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Evaluation of the use of *Olivella minuta* (Gastropoda, Olividae) and *Hastula cinerea* (Gastropoda, Terebridae) as TBT sentinels for sandy coastal habitats

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Abstract Tributyltin (TBT) contamination is still recorded in the environment even after its ban in antifouling paints. Since most biomonitors of TBT contamination, through imposex evaluation, are hard-bottom gastropods, the identification of soft-bottom sentinels has become useful for regions where rocky shores and coral reefs are absent. Thus, an evaluation of *Olivella minuta* and *Hastula cinerea* as monitors of TBT contamination was performed in two sandy beaches located under influence area of São Sebastião harbor (São Paulo state, Brazil), where previous and simultaneous studies have reported environmental contamination by TBT. In addition, the imposex occurrence in *H. cinerea* was assessed in an area with low marine traffic (Una beach), also located in São Paulo State. A moderate imposex incidence in *O. minuta* was detected in Pernambuco (% I= 9.36, RPLI=4.49 and $RPLI_{stand}$=4.27) and Barequeçaba (% I=2.42, RPLI=0.36 and $RPLI_{stand}$= 0.81) beaches, indicating TBT contamination. In contrast, more severe levels of imposex were recorded for *H. cinerea* in Una beach (% I=12.45) and mainly in Barequeçaba beach (% I=98.92, RPLI=26.65). Our results suggest that *O. minuta* and *H. cinerea* have good potential as biomonitors for TBT based on their wide geographical distribution, common occurrence in different coastal sediment habitats, easy collection, and association with TBT-contaminated sediments.

Keywords Imposex · Macrofauna · Olividae · Terebridae · São Sebastião Harbor · Brazil

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Introduction

In the early 1980s, tributyltin (TBT) exposure was related with female masculinization in neogastropods mollusks (Matthiessen and Gibbs 1998). Since then, this imposex phenomenon has been recorded for around 250 species of marine gastropods worldwide (Titley-O'Neal et al. 2011), impacting community structure and ecosystem functioning (Depledge and Billinghurst 1999). In

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this context, due to the strong dose-dependent relationship with TBT exposure, imposex has been globally used as a biomarker of contamination by TBT (Matthiessen and Gibbs 1998). Nowadays, this compound is reported as one of the most concerning pollutants in aquatic coastal systems since levels detected in the environment have the same magnitude order of those causing deleterious effects on biota (Meador et al. 2011). Even after the implementation of national and international regulatory actions (banning its use as antifouling paint biocide) in the last decade, TBT and/or imposex levels have still been detected in several South American coastal areas (Bigatti et al. 2009; Santos et al. 2009; Toste et al. 2011; Castro and Fillmann 2012). Sant'Anna et al. (2014) reported high amounts of TBT in hermit crab tissues along the Brazilian coast, which is an evidence of recent contamination (Sant'Anna et al. 2012). These results indicate that TBT pollution is an environmental problem still far from being solved, at least in developing Latin American countries (Castro et al. 2012a).

In South America, imposex and TBT environmental levels (surface sediments and biota samples) have been detected in Argentina (Penchaszadeh et al. 2001; Bigatti et al. 2009; Penchaszadeh et al. 2009; Arrighetti and Penchaszadeh 2010), Venezuela (Paz-Villarraga et al. 2015), Ecuador (Castro et al. 2012b), and Peru (Castro and Fillmann 2012). On the Brazilian coast, particularly in recent years, imposex associated to TBT has been reported in the southern (Castro et al. 2012a), southeastern (Fernandez et al. 2005; Quadros et al. 2009; Costa et al. 2009; Cardoso et al. 2009; Borges et al. 2013), and northeastern regions (Camillo et al. 2004; Castro et al. 2004; 2005; Castro et al. 2007a, b; Fernandez et al. 2007; Castro et al. 2008; Lima-Verde et al. 2010; Azevedo et al. 2012). However, most of these studies have focused on rocky-shore species such as *Stramonita haemastoma*, *Stramonita rustica*, *Thais deltoidea*, *Leucozonia nassa*, *Leucozonia ocelata*, *Cymatium parthenopeum*, *Voluta ebraea*, *Pisania auritula*, among others (Castro et al. 2012a). Thus, the use of imposex incidence in gastropods as a biomarker of TBT contamination has been often influenced by the occurrence and distribution of hard bottoms in studied coastal areas (Azevedo et al. 2012), thus hindering more general spatial evaluations. Hence, the use of soft-bottom organisms that could be obtained on sandy beaches would be highly suitable to monitor TBT pollution in coastal areas where hard bottoms are absent.

