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Imposex of *Mauritia arabica* on the south-eastern coast of China

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Imposex, the development of male sexual organs in female gastropods, is mainly induced by organotin compounds used as biocides in antifouling paints. As part of our continued efforts to report the occurrence of imposex gastropods along the coast of mainland China, this study investigates the imposex prevalence in Mauritia arabica *(Mesogastropod: Cypraeidae). A total of 529 adults were collected from 11 sites in south-eastern China between March 2001 and June 2005. Of these, 303 were females and 226 were males. Imposex is classified into six stages representing the sequence from the initial appearance of the seminal groove or small penis (S_1) to the point when this duct blocks the pallial oviduct and aborted egg capsules appear (S_6). All six stages of imposex were found in* M. arabica *with three different types (a, b and c) of expression in stages 1 and 3, two types (a and b) in stage 2, and one type in stages 4, 5, and 6. Imposex development was also measured by various indices such as the vas deferens sequence index (VDS), female penis length (FPL), percentage of affected females (%I), the relative penis length (RPL), and relative penis size (RPS—the cubed form of RPL). Imposex individuals were found in all 11 sites with %I ranged from 26% to 100%. Excluding the data from site 3 (with only one female and one male), VDS scores ranged from 1.05 to 4.07, with an average of 2.86, and the RPL ranged from 0.9 to 56.8. High correlations were observed among VDS, FPL, %I, RPL, and the RPS. Our findings suggest that* M. arabica *is an ideal bioindicator for organotin pollution along the south-eastern coast of China, and can be used in conjunction with the rock shell,* Thais clavigera*, a well-established bioindicator of tributyltin and triphenyltin contamination. To our knowledge, this is the first detailed report of imposex in* M. arabica.

Keywords: imposex, *Mauritia arabica*, organotin, bioindicator, China

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INTRODUCTION

Organotin compounds were widely used in the manufacture, agriculture, transportation and health industries (de Mora & Pelletier, 1997). Among them, tributyltin (TBT) and triphenyltin (TPT) were commonly used as antifouling paints (Dowson *et al.*, 1993). These compounds were toxic to non-target organisms and have been considered among the most harmful substances introduced to the marine environment by humans (de Mora & Pelletier, 1997). Numerous field surveys and laboratory toxicity studies have shown that organotin compounds, especially TBT and TPT, can lead to the growing of male sexual organs (penis and/or vas deferens and prostate tissue) on the reproductive system of female marine gastropods, a phenomenon termed imposex (Smith, 1971). Marine gastropod populations in many areas with heavy concentrations of organotin compounds have been seriously impacted, even resulting in local extinctions (Gibbs & Bryan, 1996). Consequently, many countries and regions have banned the use of organotin in antifouling boat-bottom paints and alternative tin-free antifoulants are under investigation (Omae, 2003). Except for Hong Kong, similar bans or legislative restrictions on the use of the organotin compounds in mainland China are lacking (Wei & Hu, 2006).

Our group has, for the first time, conducted a large-scale survey on organotin pollution and imposex of gastropods along the coast of mainland China between November 1999 and December 2005. Among the 60 species (17 families) of marine gastropods found in China, we have documented the occurrence of imposex in 40 species of 10 families, with 28 species reported for the first time, raising the total number of known imposex-affected species in the world to more than 200 (Shi *et al.*, 2005b; Zhu, 2005). In our previous studies, we have characterized the imposex expression: organ morphology, structure, degree and resulting sterility and their relationships with organotin pollution, distribution and habitat of many gastropod species, for example, *Babylonia formosae habei* (Shi *et al.*, 2003), *Thais clavigera* (Yu *et al.*, 2004), *Chicoreus brunneus* and *C. asianus* (Shi *et al.*, 2004a), *Nassarius siquijorensis, N. thersites*, and *N. hepaticus* (Shi *et al.*, 2004b), *Conus betulinus* and *C. vexillum* (Zhu *et al.*, 2005), and *Cantharus cecillei* (Shi *et al.*, 2005a). Based on these findings, we have expanded the imposex classification scheme proposed by Fioroni *et al.* (1991) and continuously modified by Oehlmann *et al.* (1991, 1992) and Stroben *et al.* (1992) (Shi *et al.*, 2005b).

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The present study continues our efforts to report the occurrence of imposex in gastropods along the coast of mainland China. Specifically, we investigated the morphological expression and classification scheme of imposex in *Mauritia arabica* Linnaeus (Mesogastropoda: Cypraeidae), and explored its potential as an indicator species to improve the bio-monitoring system for marine organotin pollution along the China coast. To our knowledge, this is the first detailed description of imposex development in *M. arabica*.

