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Imposex assessment and tributyltin levels in sediments along the Atlantic coast of South Africa

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ABSTRACT

Female marine gastropods develop imposex (growth of penis/vas deferens) when exposed to TBT (tributyltin). Ours, is the first report of an imposex survey associated with TBT in sediment along 920 km of South Africa's Atlantic coastline. We sampled and analysed 1389 individuals of 13 caenogastropod species, and sediment samples from 25 sites, presumed impacted and not impacted by TBT pollution. Imposex was detected in six species not previously reported to suffer from this phenomenon, at eight sites, with up to 100% of females affected. Butyltins were found at quantifiable concentrations at four sites, with TBT and DBT (dibutyltin) concentrations in sediments up to 20 000 μg/kg dry mass (dm) and 3740 μg/kg dm, respectively. These findings are of major concern considering that TBT has been banned globally since 2008 by the International Maritime Organisation (IMO) - more extensive research is required in areas affected by TBT and where aquaculture is present.

1. Introduction

South Africa's coastline is relatively pristine when compared to other countries (Branch et al., 2010; Wepener and Degger, 2012). However, South African marine ecosystems are under threat from many anthropogenic activities (Du Preez et al., 2018; Mead et al., 2013; Ryan et al., 2012). One of the potential threats is posed by TBT (tributyltin).

TBT is an organotin compound that has been used extensively since the 1960s, on marine vessels of all sizes, to prevent the formation of biofilms (USEPA, 2003). Its uncontrolled use in the 1970s and early 1980s had various harmful, environmental consequences on marine invertebrates (Fent, 2006) such as shell thickening in oysters, *Crassostrea gigas* (Alzieu et al., 1986), or the neogastropod *Odontocymbiola magellanica* (Márquez et al., 2010). Imposex was first reported in dogwhelks, *Nucella lapillus* (Gibbs and Bryan, 1987) and later in more than 200 gastropod species (Bigatti et al., 2009). TBT has caused reproductive alterations (Primost et al., 2015) and sterilisation in marine gastropods (Gibbs, 2009), and even immune suppression in marine mammals (Kannan et al., 1996).

The causal relationship between TBT and its harmful effects on marine organisms provided enough evidence for the IMO to call for the complete, global ban of the application of organotin compounds in antifouling systems on ships by 1 January 2008 (IMO, 2014). Once regulations came into force, TBT concentrations in coastal waters, sediments, and tissues of molluscs have declined in some areas concomitant with a recovery of affected marine ecosystems (Lahbibab et al., 2018; Laranjeiro et al., 2018; Wells et al., 2017). Nevertheless, TBT contamination and incidences of imposex continues to be reported globally, especially in developing countries (Batista et al., 2016; Batista-Andrade et al., 2018; Maciel et al., 2018).

Marine gastropods are useful bioindicators of TBT pollution (Matthiessen and Gibbs, 1998), because the relative size of the female's imposed penis can be correlated with the collection locality in relation to boating activity (Gibbs et al., 1987). TBT may induce imposex at relatively low concentrations (Bryan et al., 1986). Concentrations of more than 2–4 ng/L are capable of inhibiting breeding activity, which cause sterility, subsequent population decline, and ultimately local extinction (Gibbs and Bryan, 1987). This, in turn, may ultimately affect coastal ecology (Crowe et al., 2000). The USEPA (2003) has set the following criteria in order to protect organisms from chronic effects; levels of TBT should not exceed 0.072 μg/L in freshwater and 0.0074 μg/L in saltwater environments.

There is very limited information available regarding TBT compounds in the marine environment of Africa (Titley-O'Neal et al., 2011). Imposex in South Africa was only reported by one study (Marshall and Rajkumar, 2003), with 3.7–100% imposex incidence in the dogwhelk

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Nassarius kraussianus from Durban and Richards Bay Harbours on the Indian Ocean of South Africa. TBT concentrations were not analysed. Another study reported TBT concentrations in sediment from Cape Town Harbour (Okoro et al., 2016) of between 10 and 829 μg/kg dm. Imposex was not examined. Elsewhere in Africa, TBT concentrations in sediment ranged between 20 and 2 6500 μg/kg dm for DBT in Zanzibar Port (Tanzania), and non-detectable to 3670 μg/kg dm for TBT. In Dar es Salaam Port (Tanzania), concentrations were between 1 and 79 300 μg/kg dm for DBT, and from non-detectable to 15 900 μg/kg dm for TBT (Sheikh et al., 2007). Imposex was not investigated. In Morocco, 100% imposex incidence was found in muricids species sampled at Mdiq and Tangier Harbours, with lower prevalence (0–50%) at other harbours (Lemghich and Benajiba, 2007), but TBT was not analysed. Lopes-dos-Santos et al. (2014) found up to 100% imposexed females of the caenogastropod *Gemophos viverratus* from two sites near the Porto Grande Harbour on the islands of the Cape Verde, Atlantic Ocean. TBT was present at levels of up to a mean of 37 μg/kg dm in gastropod tissue from a 100% imposexed site.

This study is the first report of a combined imposex-sediment approach to evaluate organotin contamination along 920 km of the Atlantic coast of South Africa. This was done by sampling and analysing caenogastropods for imposex and sediment from sites along the coast from Cape Agulhas to Port Nolloth to cover presumed TBT-polluted sites (harbours with maritime traffic) and presumed pristine areas (Marine Protected Areas with low maritime traffic).

2. Materials and methods

2.1. Site selection

South Africa is located along a primary shipping route between Europe, the Americas, and Asia (Marshall and Rajkumar, 2003). Coastal sites selected for this study are from areas of high maritime traffic that are presumed polluted by TBT from ships, and areas of low maritime traffic, supposedly free of organotin pollution. High impact sites were deliberately chosen to be near or within a marina or harbour, where contamination from antifouling agents would be expected to be greatest. Low impact sites were areas, chosen as reference sites, usually on open coast or Marine Protected Areas (MPAs), such as the West Coast National Park (Zone A, B, C and Tsarsbank), Cape Agulhas, Soetwater, Jacobsbaai, Betty's Bay, Bird Island etc. (Table 1; Fig. 1) where contamination by antifouling agents is presumed to be significantly lower or absent.

The sampling sites were ranked on boating activity, using criteria adapted from Ten Hallers-Tjabbes et al. (2003), classified according to the number of vessels berthed in/or passing the specific sampling site, at the time of collection: high boating activity (> 10 vessels), medium boating activity (5–10 vessels), low boating activity (1–5 vessels) and no boating (0 vessels). We further used Ports and ships (2015) to confirm the level of boating activity for each harbour. Ports and ships (2015) is a website that provides information on the maritime activity within the harbours of South Africa at any given date and time. Through our observations at the time of collection, and information provided by the previously mentioned website, we ranked boating activity as follows: 12 sites had no boating activity, five sites had low boating activity, two were rated as medium activity, and six had high boating activity (Table 1).

