



Scientific Note

Imposex in *Nassarius vibex*: relationship with harbor and yachting activities at five beaches in Sepetiba Bay, RJ, Brazil

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Abstract. Imposex indicators for *Nassarius vibex* were markedly variable among populations from different beaches, suggesting localised effects by proximity of Yacht and Harbor. There are evidences suggesting that its population structure has been altered within Sepetiba bay contaminated areas.

Key words: Gastropods, muddy sand beaches, environment pollution, biomonitoring, Sepetiba Harbor

Resumo. Imposex em *Nassarius vibex*: relações com as atividades do Iate e do Porto em cinco praias na Baía de Sepetiba, RJ, Brasil. Indicadores do imposex para *Nassarius vibex* foram marcadamente variáveis entre as populações das diferentes praias, sugerindo efeitos da proximidade do Iate e do Porto. Evidências sugerem que a estrutura populacional tem sido alterada em áreas contaminadas na Baía de Sepetiba.

Palavras chave: Gastrópodes, Praias areno-lamosas, Poluição ambiental, Biomonitoramento, Porto de Sepetiba

Imposex, also known as pseudohermaphroditism, is characterized by the development of additional male sex organs (penis and/or vas deferens) on gastropods females. Imposex is mainly associated with tributyltin (TBT) and thiphenyltin (TPT) compounds that are used in anti-fouling paints (Fent 1996). Besides that, other compounds are known to promote imposex such as copper (Nias *et al.* 1993), nonylphenol (Evans *et al.* 2000), polychlorinated biphenyls (PCBs) and aroclor (Maran *et al.* 2006, Garaventa *et al.* 2008). This is a

widespread phenomenon known to occur in more than 190 species of marine gastropods along the world (Pessoa *et al.* 2009). In South America, it was verified in Argentina, Brazil and Chile at nearly 20 species (Gooding *et al.* 1999, Penchaszadeh *et al.* 2001, Caetano & Absalão 2002, Castro *et al.* 2007, Bigatti *et al.* 2009, Cardoso *et al.* 2009). In Brazil, imposex has been reported in seven species: *Cymatium parthenopeum*, *Leucozonia nassa*, *Nassarius vibex*, *Olivancillaria vesica*, *Stramonita haemastoma*, *S. rustica*, *Voluta ebraea* (Caetano &

Absalão 2002, Castro *et al.* 2007, 2008, Meirelles *et al.* 2007, Cardoso *et al.* 2009). This phenomenon has been used at several places around the world as a tool to monitor contamination by organotin compounds (e.g. TBT), since that methods of chemical analysis are extremely onerous (Oehlmann *et al.* 1996).

Sepetiba Bay is a flat environment fed by high flux rivers that receive wastes from over 400 industrial facilities and can be considered polluted by a wide variety of metals (Gomes *et al.* 2009) and also presents a intense yachting activities/vessels traffic mainly as a consequence of the Itacuruçá Yacht Club (with 680 ships) and Sepetiba Harbor.

Nassarius vibex is a small neogastropod snail that inhabits the intertidal area of hard and soft bottoms with wide geographical distribution that extends from Massachusetts (USA) to Santa Catarina (south Brazil) (Rios 2009, Rosenberg 2009). Demaintenon (2001) described the ontogeny of reproductive system of *Nassarius vibex* females with and without imposex and biphallia in imposexed females was recently described (Cardoso *et al.* 2009). The present investigation was designed to study the incidence of imposex in the nassariid *Nassarius vibex* at five muddy sand beaches located in Sepetiba Bay, Rio de Janeiro, Brazil.

Sepetiba Bay (22°54'-23°04'S; 44°34'-44°10'W) is a sedimentary embayment located in Rio de Janeiro State, southeastern Brazil, with an area of 520 km². It was shaped by an extensive process of sand deposition, which formed a barrier beach at its southern end. It ends in a wide confluence with the Atlantic Ocean at its western boundary (Azevedo *et al.* 2007). Five beaches (stations) - Canal do Bacalhau, Madeira, Gamboa, Flexeira and Suja (Figure 1) - were sampled in spring 2007. All these beaches could be characterized as sheltered (*sensu* McLachlan 1980) with low wave action, gentle slope, higher silt-clay content, mean salinity of 33 and maximum depth of approximately 28 m (Pessanha & Araújo 2003, Caetano *et al.* 2008).

