

Description and evaluation of imposex in *Strombus canarium* Linnaeus, 1758 (Gastropoda, Strombidae): a potential bio-indicator of tributyltin pollution

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Abstract *Strombus canarium* Linnaeus, 1758 is an important gastropod species within the study area and was traditionally collected for food by the locals. The objective of the present study is to assess the incidence of imposex and its severity in this species. Adult conchs were sampled during their main reproductive period, from October 2005 to January 2006, at Sungai Pulai estuary, Johor Straits, Malaysia. A total of 32.81% of adult females showed imposex characteristics, with varying degrees of severity though. The relative penis size (RPS) index ranged from 1.74 to 33.29 (mean = 13.40 ± 2.27 , $n = 21$), while the

relative penis length (RPL) index ranged from 6.28 to 55.19 (mean = 25.83 ± 3.33 , $n = 21$). The use of vas deferens sequence (VDS) index was however cannot be applied as the presence of egg groove obscured any vas deferens development in affected females. Sequence of imposex (male penis) development in female conch, from merely a small stump to an advance male penis homologous was therefore carefully analyzed and described, and an alternative imposex classification scheme was proposed. *S. canarium* can be a good indicator for monitoring of organotin pollution within the study area. However, more studies are needed in order to further develop and test its validity and application, such as its correlation with levels of pollutant within the tissues and the environment, as well as its application on other *Strombus* species.

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Introduction

Mollusks are very sensitive to organotins and have widely been used in marine pollutants monitoring exercises (Bright and Ellis 1990; Nishikawa et al. 2004; Ismail 2006). ‘Imposex’ (Smith 1980), which

also known as pseudohermaphroditism (Jenner 1979), is the most sensitive biological effect in response to organotins. It is an induced response of forming a penis and/or vas deferens in female gonochoristic prosobranch gastropods (deFur et al. 1999). At the moment, no other xenobiotic is known that also causes imposex, thus was recommended as a suitable bioindicator of TBT pollution (Bright and Ellis 1990; Nishikawa et al. 2004; Ismail 2006). To date, imposex has been described in more than 200 different species of gastropods belonging to 50 genera (Fioroni et al. 1991; Oehlmann et al. 1992; deFur et al. 1999; Oetken et al. 2004; Bigatti and Carranza 2007). Reproductive failure and declining populations due to imposex have been documented in several gastropods species (e.g., Bryan et al. 1986; Gibbs and Bryan 1986; Bright and Ellis 1990; Gibbs et al. 1990; Nias et al. 1993).

It was already well established that shipping activities contributed to the level of organotins, particularly TBT, in the sea and coastal environment (Smith 1981; Bright and Ellis 1990; Ellis and Pattisina 1990; Ismail 2006). A recent study along the Straits of Malacca found high concentration of TBT in bottom sediment, which ranged from 2.8 to as high as 1,100 ng/g (Sudaryanto et al. 2004). Another study reported that locations closed to port areas were the most affected (Tong et al. 1996). There were only few studies on imposex in gastropod have been conducted within the Johore Straits and The Straits of Malacca. Tan (1997, 1999) reported severe imposex incidence among the whelk such as *Thais gradata* and *Chicoreus capucinus*. Ismail et al. (2004) reported nearly 100% of female *Thais* collected along the Straits of Malacca.

Strombus canarium Linnaeus, 1758 is a true conch from the family Strombidae, a member of the order Mesogastropoda. This species was widely distributed within the coastal and sheltered islands of the tropics, and highly associated with seagrass bed ecosystem (Chuang 1973; Cob et al. 2005). This species is very important for the locals as it was traditionally collected for food (Chuang 1973; Purchon and Purchon 1981; Cob et al. 2005), and probably formed the most important gastropod fishery within the study area. However, since this traditional conch fishing ground was

very close to ports (Port of Tanjung Pelepas in Malaysia and Tuas in Singapore) and subjected to heavy shipping traffics (The Straits of Malacca), there was great concerned that TBT pollution might have significant impact on the population. However, there was no information regarding imposex cases in this species. Our objectives were to assess and described the imposex condition in *S. canarium* and to establish an imposex classification system to serve as the basis for a suitable index for TBT monitoring using this species.