2.2. Collection of gastropods

Gastropods and sediments were sampled from 25 sites along approximately 920 km of the Atlantic coastline (Fig. 1) of South Africa, from Cape Agulhas at the southern tip of Africa (S34°49′42.9"; E20°00′41.9") to Port Nolloth (S29°15′26.1"; E16°52′03.7") (Fig. 1; Table 1). Sampling occurred during two collection surveys; March–April 2013 and March–April 2014. Both surveys were scheduled during the same season (March/April) to reduce the influence of seasonality on the results. Gastropods were collected from the intertidal or sub-tidal zone by baited traps, or manually collected during low-tide/mid-tide. A sample size of 20–30 individuals of each species of gastropod was collected. This ensured that a representative proportion of females was obtained for statistical analysis. The samples were stored at −20 °C for further analysis. Since the Gastropoda are considered 'lower invertebrates,' no ethical clearance was required.

2.3. Imposex

Mature adults were used because their reproductive organs are completely developed, their sex is easily distinguishable (Gibbs et al., 1987). The sexes of all collected gastropods were determined based on the presence (females) or absence (males) of albumen, capsule, pedal and sperm-ingesting glands along with the colour of the gonads (characteristic of each sex) (Gibbs et al., 1987; Gibbs and Bryan, 1987). In some cases when the sex was not easily identifiable, sex was verified by examining gametes from a smear of the gonad. Females that showed a penis-like structure were classified as imposexed. The total penis length was measured from the base to the tip in males and imposexed females using a AZ100 Multi-Zoom Nikon compound dissection microscope. Once the necessary measurements were completed, the imposex parameters were calculated, e.g. Relative Penis Length Index (RPLI), Relative Penis Size Index (RPSI), and Imposex (%I) were used to quantify imposex and, subsequently, correlated with the concentration of TBT in co-collected sediment (Titley-O'Neal et al., 2011). The male to female ratio (M:F) was also calculated to provide supplementary information regarding imposex severity.

Percentages of imposex-affected females were calculated as the number of females with penis and/or vas deferens with respect to all females sampled of each species (Bigatti et al., 2009). The mean female penis length (mFPL) and mean male penis length (mMPL) are used to calculate the RPLI and RPSI, but they are also used independently to measure imposex severity (Titley-O'Neal et al., 2011). Since the female penis enlarges with imposex development, its length can be compared to that of the male in the same population. Length is the most useful parameter of penis size (Bryan et al., 1986; Gibbs et al., 1987; Titley-O'Neal et al., 2011). The RPLI is calculated as the mFPL/mMPL x 100. The larger the value of this index, the more serious the imposex effect is at a particular site.

Body size, including penis size can differ significantly between populations (Gibbs et al., 1987). The influence of variability is reduced by basing the RPS on a comparison of female and male penis size in individuals from the same population. The RPS index for any population is defined as the mean bulk of the female penis and is expressed as percentage of the mean bulk of the male penis from the same species and collection site. The bulk of a penis is calculated as the cube of its length: $(\mathrm{mFPL})^3/(\mathrm{mMPL})^3$ x 100 for the population. The rationale for this is that length is a convenient measure of tissue mass (Tan, 1999). An RPS value of 50% indicates that the mean penis size of the female is half the bulk of that of the male (Bryan et al., 1986; Gibbs et al., 1987).

Imposex %, RPSI, and RPLI were used together with information on boating activity, among the various sites they were sampled, to identify species that are useful as indicators of high-medium-low TBT pollution (regardless of their feeding, habitat or other features, although probably influenced by them). It was assumed that species that demonstrated a higher sensitivity towards TBT pollution were those that displayed a high percentage of imposex, and high RPLI and RPSI values at low to no boating sites. Species that displayed imposex only at high boating sites were presumed less sensitive.

2.4. Sediment collection and TBT/DBT analyses

TBT is deposited in marine sediments where it can be bioavailable for many years (Strand and Asmund, 2003), inducing imposex

Table 1
Sites, boating activity, species, total sampled per site, male to female ratio, %Imposex, RPLI, RPSI, and DBT and TBT concentrations from Cape Agulhas to Port Nolloth. The localities are indicated in Fig. 1.