MATERIALS AND METHODS

Sampling sites

We surveyed 52 sites along the coast of mainland China between March 2001 and June 2005 (Zhu, 2005), and specimens of *M. arabica* were found at 11 sites in Hainan and Guangdong provinces (Figure 1). Site 1 is located inside the fishing port of Nanao Island. It faces and has good water exchange with the South China Sea. Site 2 (outside of the harbour) and 3 (inside) are located at the Huizhou Port, a large port with heavy cargo and oil traffic. Sites 4–9 are located at Yangjiang-Zapo Fishing Port, one of the 10 largest fishing ports in China. Site 10 is located at Qiongzhou Island Fishing Port, approximately 20 km away from Zhanjiang, a large city. Site 11 is located at Sanya Port, an important port at the southern tip of Hainan province.

Specimen preparation

A total of 529 specimens (Figure 2a, b) were collected from rocks during low tide, and shell length was measured with a caliper to the nearest 0.1 mm. The animals were anaesthetized in 7% $MgCl_2$ for 12 hours, the shells were cracked with a small hammer, and the penis length was measured with a caliper to the nearest 0.1 mm. The soft bodies were fixed in 4% formaldehyde and observed with a Carl Zeiss Stemi SV11 dissecting microscope. Specimens were classified into normal male (with testes, a penis, vas deferens and a prostate), normal female (with ovaries, a capsule gland, an albumen gland and a sperm-ingesting gland), and female exhibiting the imposex phenomenon (superimposition of additional male sexual characteristics, i.e. a penis and/or vas deferens). Thirty specimens fixed in formaldehyde were embedded in paraffin and thin-sectioned (5 μm). After being stained with haematoxylin–eosin, the sections were analysed under a Carl Zeiss Axioplan 2 microscope. All photographic work under the microscope was performed with a Carl Zeiss Axiocam and analysed using Axio Vision 3.0. Shell lengths of male and female animals were compared using a Student's *t*-test with unequal sample sizes.

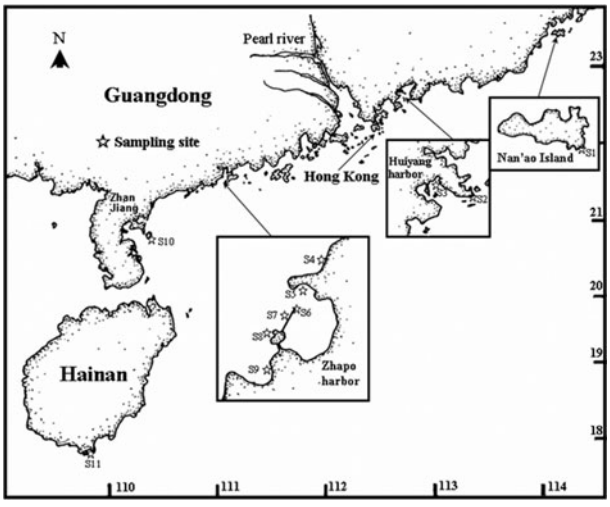

Fig. 1. Map of south-eastern China showing the sampling sites.

Imposex classification and indices

Description of imposex was based on the updated scheme proposed by Shi *et al.* (2005a, b). Briefly, the development of imposex was classified into seven stages (0–6, with S_o as normal female) and six types. Additional indices used include: (1) the average female penis length (FPL) of a sample; (2) the percentage of imposex affected females in a sample (%I); (3) the relative penis length (RPL, [(mean length of female penis)/(mean length of male penis) × 100]) (Bryan *et al.*, 1993); (4) the relative penis size (RPS, the cubed form of RPL); and (5) the vas deferens sequence index (VDS, the imposex stage average in a sample) (Gibbs *et al.*, 1988). It is worth noting that the male genital duct in *M. arabica* is represented by an open seminal groove, instead of a vas deferens observed in many neogastropods. The majority of imposex species are neogastropods, so the VDS has been widely used in the literature. To facilitate comparison, the VDS was used in this study in lieu of creating a seminal groove sequence index. Values presented were mean ± SD.

RESULTS

The female reproductive system in *M. arabica* exhibits the morphological and histological structure which is typical for Mauritania-type Cypraeinae (Kay, 1960). It includes: ovary, renal oviduct, albumen gland, seminal receptacle, capsule gland, genital papilla and vaginal opening. In the normal female, the ovary spreads over the visceral mass anteriorly from the tip of the spire. The renal oviduct leaves the gonad near the kidney aperture and joins the glandular pallial oviduct which extends over the visceral mass to the right body wall. The renal oviduct opens into the albumen gland, an elongated, bilobed structure which joins the large capsule gland, distinguished by its thick lateral walls. Dorsally, a short duct leads from the albumen gland to the seminal receptacle, a diverticulated sac, which lies over the albumen gland and the posterior portion of the capsule gland. The vaginal opening is at the tip of an elongated genital papilla (Figure 2c). A bursa copulatrix is absent in *M. arabica*.

The male genitalia consists of, from the proximal to the distal zone: the testis, the seminal vesicle (a coiled proximal portion), the prostate gland (a glandular distal portion), the seminal groove, and the penis (Figure 2d). The prostate is characterized by an elongated tube structure with sperm present in the lumen during the breeding season. Like all species in the subfamily of Cypraeinae, the duct connecting from the prostate to the penis is represented by a deep groove running along a raised muscular ridge (Figure 2e).

