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Reproductive anomaly in female marine gastropod *Babylonia spirata* along Chennai and Tuticorin coasts of Tamilnadu

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Abstract

The females of marine gastropod *Babylonia spirata* along Chennai Royapuram Fishing Harbour and Tuticorin coast showed reproductive anomaly i.e. superimposition of male sexual characters, imposex. Imposex frequency (93.8%) and the percentage of females with stage 4 of imposex (18.8%) were higher in Chennai than Tuticorin. However, no marked difference in male: female sex ratio was observed in Tuticorin (1.2: 1) and Chennai (0.9: 1). A further morphological change in the form of double penis in a female was observed in Tuticorin. Vas Deferens Sequence Index (VDS) in Tuticorin and Chennai Royapuram Fishing Harbour (1.7 and 1.8) were well below the critical index value of 4. No occlusion of genital pore was observed. This reproductive anomaly could be linked to Tributyltin (TBT) leaching from antifouling paints used in fishing boats and ships along these coasts.

Keywords: Gastropod *Babylonia spirata,* imposex

Introduction

The process of adsorption, colonization and development of living and nonliving material on an immersed substratum is known as marine biofouling (Clare, 1996). The biofouling process is initiated by the accretion of organic and/or inorganic molecules on to the submerged surfaces (molecular fouling) followed by the colonization of microbes (microfouling) and settlement of macrofoulers (macrofouling). The technical and economical consequence of the fouling of the man made structures exposed to marine environment is quite significant.

Measures to control biofoulers are going on since time immemorial. Present commercial antifouling technology includes mechanical cleaning, biocides, toxic antifouling coatings, most recently foul release, and easy clean coatings. Today's most effective antifouling coatings contain toxic additive substances known as biocides.

Antifouling paints containing tributyltin (TBT) compounds, introduced in 1960s and found wider use from mid 1970s, achieved worldwide application without any comprehensive environmental risk assessment (Champ, 2000). It has been estimated that TBT based antifouling coating save ship owners about US$ 500 million to more than US$ 1 billion annually in fuel saving and operatinal cost (Damodaran *et al.*, 1999). However, it was not until the mid 1980's that researchers in France and the United Kingdom began to suggest that the use of TBT in antifouling paints was adversely impacting a number of non-target marine organisms. TBT is an endocrine disruptor in molluscs, with females developing male sex organs (imposex) (Fent, 1996). Imposex is the superimposition of male morphological features like penis and vas deferens on female gastropods. It has been well established that TBT compounds cause imposex universally (Barroso *et al.*, 2002) at levels lower than 10 $ng l^{-1}$.

TBT has been attributed to the local extinction of some populations of dog-whelks, *Nucella lapillus* in Southwest England (Bryan *et al.*, 1986). The abnormal growth and reproductive failure in cultivated oyster, *Crassostrea gigas* in Arcachon Bay in France was linked to the leaching of TBT from antifoulants on small boats (Alzieu, 1991). Imposex is currently documented in over 118 species in 63 genera worldwide (Bettin *et al.*, 1996). In India, imposex has been recorded in *Cronia konkanensis* from Marmagoa Harbour (Vishwa Kiran and Anil, 1999), *Thais bufo, T. rudolphi, T. tissoti* and *Ocenebra bombayana* from Gujarat (Tewari *et al.,* 2002) on the west coast and *Thais biserialis* (Santhana Ramasamy and Murugan, 2002) and *Chicoreus virgineus* (Murugan and Santhana Ramasamy, 2003) from Tuticorin Harbour area on the east coast. Imposex is an important biomarker and a useful tool for assessing the impact of organotin concentration (Minchin and Minchin, 1997) since the cost of analysis of organotin concentration in water and sediment is quite high (Camillo *et al.*, 2004).