No	Site Name	Boating	Species	N	M:F	% Imposex	RPLI	RPSI	DBT (μg Sn/kg dm)	TBT (μg Sn/kg dm)
1	Cape Agulhas	No	*Afrolittorina knysnaensis* (Krauss in Philippi, 1847)	30	1:4				< 5	< 2
			Burnupena lagenaria (Lamarck, 1822)	30	1:2.3	14.3	9.8	0.09	< 5	< 2
2	Struisbaai Harbour	Medium	*Mancinella capensis* (Petit de la Saussaye, 1852)	30	8:22				< 5	< 2
3	Gansbaai	Low	*Burnupena lagenaria* (Lamarck, 1822)	30	1:2				< 5	< 2
4	Gansbaai Harbour	High	*Burnupena cincta* (Röding, 1798)	24	1:1	91.7	9.4	0.08	< 5	< 2
			Burnupena lagenaria (Lamarck, 1822)	30	1:0.3				< 5	< 2
			Clionella sinuata (Born, 1778)	30	1:29				< 5	< 2
5	Betty's Bay	No	*Bullia rhodostoma* (Reeve, 1847)	30	1:0.8	52.9	1.9	0.0006	< 5	< 2
			Burnupena cincta (Röding, 1798)	26	1:0.7				< 5	< 2
6	Gordon's Bay Harbour	High	*Burnupena cincta* (Röding, 1798)	23	1:0.8	100	80.4	52	591	20 000
7	Strand	Low	*Burnupena catarrachta* (Gmelin, 1791)	30	1:2.3				< 5	< 2
8	Simon's Town Yacht Club	High	*Afrolittorina knysnaensis* (Krauss in Philippi, 1847)	30	1:0.9				610	7930
9	Soetwater	No	*Afrolittorina knysnaensis* (Krauss in Philippi, 1847)	30	1:1.1				< 5	< 2
			Burnupena catarrachta (Gmelin, 1791)	30	1:6.5				< 5	< 2
10	Lagoon Beach	High	*Bullia digitalis* (Dillwyn, 1817)	30	1:29	6.9	14	0.25	< 5	< 2
			Burnupena rotunda (Dempster & Branch, 1999)	20	1:1.5				< 5	< 2
11	Melkbosstrand	No	*Bullia digitalis* (Dillwyn, 1817)	29	1:13.5				< 5	< 2
			Burnupena catarrachta (Gmelin, 1791)	30	1:4				< 5	< 2
12	Langebaan Zone A (WCNP)	Low	*Bullia laevissima* (Gmelin, 1791)	27	1:2.4	36.8	2.8	0.002	< 5	< 2
			Bullia rhodostoma (Reeve, 1847)	30	0:30				< 5	< 2
			Burnupena cincta (Röding, 1798)	30	1:1.3				< 5	< 2
			Burnupena lagenaria (Lamarck, 1822)	30	1:1.5				< 5	< 2
			Burnupena papyracea (Bruguière, 1789)	30	1:2.8				< 5	< 2
13	Langebaan Zone B (WCNP)	No	*Afrolittorina knysnaensis* (Krauss in Philippi, 1847)	30	1:1.7				< 5	< 2
			Nucella dubia (Krauss, 1848)	21	1:1.3				< 5	< 2
14	Langebaan Zone C (WCNP)	No	*Burnupena cincta* (Röding, 1798)	21	1:0.6				< 5	< 2
15	Tsarsbank (WCNP)	No	*Afrolittorina knysnaensis* (Krauss in Philippi, 1847)	30	1:5				< 5	< 2
			Burnupena catarrachta (Gmelin, 1791)	30	1:1.5				< 5	< 2
16	Saldanha Bay	High	*Bullia digitalis* (Dillwyn, 1817)	30	1:4	100	13.2	0.23	< 5	< 2
			Burnupena cincta (Röding, 1798)	30	1:1.7	31.6			< 5	< 2
17	Saldanha Bay Harbour	High	*Afrolittorina knysnaensis* (Krauss in Philippi, 1847)	30	1:1.5	50	48.3	11.2	3740	14 400
18	Jacobsbaai	No	*Afrolittorina knysnaensis* (Krauss in Philippi, 1847)	30	1:2.3				< 5	< 2
			Burnupena catarrachta (Gmelin, 1791)	30	1:1.7				< 5	< 2
			Clionella sinuata (Born, 1778)	30	0:30				< 5	< 2
19	Lambert's Bay Harbour	Medium	*Burnupena catarrachta* (Gmelin, 1791)	30	1:1.3				415	495
20	Bird Island (Lambert's Bay)	No	*Burnupena catarrachta* (Gmelin, 1791)	30	1:0.9				< 5	< 2
21	Strandfontein	No	*Bullia digitalis* (Dillwyn, 1817)	30	1:2.8				< 5	< 2
			Burnupena lagenaria (Lamarck, 1822)	30	1:3.3				< 5	< 2
			Burnupena rotunda (Dempster & Branch, 1999)	30	1:2				< 5	< 2
			Trochia cingulata (Linnaeus, 1771)	30	1:1.5				< 5	< 2
22	Hondeklipbaai Harbour	Low	*Burnupena catarrachta* (Gmelin, 1791)	28	1:3.7				< 5	< 2
23	Hondeklipbaai	No	*Afrolittorina knysnaensis* (Krauss in Philippi, 1847)	30	1:1.7				< 5	< 2
			Burnupena catarrachta (Gmelin, 1791)	30	1:1.3				< 5	< 2
24	Port Nolloth (McDougall Bay)	No	*Burnupen catarrachta* (Gmelin, 1791)	30	1:3.3				< 5	< 2
			Clionella sinuata (Born, 1778)	30	1:9				< 5	< 2
25	Port Nolloth Harbour	Low	*Afrolittorina knysnaensis* (Krauss in Philippi, 1847)	30	1:1.1				< 5	< 2
			Burnupena catarrachta (Gmelin, 1791)	30	1:1.3				< 5	< 2
			Burnupena rotunda (Dempster & Branch, 1999)	30	1:2				< 5	< 2

development even at concentrations as low as 0.5 μg/kg dm in the sediments inhabited by the gastropods (Ten Hallers-Tjabbes et al., 2003). Sediment was collected from three different points within each of the 25 sites using a shovel, scraping from the top 5 cm, reportedly where organotins accumulate (Clark et al., 1988). The sediment samples were placed in plastic bottles, wrapped in aluminium foil to exclude sunlight, and stored at −20 °C for further chemical analysis of TBT and its derivatives.

The EU accredited RPS Group, in the United Kingdom performed analysis for TBT and DBT concentrations in the sediment samples. The samples were freeze-dried prior to shipping. Samples were dried at 105 °C, but organic content was not determined. Organotins were extracted into acidified methanol, and reacted with sodium tetraethylborate (STEB) to produce the ethyl derivative, which was then liquid-liquid extracted into hexane. The hexane extracts were cleaned up by neutral alumina solid adsorption chromatography. Analysis was