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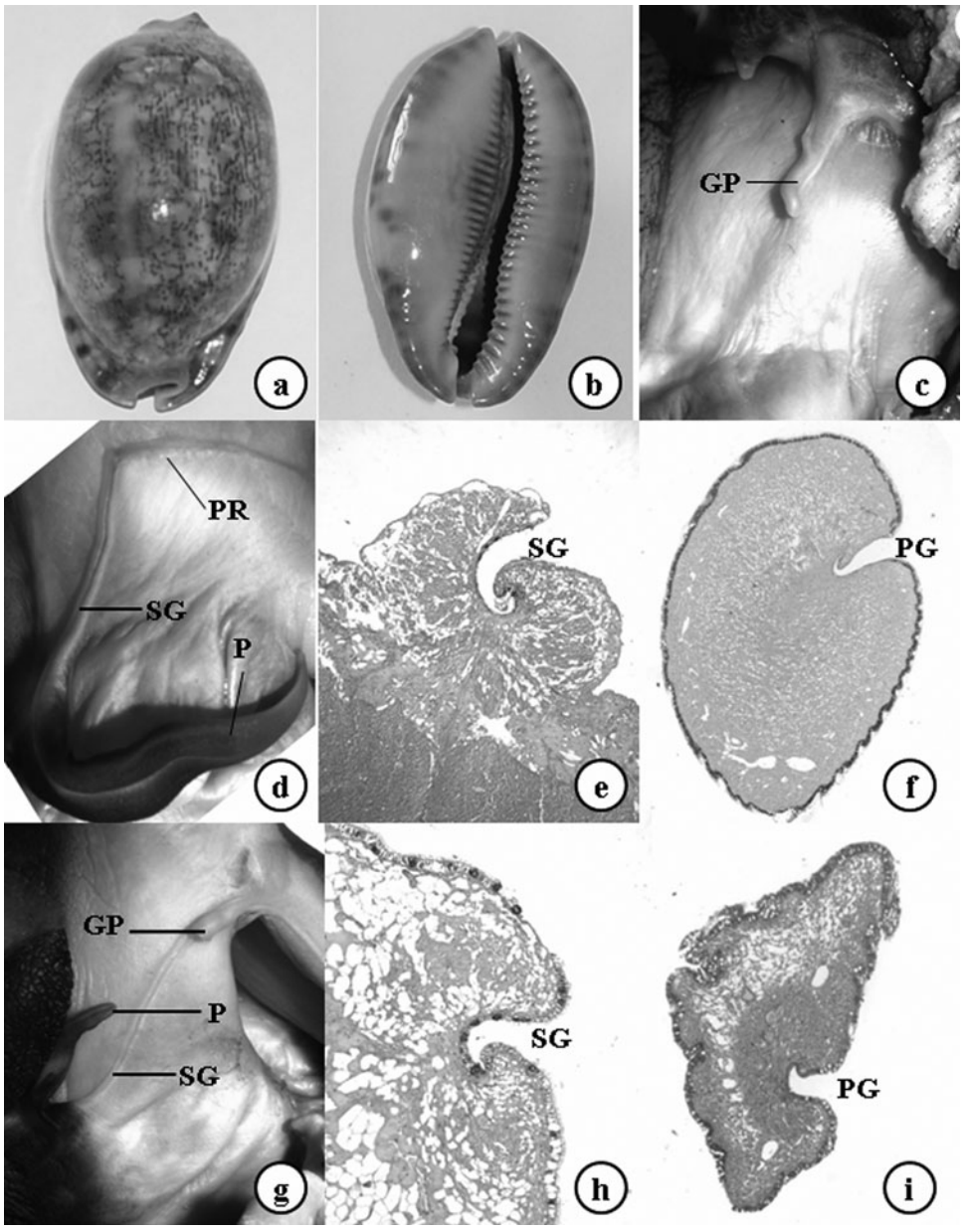

Fig. 2. *Mauritia arabica*: shell morphology (a, b); genital papilla (GP) of normal female (c, ×6); the prostate (PR), seminal groove (SG), and penis (P) of normal male (d, ×8); transverse histological section of the seminal groove (e, ×100), and penis (f, ×40); imposex affected female showing genital papilla, seminal groove, and penis (g, ×6); and transverse histological section of its seminal groove (h, ×100) and penis (i, ×40).

Similarly, the channel leading up the penis to its tip is also opened (Figure 2f). The penis is slightly flattened dorsoventrally and extends from the head posterior to the right cephalic tentacle. Length of the penis ranged from 7.5 to 17.0 mm, with an average of 14.38 ± 2.56 mm (N = 226).