Materials and methods

Sixty adult *Babylonia spirata* (Mollusca: Gastropoda: Caenogastropoda: Buccinidae) were collected each from fish landings near Tuticorin Port area (September 2004) and Chennai Royapuram Fishing Harbour (December 2004) and transported to the laboratory. They were cleaned and maintained with aeration and then narcotized with 7% $MgCl_2$ (Santhana Ramasamy and Murugan, 2002; Murugan and Santhana Ramasamy, 2003). Shell length was measured to the nearest 0.1mm. Shells were cracked open to remove the soft parts. Based on prostate gland in males and capsule gland and albumen gland in females, the sexes were separated. The females were then observed under the Motic Zoom Stereo Digital Microscope DM-143-FBGG. The penis length of males and that of females with imposex were measured using Motic Image Plus 2.0 ML software.

Proportion of females with imposex was compared with the total number of females in the sample to assess the imposex frequency (Michael Bech, 1999). The condition of vas deferens in females was assessed using the vas deferens sequence (VDS) index (Oehlmann *et al.*, 1991). Relative penis size (RPS) index is expressed as cube of mean female penis length/ cube of mean male penis length X 100. Relative penis length (RPL) index is expressed as mean female penis length / mean male penis length X 100 (Vishwa Kiran and Anil, 1999).

Results

The mean male and female shell length, penis length, the imposex stages observed and the RPS, RPL and VDS are given in Tables 1 and 2. The percentage of imposex in Tuticorin was 70.4. Almost 93.8% of females were affected by imposex in Chennai Royapuram Fishing Harbour area. Six imposex stages (1a, 1b, 1c, 2c, 3c and 4) were observed in Chennai Royapuram Fishing Harbour area and four stages (1a, 2b, 3c and 4) were observed in Tuticorin. The initial imposex stage 1a was observed in 40.6% of the females in Chennai Royapuram Fishing Harbour area. The male: female sex ratio did not deviate much in Chennai. But, it slightly varied in Tuticorin (1.2: 1). The stage 1 (1a, 1b, 1c) of imposex was represented by 56.3% of the females in Chennai. Only 29.6% females exhibited stage 1 (1b) in Tuticorin. The imposex stages 3c and 4 were observed in both places. But, the representation was high in Tuticorin (37%) than in Chennai (21.9%).

A further morphological alteration of double penis was observed in Tuticorin area in one of the females affected with imposex stage 4 (Figs. 1 and 2). The VDS index was higher in Chennai than in Tuticorin. Consequently, the RPS and RPL values were also higher in Chennai. The RPS and RPL values increased with increasing stages of imposex.

The correlation between male shell length and penis length was not significant in both Tuticorin [0.3213 (P>0.05)] and Chennai [0.1224 (P>0.05)]. The correlation between shell length and penis length of females with imposex stages 3c [0.7336 (P>0.05)] and 4 [-0.0384 (P>0.05)] in Tuticorin were not significant. Similarly, in Chennai also the correlation between female shell length and penis length of females with imposex stages of 1a [-0.1310 (P>0.05)], 3a [0.5212 (P>0.05)] and 4 [-0.4734 (P>0.05)] were not significant.

Table 1. *Reproductive anomaly in* Babylonia spirata *along Tuticorin and Chennai coasts*

No. of animals	Sex	Shell length (mm) Mean ± SD	Penis length (mm) Mean ± SD	% of imposex in females	RPS %	RPL %	VDS	M: F Sex Ratio
Tuticorin								
33	Male	44.2 ± 4.4	4.4 ± 1.2	---	---	---	---	1.2: 1
27	Female	45.6 ± 5.0	1.6 ± 0.6*	70.4	4.8	36.4	1.7	
Chennai Royapuram Fishing Harbour								
28	Male	46.1 ± 3.5	4.1 ± 1.4	---	---	---	---	0.9: 1
32	Female	46.5 ± 3.6	1.7 ± 1.1*	93.8	7.1	41.5	1.8	