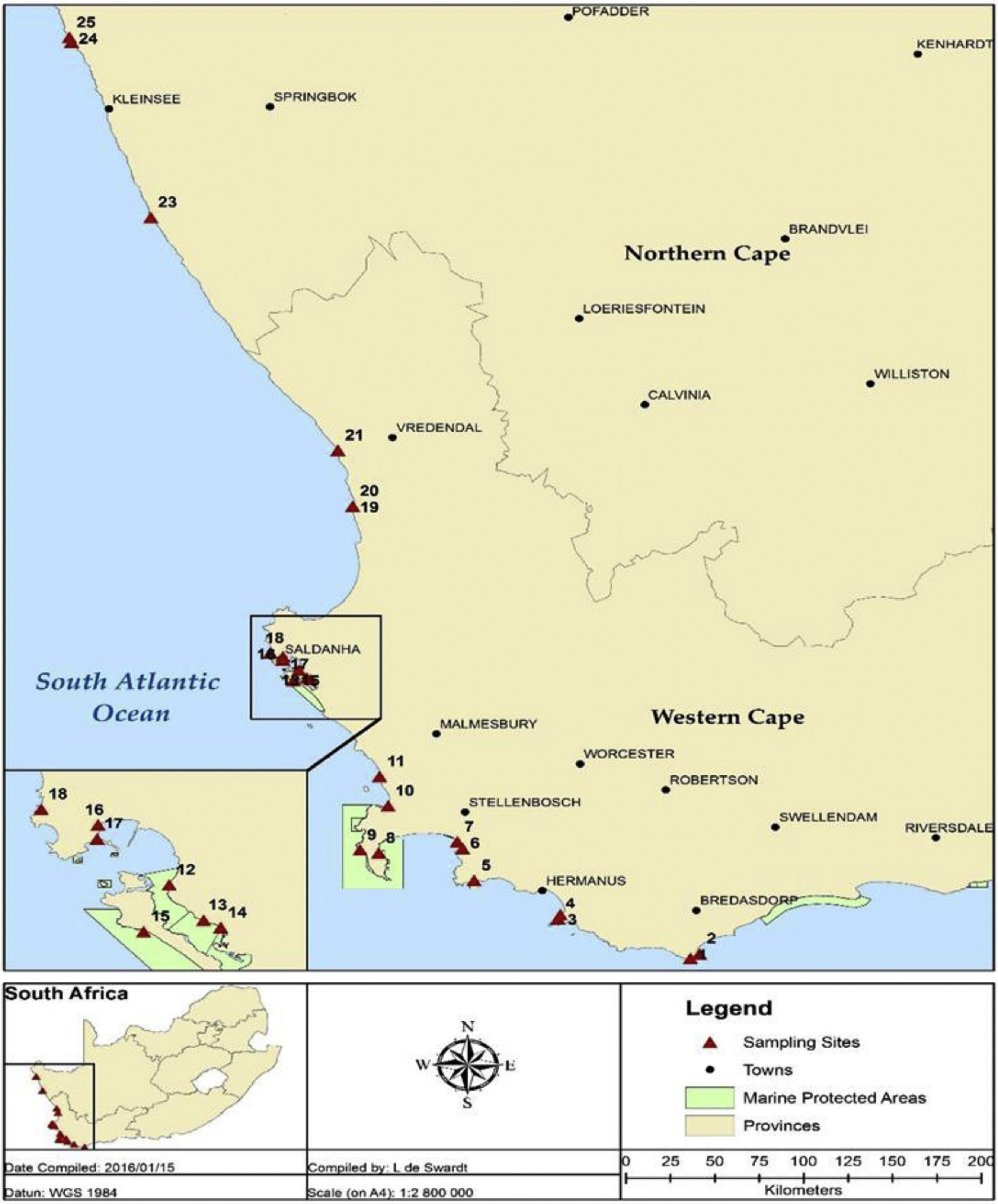

Fig. 1. Map of collection sites along the Atlantic coastline of South Africa. The site numbers correspond to Table 1.

performed by gas chromatography – tandem mass spectrometry (GC-MS/MS). TBT concentrations in sediment from Gordon's Bay Harbour, Simon's Town Yacht Club, Saldanha Bay Harbour, and Lambert's Bay Harbour were outside the dynamic range of the analytical method and required dilution to bring the results within the linear calibration range. The limits of quantification were 5 μg/kg dm, and 2 μg/kg dm for DBT and TBT, respectively.

2.5. Statistical analyses

Summary statistics, one-way ANOVA, and non-linear regressions were done using Prism (version 7.04; www.graphpad.com). Data were not normally distributed (D'Agostino and Pearson normality test). We used unpaired, non-parametric Kruskall-Wallis ANOVA for %Imposex, RPLI, and RSPI, with Dunn's post-test comparisons. Non-linear regression was done using agonist (TBT concentrations) vs. normalised response (between 0% and 100%, since all three parameters varies between 0 and 100%), with variable Hill slope (allowing different steepness of slope for each parameter, rather than 1.0 for all slopes) using least squares fitting. The concentration on the x-axis rather than log-concentration is appropriate for this model. The effect concentration (EC50 - the concentration of agonist that gives a response half way between the bottom and top of each regression) was determined for each imposex parameter using the likelihood-ratio asymmetric method. A runs test was done to determine if the fitted curves deviated from the points (GraphPad Prism, 2018).

3. Results

3.1. Collection, analysis, and boating activity

TBT concentrations in sediment ranged from 495 to 20 000 µg/kg dm. DBT concentrations ranged between 315 and 3740 µg/kg dm (Table 1; Fig. 2). The concentrations of TBT and DBT were the highest in Gordon's Bay, followed by Saldanha Bay Harbour, Simon's Town Yacht Club (False Bay), and lastly, Lambert's Bay Harbour (Fig. 2). Percentage imposex, where detected, were higher at sites with quantifiable amounts of DBT and TBT (Fig. 2).

3.2. Imposex

We collected 1389 individuals of 13 caenogastropods species. Six species displayed imposex, namely: *Afrolittorina knysnaensis, Bullia digitalis, Bullia rhodostoma, Bullia laevissima, Burnupena cincta,* and *Burnupena lagenaria* (Table 1; Fig. 3). One-way ANOVA followed by Dunn's tests showed no statistically significantly differences between the medians of none, low, and medium boating activity categories for % Imposex, RPLI, and RPSI ($p > 0.05$; Fig. 4A, B, C). On the other hand, high boating areas had significantly higher median values than all the other boating categories ($p < 0.05$). The agonist vs. normalised response with variable Hill slope non-linear regressions had r^2 values of 1, 0.9951, and 1 for %Imposex, RPLI, and RPSI, respectively, with the runs test showing no significant deviation from the model (Fig. 4D). The TBT EC50 values were 14 794 µg/kg dm for RPLI, 19 757 µg/kg dm for RPSI, and about (due to wide likelihoods for the 95% confidence intervals) 14 000 µg/kg dm for %Imposex.

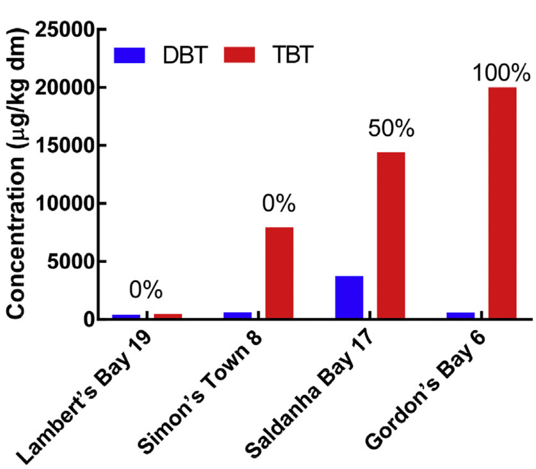

Fig. 2. Concentrations at sites with quantifiable TBT and DBT greater than their respective limits of quantification. The figures above the columns indicate percentage imposex for that site. The numbers following the site names refer to Table 1.

4. Discussion

4.1. TBT and DBT in sediments

Due to their hydrophobicity and high affinity for particulate matter, pollutants such as TBT accumulate in sediments (Hoch, 2001). We found measurable concentrations of TBT and DBT in the sediments of four out of 25 sites (Table 1; Fig. 2). These sites were associated with high to medium boating activity. TBT and DBT were not quantifiable at sites with low or no boating activity. This indicates an association between TBT in sediment and boating activity.

In the marine environment, TBT degrades in a stepwise manner to its less toxic DBT (Fent, 2006). The concentration of metabolites in relation to the parent compound may be used as an indication of recent or past usage (Diez et al., 2002). DBT and TBT concentrations were low but almost equal in Lambert's Bay Harbour (Fig. 2) suggesting that TBT has broken down to DBT. TBT at this harbour may therefore not have been from recent inputs. In contrast, TBT concentrations were considerably higher in Gordon's Bay Harbour, Saldanha Bay Harbour, and Simon's Town yacht club sediments, indicating continued inputs of TBT. There are MPAs near Saldanha Bay Harbour and Simon's Town yacht club (Fig. 1), which could pose a risk to the biota in these ecosystems. Sediments from all these sites largely exceeded the sediment quality guidelines (SQG) set for Australia and New Zealand; 9 µg/kg dm, and SQG-High = 70 µg/kg dm (Simpson et al., 2013).

