Ecotoxicology and Environmental Safety 91 (2013) 162–170

Contents lists available at SciVerse ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Is aphallic vas deferens development in females related to the distance from organotin sources? A study with *Stramonita haemastoma*

R. Toste*, I.A. Pessoa, M.P. Dore, M.A. Parahyba, M.A. Fernandez

Programa de Ecotoxicologia Marinha, Faculdade de Oceanografia, Universidade do Estado do Rio de Janeiro, São Francisco Xavier St. 524, 4018E, 20550-013 Maracanã, RJ, Brazil

ARTICLE INFO

Article history:
Received 6 June 2012
Received in revised form
29 January 2013
Accepted 30 January 2013
Available online 26 February 2013

Keywords:
Stramonita haemastoma
Imposex
VDSI
Aphally

ABSTRACT

Imposex, a syndrome characterized by the appearance of a penis and/or vas deferens in female gastropods due the presence of organotins in environment, is still observed in Brazilian gastropod populations, as in other countries, even after the tributyltin (TBT) ban. Nevertheless, the progressive controls on the use of organotins in antifouling systems at national and international levels and the consequent reduction of their environmental concentrations have led to changes in the characteristics of imposex development observed in *Stramonita haemastoma*. Populations of this species were analyzed on the coast of Rio de Janeiro (Brazil) between 2007 and 2008, and the developmental pathways associated with the syndrome were identified. Compared with previous works, it was noted that imposex expression was reduced in most of Guanabara Bay. Aphallic imposex development, on the other hand, showed a marked increase. The pathways of imposex development were also evaluated in a temporal data series from a fixed sampling station at Vermelha beach, and the incidence of aphallic imposex development was found to show a marked increase from 1998 onward. Furthermore, the observation of either the presence or absence of a penis in imposex-affected females may indicate that penis development is related to the contaminant exposure level and that the decreasing TBT concentrations in the local environment result in the predominance of an aphallic route of imposex development. These findings support the idea that imposex female aphally in this species could be a dose-dependent response, rather than a genetic anomaly.

1. Introduction

The introduction of substances by humans into the marine environment can have damaging effects in biological communities directly due to toxicant properties and/or due to synergism with other substances (Fernández-Alba et al., 2002; Takahashi et al., 1997). Tributyltin (TBT), which is considered the most efficient biocide used in antifouling paints, is a compound with largely known, well-studied effects in marine fauna. Due to its toxicity, the International Maritime Organization (IMO) established an Antifouling Systems Convention that entered into force in September 2008, completely banning the use of this compound on ships (IMO, 2001). The Brazilian Navy, in addition to suspending the use of organotin on its own ships since 2003, also established a norm forbidding the application of organotin compounds in antifouling systems in Brazil (NORMAM 23, 2007). The efficiency of these controls has been described in several studies conducted around the world (e.g., Castro et al., 2012c; Galante-Oliveira et al., 2009, 2010; Morton, 2009; Oliveira et al., 2009; Sousa et al., 2009; Tallmon and Hoferkamp, 2009), and in some instances, persistence of organotin pollution has also been reported locally (e.g., Castro et al., 2012a; Pessoa et al., 2009; Sousa et al., 2009; Swennen et al., 2009; Toste et al., 2011).

Among the effects of this compound in biota, imposex is the best known. This term was first used by Smith (1971) and describes an endocrine disruption syndrome in which female marine gastropods develop male sexual characters, such as a vas deferens and/or penis, and may become sterile (e.g., Huet et al., 1996a, 1996b; Quintela et al., 2002). In 1981, Smith correlated the occurrence of this syndrome with the presence of TBT in the water (Smith, 1981), and this phenomenon has since been described in many countries and has been identified in more than 190 species around the world. Altogether, such masculinization phenomena are known to affect a total of more than 260 species (Shi et al., 2005b; Titley-O'Neal et al., 2011). This number has continued to increase with the still growing number of studies on this topic. Imposex is a characteristic result of TBT exposure and can therefore be used as a biomarker of exposure to this organotin compound (Matthiessen and Gibbs, 1998).

The traditional imposex monitoring techniques make use of indexes of imposex expression to quantify the response of gastropod populations, making it possible to compare different

* Corresponding author.
E-mail address: rtoste@gmail.com (R. Toste).

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http://dx.doi.org/10.1016/j.ecoenv.2013.01.026

environments in this respect. The simplest approach is determination of the percentage of imposex. Other commonly used indexes are based on the relationship between female and male penis lengths, such as the RPLI (Relative Penis Length Index) and the RPSI (Relative Penis Size Index). Another way to quantify the intensity of the syndrome is based on vas deferens development in females, the VDSI (Vas Deferens Sequence Index), initially proposed by Gibbs and Bryan for *Nucella lapillus* (Gibbs and Bryan, 1987). In this species, the vas deferens begins to develop via growing from the vulva (distal section) toward the location of the penis, behind the right tentacle, and the development of a vas deferens is followed by the development of a penis. Anyway, due to the occurrence of different development of sexual characteristics in gastropod species because of this syndrome, there have been many adaptations of this initial, basic scheme. For instance, Stroben et al. (1995) described and compared the imposex response in six gastropod species from the French coast, including *N. lapillus*, and Shi et al. (2005a) modified the typical scheme for application to *Cantharus cecillei* . Subsequently, the same authors presented a general VDSI scheme, updating the previous scheme and including new degrees of imposex response, which according to the authors, was valid for all of the main types of morphological expression known in gastropods (Shi et al., 2005b).

