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Size, season and origin of gastropods matter in imposex assessments

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ABSTRACT

Through controlled exposure to tributyltin (TBT), the effects of season, size, and population origin were evaluated on imposex incidence in *Stramonita brasiliensis*. Four experiments were carried out with organisms collected from three different sites on the Brazilian coast (Torres - Rio Grande do Sul, Farol de Santa Marta - Santa Catarina, and Aracruz - Espírito Santo). *S. brasiliensis* were anesthetized, sexed, measured, classified by size in small (< 30 mm), medium (≥ 30 to ≤ 40 mm), or big (> 40 mm) and injected in the foot muscle with 0.5 μg g^{-1} of TBT. Organisms were maintained during one month in aquariums with clear marine water under controlled laboratory conditions. One month after injection, the imposex assessments showed that the population from the southeast (tropical) region was more sensitive to TBT than the population from the south of Brazil (the temperate region). A greater sensitivity in the small and medium categories was observed. Females were also more susceptible to TBT when exposed during their reproductive period. Thus, the present study highlighted the need to ensure that intrinsic biological factors related to organisms are considered in such biomonitoring studies to avoid misinterpretation of results.

1. Introduction

TBT is an organometallic compound and owing to its biocidal properties, since 1950 it has been used as an active ingredient in wood preservatives, pesticides, and marine antifouling paints to prevent biofouling (Hoch, 2001). Its toxic effects on non-target aquatic organisms, such bivalves, gastropods, fish, and marine mammals, have led to a total ban of its use in antifouling paints by the International Marine Organization (IMO), since 2003 (IMO, 2001). International regulations against the use of TBT-based antifouling paints have caused a reduction in recent TBT input. In this regard, many studies have reported a decrease in TBT concentrations and biological effects worldwide (Bray et al., 2012; Castro et al., 2012c; Langston et al., 2015). However, according to recent studies, the problem still persist mainly in Latin America (Batista-Andrade et al., 2018; Maciel et al., 2018; Mattos et al., 2017). Despite local authorities are struggling to control the use of TBT-based antifouling paints, recent assessments along the southern and southeastern coasts of Brazil and others countries in Latin America have detected OTs in sites under influence of commercial ports, marinas and shipyards (Artifon et al., 2016; Buruaem et al., 2013; Castro et al., 2012a; Sant'Anna et al., 2014). Although DBT and MBT were the predominant founded analytes, recent inputs of TBT were evident in some areas. In fact, fishing boats may be a relevant source since they were the predominant maritime activity in the most contaminated sites.

The effects of TBT on gastropods have been widely studied, and imposex is one of the best studied phenomena (Bigatti et al., 2009). Imposex has been reported to cause sterility and a consequent decline of entire populations in coastal areas. In recent years, the incidence of imposex has been widely used as a specific biomarker, especially in species showing a good correlation between morphological alterations and environmental TBT levels (Titley-O'Neal et al., 2011). Several gastropod species have been proposed worldwide as bioindicators of TBT pollution, despite their differences in size, physiology, distribution, and sensitivity to pollutants (Anastasiou et al., 2016).

Muricidae (Rafinesque, 1815) is an important family of marine gastropods that represents almost 10% of neogastropods (1600 living species and 1200 fossil) and their variety of shell shapes and morphology have created a lot of problems of taxonomic definitions, especially on subgroups (Kool, 1987; Vermeij, 2001). The *Stramonita* species complex is known to tropical latitudes of the Atlantic and eastern Pacific Oceans and, in Brazil, it was represented by the species *Stramonita haemastoma, Stramonita floridana* and *Stramonita rustica*. Claremont et al. (2011) working on molecular phylogeny of *Stramonita* and also using known distribution and morphological data (shell shape

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and number of crenulations) concluded that this muricid genus is monophyletic, restricted to the tropical and warm-temperate Atlantic and eastern Pacific (its speciation has occurred only within the Atlantic, despite its long pelagic larval duration); *S. haemastoma* is amphi-Atlantic but only found in Africa and Venezuela; *S. floridana* only found in the Mexico Gulf; and its Brazilian representants are *S. rustica* and the new species *S. brasiliensis*.

S. brasiliensis has been used for more than 16 years as an specific sentinel of TBT pollution in coastal waters from Brazil (Castro et al., 2000, 2012c; Fernandez et al., 2002). This muricid species is a carnivorous gastropod inhabitant of coastal hard substrates and presents high sensitivity to TBT exposure previously verified by field (Castro et al., 2012b; Queiroz et al., 2007; Rossato et al., 2014) and laboratory experiments (Lima et al., 2006; Limaverde et al., 2007). In addition, no reports of imposex induced by other factors, such as metal exposure (Nias et al., 1993), infestation by parasites (Morley, 2006) or the influence of other androgenic compounds (Cajaraville et al., 2000) are unknown for this specie. Thus, *S. brasiliensis* is a suitable indicator to be used in studies aiming evaluate TBT pollution.