One sector (500 m apart) were established, each 10 m wide with a 25 m base parallel to the waterline, located from the swash zone to a 15 cm water depth in the sublittoral. Specimens were hand-collected until sum a minimum of 70 individuals. In the laboratory, shell length was measured with a vernier caliper, posteriorly cracked and open in a vice, and the individuals of *N. vibex* removed and examined for sex determination. Individuals having seminal vesicle were identified as males while other ones with seminal vesicle absent as females. Females with penis were identified as imposexed

females (Demaintenon 2001). The penis length of males and imposexed females were measured under a stereoscopic microscope with a 0.01 mm precision ocular micrometer. The imposex percentage was calculated as the proportion of females with imposex compared to the total number of females in the sample. The Relative Penis Length Index (RPLI) and Relative Penis Size Index (RPSI) were calculated as follows: RPLI (%) = [(mean penis length in females)/(mean penis length in males)]*100; RPSI (%) = [(mean penis length in females)³/(mean penis length in males)³]*100 (Gibbs & Bryan 1987).

To compare the population size structure among beaches with different levels of imposex, we determined the mean shell length, L_{90%} (length representing the 90th percentile of the female population) (Velooso & Cardoso 1999). To determine the percentage of juveniles in the population we calculated the L_{5%} (length representing the 5th percentile of the juveniles population).

Each beach was classified in categories (according to the number of ships (1), distance between sampling site and Yatch/Harbor (2) and presence/absence of mooring sites (3) that express the level of yachting activities/vessels traffic. (1) High – composed by areas with more than ten ships within visible range from the sampling site; Medium – includes areas with less than ten ships in proximity to the sampling site or with harbor facilities for docking of large ships. In the medium boating activity areas, artisanal fishing was frequent; Low – areas without shipping and limited artisanal fishing. These three categories were determined according to Gooding *et al.* (1999) and information of yachting activities/vessels traffic was based on annual observations made by the authors. Each one of these categories were associated with a score (high – score = 1, medium – score = 2, low – score = 3). Similar procedures were adopted to express: (2) Distance from each sampling beach to the Itacuruçá Yatch or Sepetiba Harbor (score 1 – distances between 0 and 2.5 Km, score 2 – distances between 2.5 and 5.0 Km, score 3 – distances higher than 5.0 Km); (3) Presence (score 0) or absence (score 1) of mooring sites. After that, a sum of beach scores was generated for each beach.

Non parametric Spearman Rank test was used to correlate the sum of beach scores with imposex indicators (% imposex, RPLI, RPSI) also as with shell length of females, L_{90%} and L_{5%}. Population structure by size, represented by L_{5%} and L_{90%}, were also correlated with imposex indicators. One-way ANOVA was used to test the null hypothesis that there was no significant difference in

shell length of the *Nassarius vibex* among beaches and the Tuckey HSD *Post Hoc* test (Zar, 1999) was used in case of significant differences. An ANCOVA was used to compare the penis length among beaches,

using shell length as covariate. Data were linearized (log-transformed) in order to fulfil ANCOVA assumptions (Zar 1999).