The position, morphology and structure of the penis in imposex affected females (Figure 2g) are similar to those in normal male, but the penis length (5.99 ± 3.68 mm; N = 258) was less than half of that of the normal male. The seminal groove (Figure 2h) and penis groove (Figure 2i) in imposex affected females also resemble that of the normal male but are less pronounced. *Mauritia arabica* demonstrated all 6 imposex stages with three different types (a, b and c) of expression in stages 1 and 3, two types (a and b) in stage 2, and one type in stages 4, 5 and 6. The stages and types are defined as follows:

Stage 0: Normal female without any male sexual characteristics (Figure 3, S_o).

Stage 1: Type a: tiny penis without penis groove behind the right tentacle (Figure 3, S_{1a}); Type b: no penis but a short, distal seminal groove section (DSGS) behind the right tentacle (Figure 3, S_{1b}); Type c: no penis but a short, proximal seminal groove section (PSGS) at the vaginal opening (Figure 3, S_{1c}).

Stage 2: Type a: penis with a penis groove behind the right tentacle (Figure 3, S_{2a}); Type b: appearance of DSGS behind the right tentacle and PSGS at the vaginal opening (Figure 3, S_{2b}).

Stage 3: Type a: small penis continuing in an incomplete distal seminal groove section that is growing out successively towards the vaginal opening (Figure 3, S_{3a}); Type b: seminal groove running continuously from the right

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tentacle to the vaginal opening (Figure 3, S_{3b}); Type c: penis with a penis groove continuing in a distal portion of seminal groove, and meanwhile a short PSGS portion beginning from the vaginal opening (Figure 3, S_{3c}).

Stage 4: penis with a penis groove and a continuous seminal groove from the penis up to the vaginal opening (Figure 3, S_4). The seminal groove joined the genital papilla right beneath (Figure 4a) or connected laterally (Figure 4b) to the vaginal opening without blocking it (Figure 3, S_4).

Stage 5: Type b: vaginal opening occluded by proliferating seminal groove tissue, often forming 'nodules' (see diagram in Figure 4, S_{5b}, picture is not available).

Stage 6: Type b: in addition to the phenomenon observed for S_{5b}, there were aborted egg capsules present in the oviduct (Figure 4c).

Among the 529 specimens collected, 303 were females and 226 were males. Mean male shell length (42.9 ± 4.87 mm) was similar to that of the female (44.5 ± 5.82 mm) ($P > 0.05$). Female/male ratio from each sample varied widely from 0.8 to 4.0 (Table 1). Imposex individuals were found in every site sampled, and the %I ranged from 26 to 100%, with lower values being observed at sites 1, 2 and 10 (Table 1). The imposex affected individuals not only varied among locations, but also over time. For example, at site 7, it was 57% in 2003, but reached 100% in 2005 (Table 1). Excluding the data from site 3 (with only one female and one male analysed), the RPL value varied from 0.9 (site 1) to 56.8 (site 11), and the VDS score ranged from 1.05 (site 1) to 4.07 (site 11), with an average of 2.86. There were high correlations among VDS, FPL, %I, and the RPL and RPS (Table 2).

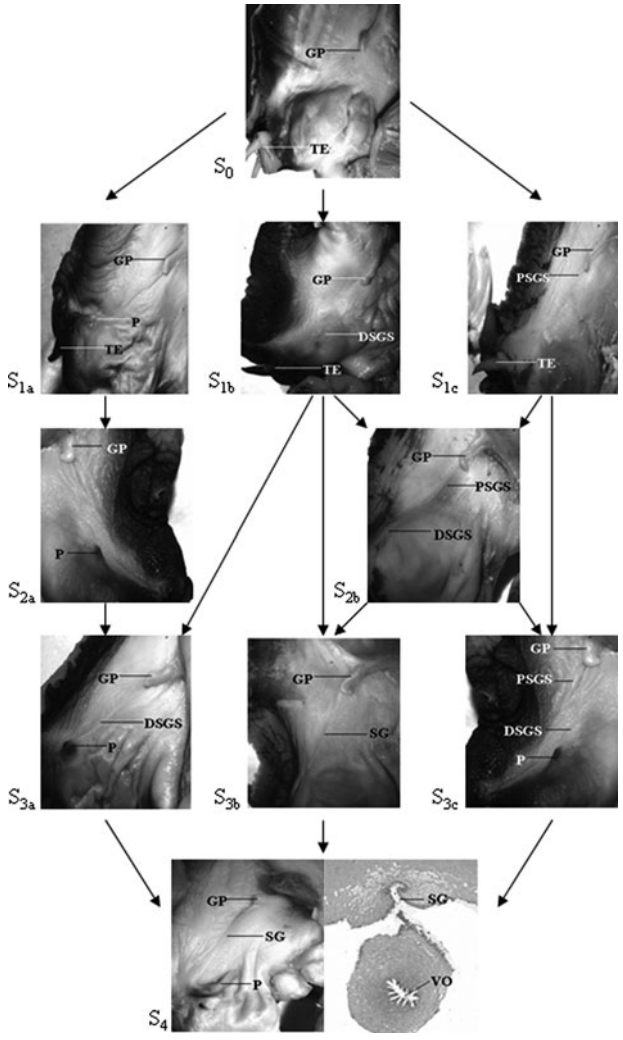

Fig. 3. Imposex development scheme in *Mauritia arabica* (GP, genital papilla; P, penis; TE, tentacle; SG, seminal groove; SGS, seminal groove section; DSGS, distal seminal groove section; PSGS, proximal seminal groove section; VO, vaginal opening).