*in females with imposex

Table 2. *Females with imposex: shell length, penis length, RPS and RPL*

Stage	Number	%	Shell length (mm) Mean ± SD	Penis length (mm) Mean ± SD	RPS %	RPL %	VDS
Tuticorin							
0	8	29.6	44.5 ± 6.2	---	---	---	---
1b	8	29.6	45.8 ± 4.2	---	---	---	0.3
2b	1	3.7	49	---	---	---	0.1
3c	5	18.5	43.8 ± 4.0	1.2 ± 0.5	2.0	27.3	0.6
4	5	18.5	48.2 ± 5.6	2.0 ± 0.5	9.4	46.4	0.7
Chennai Royapuram Fishing Harbour							
0	2	6.3	46.5 ± 2.1	---	---	---	---
1a	13	40.6	45.9 ± 4.5	1.5 ± 0.7	4.9	36.6	0.4
1b	3	9.4	45.7 ± 3.1	---	---	---	0.1
1c	2	6.3	46.5 ± 3.5	---	---	---	0.1
2c	5	15.6	48.4 ± 4.0	1.8 ± 1.8	8.5	43.9	0.3
3c	1	3.1	43.0	1.0	1.5	24.4	0.1
4	6	18.8	47.2 ± 2.2	2.2 ± 1.2	15.5	53.7	0.8

Discussion

TBT is considered as the most toxic compound ever deliberately introduced into the natural waters (Goldberg, 1986) and in fact, imposex is induced under its influence at the ppt (parts per trillion; ng/l)-level (Oehlmann *et al.*, 1991). In extreme cases, the development of male sexual characters in female gastropods occludes the genital pore leading to sterility in females. Sometimes, the buildup of egg capsules result in the rupture of capsule gland leading to premature mortality of the animal. Incidentally, this report of imposex occurrence in *B.spirata* in Royapuram Fishing Harbour is the first record along Chennai coast. Record of imposex in the marine gastropods *Thais biserialis* (Santhana Ramasamy and Murugan, 2002) and *Chicoreus virgineus* (Murugan and Santhana Ramasamy, 2003) in Tuticorin were the only earlier records pertaining to east coast of India.

The frequency of imposex among the sampled females of *B. spirata* from Tuticorin was 70.4%, which is lower than 82.4% observed for *Chicoreus virgineus* (Murugan and Santhana Ramasamy, 2003) from the same area. The frequency of imposex is also less when compared to 90–100% reported from Marmagoa Harbour (Vishwa Kiran and Anil, 1999) and Phuket, Thailand (Michael Bech, 1999). At the same time, imposex frequency in *B. spirata* was high (93.8%) at Chennai. But, this percentage of occurrence is less when compared to the high or close to 100 % in 73 locations in Japan (Horiguchi *et al.*, 2001).

The male: female sex ratio did not deviate much in *B.spirata* in the present study. The deviation is less when compared to that observed for *T. biserialis* (2.1: 1) (Santhana Ramasamy and Murugan, 2002) and *C.virgineus* (2.5: 1) (Murugan and Santhana Ramasamy, 2003) along the Tuticorin coast. The RPS index in *B. spirata* in both the places was higher than that of *T. biserialis* (0.3) (Santhana Ramasamy and Murugan, 2002) and *C. virgineus* (3.28) (Murugan and Santhana Ramasamy, 2003) reported from Tuticorin coast.

VDS index is considered as the more valid for the biomonitoring of TBT pollution and it provides information on the reproductive capability of the population (Oehlmann *et al.*, 1991). An index of 4 and above indicates the presence of sterilized females in the particular population and eventually the reproductive capacity of the population would be reduced (Gibbs *et al.*, 1987). The imposex stage 4 is also considered as the last fertile stage of imposex (Oehlmann *et al.*, 1991). However, in the present study, the observed VDS index values of 1.7 and 1.8 are well within 4 and are lower than that observed for *C.virgineus* (1.82) (Murugan and Santhana Ramasamy, 2003) and higher than that of *T. biserialis* (0.7) (Santhana Ramasamy and Murugan, 2002) from Tuticorin coast.

A unique phenomenon of double penis in a female *B. spirata,* considered as further morphological alteration, was observed (Figs. 1 and 2) in Tuticorin. Similar occurrence of double penis has been reported in stage 4 of imposex in *Nucella lapillus* (Oehlmann *et al.*, 1991).

