Ecological risk assessments of endocrine disrupting organotin compounds using marine neogastropods in Hong Kong

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Abstract

As active ingredients of anti-fouling paints that are widely used on ship hulls, organotin compounds, in particular tributyltin (TBT), are well-known endocrine disruptors causing sex changes in marine organisms and widespread in coastal waters and sediments worldwide. In this study, a comprehensive ecological risk assessment (ERA) of organotins was conducted in Hong Kong waters through determining the imposex status, sex ratio and tissue burdens of these compounds in the neogastropods, *Thais clavigera* and *Thais luteostoma* collected from 29 coastal sites. We also investigated the historical trend of organotin effects on these gastropods, and performed a probabilistic ERA based on tissue burden of TBT in the animals. Our results demonstrated that imposex indices were positively correlated with the body burden of organotins in the gastropods. Across all sites, the sex ratio (female:male) decreased significantly with increasing imposex levels or tissue burden of organotins, implying that such pollutants can result in a male-biased population, potentially leading to local extinction in extreme cases. Based on the ERA, 5.4% of all populations of *T. clavigera* are at risk due to exposure to TBT; the risks include growth inhibition, impairment of immune functions and reduced fitness. Seriously impacted areas included Aberdeen, Repulse Bay, Butterfly Beach, Mui Wo and Ha Mei Wan. A comparison with historical data revealed that there had been some improvement in the areas with low marine traffic, and distant from the major harbour/port. This could partly be due to the restriction on the use of TBT on small vessels (<25 m in length) since 1992. Nevertheless, the organotin contamination still remains severe in areas with high marine traffic or adjacent to large harbours/ports. In particular, the situation in the northeastern waters of Hong Kong has been getting worst since 1996 that is probably associated with the rapid development of the cargo container port at Yantian in China.

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1. Introduction

Organotin compounds (OTs) such as tributyltin (TBT) and triphenyltin (TPT) have been adopted in industrial applications since their biocidal properties were revealed in early 1950s (Bennett, 1996). They are used for various

purposes such as molluscicides and disinfectants, but most importantly, OTs are applied globally as wood preservatives and anti-fouling paints (de More, 1996). The toxic effects of OTs, particularly TBT on non-target organisms, have been reported since late 1970s in various marine taxa (e.g. Alzieu, 1996); among them, gastropods and bivalve molluscs are of higher concern due to the induction of toxic effects at low concentrations (Gibbs et al., 1987; Leung et al., 2001). In marine neogastropods, TBT causes

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imposex which refers to the development of vas deferens and formation of penis in female snails (Gibbs et al., 1987). The symptoms were first reported in early 1970s (Blaber, 1970; Smith, 1971), while its linkage with TBT was established a decade later (Smith, 1981a,b). This relationship meant that monitoring of TBT pollution can be effectively carried out based on the degree of imposex development in neogastropods in the receiving environment. Indeed, imposex in the female can occur at TBT concentration as low as 1 ng l^{-1} (Gibbs et al., 1988). The degree of imposex can be gauged by two main indices, namely relative penis size index (RPSI) and vas deferens stage index (VDSI) (Gibbs et al., 1988). In general, there is a strong positive correlation between the imposex indices and ambient TBT level or distance from harbour/dockyard (Bryan et al., 1987; Gibbs et al., 1988; Horiguchi et al., 1994, 1997; Chiavarini et al., 2003).