In all these studies, the intensity of imposex evaluated based on vas deferens development was closely related to the development and growth of the penis. After a certain level of imposex development, corresponding to stage 3 of the 6 stages recognized by the previously cited authors, all imposex females have developed penises. However, this is not always the case. The absence of a penis in imposex-affected females was first reported in England in Dumpton Gap, where males of *N. lapillus* showed underdeveloped genital tracts, corresponding to approximately 10 percent of adult males (Gibbs, 1993). Later, similar observations in the same species were reported by Huet et al. (1996a, 1996b) in the bay of Brest. This type of reproductive abnormality was designated Dumpton Syndrome (DS) and was demonstrated to be genetically based in these populations. This syndrome appears to be a favorable factor for species survival in highly polluted areas (Huet et al., 1996b). These observations were further confirmed in the same species in native populations of Galicia, Spain (Barreiro et al., 1999; Quintela et al., 2002). Thus, for this species, female aphally is related to a genetic phenomenon that also affects males.

In Brazil, aphally in imposex females has been widely observed in the species *Stramonita haemastoma*, a common Caenogastropod in the Brazilian littoral zone. In this species, female aphally is observed as a parallel route of imposex development, with some females showing only a pre-penis while presenting a complete vas deferens and even sterility (Fernandez et al., 2006; Lima et al., 2006; Queiroz et al., 2007; our research group, unpublished data). Thus, the use of imposex development indexes based on penis measurements in this species is meaningless. Because of these observations, Queiroz et al. (2007) proposed a new imposex development index (IDI) for *S. haemastoma*, showing three possible pathways of imposex evolution and including an aphallic route, using a similar approach to that proposed by Stroben et al. (1995) and by Barreiro et al. (1999) for DS populations of *N. lapillus*. In this new work, female aphally was also related to genetic failure by Queiroz et al. However, aphallic males are very rarely found in this species, and their numbers are irrelevant when compared to the numbers of normal males in samples, as indicated by the fact that in their study these last authors did not report any aphallic males in the analyzed samples.

Considering all of these findings, this work aims to evaluate whether the influence of environmental organotin levels could be related to the expression of imposex development in this species both temporally and spatially, using the variability in the imposex intensity in this species following enforcement of the TBT ban as a background.

2. Materials and methods

Populations of *S. haemastoma* were sampled at sixteen stations at Barra de Guaratiba, Rio de Janeiro and Niterói in Rio de Janeiro State between late 2007 and early 2008. At each sampling station, 30 specimens were collected by diving and were maintained in constantly aerated seawater until they were brought to the

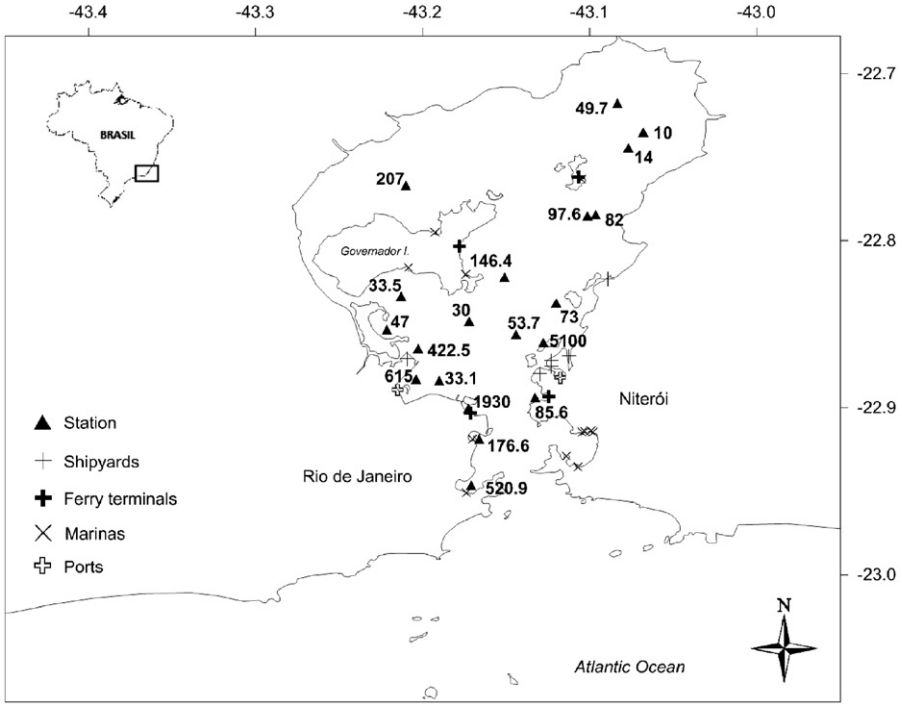

Fig. 1. TBT concentrations in sediments (ng (Sn) g^{-1}) of Guanabara Bay in 2000, and the spatial distribution of organotin sources (Almeida et al., 2004; Fernandez et al., 2005; Stringer et al., 2000).