DISCUSSION

Mauritia arabica is mainly distributed on the coast of south-eastern China, in rocky or coral reef substrate around the low tide zone. It is a dioecious species with internal fertilization. After copulation, the fertilized eggs develop into embryos inside the female body before being released into the environment where the embryos further develop into veliger larvae in about two weeks. The larvae hatch and spend several months in plankton before attaching to benthic substrate and metamorphosing into juveniles, which then grow into adults in several months (Len, 1991). Species from the subfamily Cypraeinae are characterized by an open vas deferens (seminal groove) and penis channel (Kay, 1960), which is believed to be more primitive than the closed type (Fretter, 1941). Our observations of the male and female genital system in *M. arabica* confirm the description by Kay (1960). The elongated vagina in female *M. arabica* may have evolved to compensate for the less efficient sperm transfer via an open seminal groove and penis channel in the male.

Our survey shows that imposex is common in *M. arabica* along the south-eastern coast of China. Imposex occurred in all sampling sites and on average 85% of the females collected were affected. The position, morphology and structure of the seminal groove and penis of imposex individuals of *M. arabica* at stage 4 or above are similar to those of the normal males, but not as well developed. This suggests that the male organ development process is the same for both male and imposex affected female (Gibbs, 1993). However, imposex individuals cannot function as male because their male system is defective, e.g. incomplete seminal groove, lack of prostate and testis. Though all six stages of imposex were found in *M. arabica*, stage S_4 represented the end of imposex development in most sites. In the present study, females at S_4 have shown that the seminal groove connects to the pallial oviduct laterally or right beneath it without blocking the vaginal opening, and then continues along the edge of the capsule gland. This suggests that an S_4 female will not be sterile. Samples in a more severe stage of imposex (S_5 and S_6) were only observed at the beginning of our investigation at site 11, which is a large and busy harbour at the southern tip of Hainan province. Due to the lack of image processing equipment when we first initiated this project, pictures of females at S_5 captured with a regular digital camera showed poor quality (thus not shown). Subsequent attempts to sample at the same site were hampered by the harbour construction.

Similar to all previous studies, high imposex indices were observed at the major harbours with heavy naval traffic (sites 6, 7 and 11), while low imposex indices were found in

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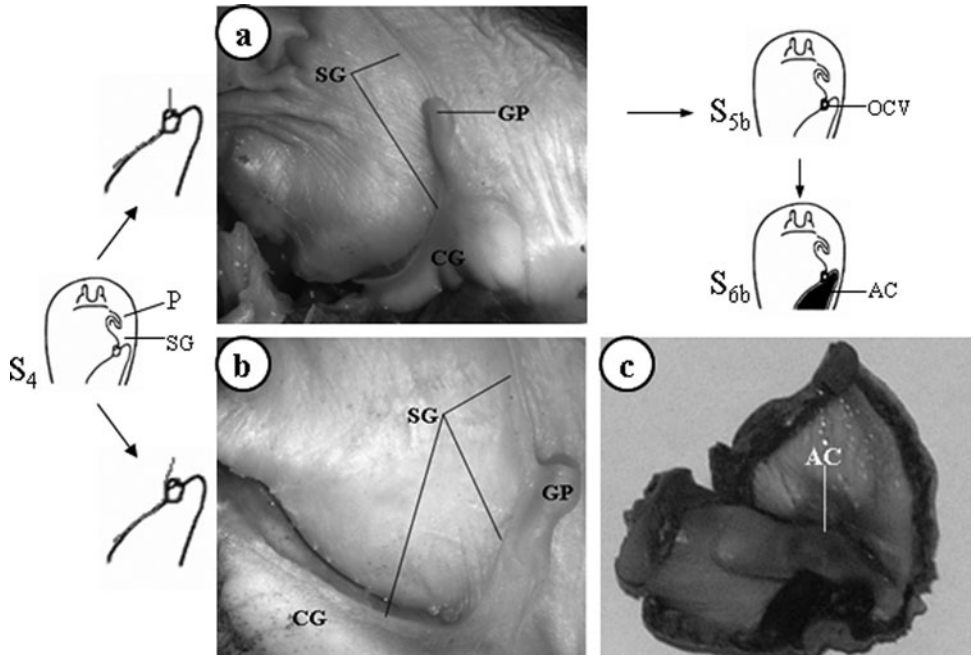

Fig. 4. Imposex development scheme in *Mauritia arabica* (continued). Seminal groove (red) passes right beneath the genital papilla (a) or connect to genital papilla laterally (b); aborted capsules in S_6 (c) (AC, aborted capsules; CG, capsule gland; GP, genital papilla; OCV, occlusion of the vulva; P, penis; SG, seminal groove).

the sites with less shipping activities and better water exchange (site 2) or away from coastal areas (sites 1 and 10). Future studies should correlate the imposex severity with the organotins contents in snails' tissues as well as in the water and sediment. Imposex indices varied temporally as well as spatially. In the present study, sites 1, 7, and 8 were sampled twice 1–2 years apart. No amelioration of imposex incidence was found at these sites over time, while increased FPL, %I,

Table 1. Biometric data of males, normal and imposex affected females of *Mauritia arabica*.

