Occurrence of imposex in *Thais haemastoma*: possible evidence of environmental contamination derived from organotin compounds in Rio de Janeiro and Fortaleza, Brazil

Ocorrência de *imposex* em *Thais haemastoma*: possíveis evidências de contaminação ambiental por compostos organotínicos no Rio de Janeiro e em Fortaleza, Brasil

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Abstract *There are indications that the widespread use of organotin compounds (TBT and TPT) as antifoulings, as stabilizers in plastic and as pesticides, has severely affected several species of marine organisms. The most striking effect of TBT and TPT as hormonal disruptors is the development of male organs in females of gastropods, currently denominated imposex. This syndrome can lead to the sterilization and death of affected organisms. The present work gives an overview of the present state of knowledge on imposex occurrence and reports results of a survey conducted in Guanabara Bay, Rio de Janeiro and in several sites along the coast of Fortaleza, Ceará State. Different stages of imposex development were verified in this survey, however, the most prominent levels appeared associated to known spot sources of TBT and TPT.*
Key words *Organotin Compounds;* Thais haemastoma*; Imposex; Endocrine Disruptors*

Resumo *Existem evidências de que a utilização amplamente disseminada de compostos organoestânicos (TBT e TPT) como antiincrustantes, estabilizadores em plásticos e como pesticidas tenha afetado severamente diversas espécies de organismos marinhos. O mais característico efeito do TBT e do TPT como desreguladores endócrinos é o desenvolvimento de caracteres sexuais masculinos em fêmeas de gastrópodos, conhecido como* imposex*. Esta síndrome pode levar à esterelização e morte dos organismos afetados. O presente trabalho apresenta uma vista geral ao estado atual do conhecimento sobre a ocorrência do* imposex*, e reporta os resultados de um estudo conduzido na Baía de Guanabara, Rio de Janeiro, e ao longo da costa de Fortaleza, no Ceará. Diferentes estágios de desenvolvimento do* imposex *foram verificados neste estudo, os mais proeminentes parecendo associados às fontes pontuais locais conhecidas de TBT e TPT.*
Palavras-chave *Compostos Orgânicos de Estanho;* Thais haemastoma*; Imposex; Desreguladores Endócrinos*

Introduction

Organotin compounds have been in use since the 1960s. Due to its powerful antifouling effect tributyltin (TBT) has been utilized as an additive in paintings applied to ships, fishing nets, marine installations and cooling systems based on seawater (Bacci & Gaggi, 1989; Fent, 1996; Horiguchi et al., 1994; Short & Thrower, 1986). Triphenyltin (TPT) has been used in association with TBT in antifouling paintings but is also effectively applied as acaricide and fungicide in agriculture and for wood preservation (Favoreto, 2000; Fent, 1996; Mensink et al., 1997). Di-substituted organotin compounds are used as plastic stabilizers in PVC production and as catalysts in some industrial processes.

Environmental problems derived from the use of organotin compounds as antifouling agents were first reported by the end of the 1970s after a sharp decline in oyster production was observed in the Basin d'Arcachon, France (Alzieu et al., 1986, 1989). The main biological alterations initially ascribed to the widespread use of organotins were balling in oysters (Alzieu et al., 1986, 1989; Axiak et al., 1995; Dyrinda, 1992), death of mollusk larvae (Horigushi et al., 1998) and imposition of male sexual characteristics to females of gastropods (Ellis & Pattisina, 1990; Fioroni et al., 1991; Gibbs & Bryan, 1987). This last effect, denominated imposex is induced in several gastropod species both in the field and in laboratory studies at very low concentrations of TBT (imposex is initiated at less than 1ng/L^{-1} TBT in *Nucella lapilus*, and at 3-5ng/L^{-1} all females may be sterilized (Gibbs & Bryan, 1994; concentrations of 1ng/L^{-1} can induce imposex development in *Thais clavigera*, Horiguchi et al., 1995). This widely observed effects suggests that TBT and TPT may also act as endocrinal disruptors (Horiguchi et al., 1995, 1997a; Liu & Suen, 1996; Mathiessen & Gibbs, 1998; Oehlmann et al., 1996).

The detrimental effects of TBT and TPT upon non-target organisms led to restrictions to the use, first in France in 1982, where antifouling paintings containing TBT were prohibited for boats of less than 25m length (Sarradin et al., 1991; Waite et al., 1991), and then in many other countries, such as the United Kingdom (1987), United States (1988), Sweden, New Zealand (1989), Australia, Japan (1990) and Denmark (1991) (Champ & Pugh, 1987; de Mora et al., 1989; Horiguchi et al., 1994; Kure & Depledge, 1994; May et al., 1993; Stewart & de Mora, 1990; Stuer-Lauridsen & Dahl, 1995). Germany and Switzerland imposed restrictions also for freshwater environments (Champ, 1986). Recently the International Maritime Organization (IMO) recommended the global banning of organotin compounds as antifoulings by 2008 and the prohibition of new application in boats by 2003 (Champ, 1999; ten Hallers-Tjabbes, 1997). In Brazil, however, there is no legislation imposing restriction or addressing permissible levels, and up to very recently no investigations have been conducted to evaluate possible environmental impacts.

By the beginning of the last decade, restrictions imposed to the use of TBT and TPT in developed countries had led to the decrease in concentration of the parent compounds and decay products, both in waters and organisms. Recoveries were observed in oyster populations of Basin d'Arcachon (Alzieu, 1986) and in gastropods from coastal environments in United Kingdom (Evans et al., 1996; Tester et al., 1996; Tester & Ellis, 1995). However, Kure & Depledge (1994) reported that in Denmark, high concentrations could still be observed in filter feeders years after establishment of the restricted use.