Imposex has been reported in approximately 150 gastropod species (Matthiessen et al., 1999). The resultant impact of such dysfunctions in the reproductive system of female snails, however, varies amongst species (Matthiessen and Gibbs, 1998). For some species, such as *Nassarius reticulatus* and *Ilyanassa obsolata*, the reproductive activity of the imposex females appeared not to be affected, while in other species, such as *Nucella lapillus* and *Thais clavigera* (Gibbs and Bryan, 1986; Horiguchi et al., 1994), the developing vas deferens may block the oviduct opening (vulva), resulting in the sterilization of the females due to the failure in egg capsule release (Bryan et al., 1986). Previously, there were various proposed mechanisms of this endocrine disruption (Matthiessen and Gibbs, 1998), including the aromatase inhibition hypothesis (Spooner et al., 1991; Bettin et al., 1996; Santos et al., 2005), testosterone excretion-inhibition hypothesis (Ronis and Mason, 1996), neuroendocrine system disruption hypothesis (Féral and LeGall, 1983) and the APGWamide hypothesis (Oberdörster and McClellan-Green, 2000, 2002). Among them, the aromatase inhibition hypothesis tended to receive more attention (Spooner et al., 1991; Bettin et al., 1996; Oehlmann et al., 1996a). In this hypothesis, it is proposed that TBT inhibits the action of cytochrome P450-dependent aromatase, which is involved in the conversion of testosterone to 17β -estradiol, and such an interruption results in a build-up of testosterone in the female to induce the male characters. However, Nishikawa et al. (2004) recently demonstrated that direct binding of TBT or TPT onto the retinoid X receptor (RXR) could induce imposex in females of *T. clavigera*. The RXR is known to be a key factor involved in the mediation of several hormone response systems in mammals. Despite more work being required to fully understand the mechanism of TBT-induced imposex, this pioneering study sheds new light on the importance of molecular controls for the hormonal systems in the gastropod.

With the better understanding of the harmful effects of OTs to marine organisms, banning of TBT-based antifoulant application on boats shorter than 25 m in length has

been enforced in several countries including France (1982), United Kingdom (1987), USA (1988), Australia (1989), Japan (1990) and European Union (1991) (Evans et al., 1995; Gibson and Wilson, 2003). In Hong Kong, a similar restriction on the use of TBT paint was enforced in 1992. Action at a global scale was also initiated as the pollution problem was recognized internationally. In October 2001, the International Maritime Organization (IMO) adopted the International Convention on the Control of Harmful Anti-Fouling Systems on Ships (CHAFSS), which called for a global prohibition on the application of OT compounds to act as biocides in anti-fouling systems on ships by 1 January 2003, and a complete prohibition by 1 January 2008. The date for the convention to enter into force has, however, not been finalized pending the minimum number of ratified states (25 required, but only 11 had agreed by June 2005), and percentage tonnage (25% required, but only 9.28% signed by June 2005) (IMO, 2005).

Hong Kong is a well-known international port, with the world's largest container throughput (Marine Department of Hong Kong SAR, 2005) and continuously increasing vessel activity (Appendix 1) within a sea boundary of about 1600 km^2 . Sea traffic is most intense around the Victoria Harbour, located between the Hong Kong Island and Kowloon Peninsula, where the sea is packed with container loading/unloading facilities and associated shipping channels, dockyards, public piers, typhoon shelters, mooring buoys, etc. Away from the harbour, the water is also utilized as shipping channels, piers, typhoon shelters, fishing grounds and mariculture zones. These activities/facilities are believed to release considerable amounts of OTs to the local marine environment. Additional vessel-based pollutants also come from across the border. For example, Yantian Port (Shenzhen) (Fig. 1.) is located less than 5 km from the boundary of Hong Kong and started its operation in 1996. With rapid investment and development, the port is now the sixth busiest container port in the world. OTs are undoubtedly an important contaminant in Hong Kong waters, and the problem is expected to be exacerbated due to the rapid development of logistic industry in local and peripheral areas, as well as the delayed ratification of the IMO International Convention of the CHAFSS.

Previous studies on the extent and trend of the OT contamination in Hong Kong are, however, limited to hydrological, chemical and biological aspects. TBT contamination in Hong Kong was first reported in 1991 (Lau, 1991). High concentrations of TBT were detected during 1988 in water and sediment samples from marinas, typhoon shelter and mariculture sites, up to 1000 ng/l (water) and 1160 ng/g (sediment) in selected sites, while the body burden of TBT as high as 672 ng/g dry wt was also detected in clams (species not specified) (Lau, 1991). The problem persisted between 1988 and 1994, as reflected by sediment analysis (Ko et al., 1995). Imposex in neogastropods, as possession of a rudimentary penis in female, was first described in *T. clavigera* in 1988 (Tong, 1988).