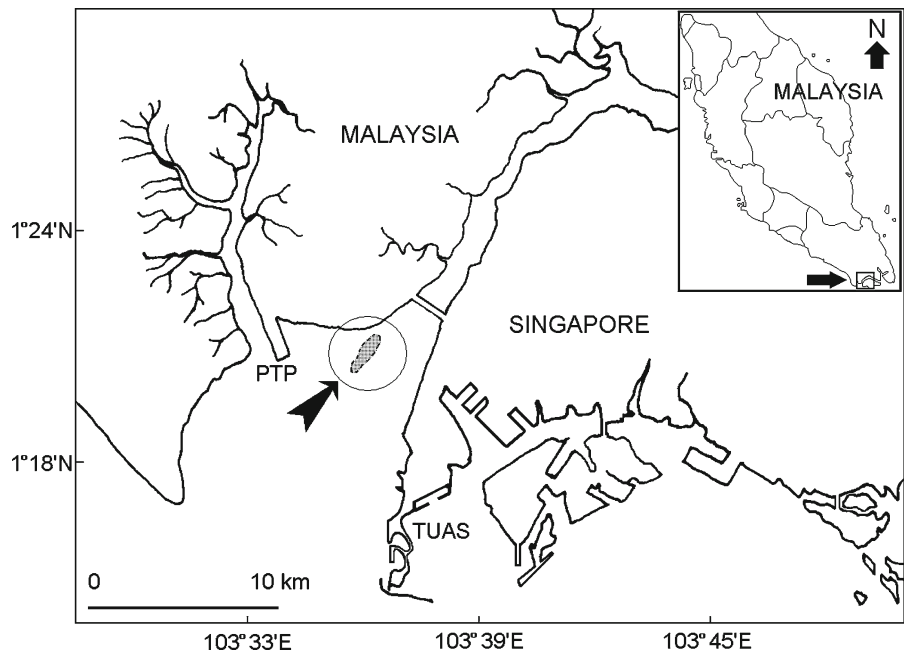
Materials and methods

The study area (Fig. 1) is a subtidal shoal located in the western part of Johor Straits (1° 19.55' N, 103° 35.57' E), and consists mainly of fine sand and mud substrate. The shoal is situated between the Port of Tanjung Pelepas, Malaysia and Port of Tuas, Singapore, and facing the Malacca Straits, which is among the world's most important international navigation waterways (Ismail 2006).

Specimen collections were conducted during the main reproductive period for the species, from October 2005 to January 2006, which was also the main conch-harvesting season for the area (Cob et al. 2005). Adult conchs, which can easily be recognized by the flared and thickened shell outer-lip (Cob et al. 2008a, b), were collected randomly along the shoal during low tides. They were manually picked and were preserved in ice, and kept frozen prior to analysis. The shell, animal and gonad tissue were then separated, blotted damp-dry and weighed (wet-weight) to the nearest 0.001 g using an analytical balance. Shell-length and shell-lip thickness were then measured to the nearest 0.01 mm using a digital vernier caliper.

The incidence of imposex in *S. canarium* was analyzed, based on the description by Cob et al. (2008b) and earlier description by Kuwamura et al. (1983) on *S. luhuanus*. The presence of male penis homologous on female individuals was noted and expressed as percent incidence (%I). The length and thickness of the verge, as well as any branching were then carefully measured using dissecting microscope equipped with an image analysis system (MOTIC™). Digital image of

Fig. 1 Sampling site (*encircled*), which was very near to two major ports of the area, Port of Tanjung Pelepas (*PTP*) and Tuas Port



the verge were captured and line drawings were made to facilitate verge area estimation using a graph paper. The relative penis length (RPL) index and relative penis size (RPS) index were then calculated, and expressed as percentage of mean male penis length and mean male penis size respectively (Bryan et al. 1986; Gibbs et al. 1987). In addition, the presence of vas deferens, prostate gland and other male characters on imposex female was also analyzed, and the alternative classification method based on the vas deferens development (vas deferens sequence index, VDS) was also evaluated (Fioroni et al. 1991; Oehlmann et al. 1991, 1992, 1996; Stroben et al. 1992).