Although TBT and TPT can be decomposed in the water column rather fast (half life time ~ 7-15 days) (Foale, 1993), estimated half life of the order of 1 year for toxic sediments, to decades for anoxic sediments, are indicative of relatively high persistence in these environments, specially in the absence of oxygen (Dowson et al., 1996). In environments with high particulate loads where photochemical reactions are less active and transport to sediments may occur before extensive degradation in the water column takes place, sediments are a potential source of TBT and TPT even after the use has been interrupted (Dowson et al., 1993, 1996; Langston & Pope, 1995; Sarradin et al., 1991, 1994). The tendency to accumulate in sediments becomes evident from the levels of TBT reported for contaminated sediments that may range from 300ng.g^{-1} up to 1,000ng.g^{-1} or more, when compared to slightly contaminated sediments, where levels are in the range of 10 to 50ng.g^{-1} (Waite et al., 1991). Values as high as 4,500ng.g^{-1} TBT in sediments where detected in Thailand (Kan-Atireklap et al., 1997a) or 3,200ng.g^{-1} TBT in Hong-Kong (Ko et al., 1995).

After 1994, reports from several authors on the presence of TBT in higher organisms including cetaceans (Iwata et al., 1994; Kannan & Falandysz, 1997; Tanabe, 1999), marine birds (Guruge et al., 1997a, 1997b) and humans (Kannan & Falandysz, 1997; Takahashi et al., 1999) demonstrated that organotin compounds are likely to be transferred among different trophic levels.

Little is known about the behavior of organotin compounds in tropical marine environments. Imposex has been observed in several species of gastropod on the coast of India (*Cronia konkanensis* – Vishwa-Kiran & Anil, 1999); New Zealand (*Lepsiella scobina, Thais orbita, Haustrum haustorium, Xymene ambiguus, Taron dubius, Cominella virgata, Amalda (Baryspira) australis* – Stewart et al., 1992); Australia (*Lepsiella vinosa* – Nias et al., 1993; *T. orbita* – Foale, 1993); Thailand (*Thais distinguenda, Thais bitubercularis, Morula musiva* – Bech, 1999a, 1999b; *Phalium bisulcatum, Distorsio reticularis, Murex occa, Murex trapa, Murex tribulus, Chicoreus capucinus, Thais gradata, Thais lacera, Babylonia areolata, Nassarius livescens, Nassarius stolatus, Hemifusus ternatanus, Voluma conchlidium, Cymbiola nobilis, Melo melo* – Swennen et al., 1997); Indonesia (*Thais kieneri, Thais savignyi, Vasum turbinellus* – Evans et al., 1995); Malaysia (*T. gradata, C. capucinus*, *T. clavigera, Thais jubilaea, T. bitubercularis* – Tan, 1997, 1999) and Hong Kong (*T. clavigera* – Blackmore, 2000). In South America, imposex occurrence is reported in Chile (*Chorus giganteus, Xanthochorus cassidiformis, Nucella crassilabrum* – Gooding et al., 1999) and Brazil (Castro et al., 2000; Fernandez et al., 1998; Magalhães et al., 1997). Information on concentration in waters (Hashimoto et al., 1998; Malaca straits), sediments (Kan-Atireklap et al., 1997a – Thailand; Ko et al., 1995 – Hong Kong; Stewart & de Mora, 1992 – Fiji Island; King et al., 1989 – New Zealand) and organisms (bivalves, *Perna viridis* – Kan-Atireklap et al., 1997b; *Mytidus edulis* and *Aequipecten irradians* – Guolan & Yong, 1995; gastropods, *T. clavigera, Thais tuberosa* and *Morula granulata* – Liu et al., 1997) are scarce, although reported levels specially in areas including intensive navigation and harbor installations have been high (over 1,000ng.g^{-1}) and of the same order as those available for temperate climate areas (Morgan et al., 1998; Ruiz et al., 1998; Swennen et al., 1997; ten Hallers-Tjabbes et al., 1994). As for the effect on humans, although organotin compounds are considered extremely toxic, environmental routes of exposure are usually not considered very critical (Schweinfurth & Günzel, 1987; Tsuda et al., 1995). However, the proved accumulation in filter feeders may be an important pathway to consumers (Guolan & Yong, 1995).

Besides causing hormonal disruption, evidences that organotins seem to act upon the immune system of mammals (cetacean, rabbits, rats and sheep) (Dacasto et al., 1994a, 1994b; Kannan & Falandysz, 1997; Kannan et al., 1997) and invertebrates (mollusks and tunicates) (Cima et al., 1995; Morcillo & Porte, 1997) call for epidemiological investigations focused upon sensitive populations.

In Brazil, imposex in *Thais haemastoma* was first reported by Magalhães et al. (1997), in Santos Bay, São Paulo, by Fernandez et al. (1998), in Guanabara Bay, Rio de Janeiro and by Castro et al. (2000), in Fortaleza, Ceará.

The present work reports recent findings on possible TBT related effects in two of these areas of the Brazilian coast.