Fig. 1. Map of Hong Kong showing the sampling sites for *Thais clavigera* and *Thais luteostoma*, locations of major shipping activities and facilities. The name of each numbered site is shown in Table 2. Dotted line represents the boundary of Hong Kong waters.

Mak (1992) studied imposex in *T. clavigera* in 1990 and recorded a 100% imposex rate for female samples from 11 locations in Hong Kong. After the partial restricted use of TBT in 1992, a few more studies on the imposex of *Thais* spp. have been conducted in 1996 (Blackmore, 2000) and 1997 (Li, 2003) without measuring the tissue burdens of OTs. Although both studies only visited a limited number of sites (6 and 17 sites, respectively), their results generally suggested high levels of TBT contamination throughout Hong Kong waters.

This study primarily aims to provide a comprehensive ecological risk assessment (ERA) of OTs, in particular TBT in Hong Kong waters through (1) determining the imposex status, sex ratio and tissue burdens of these compounds in the neogastropods, *T. clavigera* and *T. luteostoma* collected from 29 coastal sites and (2) performing a probabilistic ERA based on tissue burden of TBT in these animals. With a wider coverage of the territorial waters of Hong Kong, a more precise spatial pattern is generated. In combination of the related studies in the last 15 years, a temporal trend of the OT contamination is revealed. Relationships between body burden of OTs and imposex index or sex ratio are also established for these biomonitors. The results will provide an essential 'baseline' reference for the long-term monitoring programme in Hong Kong, and provide a methodological framework for ERA of OTs in Southeast Asia.

2. Materials and methods

2.1. Sample collection

Sampling of the two target species was conducted during June to July 2004. Adult *T. clavigera* (22.2–36.0 mm shell length) and *T. luteostoma* (31.8–43.1 mm shell length) were collected from 27 and 7 intertidal shores along the coastline of Hong Kong, respectively (Fig. 1). Both species occurred at five of the sampling sites, making up a total of 29 unique sites. The collection sites were selected to cover areas with various degree of organotin pollution. Number of collected specimen for each site ranged from 30 to 40 individuals, giving a total sample size of 1060 individuals for *T. clavigera* and 270 for *T. luteostoma* (Table 1).

2.2. Imposex identification

In the laboratory, shell length for each specimen was measured to the nearest 0.1 mm using vernier callipers. The shell was crushed in a vice, the soft body was removed, examined and measured under a binocular microscope. Sex of each specimen was determined by identification of the sperm ingesting gland and the capsule gland in females and the presence of the prostate gland in males (Horiguchi et al., 1994; Blackmore, 2000). Penis length was measured to the nearest 0.1 mm for males and females which were

Table 1

Site name, number of animals, shell length, percentage of imposex females, penis length in female, relative penis size index (RPSI), vas deferens sequence index (VDSI) and percentage of sterilized females for *Thais clavigera* and *Thais luteostoma*, respectively in this study