Olividae and Terebridae species are among the major representatives of sandy beach gastropods (Amaral et al. 2003; Denadai et al. 2005). The olivid *Olivella minuta* and the terebrid *Hastula cinerea* have wide geographical distribution along the Western Atlantic coast (Rios 1994; Simone 2009), where they inhabit coastal environments, including exposed and sheltered sandy beaches and estuaries (Amaral et al. 2003; Guisla et al. 2004; Denadai et al. 2005; Araújo and Rocha-Barreira 2012; Petracco et al. 2014). Olivid and terebrid species were previously reported as having developed imposex, including *Amalda australis* (Stewart et al. 1992), *Olivancillaria vesica* (Caetano and Absalão 2002), *Olivancillaria deshayesiana* (Teso and Penchaszadeh 2009), and *Olivella biplicata* (Jenner 1979), *Terebra dislocata*, *Terebra protexta*, and *Duplicaria spectabilis* (Shi et al. 2005). However, there are no records or assessment of imposex on *O. minuta* and *H. cinerea*.

Sandy beaches from São Sebastião Channel, northern coast of São Paulo State, and particularly from Pernambuco and Barequeçaba beaches are under the influence area of the São Sebastião Harbor, where previous (Godoi et al. 2003) as well as recent and simultaneous studies by our research group have detected high TBT concentrations in surface sediments and biota (Sant'Anna et al. 2014). Imposex incidence on the muricid *Stramonita haemastoma* has already been detected in Pernambuco and Barequeçaba beaches (Turra et al., unpublished data), indicating a higher contamination in the former. The presence of *O. minuta* in Pernambuco and Barequeçaba beaches and the co-occurrence of *H. cinerea* in the latter provides an opportunity to evaluate the development of imposex in these gastropods for the first time and their use as sentinel of TBT contamination in coastal zones dominated by soft bottoms. In addition to the assessment of imposex in *H. cinerea* in Barequeçaba beach, an area recognized as TBT contaminated, a population of this terebridae sampled previously on Una beach, located in the area with low marine nautical traffic (Jureia-Itatins Ecological Station, southern coast of São Paulo state) was evaluated. In this context, we tested the hypothesis that both species exhibit imposex on beaches of the Canal of São Sebastião, and that imposex incidence differs between beaches for *O. minuta* (Pernambuco>Barequeçaba) and *H. cinerea* (Barequeçaba>Una).

Material and methods

Study areas

São Sebastião Channel is located in the northern coast of São Paulo state. This area is subject to multiple impacts induced by intense urbanization, touristic activities, and intense ship/boat traffic (harbor terminals and several marinas) (Amaral et al. 2010). Additionally, the access to São Sebastião Harbor (SSH) is performed through this channel, leading to several environmental disturbances such as antifouling biocides inputs, oil spills, and dredging (Amaral et al. 2003). Pernambuco (23° 48′ 54″ S, 45° 24′ 26″ W) and Barequeçaba (23° 49′ 43″ S, 45° 26′ 02″ W) beaches border the São Sebastião Channel and are situated near SSH (apart ca. <1.0 and 6.0 km, respectively) (Fig. 1a). Both are sheltered beaches (sensu McLachlan 1980), have gentle slopes, and sediments composed by fine and very fine sand with low and moderate organic-matter content (~0.75 % Barequeçaba; ~2 % Pernambuco) (Nucci et al. 2001; Amaral et al. 2003; Denadai et al. 2005; Petracco et al. 2014).

Una (24° 27′ S; 47° 06′ W) is an exposed beach (sensu McLachlan 1980) situated in the Jureia-Itatins Ecological Station (JIES), in southern São Paulo state, southeastern Brazil (Fig. 1b). The JIES is located in the valley of the Ribeira de Iguape, covering an area of 80,000 ha, in which human access is restricted to a few residents and researchers (Marques and Duleba 2004). In contrast to São Sebastião Channel, this area has a low marine nautical traffic. The sampling area is situated in the northern segment of the beach, with a dissipative morphodynamic state and gentle profile, with sands ranging from fine to very fine, and very low organic-matter content (~0.5 %).

