

## Incidence of imposex in the muricid gastropod *Chicoreus virgineus* from Tuticorin, southeast coast of India

A. Murugan and M. Santhana Ramasamy

Suganthi Devadason Marine Research Institute, Tuticorin-628 001, Tamil Nadu, India

### Abstract

The muricid gastropod *Chicoreus virgineus* from Tuticorin area of Gulf of Mannar, southeast coast of India has been studied for expression of imposex. The frequency of imposex in females of *C. virgineus* collected from Tuticorin Port was 82.3%. The stage 2a, i.e. penis with closed penis duct was predominant with 64.7%. The male: female sex ratio has deviated from normal and was 2.5:1. The RPSI value of 3.2% and RPLI value of 32.0% have been recorded. 11.8% of the females with imposex exhibited the stage 4, which is considered as the last fertile stage of imposex. But no occlusion of genital pore was observed in the present study. Interestingly no imposex was observed in *C. virgineus* from Vellapatti area, which is located approximately 15 km north of Tuticorin Port.

### Introduction

Imposex is referred to the process of development of male sexual characters in female gastropods. Imposex is widespread in places close to ports, shipyards, marinas and mariculture facilities (Michael Bech, 1999) and is linked to Tributyltin (TBT), the active biocide in marine anti-fouling paints (Tan, 1997). TBT is considered as the most toxic compound ever deliberately introduced into the natural waters (Goldberg, 1986). Imposex is currently documented in over 118 species in 63 genera worldwide (Bettin *et al.*, 1996). TBT has been attributed to the local extinction of some populations of dogwhelks, *Nucella lapillus* in southwest England (Bryan *et al.*, 1986). The development of male sexual characters in female gastro-

pods in extreme cases, occludes the genital pore leading to sterility in females and sometimes results in premature mortality due to rupture of capsule gland consequent on the build up of egg capsules. Imposex has been well reported in muricid gastropods and the genus *Thais* has been used as indicators of TBT contamination in tropical waters (Bryan *et al.*, 1986). Also copper based paints are used as alternative to organotins and they both exhibit toxicity towards many non-target marine invertebrates. It has been well established that TBT compounds cause imposex universally (Barroso *et al.*, 2002).

The extent of imposex has not been studied much in India except for *Cronia konkanensis* from Marmagoa harbour (Vishwa Kiran and Anil, 1999) on the

west coast and *Thais biserialis* from Tuticorin harbour (Santhana Ramasamy and Murugan, 2002) on the east coast. Tuticorin, located at the southern end of Gulf of Mannar Marine Biosphere Reserve is a unique coral reef ecosystem. Tuticorin Port is the 10<sup>th</sup> major port in India and the vessel traffic has reached 1,236 during 2000-2001 (Fig.1.) There is no dry dock facility here and the vessels have to go around Sri Lanka as the water area linking Palk Bay and Gulf of Mannar is not deep enough for shipping activity. The muricid gastropod, *Chicoreus virgineus* has

been widely distributed in Tuticorin area of Gulf of Mannar and is being consumed by the coastal fisher community. A close look at the species revealed the existence of imposex. Hence a detailed study on this aspect was undertaken and the results are presented. The same species has also been collected from Vellapatti area, approximately 15 km north of Tuticorin Port for comparison. The possible source of TBT in this area has also been discussed.

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Material and methods

Sixty adult animals of *C. virgineus* (Mollusca: Gastropoda: Muricidae) each from Tuticorin Port and Vellapatti areas of Gulf of Mannar (Table 1), southeast coast of India were collected from fish landings, brought to the laboratory, cleaned and maintained with aeration. The animals were narcotized with 7% MgCl<sub>2</sub>. The shell length was measured to the nearest 0.1 mm. Shells were cracked and soft parts removed. The animals were sexed based on the presence of albumen gland, sperm-