No.	Site	n (m:f)	% female	Male	Female				RPSI	VDSI		% sterile female	Distance from shipping activity (km)
				Shell length (mm) Mean $\pm$ SD	Shell length (mm) Mean $\pm$ SD	% imposex	Penis length (mm) Mean $\pm$ SD	median		mean			
<i>T. clavigera</i>													
1	Kat O	40 (15:25)	62.5	29.8 $\pm$ 2.0	30.6 $\pm$ 3.0	100	3.43 $\pm$ 1.24	1.59	4.0	3.92	0.0	23.8	
2	Pak Sha Chau	40 (19:21)	52.5	31.6 $\pm$ 2.9	33.6 $\pm$ 4.4	100	2.30 $\pm$ 0.66	0.37	4.0	3.57	0.0	19.5	
3	Chek Chau	40 (12:28)	70.0	30.1 $\pm$ 3.0	29.2 $\pm$ 2.0	100	2.30 $\pm$ 0.71	0.57	3.5	3.50	0.0	17.7	
4	Wu Kwai Sha	40 (19:21)	52.5	31.0 $\pm$ 2.5	28.7 $\pm$ 2.6	100	1.72 $\pm$ 0.64	0.17	3.0	2.67	0.0	24.0	
5	Heng On Estate	40 (18:22)	55.0	36.0 $\pm$ 3.3	34.8 $\pm$ 3.1	100	3.85 $\pm$ 1.10	0.84	4.0	3.64	0.0	27.0	
6	Wong Mau Chau	40 (10:30)	75.0	31.1 $\pm$ 2.0	32.2 $\pm$ 2.5	100	1.68 $\pm$ 0.50	0.11	3.0	3.03	0.0	21.0	
7	Kong Tau Pai	40 (11:29)	72.5	28.8 $\pm$ 2.3	29.8 $\pm$ 3.0	100	2.30 $\pm$ 0.71	0.06	2.0	2.38	0.0	15.3	
8	Sai Kung Pier	30 (20:10)	33.3	29.0 $\pm$ 3.8	33.4 $\pm$ 2.6	100	8.75 $\pm$ 1.22	34.38	4.0	4.70	40.0	0.0	
9	UST	40 (22:18)	45.0	33.7 $\pm$ 2.7	34.6 $\pm$ 3.0	100	7.42 $\pm$ 1.82	8.85	4.0	4.17	11.1	3.0	
10	Clear Water Bay	40 (12:28)	70.0	30.4 $\pm$ 2.6	32.3 $\pm$ 3.4	100	1.95 $\pm$ 0.65	0.53	3.0	3.04	0.0	13.0	
11	Shek Mei Tao	30 (7:23)	76.6	22.2 $\pm$ 1.7	23.8 $\pm$ 2.4	100	1.14 $\pm$ 0.26	0.15	3.0	3.22	0.0	6.7	
12	Tung Lung Island	40 (17:23)	57.5	31.4 $\pm$ 2.1	30.9 $\pm$ 2.7	100	5.98 $\pm$ 1.20	12.59	4.0	4.00	0.0	6.0	
13	Waglan Island	40 (14:26)	65.0	32.1 $\pm$ 3.9	35.4 $\pm$ 5.6	100	1.78 $\pm$ 0.56	0.18	3.0	3.00	0.0	12.6	
14	Po Toi	40 (15:25)	62.5	29.6 $\pm$ 2.1	30.0 $\pm$ 2.5	100	2.89 $\pm$ 0.99	2.32	4.0	3.52	0.0	13.6	
15	Shek O	40 (20:20)	50.0	30.8 $\pm$ 1.9	31.5 $\pm$ 2.0	100	6.73 $\pm$ 1.24	29.65	4.0	3.90	5.0	6.0	
16	Turtle Cove	40 (16:24)	60.0	31.9 $\pm$ 2.0	33.5 $\pm$ 2.6	100	2.45 $\pm$ 0.80	0.43	3.0	3.33	0.0	13.7	