Sampling

In São Sebastião Channel's beaches, samples of *O. minuta* (Barequeçaba and Pernambuco) and *H. cinerea* (only Barequeçaba) were taken monthly during spring low tides, from November 2009 through October 2010. Five transects perpendicular to the waterline were established randomly within a segment of 100 m on each beach. On Una beach (Jureia-Itatins Ecological Station), *H. cinerea* samples were obtained monthly during spring low tides from December 2003 through November 2004. Six fixed transects were sampled from the base of the foredunes to the waterline. On the three above-mentioned beaches, the sediment was sampled in quadrats (50 cm on a side) and at a depth of 10 cm. The samples were sieved through a 1.0-

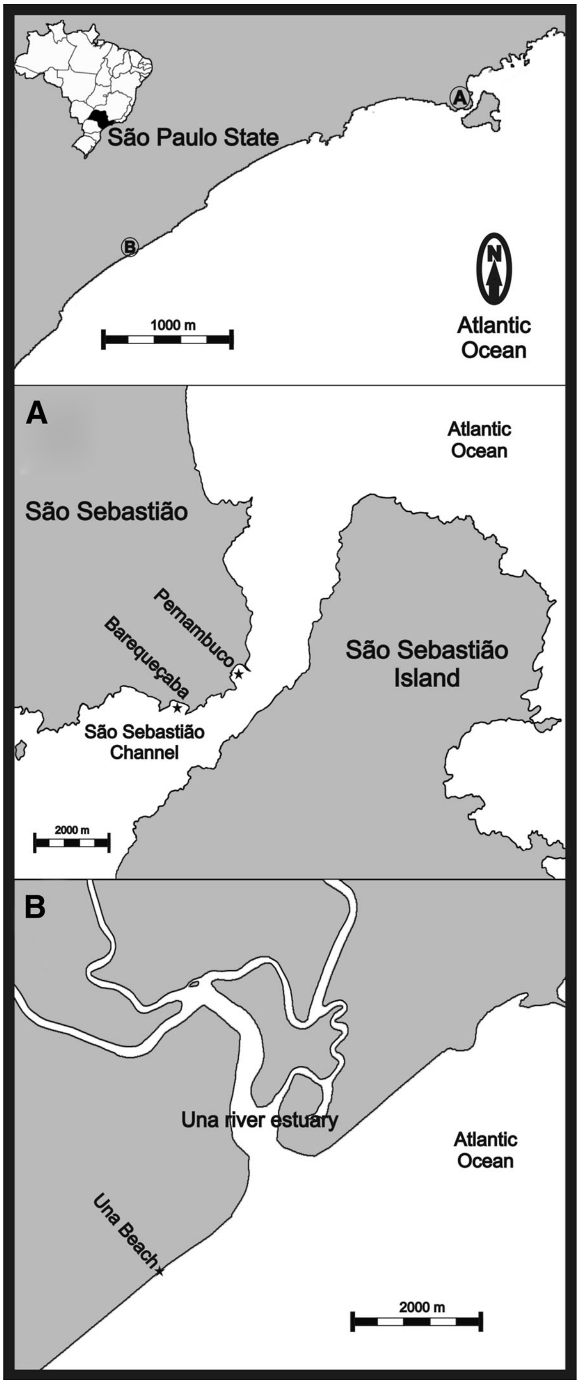

Fig. 1 Study area and location of Pernambuco and Barequeçaba beaches (**a**) and Una beach (**b**)

mm mesh, and the gastropods retained were preserved in 70 % ethanol.