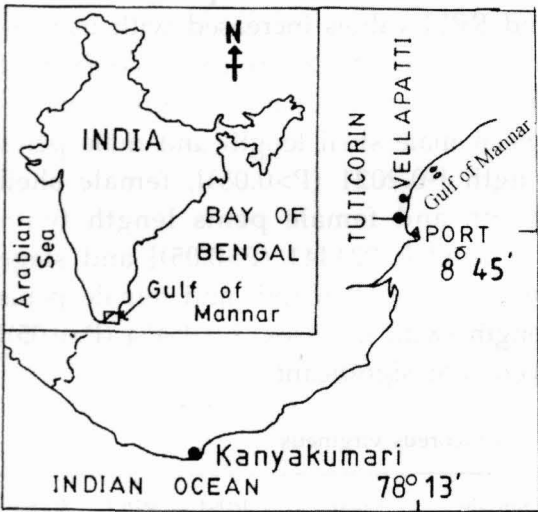


Fig 1. Map showing the study area

Table1. Sample size, sex ratio and imposex frequency in *Chicoreus virgineus*

Sl. No.	Area	Location	Sample size	Male	Female	Sex ratio	Frequency of imposex in females
1.	Tuticorin Port	Lat.8°45'N; Long.78°13'E	60	43	17	2.5:1	82.35%
2.	Vellapatti	Lat.8°51'54"N; Long.78°10'E	60	31	29	1.1:1	—

ingesting gland and capsule gland in females and prostate gland in males. Using binocular microscope and oculometer the length of penis in both males and females with imposex were measured. Imposex frequency was calculated as the proportion of females with imposex compared with total number of females sampled (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 and penis length both in Vellapatti and Tuticorin Port are shown in Table 2. The male: female sex ratio in *C. virgineus* at

devoid of imposex. The Vas deferens Sequence index (VDS) in Tuticorin Port was 1.8. The RPSI and RPLI values are shown in Table 2. Three imposex stages were recorded during the present study in females from Tuticorin Port. Stage 1a, i.e., penis without a penis duct was observed in 5.9% of the females, Stage 2a, i.e., penis with closed penis duct was predominant and was observed in 64.7% of the females and Stage 4, i.e., penis with a penis duct and continuous vas deferens from penis to vulva was observed in 11.8% of the females. Only 17.6% of the females were normal without imposex. The RPSI and RPLI values increased with increasing stages. The correlation coefficient analysis showed that the correlation between male shell length and male penis length [-0.2021 ( $P > 0.05$ )], female shell length and female penis length (with imposex) [0.021441 ( $P > 0.05$ )] and stage 2a female shell length and female penis length (with imposex) [-0.05454 ( $P > 0.05$ )] were not significant.

Table 2. Mean shell and penis length with imposex index in *Chicoreus virgineus*

Area	Mean male shell length (mm)	Mean male penis length (mm)	Mean female shell length (mm)	Mean female penis length (mm)	RPSI	RPLI	VDSI
Tuticorin Port	52.23±10.78	7.06±2.17	45±7.63	2.26±0.53	3.28	32.01	1.82
Vellapatti	58.77±14.01	6.93±1.76	45.21±7.66	—	—	—	—

Vellapatti was 1.1:1 and whereas in Tuticorin Port it showed a marked deviation and was 2.5:1. The frequency of imposex in females at Tuticorin Port was 82.4% and the females in Vellapatti were

## Discussion

Today's most effective antifouling coatings contain toxic additive substances known as biocides. The tributyltin (TBT) and copper based paints are most com-

monly applied to marine structures to deter fouling organisms. TBT is effective in controlling fouling and remain active for about seven years. TBT based antifouling coating are estimated to save ship owners from US\$ 500 million to more than US\$ 1 billion annually in fuel avoidance and operations cost (Damodaran *et al.*, 1999). TBT based antifouling technology was so successful that it achieved worldwide application without any comprehensive environmental risk assessment during its 40 years of use as a marine antifoulant (Champ, 2000). The toxic effect of such paints to marine organisms especially to non-target organisms has been recognised and many countries imposed legislations to restrict their use. Even in Icelandic waters of sub arctic, the dogwhelk *Nucella lapillus* exhibited imposex due to TBT (Svavarsson and Skarphedinsdottir, 1995). Imposex is induced under the influence of TBT (tributyltin) compounds at the ppt (parts per trillion; ng/l)-level (Oehlmann *et al.*, 1991). TBT was an endocrine disruptor in molluscs, with females developing male sex organs (imposex) at levels lower than 10 ng l<sup>-1</sup> (Fent, 1996). So, International Maritime Organization (IMO) has proposed a total ban on TBT based paints by the year 2008.