17	Chung Hum Kok	40 (19:21)	52.5	31.9 $\pm$ 1.7	32.6 $\pm$ 1.8	100	4.74 $\pm$ 1.38	9.01	4.0	4.10	4.8	7.0	
18	Repulse Bay	40 (21:19)	42.5	31.7 $\pm$ 2.5	32.5 $\pm$ 3.3	100	5.87 $\pm$ 1.67	11.84	4.0	3.90	10.5	7.5	
19	Deep Water Bay	40 (23:17)	47.5	30.9 $\pm$ 1.6	30.8 $\pm$ 1.7	100	4.95 $\pm$ 1.70	7.72	4.0	4.35	29.4	6.9	
20	Aberdeen	40 (34:6)	15.0	29.9 $\pm$ 3.0	30.2 $\pm$ 2.6	100	8.45 $\pm$ 1.68	64.22	5.0	5.00	83.3	0.0	
21	Sok Kwu Wan	40 (26:14)	35.0	32.7 $\pm$ 1.9	33.3 $\pm$ 3.2	100	7.32 $\pm$ 1.51	12.07	6.0	5.36	71.4	0.0	
22	Ha Mei Wan	40 (17:23)	57.5	29.2 $\pm$ 2.5	29.6 $\pm$ 2.6	100	3.71 $\pm$ 0.89	1.89	4.0	3.65	0.0	3.3	
23	Mui Wo	40 (17:23)	57.5	31.8 $\pm$ 2.6	31.3 $\pm$ 2.4	100	5.06 $\pm$ 2.36	3.05	4.0	4.00	0.0	0.0	
24	Cheung sha	40 (15:25)	62.5	29.9 $\pm$ 1.7	30.6 $\pm$ 2.1	100	3.38 $\pm$ 1.45	1.26	4.0	4.00	0.0	14.0	
25	Tai O	40 (21:19)	47.5	24.6 $\pm$ 2.4	26.6 $\pm$ 2.9	100	3.31 $\pm$ 1.31	3.18	4.0	3.79	0.0	13.4	
26	Butterfly Beach	40 (31:9)	22.5	30.0 $\pm$ 3.0	28.3 $\pm$ 1.8	100	6.57 $\pm$ 1.85	15.56	4.0	4.11	33.3	1.4	
27	Kadoorie Beach	40 (25:15)	37.5	29.1 $\pm$ 1.4	29.3 $\pm$ 1.8	100	9.05 $\pm$ 1.48	38.43	5.0	4.87	53.3	1.6	
Total		1060											
<i>Thais luteostoma</i>													
4	Wu Kwai Sha	40 (18:22)	55.0	39.7 $\pm$ 3.3	41.4 $\pm$ 3.5	100	2.71 $\pm$ 0.84	0.17	3.0	2.96	0.0	24.0	
13	Waglan Island	30 (16:24)	60.0	31.9 $\pm$ 2.9	33.2 $\pm$ 3.7	100	1.49 $\pm$ 0.66	0.93	3.0	3.11	0.0	13.6	
14	Po Toi	40 (11:19)	63.0	32.7 $\pm$ 4.5	35.8 $\pm$ 5.4	100	3.04 $\pm$ 2.27	0.19	3.0	2.92	0.0	12.6	
21	Sok Kwu Wan	40 (18:22)	55.0	40.7 $\pm$ 3.4	41.0 $\pm$ 3.4	100	6.66 $\pm$ 2.49	4.37	5.0	4.82	54.5	0.0	
22	Ha Mei Wan	40 (13:27)	66.0	31.8 $\pm$ 3.4	32.9 $\pm$ 4.5	100	4.09 $\pm$ 4.42	2.01	3.0	3.33	0.0	3.3	
28	Central Pier	40 (27:13)	33.0	36.8 $\pm$ 4.1	37.1 $\pm$ 4.0	100	9.71 $\pm$ 2.20	14.13	5.0	4.92	76.9	0.0	
29	Tsim Sha Tsui	40 (14:26)	65.0	43.1 $\pm$ 5.1	41.0 $\pm$ 3.7	100	12.88 $\pm$ 1.78	23.44	5.0	5.31	84.6	0.0	
Total		270											