Laboratory procedures

For each *O. minuta* population (Pernambuco and Barequeçaba beaches), a subsample of 500 individuals obtained randomly from the total individuals sampled was used to assess the biometrics and imposex parameters. For *H. cinerea* populations, all individuals sampled were used in the analysis. In the laboratory, the shell length (SL) of *O. minuta* and *H. cinerea* was measured using, respectively, a stereoscopic microscope with an ocular micrometer and a caliper. During this step, juveniles (*O. minuta*: SL<5 mm; *H. cinerea*: SL<22 mm) and organisms with no visible sexual characteristics were discarded from analysis. Thus, the sample used was comprised of 91 and 300 adult individuals of *O. minuta* and *H. cinerea* respectively. Subsequently, the shells were gently broken for the analyses of soft tissues, and the gender identification was performed based on the presence or absence of seminal vesicle (males and females, respectively) (Fig. 2a–d). For both *O. minuta* populations, penis length (PL) of 30 randomly selected males and all imposexed females were measured (Fig. 2e) under a stereoscopic microscope with an ocular micrometer. For *H. cinerea*, the PL was determinate only for the Barequeçaba population (Fig. 2f–h) because imposex affected females from Una showed only vestigial penis. In this case, the PL of all males and imposexed females was measured using the same procedure describe for *O. minuta*. The vas deferens characters were not used to assess the imposex due to the difficulty to visualize this structure accurately in both species.

Data analysis

O. minuta

The imposex levels of *O. minuta* were assessed and compared between Pernambuco and Barequeçaba beaches initially using two traditional indices: the imposex percentage (% *I*), which was calculated as the proportion of imposexed females affected compared to the total number of females in the sample and the relative penis length index (RPLI) obtained by Eq. (1) (Gibbs et al. 1987).

$$\mathrm{RPLI} = \left[\frac{\text{mean female PL}}{\text{mean male PL}}\right] \times 100 \quad (1)$$

The occurrence of a statistical relationship between shell length (SL) and penis length (PL) was investigated by an exponential regression. Afterwards, an independent *t* test was performed to compare shell sizes between organisms from Pernambuco and Barequeçaba beaches. Thus, considering that there is a significant correlation between PL and SL in *O. minuta,* and that the studied populations presented different sizes (see results), a SL standardized $\mathrm{RPLI_{stand}}$ (Eq. 2) was calculated according to Castro and Fillmann (2012).

$$\mathrm{RPLI_{stand}} = \left[\frac{\text{mean female PL/mean female SL}}{\text{mean male PL/mean male SL}}\right] \times 100 \quad (2)$$

Finally, in order to verify the sex ratio alterations in the studied *O. minuta* populations a chi-square test (χ^2) with Yates correction for continuity (Zar 1999) was used. Prior to the statistical analyses, the normality and homogeneity of data (SL and PL) were verified using Shapiro-Wilk and Levene tests, respectively. All statistical analyses were performed using Statistica® (version 12.0 (Statsoft)) with a significance level of 0.05.

H. cinerea

For *H. cinerea*, the imposex percentage (% *I*) was calculated for both populations, while the RPLI was estimated only for the Barequeçaba population, since the penis length of males and imposexed females were not determined to the Una population. According to Gibbs and Bryan (1987), the early imposex stages can be adequately expressed as the percentage of females bearing a penis. As already mentioned, females from Una presented trace penis and in low frequency, characterizing an early imposex stages. An independent *t* test was performed to compare shell sizes of males and females on Barequeçaba Beach. Given the absence of differences in shell size (see results), the common relative penis length index (RPLI: Eq. 1) was used for this population. The sex ratio of *H. cinerea* populations