The comparison for expression of imposex by *C. virgineus* from two locations of Tuticorin Port and Vellapatti in Gulf of Mannar area along Southeast coast of India indicated a marked variation. The expression of imposex was prominent in Tuticorin Port area and was absent in Vellapatti, located approximately 15 kms.

north of Tuticorin Port. This is in coincidence with the report of Evans *et al.* (1995) that the imposex was virtually absent in gastropods collected from outside the harbour area of Ambon Bay (Maluku), Indonesia. Smith (1981) and Gibbs *et al.* (1987) have also correlated the incidence of imposex to ambient water TBT contamination with higher induction linked to yacht docking facilities, ship repair docks and marine traffic. So, the localization of imposex to Tuticorin Port area may be linked to the shipping activities in the Port.

The severity of imposex was high and prominent in *C. virgineus* than in *T. biserialis* (Santhana Ramasamy and Murugan, 2002) from the same area. This observation is in contrast to the earlier reports that species belonging to the *Thais* species have been used as indicators of TBT contamination in tropical waters (Bryan *et al.*, 1986). *C. virgineus* seems to be sensitive than *T. biserialis* in the study area. The other possible reason may be due to their distribution. *T. biserialis* were found distributed near the shore and that of *C. virgineus* away from the shore.

The VDS index of 4 and above indicates that the particular population has sterilized females and with reduced reproductive capacity (Gibbs *et al.*, 1987). But the Vas Deferens Sequence Index in the present study has not exceeded 4 and was 1.82. It is to be noted that 11.8% of the females from Tuticorin Port were found to have stage 4 of the imposex. According to Oehlmann *et al.* (1991), the stage 4 represents the last fertile stage of imposex. Interestingly, the 4<sup>th</sup> stage has not been

observed in *T. biserialis* from the same location (Santhana Ramasamy and Murugan, 2002). RPSI value of 3.28 was observed in the present study. It is comparable to RPSI values of 3 - 64% observed in Indonesian coastal waters (Pandey and Evans, 1996) and in *T. clavigera* (upto 88%) from Japan (Tan, 1997).

The *C. virgineus* is being consumed in the coastal areas of Tuticorin. In this context, the widespread occurrence of organotin pollution has raised concerns over the accumulation of organotin compounds in the food chain and the associated risks related to the presence of contaminants (Belfroid *et al.*, 2000). 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 toxins from antifouling coatings either directly or indirectly threaten much of the world's seafood supply. Kannan *et al.* (1995) reported that fish samples from India contained butyltin concentration of upto 79 ng g<sup>-1</sup> (wet wt.) and that of TBT alone upto 1.6 ng g<sup>-1</sup> wet wt. and predicted the human dietary intake of <27-710 ng person<sup>-1</sup> day<sup>-1</sup> of butyltins. The present report on imposex, therefore, assumes significance in this context.

Santhana Ramasamy and Murugan (2002) reported the possibility of TBT contamination in Tuticorin Port through leaching from antifouling paints from ships.

The present observation of imposex in *C. virgineus* in Tuticorin Port and its absence from the nearby Vellapatti area indicate that the possible source may be TBT in Tuticorin Port as no other contaminant has been envisaged yet.

Apart from acute and chronic toxicity towards sessile adult invertebrates such as corals, the anti-foulant contamination could adversely affect the recruitment of prospects of many other invertebrates as well in the damaged site and could slow down subsequent ecosystem recovery. In this context, the report of incidence of imposex in two species of *C. virgineus* and *T. biserialis* from Tuticorin Port has to be viewed with great concern as the Gulf of Mannar ecosystem is already under stress due to coral mining, unsustainable resource exploitation, pollution and other anthropogenic interventions.

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