The use of imposex in gastropods as indicators of TBT pollution has some advantages over chemical analysis of TBT in environmental matrices. Biomonitoring may overcome some of the difficulties related to the chemical measurements of TBT in environmental samples. Such difficulties mainly consist of the large temporal variations in the levels of this compound at different locations. Furthermore, often imposex may occur at TBT concentrations below the limit of detection (Bryan et al., 1988; Gibbs et al., 1987; Gooding et al., 2003). Moreover, the use of imposex as an indicator is a low-cost technique and a biologically meaningful measurement that reveals the effects of a pollutant on an ecosystem at the individual, population, and community levels (Barroso et al., 2000). Traditional TBT imposex monitoring techniques use imposex parameters to quantify the response in gastropod populations (Gibbs and Bryan, 1994; Oehlmann et al., 1996), allowing the comparison of different environments (Toste et al., 2013). However, several experimental studies have demonstrated the deferential sensitivity of gastropod species to TBT exposure. Furthermore, a few studies have reported variations in imposex responses among organisms of different sizes and/or ages (Mensink et al., 2002, 1996; Santos et al., 2006), and stages of reproductive maturation (McClellan-Green et al., 2007). Thus, the use of organisms from different sizes classes or even of different populations can bias the results of biomonitoring imposex-based. Mensink et al. (1996) showed that in *Buccinum undatum,* juveniles were the most sensitive than adult females exposed to TBT. In addition, considering the already reported influence of hormonal imbalances over imposex development (Rossato et al., 2016), it is plausible that seasonality may also influence the acuteness of imposex responses. At this sense, surveys using imposex to assess TBT pollution and disregard these confusing effects may lead to misinterpretations. In the present study, the effects of season, size and origin on imposex responses were experimentally evaluated in *S. brasiliensis* from the Brazilian coast (south and southeast) through controlled exposure to TBT. The main results are discussed in therms of sensitivity of gastropods to TBT with regard to the use of the different populations, seasonal variation and influence of the size in imposex parameters as imposex as a biomarker of TBT pollution.

2. Material and methods

2.1. Gastropod sampling

At least 344 individuals of *S. brasiliensis* (Mollusca: Prosobranchia: Muricidae) (> 19 mm in length) were manually caught from three different coastal sites in South and Southeast Brazil (Torres, Rio Grande do Sul (RS); Farol de Santa Marta, Santa Catarina (SC), and Aracruz, Espírito Santo (ES)) (Fig. 1). Torres (RS) and Farol de Santa Marta (SC) are in a temperate area and Aracruz (ES) is a tropical area and both sites

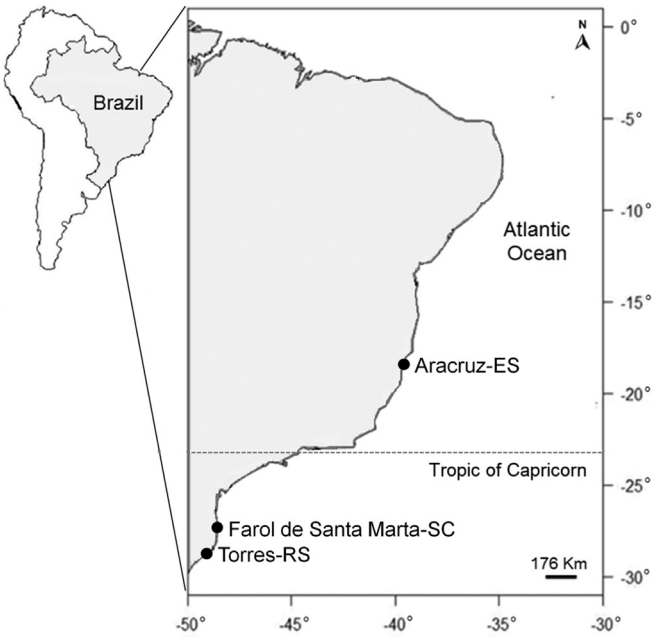

Fig. 1. Location of sampling sites on the Brazilian coast.

presented rocky coasts. All the sites were considered a reference areas based on the lack of ships, harbors and marinas. During the sampling, imposex assessments were performed (see Section 2.4) in all sites using an aliquot of 30 organisms per site. The absence of imposex of all animals collected in these areas confirmed the absence of OT pollution.

After sampling, the gastropods were acclimated under laboratory conditions over 2 weeks. Posteriorly, all organisms were narcotized by immersion in a 7% $MgCl_2$ solution (1:1 seawater/distilled water) for 2 h and sexed by pulling gently the operculum until it was possible to visualize the entrance of the palial cavity and the presence or absence of penis. Only females were used in the experiment.

2.2. Exposure experiment

Tributyltin (TBT – CAS number 1461-22-9) was purchased from Sigma Aldrich. A stock solution (4.8 $\mu g\,L^{-1}$ of TBT) was prepared in ethanol and stored at 4 °C in the dark before use. The same solution was used in all exposure experiments.

The gastropods were narcotized using magnesium chloride as described above, dried with an absorbent paper, weighed (total weight), and injected in the foot muscle with a unique dose comprising an ethanolic solution of TBT (0.5 $\mu g\,g^{-1}$ wet weight (ww) as TBT – or 0.18 $\mu g\,g^{-1}$ ww as Sn). The control treatments included a group that received no injection and a second group injected with an equal volume of pure ethanol. The TBT dose was selected aiming to induce quickly the imposex responses in laboratory conditions. In addition, the dose is in accordance with environmental relevant levels recently reported in tissues of muricid species from south America (Mattos et al., 2017). After injections, the gastropods were kept for 30 days in aerated 10-L aquariums (separated according to treatment) supplied with filtered saltwater collected in the same sites. Laboratory conditions are maintained at 20 °C, under a 12-h light-dark cycle. Before and during the experiments, the gastropods collected at Torres (RS) and Farol de Santa Marta (SC) were fed once a week, *ad libitum*, with mussels (*Perna perna*) caught from the same site as the *S. brasiliensis* snails and the animals collected at Aracruz (ES) were fed with small gastropods (*Tegula viridula*) collected in the same site. The aquarium saltwater was renewed twice per week.