Date	Site	Sex	No. of specimens	Shell length (mm)	Penis length (mm)	%I	RPL (%)	VDS	SR (F/M)
03/01	11	F	14	40.6 ± 3.0	8.8 ± 1.5	100	56.8	4.07	2.3
		M	6	38.8 ± 2.9	15.5 ± 2.3				
04/02	2	F	8	41.2 ± 3.1	1.2 ± 0.3	63	8.4	1.63	1.6
		M	5	39.1 ± 2.9	14.3 ± 2.1				
10/02	1	F	9	43.1 ± 3.5	0.7 ± 0.4	56	4.9	1.33	1.5
		M	6	49.8 ± 3.2	14.2 ± 1.6				
06/03	6	F	24	46.8 ± 4.0	7.8 ± 2.8	100	48.4	4.00	1.3
		M	18	48.5 ± 3.2	16.1 ± 1.5				
06/03	7	F	23	44.8 ± 3.7	2.4 ± 1.0	57	16.3	1.57	1.4
		M	16	47.2 ± 3.0	14.7 ± 1.7				
06/03	8	F	19	45.4 ± 3.8	6.4 ± 2.4	100	56.6	3.89	0.8
		M	25	47.6 ± 3.2	11.3 ± 1.3				
07/03	3	F	1	42.3	9.3	100	124.0	4.00	1.0
		M	1	46.9	7.5				
08/03	10	F	4	56.8 ± 4.6	0.3 ± 0.2	50	3.0	2.00	4.0
		M	1	57.3 ± 3.6	10.0 ± 1.2				
10/03	1	F	19	45.9 ± 3.5	0.1 ± 0.1	26	0.9	1.05	1.4
		M	14	52.5 ± 3.5	11.0 ± 1.5				
06/05	4	F	16	38.3 ± 2.7	4.6 ± 2.3	100	35.7	3.50	1.1
		M	15	39.5 ± 2.5	12.9 ± 2.0				
06/05	5	F	70	40.8 ± 3.0	7.2 ± 3.0	97	46.5	3.50	0.9
		M	77	42.1 ± 2.9	15.5 ± 2.2				
06/05	7	F	24	39.7 ± 2.9	8.1 ± 3.2	100	52.6	4.00	3.0
		M	8	40.6 ± 2.8	15.4 ± 1.8				
06/05	8	F	49	46.3 ± 3.7	6.5 ± 2.8	98	41.9	3.51	2.0
		M	24	47.6 ± 3.1	15.5 ± 1.9				
06/05	9	F	23	37.4 ± 2.5	0.3 ± 0.2	70	2.2	2.00	2.3
		M	10	38.1 ± 2.4	13.8 ± 1.8				

F, female; M, male; %I, percentage of affected females; RPL, relative penis length; VDS, vas deferens sequence index; SR, sex ratio.

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Table 2. Correlations (r, correlation coefficient) among various imposex indices.

Imposex indices	FPL	%I	RPL	RPS
VDS (vas deferens sequence index)	0.945	0.950	0.956	0.895
FPL (female penis length)		0.894	0.981	0.913
%I (percentage of affected females)			0.907	0.800

RPL, relative penis length; RPS, relative penis size (the cubed form of RPL).
Note: data from site 3 were excluded for correlation calculation because the small sample size (one male and one female).

RPL, and VDS were found at site 7 in 2005. This was expected because there were no legislative restrictions on the use of the organotin compounds in mainland China.

Among various imposex indices, the VDS is recommended as the most suitable biological parameter for effective monitoring because this index is characterized by the lowest deviation from the calculated concentration-effect-equations and shows no seasonal variations (Stroben *et al.*, 1996). However, in the present study, high correlations were found among VDS, FPL, %I, RPL, and the RPS. This suggests that any of these could be used as a valid index in *M. arabica*. It also implies that there is less individual variation (within the same sites) and population variation (among different geographical sites) of female *M. arabica* response to orgonotins contamination, or their specificity to one particular type of orgonotin compound. This has the additional advantage that this species could be used as a bioindicator for organotin pollution, because a smaller sample size could be used for an accurate estimate, thus also minimizing the effect of sampling on the population. Although the RPS has been considered as an invalid index for *Hinia reticulate* because of its great seasonal variations (Stroben *et al.*, 1992), findings with *M. arabic* (the present study) and *Cronia konkanensis* (Vishwa kiran & Anil, 1999) all showed high correlations between RPL, RPS, and VDS. This discrepancy mainly resulted from the seasonal variation of normal male penis length. For example, in the present study, the male penis length varied little over time with the exception of July 2003 (this data point was excluded from correlation analysis because of the small sample size). The collection of imposex *C. konkanensis* was confined to April and May (Vishwa kiran & Anil, 1999). On the contrary, studies with *H. reticulate* have shown great seasonal variations of normal male penis length (Stroben *et al.*, 1992). Thus, whether RPL or RPS is a valid imposex index depends on whether a species exhibits seasonal variation of male penis length. In addition, appropriate imposex indices may vary with species as well (see below). For *M. arabica*, more research is needed to determine if there is seasonality of male penis length.