Bifurcate and double penis has also been reported in *Hinia reticulata* of Buccinidae (Stroben *et al.*, 1992). Similarly, ovarian dysmaturity accompanied by imposex was observed in the ivory shell *Babylonia japonica* in Japan (Horiguchi *et al.*, 2001). The decrease in its catch and spawning are correlated to triorganotin concentration in the ovaries and the subsequent imposex associated ovarian insufficiency (Horiguchi *et al.*, 2001).

The occurrence of imposex in *B.spirata* in Chennai Royapuram Fishing Harbour and Tuticorin could be linked to the effect of TBT from antifouling paints. The earlier observation linking imposex occurrence to leaching of TBT from antifouling paints from ships in Tuticorin (Santhana Ramasamy and Murugan, 2002) and the high contaminated categorization of the sediment butyltin levels of Chennai and Tuticorin harbours (Babu Rajendran *et al.*, 2001) substantiate the present observation of imposex in Tuticorin and Chennai.

TBT is toxic not only to shellfish but also to a variety of marine species and humans (Baumann, 1991). It has been observed that the tolerable average residue levels (TARL) for TBT in seafood have exceeded in one or more samples in nine of the 22 countries for which data were available i.e. Canada, France, Italy, Japan, Korea, Poland, Taiwan, Thailand and USA (Belfroid *et al.*, 2000). The fish samples from India have been reported to contain butyltin concentration of up to 79 ng g^{-1} (wet wt.) and that of TBT alone upto 1.6 ng g^{-1} wet wt. and a human dietary intake of <27-710 ng $person^{-1}$ day^{-1} of butyltins has been predicted (Kannan *et al.*, 1995). The reported high tributyltin concentration in some stations of Chennai and Tuticorin harbours exceeded the toxicity level to many aquatic species and the environmental quality standards (Babu Rajendran *et al.*, 2001). *B.spirata* is an edible gastropod and the occurrence of imposex and the reported tissue concentration in other marine animals is really a cause for concern. This species is considered as one of the sensitive marine gastropods which could be used as a possible bio-indicator species for TBT contamination.

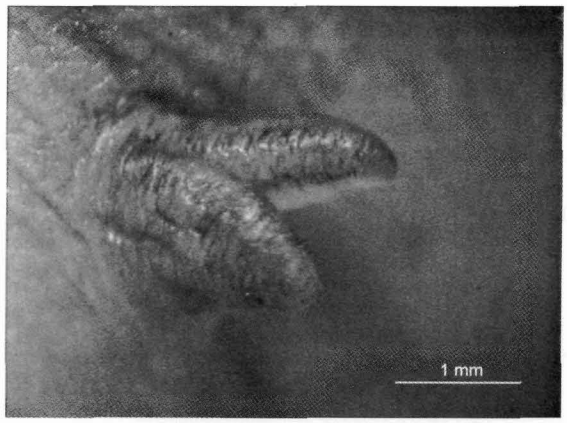

Fig.1. Close-up view of double penis observed in a female *B. spirata* at Tuticorin

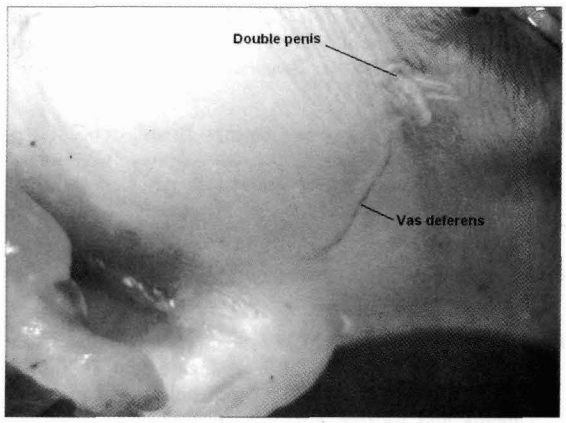

Fig.2. Imposex stage 4 in *B. spirata* with double penis and complete vas deferens observed at Tuticorin

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