Methodology

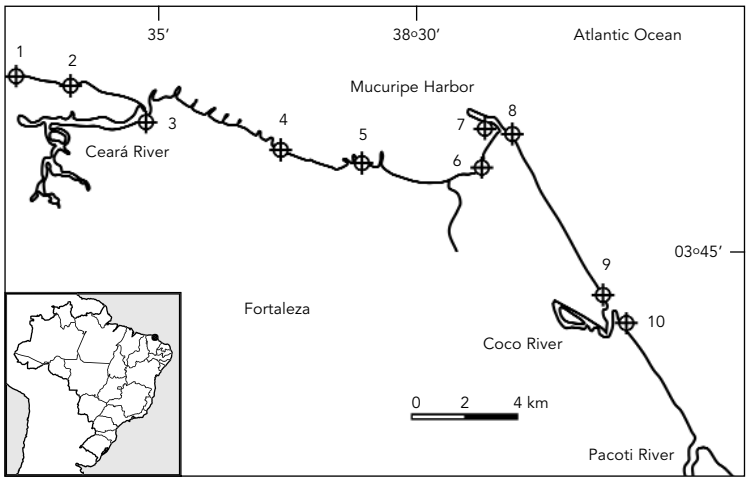
The development of imposex in *T. haemastoma* was used as a tool to search for indications of biological stress derived from TBT and TPT in Guanabara Bay and in coastal areas of Fortaleza. The bioindicator was selected based on the following properties: (1) ubiquity along the Brazilian coast (Leal, 1984); (2) tendency to imposex development (Spence et al., 1990); (3) existing scientific evidences of imposex related to TBT and TPT for organisms of the same genus (*T. clavigera* and *Thais bronni*) (Horiguchi et al., 1997a; Liu & Suen, 1996).

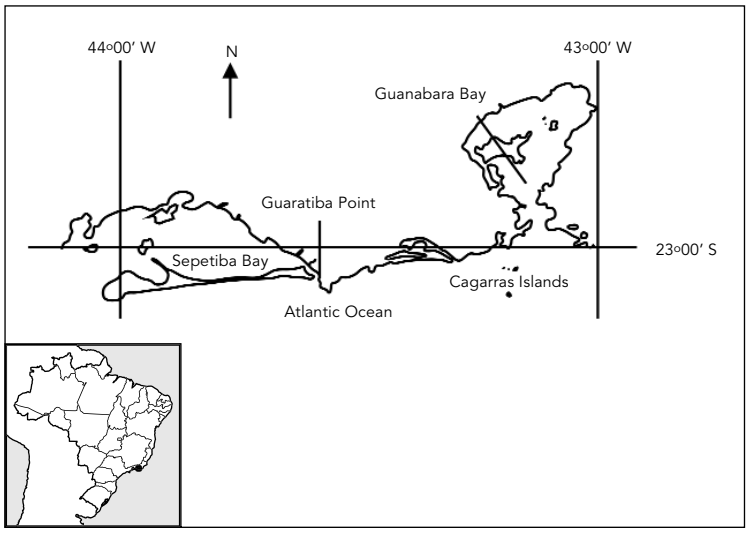
Figures 1 and 2 show the sampling stations from which 20 to 50 specimens of *T. haemastoma* were collected. Once in the laboratory, the specimens were narcotized by immersion in a 1:1 solution of local seawater and $MgCl_2$ 7%. Shell length was measured with the aid of a vernier calliper and after removing the soft parts from the shell, sex was determined based on the presence (female) or absence (male) of albumen and sperm ingestion glands (Figure 3) (Gibbs & Bryan, 1987; Horiguchi et al., 1994; Spence et al., 1990). Penis length was also measured in males and imposexed females as in Axiak et al. (1995) e Stewart et al. (1992).

Imposex occurs at different levels which are related to TBT concentrations in water and in gastropod food; i.e., to the presence of this compound in the local environment. Therefore, different levels of alterations may be discriminated, ranging from apparently unaffected females to those showing male attributes. In the present work, four indicators of imposex were applied to the determine different level of imposex: (1)% of imposexed females; (2) relative penis length index (RPLI); (3) relative penis size index (RPSI), and (4) vas deferens sequence index (VDSI). RPLI and RPSI were calculated by using the relations ($[C_{FI}/C_M].100$) and ($[C_{FI}^3/C_M^3].100$) (Gibbs & Bryan, 1987; Huet et al., 1996; Minchin & Minchin, 1997), respectively,

Figure 1

Location of Guanabara Bay area and reference station in the coast of Rio de Janeiro and monitoring stations along the coast of Fortaleza, Ceará State.





where C_{FI} is the average penis length in imposexed females and C_M is the average penis length in males at each sampled station.

The first index is a good indicator of imposex response at low environmental expositions, when not all females show male attributes (Curtis, 1994; Gibbs & Bryan, 1994). The penis development indexes, RPLI and RPSI, are more adequate for medium (RPLI) or high (RPSI) imposex levels.

In this last situation, penis length of imposexed females approaches those of males, and the RPLI index becomes less effective in discriminating between different levels of alterations. VDSI is the only index that responds effectively over a broad range of imposex. It is useful in assessing the reproductive capacity of the studied population; populations with a mean VDSI above IV are comprised of sterile females, and show reduced reprodutive capacity (Gibbs & Brian, 1994; Oehlmann et al., 1996, 1998; Stroben et al., 1995).

VDSI was obtained through two different approaches: (1) by using the scale of Gibbs (Gibbs & Bryan, 1987), and (2) by using a modified scale of seven levels of imposex, developed by Fernandez et al. (1998). The development of a modified scale was required because of the difficulty to discriminating among initial stages of the Gibbs scale without using microscopy and histological analysis. The new scale provided an easy field method for assessing vas deferens sequence using only a magnifying glass. In this scale, level I of imposex was determined as in Solé et al. (1998) while discrimination between level III and IV was set according to Horiguchi et al. (1994). The other imposex levels were determined following the original scale of Gibbs. It should be emphasized that this scale was originally developed for *N. lapilus*. The same approach, however, could be adapted for other genera with little modifications (Fioroni et al., 1991; Horiguchi et al., 1994; Solé et al., 1998; Stewart et al., 1992; Stroben et al., 1995). The modified scale shows a slight tendency to underestimated results, however, there is in general, general agreement with the Gibbs scale (Table 1). The more detailed VDSI development scheme proposed by Fioroni et al. (1991) and Stroben et al. (1995), was tested, but histological analyses are required for its application.