Total organic carbon contents and grain size distributions were not measured, and may have added more insight regarding TBT pollution.

4.2. Imposex

We found imposex at eight of the 25 collection sites (Table 1). Two sites had no boating activity, one had low boating activity, and the other five sites had high boating activity.

In the sites with no boating activity, a low percentage of imposex, RPLI and RPSI was recorded in *B. lagenaria* from Cape Agulhas. *B. rhodostoma* sampled from Betty's Bay, displayed 53% imposex percentage, however the RPLI and RPSI values were low. Both these sites had undetectable TBT and DBT concentrations in sediments. The amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable (ATSDR, 2005). The half-lives of organotin compounds found in sediment, especially anaerobic sediments, are several years (Dowson et al., 1996). It is also possible that the gastropods have experienced TBT exposure, developed imposex, but at the time of sampling, the TBT concentrations have dropped to below detectable concentrations. The incidence of imposex in these sites indicates that areas presumed to be pristine may also be impacted by TBT pollution as found by Negri and Marshall (2009).

The site with low boating activity, Langebaan Zone A, is situated in the West Coast National Park in the same bay as Saldanha Bay. Only 37% of *B. laevissima* females exhibited imposex. Low RPLI and RPSI values were recorded, indicating low TBT exposure. Due to the site's situation within a bay, the gastropods may have been exposed to TBT from the nearby Saldanha Bay Harbour.

Gansbaai Harbour, Gordon's Bay Harbour, Saldanha Bay, Saldanha Bay Harbour, and Lagoon Beach were categorised as having high boating activity. In Gansbaai Harbour, 92% of *B. cincta* females developed imposex. *B. cincta* females sampled from Gordon's Bay Harbour displayed a 100% imposex. The RPLI and the RPSI at this site were the highest found in the present study, with RPLI values of 80.37, and RPSI of 51.92, respectively. The RPLI and RPSI values were indicative of advanced penis development, approaching that of the male population.

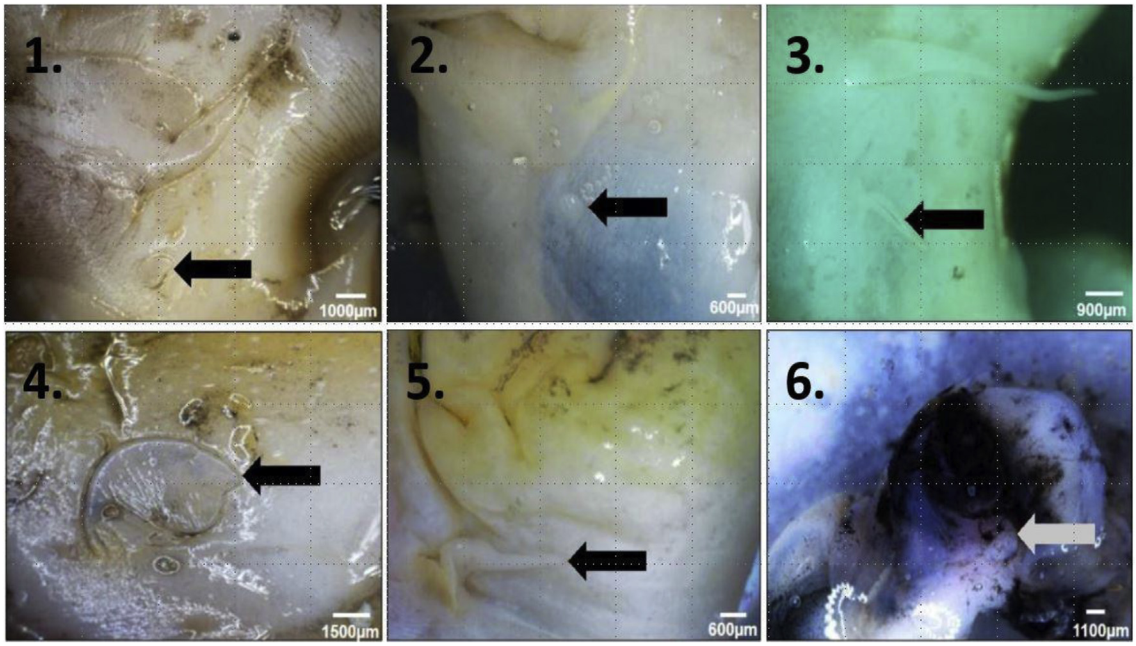

Fig. 3. Female specimens that displayed imposex. Arrows indicate the pseudo-penis of *Bullia laevissima* (1); *Bullia rhodostoma* (2); *Bullia digitalis* (3); *Burnupena cincta* (4); *Burnupena lagenaria* (5); and *Afrolittorina knysnaensis* (6).