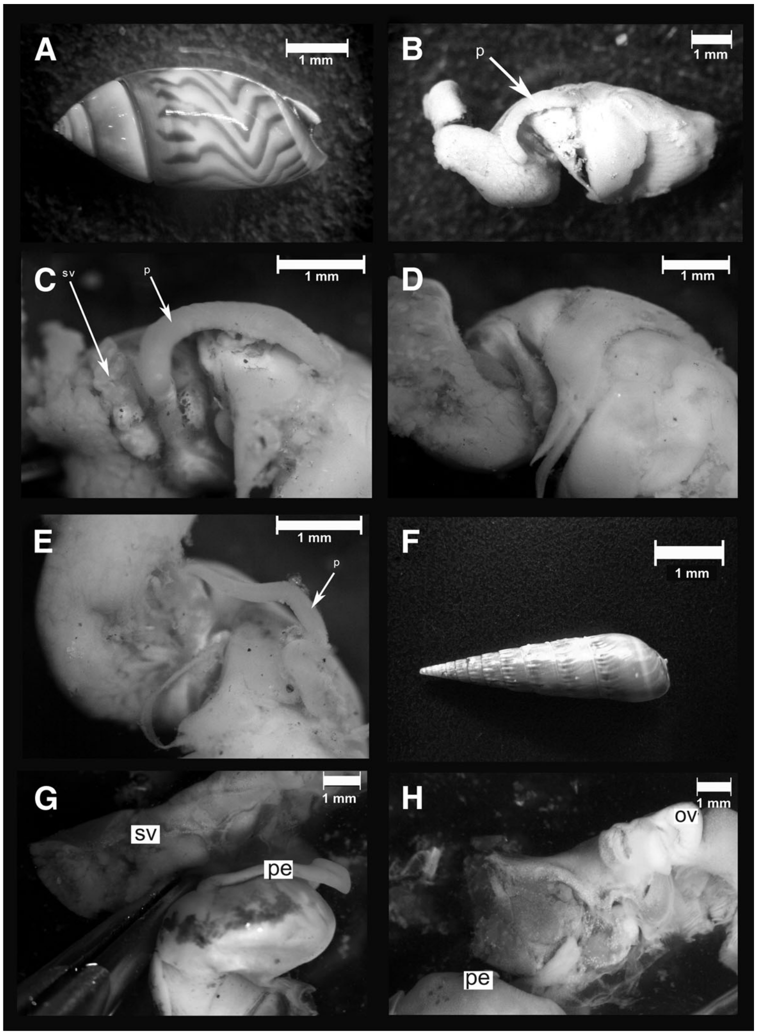

Fig. 2 *Olivella minuta*: **a** shell; **b** soft parts of a male with penis indicated (*p*); **c** detail of the penis (*p*) and the seminal vesicle (*sv*) of a male; **d** soft parts of a female without imposex (absence of seminal vesicle); **e** detail of the penis of an imposex affected female. *Hastula cinerea*: **f** shell; **g** detail of the penis (*p*) and the seminal vesicle (sv) of a male; **h** detail of the oviduct (*ov*) and the penis (*p*) of an imposex affected female

was also compared using a chi-square test (χ^2) with Yates correction for continuity.

Results and discussions

O. minuta individuals obtained on Pernambuco beach were distributed in 35 juveniles, 292 females, and 173 males. For the samples from Barequeçaba Beach, 254 were females, 246 males, and no juveniles were found. The penis length in males and females considering pooled *O. minuta* populations proved to be dependent on shell size (Fig. 3). The PL and SL values were pooled by gender since the slope of the relationships between these variables were not different between males (*t* test: $t_{1,56}$=0.10, p>0.05) or females (*t* test: $t_{1,27}$=0.49, p>0.05). The relatively lower regression coefficient (r^2) for females was due to the low imposex levels observed in the study areas, since PL in imposexed females is TBT dependent and not SL dependent (see below). Additionally, significant variations were also detected between *O. minuta* shell sizes (*t* test; $t_{1.185}$= −4.12, p<0.001) from Pernambuco and Barequeçaba. The RPLI considers biometric parameters that may be influenced by differences in organism sizes (Galante-Oliveira et al. 2010). Therefore, in the present study, RPLI values were standardized by SL to reduce bias, and allow comparison between imposex parameters obtained for Peranambuco and Barequeçaba (Table 1). Similar situations were previously reported for the muricids *Bolinus brandaris* from Ria Formosa