Results and discussion

Imposex in Guanabara Bay, Rio de Janeiro

The results for the biomonitoring in Guanabara Bay are shown in Table 2. Figures 2 and 4 show a comparison of the actual distribution of *T. haemastoma* with that in the 1960s, and the geographic incidence of imposex, respectively. The potential sources of TBT and TPT are depicted in Figure 3.

As can be observed from Figure 2, the actual distribution of *T. haemastoma* differs significantly from that reported for the 1960s when the use of TBT in several areas of the globe was initiated. The species nowadays is absent in all stations at the inner areas of the bay (stations

1, 2, 3 and 4) even though the past occurrence of *T. haemastoma* in stations 2, 3 and 4 have been reported by Leal (1984). In the sampling of August 1999, only empty shells were found in station 1.

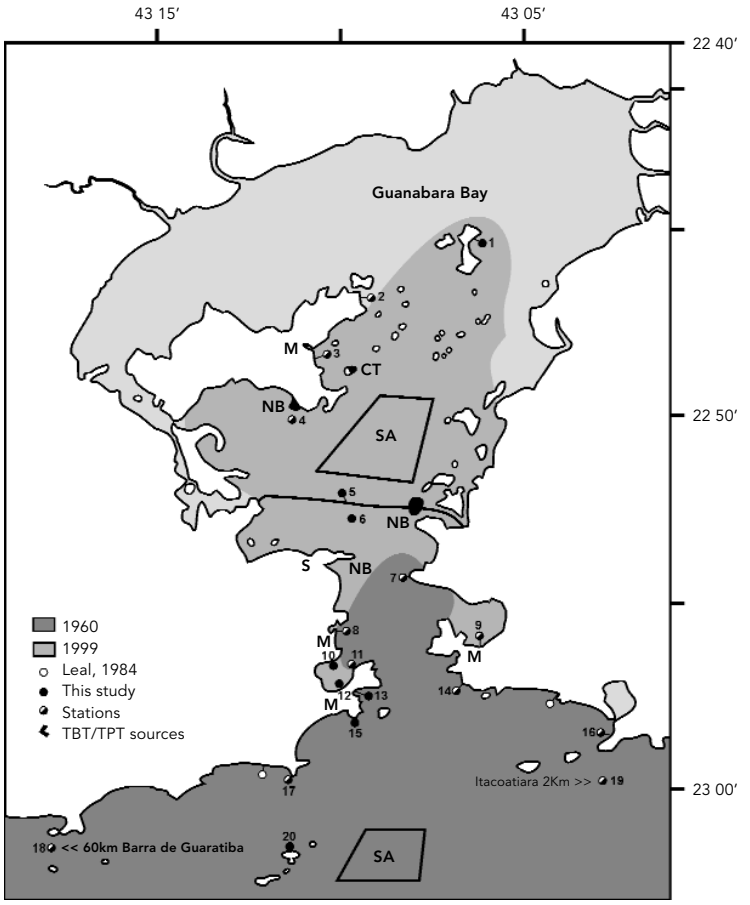
Organisms were not found during the sampling campaigns of February and August 1999 in station 5, located under the central part of the Rio-Niterói Bridge, and in station 9 (1998) located in a sewage contaminated area near a marina. However, while in all these areas *T. haemastoma* was absent, the mussell *Perna perna,* a common prey for the gastropod, was abundant, unless for the bridge site where mussel is frequently harvested for human consumption. The same feature was observed in stations 6 (presence of only one juvenile specimen), 8 (presence of only two young organisms) and 12, which are located near a harbor and two marinas. The presence of young organisms may indicate that a recolonization of the areas is possible, as reported by Spence et al. (1990) and Blackmore (2000) for species having planktonic larvae. It should be noted that stations 9 and 12 are in sites of restricted water circulation, a condition that favors the build up of critical TBT levels in the water (Gibbs & Bryan, 1994; Horiguchi et al., 1994, 1997b; Nicholson et al., 1998).

Imposex indexes were generally high in the stations located inside Guanabara Bay (Figure 4). The relative abundance of imposexed females in a certain site is a tool for impact assessment when concentrations of TBT in water are relatively low and only a fraction of the females is masculinized (Curtis, 1994; Gibbs & Bryan, 1994). For all sites inside Guanabara Bay 100% of the analyzed females showed some degree of sexual alteration, and only in four stations outside the bay, normal and less imposexed females were present (Ponta do Arpoador, Itacoatiara, Cagarras Island and the control station at Barra de Guaratiba) (Figure 4).

RPLI and RPSI levels are shown in Figure 4. Except for some stations (RPLI: 17b, 19 and 20; RPSI: 15, 17b, 18, 19 and 20) where due to the small penis size in the females, indexes could not be determined, all other stations inside Guanabara Bay showed measurable indexes. RPSI and RPLI values above 30 and 70, respectively, obtained for some inner sites of the bay have been considered high in other regions known as polluted by TBT. For instance, values ranging from 60.9 to 127.3 for RPLI and from 22.2 to 206.2 for RPSI were recorded in *T. clavigera* and *T. bronni* (Horiguchi et al., 1994, 1997b) from the most polluted sites in Japan, around Aburatsubo in the Miura Peninsula.

Figure 2

Map showing the sampling stations and main sources of TBT and TPT in Guanabara Bay.