not narcotized. The degree of the imposex was estimated using the relative penis size index (RPSI) and vas deferens sequence index (VDSI) (Gibbs et al., 1987; Horiguchi et al., 1994). The RPSI was calculated as

$$\text{RPSI} = (\text{mean length of female penis})^3 / (\text{mean length of male penis})^3 \times 100$$

The VDSI, which was firstly developed by Gibbs et al. (1987) for the dogwhelk *Nucella lapillus* and later adopted for other neogastropod species like *T. clavigera* (Blackmore, 2000), has seven stages (0–6), with stage 0 indicating

no effect. At stage 1, the proximal section of the vas deferens had developed; at stage 2, a penis had formed and the vas deferens had increased in length; at stage 3, a vas deferens had developed posterior to the penis, and the posterior vas deferens outgrowth had developed further. At stage 4, the two outgrowths had fused into a continuous vas deferens. At stages 5 and 6, the females were sterile; at stage 5, the outgrowth had blocked the genital papilla while aborted capsules were seen in the capsule gland at stage 6 (Gibbs et al., 1987; Blackmore, 2000). Recent microscopic observation for the development of the vas deferens and penis in imposex-exhibiting females of *T. clavigera* revealed

that the VDSI of *T. clavigera* was only a little different from that of *N. lapillus* (Horiguchi et al., unpublished data). Mean VDSI was computed for each site. To maintain consistency, the imposex identification was conducted by the same person (i.e. R.P.Y. Kwong) throughout the study. Randomly selected samples (46 *T. clavigera* and 13 *T. luteostoma*) were re-examined by T. Horiguchi for cross-checking the VDSI. Agreement of the identification was subsequently tested using a paired *t*-test. The historical trend of imposex levels in these gastropods, between 1990 and 2004, was also compared based on the data reported in Mak (1992), Blackmore (2000), Li (2003) and the present study.

2.3. Organotin analysis

The concentrations of organotin species, monobutyltin (MBT), dibutyltin (DBT) and tributyltin (TBT), were quantified in the whole soft-body tissues of pooled animals. Two replicates of ten pooled animals were analysed for each study site. These organotin compounds were extracted using the method described in Sudaryanto et al. (2002) and Kan-Atireklap et al. (1997). The analytical procedures followed those described in Szpunar et al. (1996). To avoid the possibility of bias, samples were coded so that the analysts did not know the collection locations of the samples prior to analysis. Briefly, about 2 g (wet wt) of snail tissue sample was homogenized with 70 ml of 0.1% tropolone/acetone and 5 ml of 2 N HCl. After centrifugation, the supernatant was transferred to 100 ml of 0.1% tropolone/benzene, and moisture in the extract was removed with anhydrous sodium sulphate. After concentrating the extract using a rotary evaporator, all BTs in the extract were propylated by adding 10 ml of *n*-propyl magnesium bromide (~1 mol/l in tetrahydrofuran, Kanto Chemical, Tokyo, Japan) as Grignard reagent. Excess reagent was destroyed with 1 N sulphuric acid, and the propylated mixture was transferred to 10% benzene/hexane. The solution was purified on a florisil-packed glass column and eluted with hexane. The final hexane solution was concentrated and subjected to analysis by a Gas-Chromatograph (Hewlett-Packard 5890 Series II, CA, USA) equipped with Flame Photometric Detector (GC-FPD). A fused silica capillary column (30 m length $\times$ 0.25 mm i.d. $\times$ 0.25 μ m film thickness) coated with 100% dimethyl polysiloxane (J&W Scientific, Folsom, CA, USA) was used in the GC oven for separation. The flame photometer was equipped with a 610 nm band-pass filter that is selected for tin compounds. Known amounts of monobutyltin trichloride, dibutyltin dichloride and tributyltin chloride (0.1 μ g each) were used as the external standard mixture. Trihexyltin (0.16 μ g ml⁻¹ in acetone) was used as the internal standard. The procedure was validated using the certified reference material (CRM) BCR 477 (mussel tissue). The mean recoveries through the entire analytical procedure for MBT, DBT and TBT were 94.3%, 82.2% and 79.8%,

respectively. The detection limit was 0.02–0.05 ng g⁻¹ dry weight (dw). The results are reported as ng Sn g⁻¹ dw where appropriate.

2.4. Probabilistic ecological risk assessments

In order to assess the ecological risk of TBT on the Hong Kong populations of *T. clavigera*, a probabilistic risk assessment approach based on tissue burden of TBT was developed for this purpose. The risk can be described using the following equation:

Risk quotient(RQ)

$$= \frac{\text{Measured tissue concentration(MTC)}}{\text{Predicted no effect tissue concentration(PNETC)}}$$