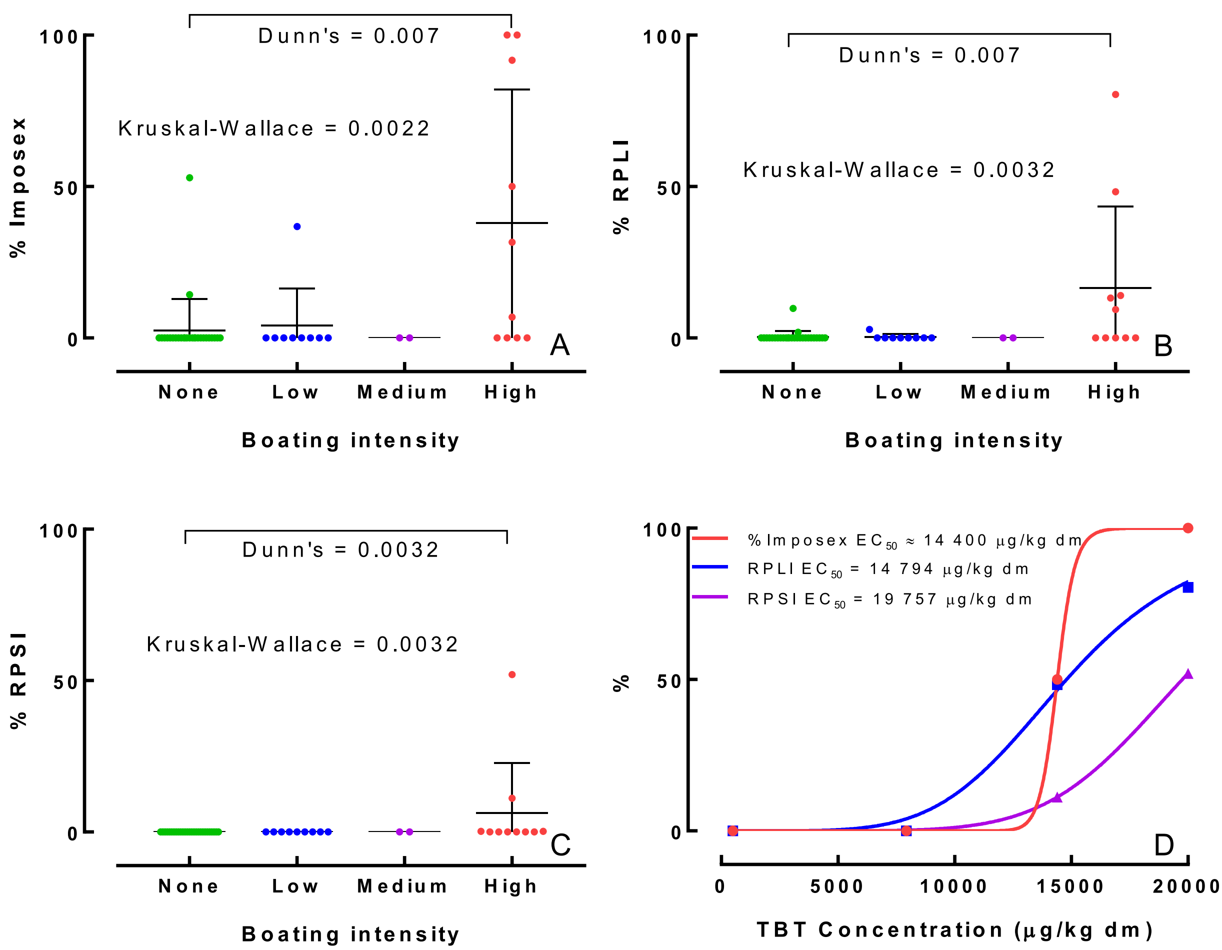

Fig. 4. A – Percentage imposex parameters according to boating intensity. B – Percentage RPLI according to boating intensity. C – Percentage RPSI according to boating intensity. D – Non-linear regressions (agonist vs. normalised response with variable Hill slope, with least squares fitting) of %Imposex, %RPLI, and %RPSI against TBT concentrations from sites where both quantifiable TBT concentrations and imposex data were available.

Lagoon Beach displayed low %Imposex, and RPLI/RPSI values in *B. digitalis* species. Concomitantly, TBT and DBT concentrations were below the limit of quantification at this site. Saldanha Bay and Saldanha Bay Harbour are situated within the same bay. There are various maritime and aquaculture activities taking place within the bay. This may account for the imposex incidence in both *B. digitalis* and *B. cincta* within Saldanha Bay. *B. digitalis* females displayed 100% imposex with low RPLI and RPSI values, while *B. cincta* displayed 32% imposex (Table 1). Zero values were assigned to RPLI and RPSI for *B. cincta.* Although imposex was evident, its development was only in the early stages. *B. cincta* females did not develop a penis, but only developed a so-called 'bud penis' (beginning stage of imposex), which suggests recent TBT exposure. Fifty percent of *A. knysnaensis* females were affected by imposex from Saldanha Bay Harbour. The RPLI was 48.27 indicating that the penis length was almost half that of the male population's. The RPSI was 11.24. These females inhabit rocky shore where there is presumably less exposure to TBT pollution from sediments. Littorinds are also known for their lower sensitivity towards TBT pollution (Oehlmann et al., 1998). For this species to display imposex to such an extent probably reflects the high concentrations of TBT at this site.

The coincidence between imposex and proximity to harbours and marinas suggests that imposex is associated with TBT from shipping or harbour activity (Fig. 3A, B, and C) as found by Bigatti et al. (2009) in an imposex survey at the South-western Atlantic coast in Argentina. The non-linear regressions between TBT concentrations and %Imposex, RPLI, and RPSI also reflect the observations by others that %Imposex develops first (14 400 μg/kg TBT dm in sediment), followed by a subsequent penis length increase (RPLI, at 14 794 μg/kg TBT dm), followed by a bulk increase (RPSI; 19 757 μg/kg TBT dm) relative to male penises from the same population, irrespective of species (Fig. 4D).

4.3. Species sensitivity

The comparison of species that share habitats with different exposures to TBT levels provides us with an estimation of their sensitivity (Oehlmann et al., 1996) and, thus, to select taxa best suited as bioindicators of TBT pollution. In this sense, TBT impacts were better described by species inhabiting soft bottom environments where TBT is known to accumulate and those with a higher sensitivity. The genus *Bullia* appears to be a good bioindicator of TBT in a South African context as imposex developed in these species at low concentrations. Species belonging to the genus *Burnupena* are also potential bioindicators. In areas of high TBT pollution, where *Bullias* and *Burnupenas* may be absent, littorinids such as *A. knysnaensis* may be more suitable to determine TBT-associated effects. *A. knysnaensis* displayed a lower sensitivity because they only developed imposex at high levels of boating activity/concentrations. All of these species are commonly found and easy to collect in the studied sites.

4.4. Implications of our findings for the Saldanha Bay aquaculture development

Saldanha Bay is South Africa's largest and deepest natural port and undergone extensive harbour development (Clark et al., 2012; Transnet, 2015). It is also a location of current shellfish aquaculture, earmarked for large extensions (SRK, 2017). The presence of a shipyard, harbours, fishing ports, marinas, ore terminal, oil terminal, together with some ship mooring areas and dredging are potential sources of TBT pollution. The South African government sees great potential in the development of marine aquaculture to alleviate poverty in certain communities. Saldanha Bay is one of the areas that demonstrates great potential. Saldanha Bay is already an important site used for the culture of Pacific oysters (*Crassostrea gigas*) and the Mediterranean mussel (*Mytilus galloprovincialis*) (Olivier et al., 2013) Seafood such as fish, mussels, and crabs collected from aquatic environments contain various amounts of butyltins and humans are likely exposed via dietary intake (ATSDR, 2005; Hoch, 2001). The presence of imposex and measured TBT pollution in Saldanha Bay Harbour is of major concern. Bivalves have the ability to bioaccumulate tributyltin under low pollution levels (Tang et al., 2010). Strand and Asmund (2003) found the highest concentration of TBT in mussels (*Mytilus edulis*) sampled from the Nuuk Harbour (Greenland). In the presence of TBT, oysters may be severely affected by a complete lack of reproduction and juvenile recruitment and the appearance of shell calcification of adult oysters lead to stunted growth (Alzieu et al., 1986). The lethal dose of TBT to the Pacific oyster, *Crassostrea gigas*, larvae is 1.557 μg/L, whereas the value for adults is 282.2 μg/L (USEPA, 2003).