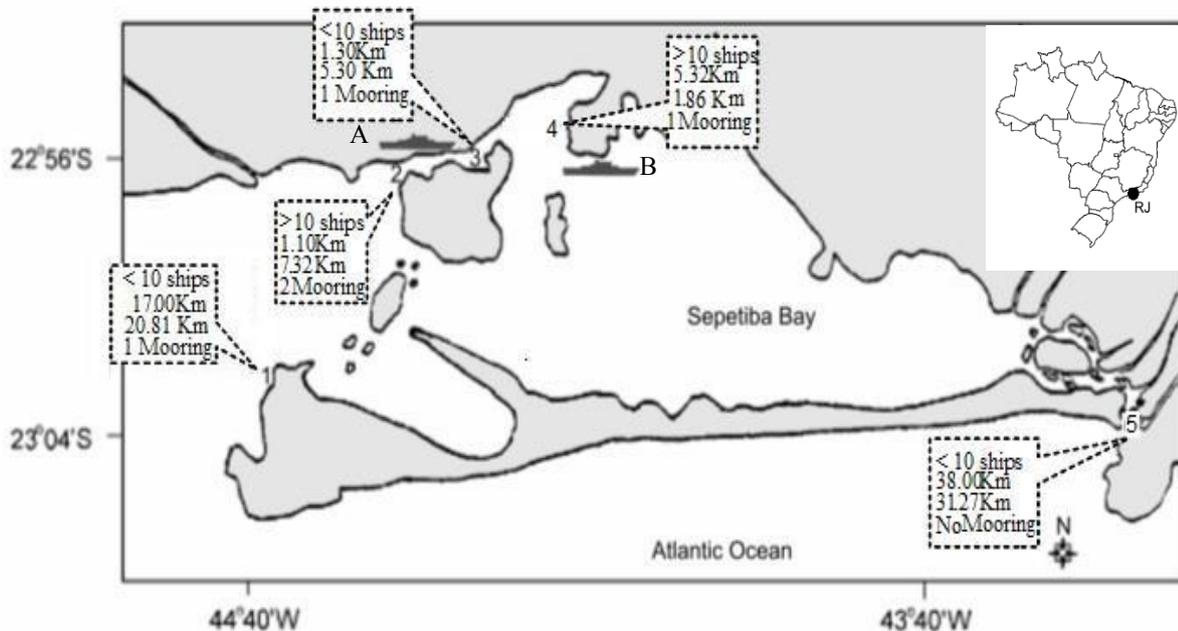


Figure 1. Map from Sepetiba Bay showing sampling sites/beaches. (1) Suja; (2) Flexeiras; (3) Gamboa; (4) Madeira; (5) Canal do Bacalhau. Information inside the boxes are on shipping intensity, distance from Itacuruçá Yacht, distance from Sepetiba Harbor, and number of mooring sites. “Black ship” – indicates the relative position of (A) Itacuruçá Yacht Club and (B) Sepetiba Harbor.

The highest imposex percentage was verified at Flexeira beach (94.55%), followed by Madeira beach (92.50%), Canal do Bacalhau (17.31%), Suja beach (14.78%) and Gamboa beach (13.97%). Penis length based indexes (RPLI and RPSI) showed similar pattern and were ordered in the following sequence: Madeira beach (RPLI = 35.64%, RPSI = 4.53%), Flexeira beach (RPLI = 32.13%, RPSI = 3.32%), Gamboa beach (RPLI = 5.64%, RPSI = 0.01%), Suja beach (RPLI = 4.40%, RPSI = 0.01%) and Canal do Bacalhau (RPLI = 4.95%, RPSI = 0.01%). The differences between the penis length based indexes are not significant and continue to show that both Madeira and Flexeiras beaches are most affected in terms of imposex on this species (see Table I).

The sum of beach scores demonstrated that Canal do Bacalhau (score sum = 10) and Suja (score sum = 8) beaches clearly contrast with the other three beaches, Gamboa, Flexeiras and Madeira (all with score sum = 5) in respect to level of yachting activities/vessels traffic and other possible sources of contamination (Table I).

The relationship between penis length based indexes (RPLI and RPSI) and score sum was not significant. However, a significant positive

correlation was also observed for score sum with $L_{90\%}$ ($R_s = 0.89$). Correlations were not significant with imposex percentage, mean penis length of imposexed females, mean shell length and $L_{5\%}$ ($p > 0.05$). In respect to population structure by size, $L_{5\%}$ was significantly correlated ($R_s = 0.9$) with RPLI index. No significant correlation was verified between $L_{90\%}$ and imposex indicators - % imposex, RPLI and RPSI.

There were no differences (ANCOVA $F_{4,128} = 1.29$, $p = 0.27$) in penis length of imposexed females among beaches.

There were significant inter-beaches differences in female shell length (ANOVA $F_{5,377} = 12.15$, $p = 0.00$). The Tuckey HSD *Post Hoc* test revealed that females of Flexeiras beach were smaller (shell length) than those from the other beaches.

Imposex was registered in all of the sampling sites/beaches in Sepetiba bay and its intensity being evaluated by three different indicators: imposex percentage, RPLI and RPSI (Table I). Suja and Canal do Bacalhau beaches showed the lowest degree of embayment, thus receiving much less shipping and boating. Imposex incidence is, therefore low in these beaches when

compared with other inner beaches located in Sepetiba Bay (such as Gamboa, Flexeiras and Madeira). High shipping activity is the probable cause of increased metal contamination and the problem is exacerbated by: (1) reduced tidal exchange in the enclosed area of Sepetiba Harbor and (2) discharge of rivers that could bring pollutants to this area of the Bay. Besides that, Suja and Canal do Bacalhau are further away from

potential sources of metal contamination. However, Gamboa with lower imposex percentage (11.94%) is inner located in Sepetiba bay where higher source contamination could be expected (Table I).