Similar to the dog whelk *Nucella lapillus* in Europe, the rock shell, *Thais clavigera* is very sensitive to TBT/TPT. It is also widespread and abundant in Asia, thus it has been used as a bioindicator for organotin pollution in the coastal waters of Singapore (Tan, 1997), Japan (Horiguchi *et al.*, 1998), Korea (Shim *et al.*, 2000), Hong Kong (Blackmore, 2000), Taiwan (Hung *et al.*, 2001), and mainland China (Yu *et al.*, 2004). Despite this, studies have reported the unclear and variable development of the vas deferens in females of this species making the use of VDS an inappropriate index (Tan, 1997). Instead, most studies use RPL for imposex expression in *T. clavigera*, which is considered to be inferior to VDS due to its seasonal variation (Oehlmann *et al.*, 1998). In addition, our previous reports have revealed the absence of *T. clavigera* in many sites, especially those with heavy organotin contamination, possibly indicating a population decline (Zhu, 2005). *Mauritia arabica* is common and widely distributed along the coast of south-eastern China. The present study has shown that it exhibits imposex development comparable to *T. clavigera* but may be more tolerant to high organotin concentrations as evidenced by the absence of advanced imposex stages of S_5 and S_6 in most sites. As indicated above, various indices could be used as valid estimates for imposex expression in this species. In those sites where *T. clavigera* was absent, the biological effects of organotin pollution could be assessed using *M. arabica*.

In addition to the attributes and advantages mentioned above, *M. arabica* is also one of the few large snails that are not consumed by humans in China; therefore its populations are not likely to be over-fished. The species, sex and degree of imposex are relatively easy to identify. It normally inhabits a rocky or coral reef substrate and is easy to collect. Therefore, we propose that *M. arabica* can be used in conjunction with *T. clavigera* to provide a more complete organotin pollution bio-monitoring system for south-eastern China. Analysis of multiple species in biomonitoring programmes would result in a broader data base, and may eventually lead to implementation of legislative restrictions on TBT use in China.

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REFERENCES

Blackmore G. (2000) Imposex in *Thais clavigera* (Neogastropoda) as an indicator of TBT (tributyltin) bioavailability in coastal waters of Hong Kong. *Journal of Molluscan Studies* 66, 1–8.

Bryan G.W., Burt G.R., Gibbs P.E. and Pascoe P.L. (1993) *Nassarius reticulatus* (Nassariidae: Gastropoda) as an indicator of tributyltin pollution before and after TBT restrictions. *Journal of the Marine Biological Association of the United Kingdom* 73, 913–929.

de Mora S.J. and Pelletier E. (1997) Environmental tributyltin research; past, present, future. *Environmental Technology* 18, 1169–1177.

Dowson P.H., Bubb J.M. and Lester J.N. (1993) Temporal distribution of organotins in the aquatic environment: five years after the 1987 UK retail ban on TBT-based anti-fouling paints. *Marine Pollution Bulletin* 26, 487–494.

Fioroni P., Oehlmann J. and Stroben E. (1991) The pseudohermaphroditism of prosobranchs, morphological aspects. *Zoologischer Anzeiger* 226, 1–26.

Fretter V. (1941) The genital ducts of some British stenoglossan prosobranchs. *Journal of the Marine Biological Association of the United Kingdom* 25, 173–211.

Gibbs P.E. (1993) A male genital defect in the dog-whelk, *Nucella lapillus* (Neogastropoda), favouring the survival of a population in

a TBT-polluted area. *Journal of the Marine Biological Association of the United Kingdom* 73, 667–678.

Gibbs P.E. and Bryan G.W. (1996) TBT-induced imposex in neogastropod snails: masculinization to mass extinction. In de Mora S.J. (ed.) *Tributyltin: case study of an environmental contaminant*. Cambridge: Cambridge University Press, pp. 212–236.

Gibbs P.E., Pascoe P.L. and Burt G.R. (1988) Sex change in the female dog-whelk *Nucella lapillus*, induced by TBT from anti-fouling paints. *Journal of the Marine Biological Association of the United Kingdom* 68, 715–731.