Results

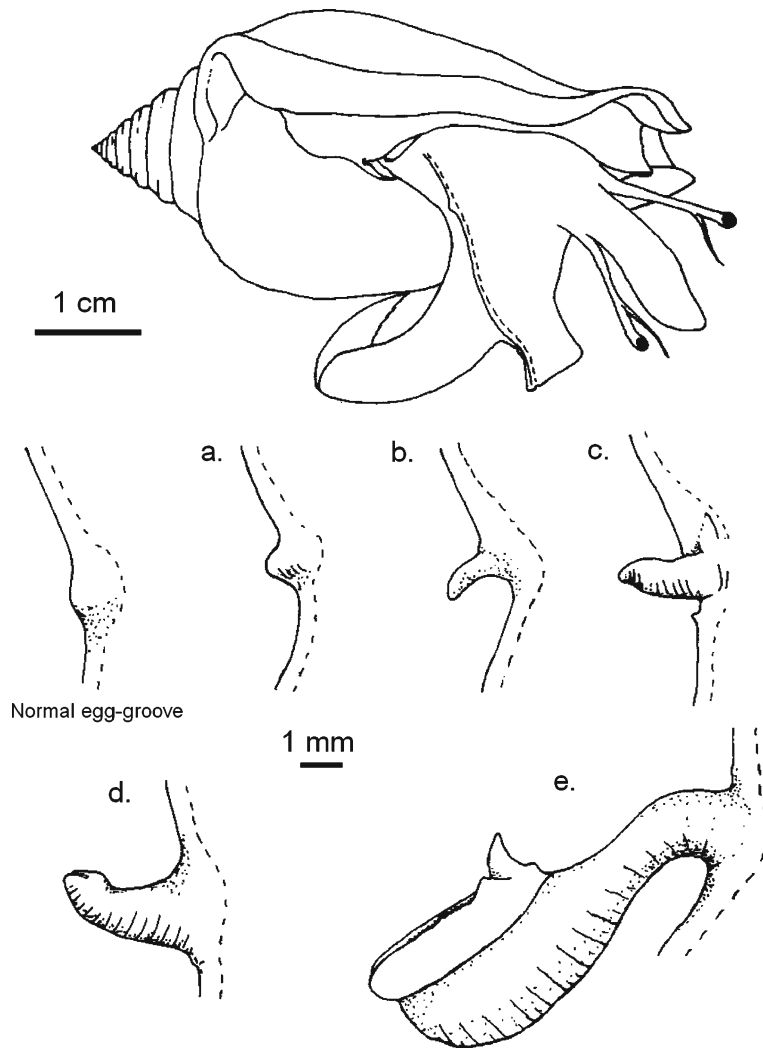
The incidence of imposex was observed in *S. canarium* population where 32.81% of adult females were affected. Sequence of imposex development in female conch, from merely a small stump at clitoris area to an advance male penis homologous, is presented in Fig. 2. Both RPS and RPL indices were calculated by comparing female penis with the penis of fully matured male, with shell length ranged from 51.29 to 64.92 mm. The

male penis parameters (mean length and mean size) that were used in the RPS and RPL index calculations were pooled from October 2005 to January 2006 samples. Sample collections were conducted within their main spawning season (Cob et al. 2008c), thus it was assumed that no significant difference in mean male penis length occurs within the sampling period (Tan 1999).

Furthermore, regression analyses found no significant correlation between shell-length and penis length ($F = 1.42, R^2 = 0.08, P = 0.25$); between shell-length and penis size ($F = 0.001, R^2 = 0.00007, P = 0.97$); between lip-thickness and penis length ($F = 0.68, R^2 = 0.04, P = 0.42$); and between lip-thickness and penis size ($F = 4.70, R^2 = 0.22, P = 0.05$). Therefore the pooled data were considered valid as references for imposex index calculation (Tan 1999). The RPS index ranged from 1.74 to 33.29 (mean = $13.40 \pm 2.27, n = 21$), while the RPL index ranged from 6.28 to 55.19 (mean = $25.83 \pm 3.33, n = 21$; Table 1).

The alternative method based on the vas deferens development (vas deferens sequence (VDS) index; Fioroni et al. 1991; Oehlmann et al. 1991, 1992, 1996; Stroben et al. 1992) as a measure of imposex was not observed in this species. Therefore a different method that could give some

Fig. 2 Evolution of female penis development in *S. canarium*. (a) normal female reproductive organ, (b–f) different stages of female's penis development. Scale bar, top = 1 cm, bottom = 1 mm



information on stages of imposex and its severity was proposed.