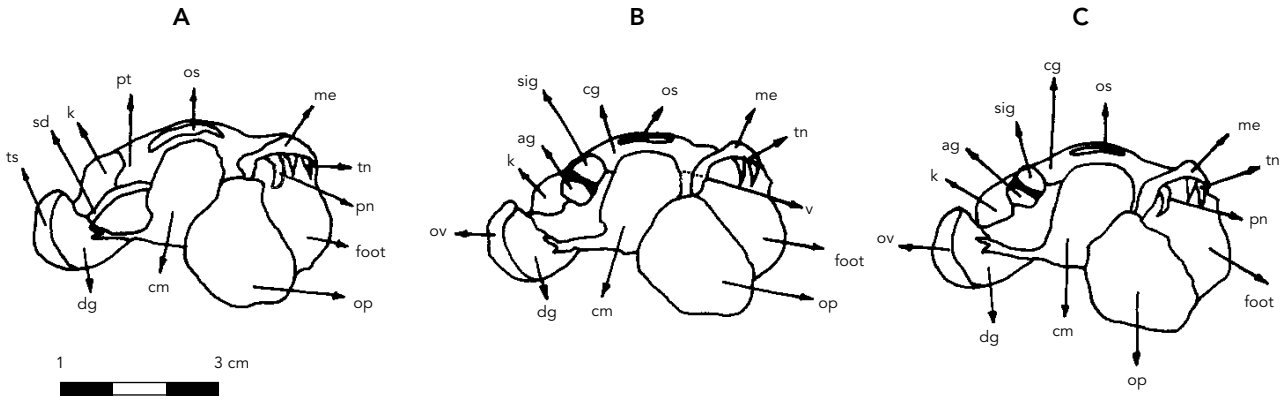
M = marine; CT = commercial terminal; S = shipyard; NB = naval base; SA = ship anchoring area. Comparison between the actual occurrence of *T. haemastoma* and the distribution observed in the 1960s in Guanabara Bay is also showed as shaded areas.

Values over 100 indicate that imposexed females mean penis lenghts (RPLI), or volumes (RPSI) are greater than those of males from the same locations. Nicholson et al. (1998) reported that values of RPSI in *N. lapilus* were in the range 37.9 to 44.8 near Sollun Voe, an oil tanker port, and Spence et al. (1990) recorded values as high as 48.3 to 81.7 in *T. haemastoma* collected from harbors and marinas in the Azores.

An increasing gradient in imposex intensity towards inner areas can be observed both in the western (RPLI from N.A. to 106.3 and RPSI from N.A. to 107.6 in stations 11, 13, 15, 17, 20) and eastern (RPLI from N.A. to 107.6 and RPSI from N.A. to 125 in stations 7, 14, 16, 19) sides of the bay entrance. Those gradients include all imposex levels so far detected in Guanabara Bay.

Figure 3

Schematic representation of *T. haemastoma*. (A) male; (B) healthy female; (C) imposexed female.



tn – tentacles; me – mantle edge; os – osphradium; cm – collumela muscle; op – operculum; dg – digestive gland; k – kidney; pn – penis; ts – testicle; sd – spermatic duct; pr – prostate; v – vulva (inside mantle cavity); ov – ovary; ag – albumem gland; sig – sperm ingesting gland; cg – capsule gland.

Table 1

Comparison between the Gibbs scale and the adapted scale for vas deferens sequence index (VDSI).

Gibbs scale	Characteristics of imposex development	Modified scale
0	Normal Female	0
I	Beginning of penis formation (A),	
	Beginning of vas deferens formation (G)	I
II	Formed penis, length < 2mm	II
III	Formed penis, length > 2mm, distal vas deferens present (G)	III (RPLIi < 0.5)
IV	Completely developed vas deferens	IV (RPLIi > 0.5)
V	Vulva blocked by growth of vas deferens tissues	V
VI	Dark mass of aborted capsules in capsule gland	VI

RPLIi = Relative Penis Length Index of female i = $[C_{Fl\ i} / C_M] * 100$ (adapted from Gibbs & Bryan, 1987).

The penis length is a better tool to characterize the first and medium stages of imposex development than the penis volume, therefore, RPLI values seems to be more adequate for discriminating between stations than RPSI, in Guanabara Bay. Similar findings were reported by Horiguchi et al. (1994), in *T.clavigera* and *T. bronni* along the Japanese coast.

The observed VDSI values and existing imposex gradients (Figure 4) confirmed the observations given above. In stations 6 and 11 sterile organisms with VDSI levels V and VI were found. In station 6 in Boa Viagem, Niterói, only a female was found amongst 15 adults harvested during February 1999 while in August 1999 only a few young organisms were available for sampling. In station 11, organisms of VDSI V and VI were found in 1997 and in January 2000, only a single female amongst 18 adults was present showing VDSI of IV. The unbalanced sex distribution in those stations may result from selective extinction of females due to the blockage of capsule glands, a phenomenon observed in other regions and described by Spence et al. (1990), Nicholson et al. (1998) and Blackmore (2000).

Based on the results obtained for the several indexes given above, we inferred that three

Table 2

Results of biological monitoring in Guanabara Bay.