5. Conclusion

The present study found that increasing %Imposex, RPLI, and RPSI were associated with increasing boating activity (Figs. 2 and 4A, B, C) and increased TBT concentrations in sediment (Fig. 4D). Populations that inhabit areas with high boating activity showed the highest degree of imposex. The relationship between high degrees of imposex and proximity to harbours and marinas indicates that the pollutant is almost certainly associated with shipping or boating activity. Only four sites had quantifiable concentrations of TBT in sediment. However, these concentrations were relatively high, which is a major cause for concern. Tributyltin is known to have had significant economic impacts in important breeding waters due to contamination and this has jeopardised the sustainable development of some marine activities (Alzieu, 1998). Seafood contaminated by TBT can cause a risk to local population and tourism as there are potential human health implications associated with the consumption of these marine organisms (ATSDR, 2005). The undesirable effects of TBT have affected biota in protected areas and it should be monitored in order to understand its implications on these ecosystems. Imposex was recorded in gastropods where mussels were farmed, in Saldanha Bay. Although TBT is largely banned by all nations Party to the TBT protocol, there are others that are not, and illegal use cannot be discounted. The situation regarding TBT contamination and associated imposex prevalence along the Atlantic coast of South Africa reported in this study must be taken seriously in order to conduct further research and monitoring at identified hotspots, and implement regulations and policies regarding TBT usage and its management.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2018.09.016.

References

Alzieu, C., 1998. Tributyltin: case study of a chronic contaminant in the coastal environment. Ocean Coast Manag. 40, 23–36.
Alzieu, C.L., Juan, J., Deltreil, J.P., Borel, M., 1986. Tin contamination in Arcachon Bay: effects on oyster shell anomalies. Mar. Pollut. Bull. 17, 494–498.
Agency for Toxic Substances and Disease Registry (ATSDR), 2005. Toxicological Profile for Tin. U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA. http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=543andtid=98, Accessed date: 21 March 2016.
Batista, R.M., Castro, I.B., Fillmann, G., 2016. Imposex and butyltin contamination still evident in Chile after TBT global ban. Sci. Total Environ. 566–567, 446–453.
Batista-Andrade, J.H., Caldas, S.S., Batista, R.M., Castro, I.B., Fillmann, G., Primel, E.G., 2018. From TBT to booster biocides: levels and impacts of antifouling along coastal

areas of Panama. Environ. Pollut. 234, 143–252.

Bigatti, G., Primost, M.A., Cledón, M., Averbuj, A., Theobald, N., Gerwinski, W., Arntz, W., Morriconi, E., Penchaszadeh, P.E., 2009. Biomonitoring of TBT contamination and imposex incidence along 4700 km of Argentinean shoreline (SW Atlantic: from 38S to 54S). Mar. Pollut. Bull. 58, 695–701.

Branch, G.M., Griffiths, C.L., Branch, M.L., Beckley, L.E., 2010. Two Oceans: a Guide to Marine Life of Southern Africa. Struik Nature, South Africa.

Bryan, G.W., Gibbs, P.E., Hummerstone, L.G., Burt, G.R., 1986. The decline of the gastropod *Nucella lapillus* around southwest England evidence for the effect of tributyltin from antifouling paints. J. Mar. Biol. Assoc. U. K. 66, 611–640.

Clark, E.A., Sterritt, R.M., Lester, J.N., 1988. The fate of tributyltin in the aquatic environment. Environ. Sci. Technol. 22, 600–604.

Clark, B.M., Tunley, K. Hutchings, Steffani, N., Turpie, J., Jurk, C., Gericke, J., 2012. Technical Report: Saldanha Bay and Langebaan Lagoon State of the Bay 2011. Saldanha Bay Water Quality Trust, Saldanha Bay.

Crowe, T.P., Thompson, R.C., Bray, S., Hawkins, S.J., 2000. Impacts of anthropogenic stress on rocky intertidal communities. J. Aquatic Ecosyst. Stress Recovery 7, 273–297.

Diez, S., Abalos, M., Bayona, J.M., 2002. Organotin contamination in sediments from the Western Mediterranean enclosures following 10 years of TBT regulation. Water Res. 36, 905–918.

Dowson, P.H., Bubb, J.M., Lester, J.N., 1996. Persistence and degradation pathways of tributyltin in freshwater and estuarine sediments. Estuar. Coastal Shelf Sci. 42, 551–562.

Du Preez, M., Nel, R., Bouwman, H., 2018. First report of metallic elements in loggerhead and leatherback turtle eggs from the Indian Ocean. Chemosphere 197, 716–728.

Fent, K., 2006. Worldwide occurrence of organotins from anti-fouling paints and effects in the aquatic environment. In: Konstantinou, I.K. (Ed.), Handbook of Environmental Chemistry: Antifouling Paint Biocides. Springer-Verlag, Berlin.

Gibbs, P.E., 2009. Long-term tributyltin (TBT)-induced sterilization of neogastropods: persistence of effects in *Ocenebra erinacea* over 20 years in the vicinity of Falmouth (Cornwall, UK). J. Mar. Biol. Assoc. U. K. 89, 135–138.

Gibbs, P.E., Bryan, G.W., 1987. TBT paints and the demise of the dog-whelk, *Nucella lapillus* (Gastropoda). Oceans 1482–1487.

Gibbs, P.E., Bryan, G.W., Pascoe, P.L., Burton, G.R., 1987. The use of the dogwhelk, *Nucella lapillus*, as an indicator of tri-n-butyltin (TBT) contamination. J. Mar. Biol. Assoc. U. K. 67, 507–523.

GraphPad Prism. 2018. https://www.graphpad.com/guides/prism/7/curve-fitting/index.htm?REG_DR_stim_variable_normalized_2.htm. Accessed: 2 September 2018.

Hoch, M., 2001. Organotin compounds in the environment: an overview. Appl. Geochem. 16, 719–743.

IMO, International Maritime Organisation, 2014. Anti-Fouling Systems. http://www.imo.org/en/OurWork/Environment/Anti-foulingSystems/Pages/Default.aspx, Accessed date: 28 November 2015.

Kannan, K., Corsolini, S., Focardi, S., Tanabe, S., Tatsukawa, R., 1996. Accumulation pattern of butyltin compounds in dolphin, tuna, and shark collected from Italian coastal waters. Arch. Environ. Contam. Toxicol. 31, 19–23.

Lahbibab, Y., Abidlia, S., Trigui-El Menifa, N., 2018. First assessment of the effectiveness of the international convention on the control of harmful anti-fouling systems on ships in Tunisia using imposex in *Hexaplex trunculus* as biomarker. Mar. Pollut. Bull. 128, 17–23.