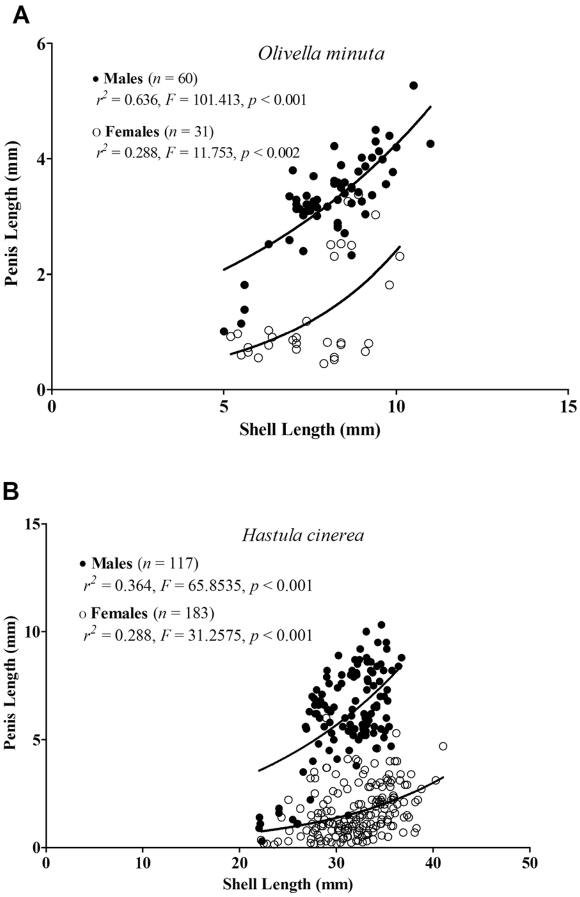

Fig. 3 Exponential regression between shell length and penis length for **a** males (y=0.7046e$^{0.1854x}$) and females (y=0.1918e$^{0.2267x}$) of *O. minuta* from Pernambuco and Barequeçaba beaches, and for **b** males (y=0.2917e$^{0.0403x}$) and females (y=0.0645e$^{0.0140x}$) of *H. cinerea* from Barequeçaba beach

(Algarve, southern Portugal) (Vasconcelos et al. 2011) and *Thais chocolata* from Peru (Castro and Fillmann 2012). These studies suggest that the assessment of imposex spatial variations should include robust and reliable indices, such as standardized RPLI.

TBT concentrations in environmental samples are frequently well correlated with RPLI values (Galante-Oliveira et al. 2006) and also SL standardized data (Castro and Fillmann 2012). The RPLI is an imposex parameter that roughly balances the penis sizes of males and females at each site, being less affected by interindividual variability than by vas deferens sequence index (VDSI) (Stroben et al. 1992). Thus, relative penis indices have been confirmed to be more responsive to TBT contamination than VDSI. This stems partially from the use of adult specimens, which are known to be scarcely susceptible to change of VDSI stages (Quintela et al. 2000). As the observation of vas deferens characters in *O. minuta* and *H. cinerea* was not possible in the present study, no vas deferens sequence index (VDSI) was calculated. However, as previously mentioned, not using the VDSI did not impair the evaluation of the viability of *O. minuta* and *H. cinerea* as a sentinel of TBT contamination.

Higher imposex levels in *O. minuta* were observed on Pernambuco (%I=9.36, RPLI=4.49 and RPLI$_{stand}$=4.27) than on Barequeçaba (%I=2.42, RPLI=0.36 and RPLI$_{stand}$=0.81) (Table 1). In fact, Pernambuco beach is located in the vicinity of the São Sebastião harbor (<1.0 km) (Fig. 1a) where a recent study detected TBT concentrations of up to 105 ng Sn g^{-1} in surface sediments. The very sheltered conditions on Pernambuco Beach in the inner part of Araçá Bay, combined with the higher proportion of silt and organic matter, probably also led to a higher adsorption and longer residence time of TBT in the sediments than on Barequeçaba (Langston and Pope 1995; Pinochet et al. 2009; Sant'Anna et al. 2014). The lower imposex levels on Barequeçaba beach, located farther (ca. 6.0 km) from SSH and from local marinas suggest a gradient of TBT contamination related to ship and/or boat activities. Our research group has recently detected a higher imposex percentage for the muricid *Stramonita haemastoma* on Pernambuco beach than on Barequeçaba beach (Turra et al., unpublished data), indicating a lower contamination in the latter and showing a similar trend observed in present study. Similar findings have been frequently reported in several coastal areas, always pointing out harbors and/or marinas as hotspots of TBT contamination (Titley-O'Neal et al. 2011).

