors. Ecotoxicology of Antifouling Biocides. Tokyo: Springer, p 111–123.

Huet M, Fioroni P, Oehlmann J, Stroben E. 1995. Comparison of imposex response in 3 prosobranch species. Hydrobiologia 309:29–35.

Huet M, Paulet YM, Glemarec M. 1996. Tributyltin (TBT) pollution in the coastal waters of west Brittany as indicated by imposex in *Nucella lapillus*. Marine Environmental Research 41:157–67.

Lahbib Y, Boumaiza M, Trigui El Menif N. 2008. Imposex expression in *Hexaplex trunculus* from the North Tunis Lake transplanted to Bizerta channel (Tunisia). Ecological Indicators 8:239–245.

Lahbib Y, Abidli S, Chiffoleau JF, Averty B, Menif NTE. 2010. Imposex and butyltin concentrations in snails from the lagoon of Bizerta (Northern Tunisia). Marine Biology Research 6:600–07.

Limaverde AM, de LRW, Fernandez MA, Schofield AdL, Coutinho R. 2007. *Stramonita haemastoma* as a bioindicator for organotin contamination in coastal environments. Marine Environmental Research 64:384–98.

Liu LL, Chen SJ, Peng WY, Hung JJ. 1997. Organotin concentrations in three intertidal neogastropods from the coastal waters of Taiwan. Environmental Pollution 98:113–18.

Matthews HR. 1968. Notes on the genus *Thais* Roding, 1798 in Northeast Brazil. Archives of Marine Biological Station of Ceará Federal University 8:37–41.

Matthiessen P, Gibbs PE. 1998. Critical appraisal of the evidence for tributyltin-mediated endocrine disruption in mollusks. Environmental Toxicology and Chemistry 17:37–43.

Mensink BP, Kralt H, Vethaak AD, ten Hallers-Tjabbes CC, Koeman JH, van Hattum B, et al. 2002. Imposex induction in laboratory reared juvenile *Buccinum undatum* by tributyltin (TBT). Environmental Toxicology and Pharmacology 11: 49–65.

Mohamat-Yusuff F, Zulkifli S, Ismail A, Harino H, Yusoff M, Arai T. 2010. Imposex in *Thais gradata* as a biomarker for TBT contamination on the southern coast of peninsular Malaysia. Water Air and Soil Pollution 211:443–57.

Oliveira CR, Santos DM, Santos Madureira LA, Marchi MRR. 2010. Speciation of butyltin derivatives in surface sediments of three southern Brazilian harbors. Journal of Hazardous Materials 181:851–56.

Quintela M, Barreiro R, Ruiz JM. 2000. The use of *Nucella lapillus* (L.) transplanted in cages to monitor tributyltin (TBT) pollution. Science of the Total Environment 247:227–37.

Rilov C, Gasith A, Evans SM, Benayahu Y. 2000. Unregulated use of TBT-based antifouling paints in Israel (eastern Mediterranean): High contamination and imposer levels in two species of marine gastropods. Marine Ecology Progress Series 192:229–38.

Santos DM, Araújo IP, Machado EC, Carvalho-Filho MAS, Fernandez MA, Marchi MRR, et al. 2009. Organotin compounds in the Paranaguá Estuarine Complex, Paraná, Brazil: Evaluation of biological effects, surface sediment, and suspended particulate matter. Marine Pollution Bulletin 58: 1926–31.

Shi HH, Huang CJ, Zhu XS, Yu XJ, Xie WY. 2005. Generalized system of imposex and reproductive failure in female gastropods of coastal waters of mainland China. Marine Ecology Progress Series 304:179–89.

Smith AJ, Thain JE, Barry J. 2006. Exploring the use of caged *Nucella lapillus* to monitor changes to TBT hotspot areas: A trial in the River Tyne estuary (UK). Marine Environmental Research 62:149–63.

Smith BS. 1971. Sexuality in the American mud-snail *Nassarius obsoletus* Say. Proceedings of the Malacological Society of London 39:377–78.

Spence SK, Hawkins SJ, Santos RS. 1990. The mollusk *Thais haemastoma* – An exhibitor of imposex and potential biological indicator of tributyltin pollution. Marine Ecology – Pubblicazioni Della Stazione Zoologica di Napoli I 11:147–56.

Sternberg R, Gooding M, Hotchkiss A, LeBlanc G. 2010. Environmental-endocrine control of reproductive maturation in gastropods: Implications for the mechanism of tributyltin-induced imposex in prosobranchs. Ecotoxicology 19:4–23.

Stewart C, Demora SJ, Jones MRL, Miller MC. 1992. Imposex in New Zealand Neogastropods. Marine Pollution Bulletin 24:204–09.

Stroben E. 1996. The organotin pollution at the bay of Morlaix with special reference biomonitoring with prosobranchs. Molluscan Reproduction 6:163–71.

Tan KS. 1999. Imposex in *Thais gradata* and *Chicoreus capucinus* (Mollusca, Neogastropoda, Muricidae) from the Straits of Johor: A case study using penis length, area and weight as measures of imposex severity. Marine Pollution Bulletin 39:295–303.

Tan KS, Liu LL. 2001. Description of a new species of *Thais* (Mollusca: Neogastropoda: Muricidae) from Taiwan, based on morphological and allozyme analyses. Zoological Science 18:1275–89.

Tewari A, Raghunathan C, Joshi HV, Khambhaty Y. 2002. Imposex in rock whelks *Thais* and *Ocenebra* species (Mollusca, Neogastropoda, Muricidae) from Gujarat coast. Indian Journal of Marine Sciences 31:321–28.

Toste R, Fernandez MA, Pessoa IdA, Parahyba MA, Dore MP. 2011. Organotin pollution at Arraial do Cabo, Rio de Janeiro State, Brazil: Increasing levels after the TBT ban. Brazilian Journal of Oceanography 59:111–17.

Vasconcelos P, Gaspar MB, Castro M. 2006. Imposex in *Hexaplex (Trunculariopsis)* trunculus (Gastropoda: Muricidae) from the Ria Formosa lagoon (Algarve coast – southern Portugal). Marine Pollution Bulletin 52:337–41.

Vasconcelos P, Moura P, Barroso C, Gaspar M. 2011. Size matters: Importance of penis length variation on reproduction studies and imposex monitoring in *Bolinus brandaris* (Gastropoda: Muricidae). Hydrobiologia 661:363–75.

Editorial responsibility: Ketil Hylland