Based on the development of female penis the severity of imposex in *S. canarium* can be divided into four stages, as outlined in Fig. 3. Stage-0 represent normal female while imposex females were grouped into stage 1, 2, and 3. Stage-1 represent

female with the appearance of rudimentary penis but without penis duct; Stage-2 represent female with simple prong of penis with penis duct but without accessory; and Stage-3 represent female with penis complete with accessories similar to those of adult male (i.e., penis keel, accessory pad, and auxiliary prongs). In this study, stage-1

Table 1 Relative penis size index (RPS) and relative penis length index (RPL) of *S. canarium* at Sg. Pulai estuary

	S.L	Lip	P-Len	P-size	RPS	RPL
Female ($n = 21$)	57.78 ± 0.73	4.39 ± 0.28	6.87 ± 0.89	15.67 ± 2.65	13.40 ± 2.27	25.83 ± 3.33
Male ($n = 19$)	56.81 ± 0.93	3.79 ± 0.32	26.60 ± 0.72	116.96 ± 5.16		

Values are mean \pm standard error

S.L shell length (mm); Lip shell-lip thickness (mm); P-Len penis length (mm); P-size penis size (mm²)

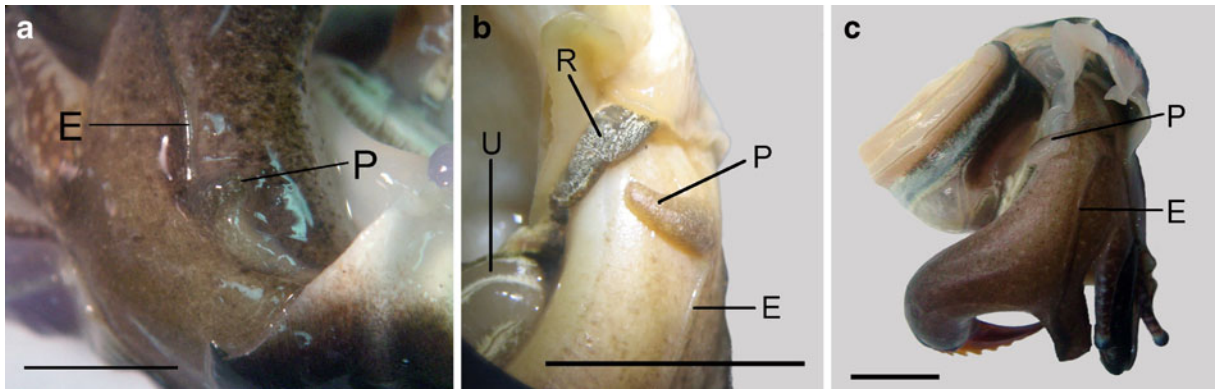


Fig. 3 Stages of imposex scheme based on female's penis development in *S. canarium*: **a** Stage 1; **b** Stage 2; **c** Stage 3. *E* egg groove, *P* penis, *R* rectum, *U* primary uterus. Scale bar, 1 cm

accounted for 19.05%, Stage-2 accounted for 42.86% and Stage-3 accounted for 38.10%, of the imposex females.

Discussions

The present study for the first time described the incidence, and morphological expression of imposex in *S. canarium*. Although the Strombidae is an important group of gastropod, both ecologically and economically (Poutiers 1998; Stoner 1997), very few studies have adopted this family in coastal bio-monitoring and pollution assessment, particularly concerning the impact of TBT and heavy metal accumulations. *S. canarium* is the most dominant herbivorous gastropod within the study area, traditionally harvested and most importantly are consumed by the locals (Poutiers 1998; Cob et al. 2005). Thus, assessment on the impact of pollution on this species is very important.

Androgenic activity in female gastropod affected by organotin can be expressed either by the development of vas deferens, penis, prostate gland, seminal vesicle and/or testes, in accordance with level of exposure. At low concentrations female might grow a small non functional penis, and an incomplete vas deferens (Gibbs et al. 1987, 1990). At high concentrations females might have a fully grown penis and a vas deferens comparable with those of mature males (Gibbs and Bryan 1986). Further, in the most advanced form, the

development of prostate gland, seminal vesicle, and testes might also occur in affected females (Gibbs et al. 1988).