H. cinerea individuals obtained on Barequeçaba and Una beaches were distributed, respectively, in 95 juveniles, 186 females, and 117 males, and 828 juveniles, 199 females, and 181 males. As verified for *O. minuta*, penis length of males and females of *H. cinerea* in the Barequeçaba population was exponentially and significantly related to shell size (Fig. 3b). The shell length, however, did not differ between sexes (t test; $t_{1.301}$=1.52, p>0.05). Supporting the hypothesis of this study, the imposex percentage of *H. cinerea* on Barequeçaba (%I=98.92) was higher than on Una beach (%I=12.45) (Table 1). Given the low marine nautical traffic in the vicinity of Jureia-Itatins Ecological Station (JIES), the occurrence of imposex in this *H. cinerea* population is initially unexpected, although moderate. Nevertheless, Sant'Anna et al. (2014) recently showed high TBT

Table 1 Mean shell length and penis length of males and females, imposex percentage (% imposex), relative penis length index (RPLI), relative penis length index standardized ($RPLI_{stand}$), of *O. minuta* (Pernambuco and Barequeçaba beaches) and *H. cinerea* (Barequeçaba and Una beaches). Note: on Una beach, the penis length and the RPLI of *H. cinerea* were not obtained

Imposex indicator/species/beaches	*Olivella minuta*		*Hastula cinerea*	
	Pernambuco	Barequeçaba	Barequeçaba	Una
Male shell length (mm)	7.38 (±1.01)	8.95 (±0.86)	31.91 (±3.95)	–
Female shell length (mm)	7.77 (±1.30)	6.92 (±1.63)	31.27 (±3.95)	–
Male penis length (mm)	2.86 (±0.81)	4.04 (±0.64)	6.21 (±2.07)	–
Female penis length (mm)	0.13±(0.98)	0.03 (±0.71)	1.68 (±1.18)	–
% imposex	9.36	2.42	98.92	12.45
RPLI	4.49	0.36	26.65	–
$RPLI_{stand}$	4.27	0.81	–	–

contamination in surface sediments from several estuaries of the Brazilian coast, including Iguape estuary, where high TBT concentration (200 ng Sn g^{-1}) was detected on the mouth of the Ribeira de Iguape River. Despite the distance between the Iguape estuary and Una beach (~35 km), this TBT source can be responsible by the development of imposex in this *H. cinerea* population.

The imposex incidence in gastropods is TBT dependent (Matthiessen and Gibbs 1998) but also related to environmental concentrations (Gibbs 2009), exposure pathway (Lima and Castro 2006; Rossato et al. 2014), exposure time (Fernandez et al. 2007), and species sensibility (Castro et al. 2012c). Thus, different species can develop different imposex stages, even in similar exposure conditions (Bech 1999). The simultaneous sampling of *H. cinerea* and *O. minuta* species on Barequeçaba beach, where they coexist in the same intertidal zone, allow for an adequate comparison in the TBT sensitivity between these species. The higher levels of imposex in *H. cinerea* on Barequeçaba beach (% I=98.92; RPLI=26.65) than in both *O. minuta* populations studied indicates that *H. cinerea* is much more TBT-sensitive than *O. minuta*. In fact, *H. cinerea* seems to be a very TBT-sensitive species since the imposex percentage was also higher than the detected for the muricid *Stramonita haemastoma*, a species widely used as TBT biomonitor on Barequeçaba beach (% I=~45 %) and close to that estimated for S. *haemastoma* on Pernambuco beach (% I=~95 %) (Turra et al., unpublished data). Regarding the use of *O. minuta* and *H. cinerea* as TBT biomonitor species, both show advantages and disadvantages. On one hand, *H. cinerea* has the advantage of greater sensitivity to exposure to TBT than *O. minuta*; on the other hand, there are no records of *H. cinerea* on exposed beaches with reflective morphodynamic state, unlike *O. minuta*. In addition, despite the general scarcity of information on the ecology of *H. cinerea* (Petracco 2008), the occurrence of this terebridae has been frequently recorded in sandy environments (e.g., Rios 1994; Trueman and Brown 1992), particularly on sandy beaches. Therefore, although this gastropod can also inhabit estuarine sandy beaches (Petracco, personal observation), its occurrence seems to be more restricted than O. *minuta*. In fact, besides the frequent occurrence in sheltered, and in reflective and dissipative exposed sandy beaches (Denadai et al. 2005; Petracco et al. 2014; Rocha-Barreira et al. 2005), *O. minuta* is also frequently recorded in tidal flats and estuaries (e.g., Boehs et al. 2004; Rolemberg et al. 2008).