Station	Date	Location	n (males/females)	% imposex	RPLI	RPSI	VDSI
1	August 1999	Paquetá Island (*)	NF	NA	NA	NA	NA
2	March 1998	Valente Inlet (*)	NF	NA	NA	NA	NA
3	March 1998	Zumbi Beach (*)	NF	NA	NA	NA	NA
4	March 1998	Matoso Point (*)	NF	NA	NA	NA	NA
5a	February 1999	Rio-Niterói Bridge (*)	NF	NA	NA	NA	NA
5b	August 1999	Rio-Niterói Bridge (*)	NF	NA	NA	NA	NA
6	February 1999	Feiticeiras Rocks	1 (NA)	NA	NA	NA	NA
7a	February 1999	Boa Viagem Island (*)	15 (14/1)	100.0	107.6	125.0	V
7b	August 1999	Boa Viagem Island (*)	10 (NA)	NA	NA	NA	NA
8	September 1997	Glória Marine (*)	2 (1/1)	NA	NA	NA	NA
9	March 1998	São Francisco Inlet (*)	NF	NA	NA	NA	NA
10	September 1997	Flamengo Beach	8 (5/3)	100.0	106.3	120.0	IV
11a	April 1997	Urca, São João Fortress (*)	27 (18/9)	100.0	90.3	79.0	IV
11b	July 1997	Urca, São João Fortress (*)	29 (15/14)	100.0	90.8	75.0	V
11c	January 2000	Urca, São João Fortress (*)	18 (17/1)	100.0	81.6	54.4	IV
11X	January 2000	Urca, São João Fortress, internal area	21 (9/12)	100.0	21.8	1.1	I-III
12a	April 1997	Rio de Janeiro Yatch Club	NF	NA	NA	NA	NA
12b	January 2000	Rio de Janeiro Yatch Club	1 (1/0)	NA	NA	NA	NA
13	May 1998 – March 2000	Vermelha Beach	188 (120/68)	100.0	44.0	13.0	III
14	July 1998	Adão Beach (*)	17 (12/5)	100.0	83.1	57.0	IV
15	July 1997	Leme Beach	17 (11/6)	100.0	8.5	< 0.1	I
16a	July 1998	Itaipú Point (*)	15 (10/5)	100.0	32.7	1.4	II
16b	July 1998	Itaipú Point (*)	17 (11/6)	100.0	37.6	13.0	II
17a	May – August 1998	Arpoador Point (*)	26 (16/10)	30.0	44.1	8.6	II
17b	January 2000	Arpoador Point (*)	20 (11/9)	22.2	< 0.1	< 0.01	I-II
18	May 1997	Barra de Guaratiba Beach	51 (26/25)	19.0	5.6	<0.1	I
19	March 2000	Itacoatiara Beach (*)	30 (15/15)	53.3	< 0.1	< 0.01	0-I
20	April 2000	Cagarras Islands	27 (12/15)	40.0	< 0.1	< 0.01	0-I

NF = Animal not found; NA = Not analyzed; (*) = Animal reported before in this station;
RPLI = Relative penis length index; RPSI = Relative penis size index; VDSI = Vas deferens sequence index.

regions, with different degrees of impact exist in Guanabara Bay: (1) the region extending from the Rio-Niterói Bridge to the northern area of the bay (stations 1 to 4) where presently the bioindicator is not available. Here, at the present stage of knowledge, it is difficult to attribute the absence of organisms to TBT or TPT contamination because this region receives heavy loads of other pollutants derived from domestic and industrial sources; (2) the region between the Rio-Niterói Bridge and the mouth of the bay (stations 5 to 14) where high indexes of imposex and strong imposex gradients were found, and (3), regions outside of Guanabara Bay (stations 15 to 20) in which only organisms with low imposex levels were found, and lesser impact is to be expected.

The results of the two samplings in Arpoador (station 17) showed a wide range of variability that can be ascribed to the difference in size and sexual development of the organisms available for sampling. This was the greatest variability observed in repeated sampling of the same station.

The water circulation pattern and the proximity to possible sources of TBT seem to be important factors controlling the degree of imposex in the studied area. This trend to higher imposex indexes (RPSI > 10; RPLI >30; VDSI ≥ II) with increasing boat traffic or anchoring and water stagnation is a typical feature of TBT contamination (Axiak et al., 1995; Smith & McVeagh, 1991; Spence et al., 1990; Stewart et al., 1992) that has been used to map zones of influence in a regional (Minchin et al., 1996; Minchin & Minchin, 1997) and national scale (Horiguchi et al., 1994, 1997b; ten Hallers-Tjabbes et al., 1994, 1996). A few authors

Figure 4a

Intensity of imposex in Guanabara Bay as determined according to RPLI index.
Displayed area corresponds to the actual area where *T. haemastoma* is still found in the bay.