Laranjeiro, F., Sánchez-Marín, P., Oliveira, I.B., Galante-Oliveira, S., Barroso, C., 2018. Fifteen years of imposex and tributyltin pollution monitoring along the Portuguese coast. Environ. Pollut. 232, 411–421.

Lemghich, I., Benajiba, M.H., 2007. Survey of imposex in prosobranch mollusks along the northern Mediterranean coast of Morocco. Ecol. Indicat. 7, 209–214.

Lopes-dos-Santos, R.M.A., Almeida, C., de Lourdes Pereira, M., Barroso, C.M., Galante-Oliveira, S., 2014. Morphological expression and histological analysis of imposex in *Gemophos viverratus* (Kiener, 1834) (Gastropoda: Buccinidae): a new bioindicator of tributyltin pollution on the West African coast. J. Molluscan Stud. 80, 412–419.

Maciel, D.C., Castro, Í.B., Souza, J. R. B. de, Yogui, G.T., Fillmann, G., Zanardi-Lamardo, E., 2018. Assessment of organotins and imposex in two estuaries of the northeastern Brazilian coast. Mar. Pollut. Bull. 126, 473–478.

Márquez, F., Gonzalez, R., Bigatti, G., 2010. Combined methods to detect pollution effects on shell shape and structure in neogastropods. Ecol. Indicat. 11, 248–254.

Marshall, D.J., Rajkumar, A., 2003. Imposex in the indigenous *Nassarius kraussianus* (Mollusca: neogastropoda) from South African harbours. Mar. Pollut. Bull. 46, 1150–1155.

Matthiessen, P.M., Gibbs, P.E., 1998. Critical appraisal of the evidence for tributyltin-mediated endocrine disruption in mollusks. Environ. Toxicol. Chem. 17, 37–43.

Mead, A., Griffiths, C.L., Branch, G.M., McQuaid, C.D., Blamey, L.K., Bolton, J.J., Anderson, R.J., Dufois, F., Rouault, M., Froneman, P.W., Whitfield, A.K., Harris, L.R., Nel, R., Pillay, D., Adams, J.B., 2013. Human-mediated drivers of change — impacts on coastal ecosystems and marine biota of South Africa. Afr. J. Mar. Sci. 35, 403–425.

Negri, A., Marshall, P., 2009. TBT contamination of remote marine environments: ship groundings and ice-breakers as sources of organotins in the Great Barrier Reef and Antarctica. J. Environ. Manag. 90, 31–40.

Oehlmann, J., Stroben, E., Schulte-Oehlmann, U., Bauer, B., Fioroni, P., Markert, B., 1996. Tributyltin biomonitoring using prosobranchs as sentinel organisms. Fresen. J. Anal. Chem. 354, 540–545.

Oehlmann, J., Bauer, B., Minchin, D., Schulte-Oehlmann, U., Fioroni, P., Markert, B., 1998. Imposex in *Nucella lapillus* and intersex in *Littorina littorea*: interspecific comparison of two TBT-induced effects and their geographical uniformity. Hydrobiologia 378, 199–213.

Okoro, H.K., Fatoki, O.S., Adekola, F.A., Ximba, B.J., Snyman, R.G., 2016. Spatio-temporal variation of organotin compounds in seawater and sediments from Cape Town Harbour, South Africa using gas chromatography with flame photometric detector (GC-FPD). Arab. J. Chem. 9, 95–104.

Olivier, D., Heinecken, L., Jackson, S., 2013. Mussel and oyster culture in Saldanha Bay, South Africa: potential for sustainable growth, development and employment creation. Food Secur. 5, 251–267.

Ports and ships, 2015. Ship Movements. (Accessed 2015/06/17). http://ports.co.za/shipmovements/articles.php.

Primost, M., Averbuj, A., Bigatti, G., 2015. Variability of imposex development and reproductive alterations in the Patagonian gastropod *Buccinanops globulosus* inhabiting a polluted harbour area. Revista del Museo Argentino de Ciencias Naturales ns 17, 167–171.

Ryan, P.G., Bouwman, H., Moloney, C.L., Yuyama, M., Takada, H., 2012. Long-term decreases in persistent organic pollutants in South African coastal waters detected from beached polyethylene pellets. Mar. Pollut. Bull. 64, 2756–2760.

Sheikh, M.A., Noah, N.M., Tsuha, K., Oomori, T., 2007. Occurrence of tributyltin compounds and characteristics of heavy metals. Int. J. Environ. Sci. Technol. 4, 49–59.

Simpson, S.L., Batley, G.B., Chariton, A.A., 2013. Revision of the ANZECC/ARMCANZ Sediment Quality Guidelines. CSIRO Land and Water Science Report 08/07. CSIRO Land and Water.

SRK, 2017. Executive Summary: Final Basic Assessment Report. BA Process for a Proposed Sea-based Aquaculture Development Zone in Saldanha Bay. SRK Project Number 499020.

Strand, J., Asmund, G., 2003. Tributyltin accumulation and effects in marine molluscs from West Greenland. Environ. Pollut. 123, 31–37.

Tan, K.S., 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. Mar. Pollut. Bull. 39, 295–303.

Tang, C.-H., Hsu, C.-H., Wang, W.-H., 2010. Butyltin accumulation in marine bivalves under field conditions in Taiwan. Mar. Environ. Res. 70, 125–132.

Ten Hallers-Tjabbes, C.C., Wegener, J.-W., Van Hattum, B.(A.G.M.)., Kemp, J.F., Ten Hallers, E., Reitsema, T.J., Boon, J.P., 2003. Imposex and organotin concentrations in Buccinum undatum and Neptunea antiqua from the North Sea: relationship to shipping density and hydrographical conditions. Mar. Environ. Res. 55, 203–233.

Titley-O'Neal, C.,P., Munkittrick, K.R., MacDonald, B.A., 2011. The effects of organotin on female gastropods. J. Environ. Monit. 13, 2360–2388.

Transnet, 2015. Transnet National Ports Authority. Cape Town.http://www.transnetnationalportsauthority.net/OurPorts/Cape%20Town/Pages/Overview.aspx, Accessed date: 3 April 2016.

USEPA, 2003. In: U.S.E.P. Agency (Ed.), Ambient Aquatic Life Water Quality Criteria for Tributyltin (TBT)—Final. USEPA, Washington D.C.

Wells, F.E., Keesing, J.K., Brearleye, A., 2017. Recovery of marine Conus (Mollusca: caenogastropoda) from imposex at rottnest island, western Australia, over a quarter of a century. Mar. Pollut. Bull. 123, 182–187.

Wepener, V., Degger, N., 2012. Status of marine pollution research in South Africa (1960–Present). Mar. Pollut. Bull. 64, 1508–1512.