The sex ratio of *O. minuta* (Pernambuco F:M= 1.71:1, Barequeçaba F:M=1.03:1) and *H. cinerea* (Barequeçaba F:M=1.59:1, Una F:M=1.10:1) differed between beaches (χ^2 test= 6.05 and 5.21, $p<0.05$, respectively). In this context, although some studies have found that imposex can lead to a decrease of female proportion in some gastropod populations due to premature female mortality (Gibbs and Bryan 1987; Evans 1999), the higher proportion of females of *O. minuta* and *H. cinerea* in areas with lower imposex levels indicate that this TBT effect on the females of the populations studied is highly unlikely. These differences in the sex ratios between the populations of both species probably result from some differences in population structure features, such as across-shore distribution, and size structure. For instance, the lower proportion of females on Barequeçaba than on Pernambuco is probably related to the different spatial distributions of *O. minuta* on these beaches (Petracco et al. 2014). While on Pernambuco beach all size classes of *O. minuta* occurred in the intertidal zone, on Barequeçaba, a sharp size stratified across-shore distribution occurred (Petracco et al. 2014). The smaller shell size of females than males on Barequeçaba (*t* test; $t_{1,498}=2.31$, $p<0.05$) associated with the size stratified distribution may have led to a lower proportion of females in the *O. minuta* population on Barequeçaba than on Pernambuco. On the other hand, since females of *H. cinerea* reached larger size than males on Barequeçaba (see Fig. 3b) and Una (Petracco 2008) and that *H. cinerea* presents a larger size on Barequeçaba (40 mm) than on Una Beach (31 mm), the sex ratio displaced to the females on Barequeçaba can derive from these differences in the size structure between the populations.

After the recent national and international restrictions to the use of antifouling TBT-based paints (Borges et al. 2013), imposex recovery and reductions in TBT environmental levels have been observed in some areas in the Brazilian coast (Fernandez 2006; Castro et al. 2012d). However, antifouling TBT-based paints are still sold in Brazil (Toste et al. 2011), which may be contributing to recent TBT inputs. In addition, previous studies demonstrated high TBT contamination in surface sediments from São Sebastião Harbor (Godoi et al. 2003; Sant'Anna et al. 2014). Hence, the imposex occurrence in *H. cinerea* and *O. minuta* in São Sebastião Channel reported in the present study may be due to fresh TBT inputs near the study area and/or to the high persistence of TBT in the sediment layers (Choi et al. 2010). Therefore, based on imposex incidence in *O. minuta* and *H. cinerea,* it was not possible to determine if the TBT contamination observed in the present study is recent or not. However, TBT contamination in São Sebastião Channel and Jureia-Itatins Ecological Station must be monitored by further studies on the imposex incidence in *O. minuta* and *H. cinerea* populations to verify the effectiveness of international and local TBT restrictions in these areas.

Considering the wide geographical distribution (Rios 1994; Simone 2009), high abundance, ease of collection, and the ability to develop imposex, *O. minuta* and *H. cinerea* fulfill all requirements for a suitable sentinel of coastal contamination by TBT. In addition, their occurrence in different unconsolidated coastal environments, including sandy beaches (sheltered and exposed) and estuaries (mainly *O. minuta*) (Denadai et al. 2005; Petracco 2008; Petracco et al. 2014), has potential to fill current gaps in biomonitoring studies of the TBT pollution in South American areas. This finding is especially important since soft-bottom coastal environments restrict the occurrence of typical TBT biomonitor species.

In summary, the present study points out moderate and high imposex incidence, respectively in *O. minuta* and *H. cinerea* from São Sebastião Channel, indicating TBT contamination in the area, which was confirmed (in the case of Pernambuco beach) by chemical determinations of TBT in surface sediments. For *O. minuta* populations, the imposex levels were higher on Pernambuco than on Barequeçaba, suggesting a gradient of TBT contamination, whose sources are probably harbors and marinas located in the northern part of São Sebastião channel. The imposex incidence in *H. cinerea* on Una beach, located in an area with low marine nautical traffic, probably results from TBT contamination from the Iguape estuary and reinforces the need for assessing TBT contamination on both marine and estuary environments. In the perspective of a more suitable TBT sentinel of soft bottoms, *H. cinerea* has the advantage of being a more TBT-sensitive species than *O. minuta*, although the latter seems to occur in a wider range of coastal environments. In addition, the results obtained here can act as a useful baseline for the assessment of future trends in TBT contamination in the area, contributing to verify the effectiveness of restrictive regulations on the use of TBT-based antifouling paints.

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