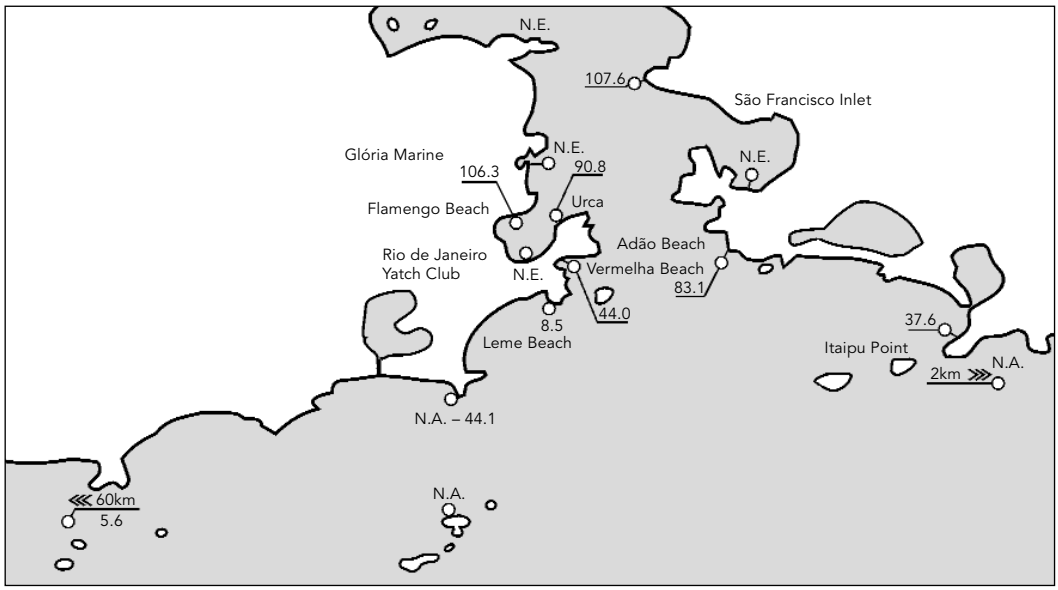


Figure 4b

Intensity of imposex in Guanabara Bay as determined according to RPSI index.
Displayed area corresponds to the actual area where *T. haemastoma* is still found in the bay.

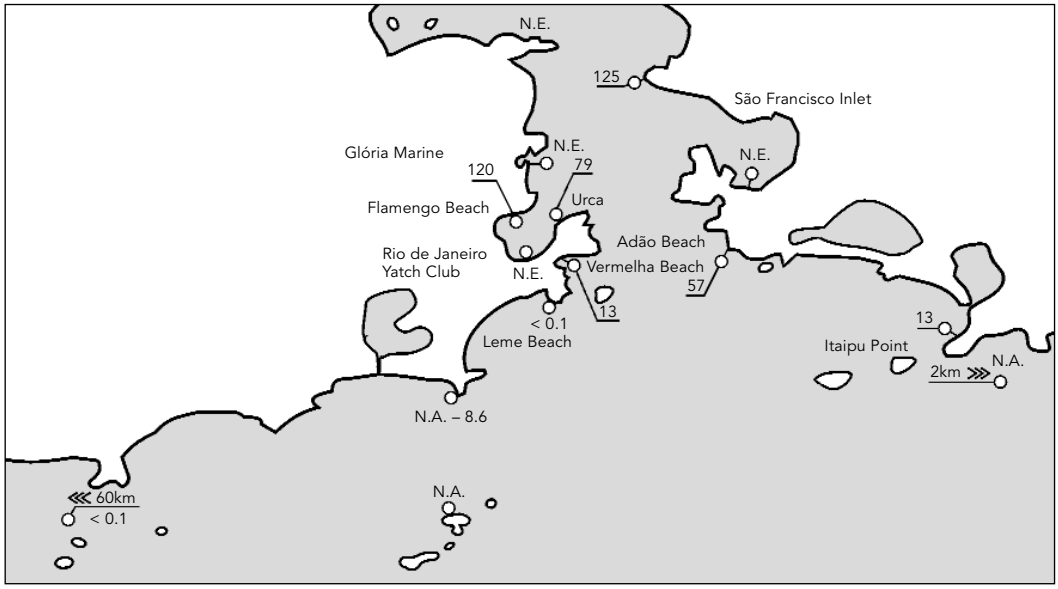
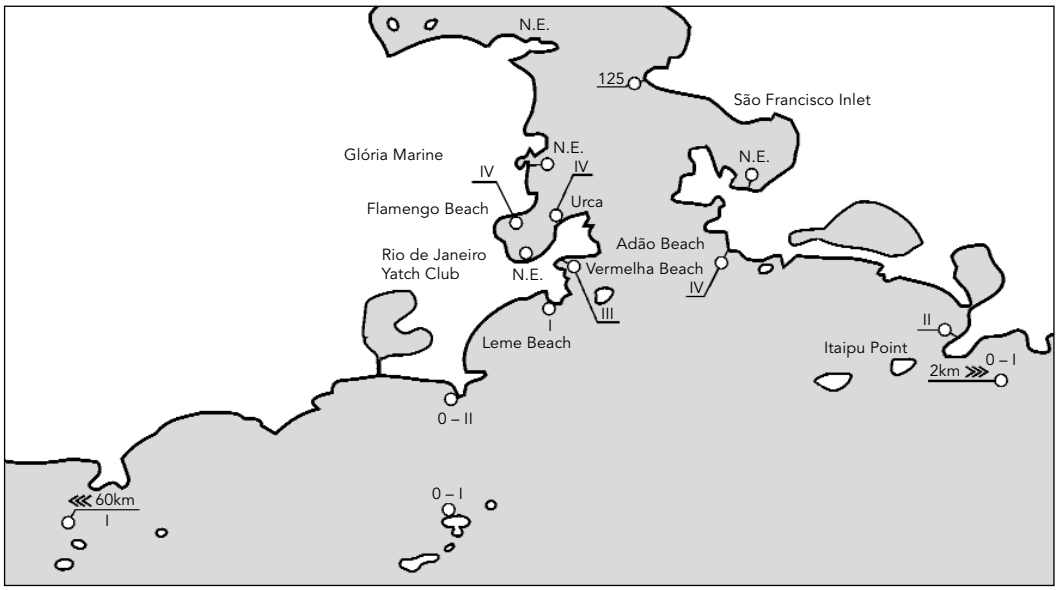


Figure 4c

Intensity of imposex in Guanabara Bay as determined according to VDSI index.
Displayed area corresponds to the actual area where *T. haemastoma* is still found in the bay.



(Davies et al., 1987; Evans, 1999; Nicholson et al., 1998) based on experimental observations have proposed that TBT impact would be restricted to sites directly under influence of specific sources showing, therefore, little tendency to migrate over long distances from the input area. Our results seems to confirm this observation. In Guanabara Bay the actual absence of the organism in previously reported sites is an additional evidence of impact (Huet et al., 1996; Minchin & Minchin, 1997; Smith, 1996) that may be derived from TBT, especially when mussels are abundant (Morcillo & Porte, 1997).