It may be noted that there are similar trends in the values of the RPLI and RPSI indices. The values were low at Canal do Bacalhau, Suja and Gamboa while were relatively higher in Madeira and Flexeira (Table I).

Table I. Beach scores, imposex indicators (imposex percentage, RPLI, RPSI), Shell length and Penis length of *Nassarius vibex* from five beaches in Sepetiba bay

Beaches features	Madeira	Gamboa	Flexeiras	Suja	Canal do Bacalhau
	22°55'10" S 43°51'05" W	22°55'45" S 43°53'24" W	22°56'08" S 43°52'19" W	23°03'30" S 43°59'19" W	23°03'34" S 43°34'02" W
% Imposex	92.50	13.97	94.55	14.78	17.31
RPLI	35.64	5.64	32.13	4.40	4.95
RPSI	4.53	0.01	3.32	0.01	0.01
Beach Score	5	5	5	8	11
Male shell length (mm)	14.00 (±1.1)	14.38 (±0.84)	13.41 (±1.1)	15.32 (±1.3)	16.48 (±1.2)
Female shell length (mm)	14.82 (±1.3)	14.68 (±1.21)	12.75 (±1.9)	14.14 (±2.6)	15.27 (±2.6)
Male penis length (mm)	6.84 (±1.1)	8.57 (±1.50)	7.95 (±1.3)	7.83 (±1.3)	8.95 (±1.3)
Female penis length (mm)	2.63 (±1.2)	4.05 (±1.70)	2.70 (±1.6)	2.33 (±1.2)	2.53 (±1.1)
L _{5%}	12.4	11.6	10.6	9.9	10.5
L _{90%}	16.3	16.3	14.7	17.5	18.1

Vas deferens sequence index (VDSI) has been considered a better descriptor of imposex development at heavy polluted cenarios. However, we were unable to examine the VDSI for imposex in *N. vibex*, because the vas deferens observation requires a more detailed morphological study including histological techniques (Simone, L.R.L., pers. comm.). On the other hand, 100% imposex was not found in any of the studied sites suggesting that imposex indicators (% imposex, RPLI and RPSI) used in this work were satisfactory.

In general, specimens collected at sites/beaches near marinas and harbours (*e. g.*, Sepetiba harbor and Itacuruçá Yacht) with heavy shipping traffic (*e.g.*, Flexeira and Madeira) presented higher imposex percentage and RPLI-RPSI values. It is thus suggested that the imposex development in *Nassarius vibex* reflect the degree of pollution. Similar trends were observed in many others localities around the world (Horiguchi *et al.* 1994, Blackmore 2000, Fernandez *et al.* 2002, Castro *et al.* 2007). Bech (2002) demonstrated the importance of pollution caused by the marinas of Phuket Island, Thailand, where imposex level was as high as those found in big Harbors around the world.

Alternatively to the use of standard imposex indicators (% imposex, RPLI, RPSI), we could

investigate the influence of imposex development in the population size structure. We assume that higher shell length of juveniles would be expected in sites/beaches with higher imposex levels as a result of reduced reproduction. This can result in a marked decrease in the proportion of juveniles, which was measured in this study by means of L_{5%}. Higher values of L_{5%} indicate lower proportion of juveniles. Thus, in beaches such as Madeira and Flexeiras, the higher values of both L_{5%} and penis length based indexes suggest a reduced reproduction in these populations. Statistical analysis (Non parametric Spearman Rank test) showed significant correlation between L_{5%} and RPLI.

The results from non-parametric correlations showed a significant increase in L_{90%} with the increase in sum of beach scores sum, which support the influence of imposex development in size structure of studied populations. It indicates that in environment less impacted the populations presented higher length.

Most population descriptors gave support for the hypothesis that *N. vibex* can be used as an indicator of environmental pollution or stress. Imposex indicators were markedly variable among populations from different beaches, suggesting localised effects by proximity of Yacht and Harbor. Besides that, some evidences suggest that the population structure of *Nassarius vibex* has been

altered in Sepetiba bay contaminated areas with lower proportion of juveniles in affected beaches.

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