Horiguchi T., Hyeon-Seo C., Shiraishi H., Shibata Y., Soma M., Morita M. and Shimizu M. (1998) Field studies on imposex and organotin accumulation in the rock shell, *Thais clavigera*, from the Seto Inland Sea and the Sanriku region, Japan. *The Science of the Total Environment* 214, 65–70.

Hung T.C., Hsu W.K., Mang P.J. and Chuang A. (2001) Organotins and imposex in the rock shell, *Thais clavigera*, from oyster mariculture areas in Taiwan. *Environmental Pollution* 112, 145–152.

Kay A. (1960) Generic revision of the Cypraeinae. *Proceedings of the Malacological Society of London* 33, 278–287.

Len S. (1991) *Invertebrate biology*. Beijing: Beijing University Press.

Oehlmann J., Stroben E. and Fioroni P. (1991) The morphological expression of imposex in *Nucella lapillus* (Linnaeus) (Gastropoda: Muricidae). *Journal of Molluscan Studies* 57, 375–390.

Oehlmann J., Stroben E. and Fioroni P. (1992) The rough tingle *Ocenebra erinacea* (Neogastropoda: Muricidae): an exhibitor of imposex in comparison to *Nucella lapillus*. *Helgoland Marine Research* 46, 311–328.

Oehlmann J., Stroben E., Schulte-Oehlmann U. and Bauer B. (1998) Imposex development in response to TBT pollution in *Hinia incrassata* (Strom, 1768) (Prosobranchia, Stenoglossa). *Aquatic Toxicology* 43, 239–260.

Omae I. (2003) Organotin antifouling paints and their alternatives. *Applied Organometallic Chemistry* 17, 81–105.

Shi H., Huang C. and Lei Z. (2003) Organotin compound pollution in coastal water as indicated by imposex in *Babylonia formosae* habei. *Ocean Technology* 22, 82–86. [In Chinese with English abstract.]

Shi H., Huang C., Yu X. and Zhu S. (2004a) Imposex and female reproductive failure of *Chicoreus brunneus* and *C. asianus*. *Journal of Tropical Oceanography* 23, 82–87. [In Chinese with English abstract.]

Shi H., Huang C., Yu X., Zhu S. and Zhang Y. (2004b) Development of imposex and structural effect on males in three *Nassarius* spp. *Marine Sciences* 28, 36–41. [In Chinese with English abstract.]

Shi H., Huang C., Yu X. and Zhu S. (2005a) An updated scheme of imposex for *Cantharus cecillei* (Gastropoda: Buccinidae) and a new mechanism leading to the sterilization of imposex-affected females. *Marine Biology* 146, 717–723.

Shi H., Huang C., Zhu S., Yu X. and Xie W. (2005b) Generalized system of imposex and reproductive failure in female gastropods of coastal waters of mainland China. *Marine Ecology Progress Series* 304, 179–189.

Shim W.J., Kahng S.H., Hong S.H., Kim N.S., Kim S.K. and Shim J.H. (2000) Imposex in the rock shell, *Thais clavigera*, as evidence of organotin contamination in the marine environment of Korea. *Marine Environmental Research* 49, 435–451.

Smith B.S. (1971) Sexuality in the American mud snail, *Nassarius obsoletus* Say. *Proceedings of the Malacological Society of London* 39, 377–378.

Stroben E., Oehlmann J. and Fioroni P. (1992) The morphological expression of imposex in *Hinia reticulata* (Gastropoda: Buccinidae): a potential biological indicator of tributyltin pollution. *Marine Biology* 113, 625–636.

Stroben E., Oehlmann J., Schulte-Oehlmann U. and Fioroni P. (1996) Seasonal variations in the genital ducts of normal and imposex-affected prosobranchs and its influence on biomonitoring indices. *Malacological Review* Suppl. 6, 173–184.

Tan K.S. (1997) Imposex in three species of *Thais* from Singapore, with additional observations on *T. clavigera* (Kuster) from Japan. *Marine Pollution Bulletin* 34, 577–581.

Vishwa kiran Y. and Anil A.C. (1999) Record of imposex in *Cronia konkanensis* (Gastropoda, Muricidae) from Indian waters. *Marine Environmental Research* 48, 123–130.

Wei A. and Hu J. (2006) Effects of endocrine disrupting chemicals on China's rivers and coastal waters. *Frontiers in Ecology and Environment* 4, 378–386.

Yu X., Huang C., Zhu S., Shen J. and Dong Q. (2004) Anatomical and histological studies on imposex of *Thais clavigera*. *Oceanologia et Limnologia Sinica* 35, 149–155. [In Chinese with English abstract.]

Zhu S. (2005) *A large-scale investigation on imposex in the coastal waters of mainland China*. Masters thesis. Shantou University, China [In Chinese with English abstract.]

and

Zhu S., Dong Q., Shen J., Yu X., Du H. and Huang C. (2005) Imposex of *Conus betulinus* and *Conus vexillum*. *Acta Ecologica Sinica* 25, 289–297. [In Chinese with English abstract.]

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