In this study, imposex in *S. canarium* was principally characterized by the growth of penis homologous on female individuals. Development of other male characters such as the vas deferens, prostate gland, seminal vesicle, and testis was not observed on the affected females. Thus, the general scheme of imposex evolution in prosobranch gastropods (Fioroni et al. 1991; Oehlmann et al. 1991; Stroben et al. 1992), which incorporated various imposex characters, could not be satisfactorily applied to this species. The presence of female's egg groove (see Figs. 2 and 3) obscured the observations on the development and/or progression of vas deferens (if any) in affected females. This was in agreement with Fioroni et al. (1991) who reported that although the VDS index was widely employed to evaluate imposex, not all of the morphological variations can be described. There are few other species that cannot be described by the VDS index such as *Thais gradata* and *Chicoreus capucinus* (Tan 1997); *Dicathais orbita* and *Lepsiella vinosa* (Foale 1993; Nias et al. 1993), *Thais clavigera* and *Thais bronni* (Horiguchi et al. 1994) and others.

Therefore, a new scheme of imposex development in females *S. canarium* was defined, which can be categorized into four stages: Stage-0 without any male genital system (normal female); Stage-1 with the appearance of rudimentary penis,

but without penis duct; Stage-2 with simple prong of penis and penis duct but without accessory; and Stage-3 with male penis complete with accessories similar to those of adult male (i.e. penis keel, accessory pad, and auxiliary prongs). This is a new scheme for assessing imposex severity in *S. canarium*, and hopefully could be applied for other *Strombus* species in bio-monitoring exercise. The scheme described imposex severity according to penis development, which was assumed to reflect the level of exposures to organotin substances. However, more studies are needed in order to further develop and test its validity and application, such as its correlation with levels of pollutant within the tissues and the environment, as well as its application on other *Strombus* species.

Nevertheless, although female *S. canarium* penis was quite similar in shape and appearance to male penis it was much smaller, which was not described by the proposed scheme. Therefore, for better evaluation, imposex assessment using this new scheme should also include other parameters, e.g., the RPS and RPL indices. According to Tan (1999), accurate assessment of the penis size is a necessary step towards determining the severity of imposex. The penis (or verge) morphology of *S. canarium* was rather complex where it grows in more than one dimension. Therefore volume is more appropriate than length for comparison purposes. Nevertheless, the complexity in shapes makes accurate volume measurement difficult, thus area measurement was a good substitution (Tan 1999). Tan (1999) also used penis area as indication of size in RPS index calculation for two muricid gastropods in his study, and found high correlation between penis areas and penis dry weight.

The RPS and RPL indices recorded in the current study were much higher compared with data on other species within the sampling area. Tan (1999) reported RPL and RPS for *T. gradata* ranged from 0.02% to 11% and 1.5% to 25.5%, respectively. He also measured imposex incidence in *C. capucinus*, where its RPL index ranged from 0.2% to 2.1%; and RPS from 7.9% to 23.9% (Tan 1997, 1999). However, despite of the high index (RPS and RPL) values, the general impact might still considered minimal as there was no indication of sterility observed in *S. canarium*. The ovary,

pallial glands and vaginal opening were appeared normal, similar to those of a normal female. There was also no case of accumulating eggs in oviduct due to blockage by vas deferens, and thus no eventual rupture of capsule gland or oviducts was observed (Gibbs and Bryan 1986; Gibbs et al. 1987). Reproductive failure and declining populations due to imposex have been reported in several gastropod species (Nias et al. 1993; Bright and Ellis 1990; Bryan et al. 1986; Gibbs and Bryan 1986; Gibbs et al. 1990). The declining populations usually contained an abnormally low percentage of females and a large portion of which are sterile (Bryan et al. 1986; Gibbs and Bryan 1986). The muricid snails for example (Tan 1997), although the RPS index was less than 25% their oviduct was already occluded and aborted capsules were found in the capsule gland. In addition, the presence of other male characters, e.g., prostate, seminal vesicle and testes was also not observed in the species studied.

Conclusion

The percentage incidence of imposex (%I) in *S. canarium* population was lower when compared with other prosobranch gastropod present within the study area. Although high value of RPS and RPL indices were recorded, the impact of organotins to the population was still considered low as no case of sterility ever observed. Together with other mollusks species, *S. canarium* can be a good indicator for monitoring of organotin pollution. The species is abundant, present all year round, commercially harvested and most importantly the presence of penis on affected females can easily recognized make it a good candidate for indicator species. However, further studies are greatly needed, e.g., in assessing the correlation between the morphological expression of imposex and levels of organotin, as well as the effect of organotin on its reproduction and larval development.

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