Imposex in the coast area of Fortaleza, Ceará

Imposex was registered in several of the sampling sites (Figure 1 and Table 3) and the higher indexes were found in females collected from areas near the Mucuripe Harbor. In the Pacheco Beach and in Titanzinho, Caça e Pesca and Sabiaguaba, beaches where boat cruises are virtually absent, no imposex or related anomalies were observed. The absence of imposex in the last three beaches located east of Mucuripe Harbor is due to the water circulation in the region that prevents contamination predominantly flowing from east to west (Maia, 1998). In station 2 (Dois Coqueiros Beach) evidence of imposex in stage I (VDSI) was observed in females that showed a small protuberance as a first step towards penis development. Because in this initial stage, the penis dimensions cannot be measured, RPSI was not determined for this station, neither was it for Formosa and Poço da Draga, where females displayed similar alterations. In station 3 (Barra do Ceará), advanced stages of imposex were found with VDSI ranging between II and III, a RPLI of 31.9 and a RPSI of 3.2. In this area there is a small shipyard and boat cruising is intensive. In Mucuripe Beach (station 6), near Mucuripe Harbor and Mansa Beach (station 7), also close to the harbor, where boat traffic associated to fishing and tourism as well as large ship traffic are very intense, VDSI was V, except for a single female in Mansa Beach that had VDSI equal to IV. The values of the RPLI and RPSI obtained for both station 6 (RPLI 63.6 and RPSI 25.7) and station 7 (RPLI 82.8 and RPSI 61.7) are comparable to those found in Guanabara Bay and elsewhere, and support the conclusions drawn from the vas deferens index.

Contrary to the observations of Bryan et al. (1986) in *N. lapillus* whose populations had severely decreased prior to the establishment of restrictions to TBT use, populations of *T. heamastoma* in Fortaleza were apparently stable and abundant in all sampling sites. The intra-

Table 3

Results of biological monitoring in Fortaleza, Ceará.

Station	Date	Location	n (males/females)	% imposex	RPLI	RPSI	VDSI
1	March 2000	Pacheco Beach	27/23	0.0	0.0	0	0
2	March 2000	Dois Coqueiros Beach	20/30	100.0	< 0.1	< 0.01	I-II
3	October 2000	Barra do Ceará Beach	15/35	100.0	31.9	3.2	III
4	November 1999	Formosa Beach	26/24	100.0	< 0.1	< 0.01	I
5	December 1999	Poço da Draga Beach	32/18	100.0	< 0.1	< 0.01	I
6	September 1999	Mucuripe Beach	36/14	100.0	63.6	25.7	IV
7	March 2000	Mansa Beach	32/18	100.0	82.8	61.7	V
8	January 2000	Titanzinho Beach	25/25	0.0	0.0	0.0	0
9	January 2000	Caça e Pesca Beach	22/28	0.0	0.0	0.0	0
10	December 1999	Sariguaba Beach	20/30	0.0	0.0	0.0	0

RPLI = Relative penis length index; RPSI = Relative penis size index; VDSI = Vas deferens sequence index.

capsular development of *N. lapillus* (Bauchet, 1989; Feare, 1970) seems to favor population decline in polluted sites because organisms are directly exposed to the contaminant since the early stages of life. As *T. haemastoma* development presents a stage of planktonic larvae, areas can be re-colonized by organisms imported from uncontaminated sites (Spence et al., 1990) promoting constant provision of healthy individuals.

T. haemastoma spawning was observed even in sites where stage IV of VDSI occurred, both in Fortaleza and in Guanabara Bay. A similar observation was reported by Gibbs & Bryan (1986), Fioroni et al. (1991) and Oehlmann et al. (1996) that concluded reproductive failure will only occur in organisms displaying VDSI above V, a limit above which blockage of the female genitals initiates. In Mansa Beach, however, the majority of the sampled organisms showed VDSI V with penis and vas deferens completely formed and blocked genital cavity. In this case spawning was not observed, suggesting that affected females were unable to reproduce.

Conclusions

In the present investigation, sexual alterations leading to imposex in females of neogastropod *T. Haemastoma* were observed in Rio de Janeiro and in Ceará coastal areas. This reprodutive anomaly has been attributed to the presence of TBT and TPT in the water (Gibbs & Bryan, 1994; Horiguchi et al., 1997a, 1997b; ten Hallers-Tjabbes et al., 1994). Although concentration levels in water are not yet available for the observed sites, the distribution of imposex levels points towards a possible cause-effect relation with TBT and/or TPT.

The biomonitoring results for Guanabara Bay and Fortaleza show that higher imposex indexes are occurring in areas of harbor and marina activities, confirming the trend observed by other authors in several regions of the globe. Because Guanabara Bay is subjected to various sources of organic and inorganic pollutants, it is difficult to establish unambiguous cause-effect relationships. However, the actual absence of *T. Haemastoma* in areas where it was commonly found in the past, raises concern on the extension of possible effects of TBT contamination in the bay.

All sampled females from Guanabara Bay displayed some level of imposex. Differently, in Fortaleza some sites were unaffected and, possibly due to the lower level of contamination of the marine environment, no evidence of extinction were registered.

The wide distribution of *T. haemastoma* along the Brazilian coast makes the species a good candidate for large scale biological screening of "hot spots" of TBT and TPT pollution, although further laboratory and field studies are required to establish a quantitative relation between imposex level and concentration of TBT and/or TPT in the marine environment.

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