If RQ < 1, there is low risk to *T. clavigera* population. If RQ $\geq$ 1, the population of *T. clavigera* is at certain risk due to TBT. The PNETCs were extracted from peer-reviewed literature on the chronic and sub-chronic effects of TBT on gastropod and bivalve molluscs. As most of the studies only measured the predicted no effect concentration (PNEC) of TBT in water, appropriate bioconcentration factors (BCFs) were applied to convert the TBT concentration in water into tissue concentration value, i.e. PNETC = PNEC/BCF. The corresponding BCF values were extracted from relevant peer-reviewed literature (Appendix 4). A geometric mean was used as a PNETC if there were multiple data for the same species. All available PNETC values were plotted as a species sensitivity distribution to serve as an effect distribution (Wheeler et al., 2002). The MTC was the mean tissue concentration of TBT obtained from each site in Hong Kong (Section 2.3). All MTC values were also plotted as a cumulative function to serve as an exposure distribution. A distribution of RQ values was finally computed using Monte Carlo simulation with 10,000 iterations, based on the distributions of MTC and PNETC. The simulation was carried out using @Risk software (version 4.5.2, professional edition, 2002, Palizade Corporation). The probability of *T. clavigera* populations in Hong Kong, being at risk (i.e. RQ $\geq$ 1), was subsequently obtained from the RQ frequency histogram.

3. Results

3.1. Imposex level

Agreement between two observers (i.e. actual measurement and validation check) in the identification of VDSI was good for *T. luteostoma* (paired *t*-test, *t* = 2.179, *p* = 0.096). For *T. clavigera*, there was a significant difference in VDSI assessment between the two observers (*t* = 2.014, *p* = 0.049). Nevertheless, the mean difference was only -5.3% between the actual measurement and the validation check, indicating a very slight underestimation

of VDSI in the actual assessment. Therefore, the precision of the current VDSI assessment was regarded as acceptable.

Sex ratio varied among the sampling sites for *T. clavigera* (ranging from 15% to 77% female) and *T. luteostoma* (33–66% female) (Table 1). Examination of the RPSI and VDSI showed that imposex occurred in all specimens across all sites for both species. The RPSI ranged from 0.06 to 64.22 for *T. clavigera*, and from 0.17 to 23.44 for *T. luteostoma* (Table 1). Sites with high RPSI included Aberdeen (RPSI = 64.22, *T. clavigera*), Kadoorie Beach (38.43, *T. clavigera*), Sai Kung Pier (34.38, *T. clavigera*), Shek O (29.65 *T. clavigera*), Tsim Sha Tsui (23.44, *T. luteostoma*), and Central Pier (14.13, *T. luteostoma*).

The mean VDSI ranged from 2.38 to 5.36 for *T. clavigera* and from 2.92 to 5.31 for *T. luteostoma* (Table 1). Sites with high VDSI included Sok Kwu Wan (mean VDSI = 5.36, *T. clavigera*; 4.82, *T. luteostoma*), Aberdeen (5.00, *T. clavigera*), Kadoorie Beach (4.87, *T. clavigera*), Tsim Sha Tsui (5.31, *T. luteostoma*), Central Pier (4.92, *T. luteostoma*) and Sai Kung Pier (4.70, *T. clavigera*). The lowest individual VDSI recorded was 1, which occurred only in *T. clavigera*, representing the commencement of vas deferens development. Specimens with VDSI of 1 were identified in four sites, namely Wu Kwai Sha, Wong Mau Chau, Kong Tau Pai and Clear Water Bay; all of them were located at relatively clean eastern waters. Sterile females, represented by VDSI of 5 or 6, were

Table 2
Site name, mean and SD of organotin concentration (ng Sn g⁻¹ dw) in tissue of *Thais clavigera* and *Thais luteostoma*

No.	Site	MBT		DBT		TBT		Total OT	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
<i>Thais clavigera</i>									
1	Kat O	30.2	7.2	32.4	2.0	9.3	2.2	72.0	11.3
2	Pak Sha Chau	21.1	3.2	20.3	3.6	8.5	1.4	49.8	8.2
3	Chek Chau	<d.l.	0.0	<d.l.	0.0	<d.l.	0.0	<d.l.	0.0
4	Wu Kwai Sha	107.6	2.4	<d.l.	0.0	3.8	0.4	111.4	2.8
5	Heng On Estate	<d.l.	0.0	16.9	4.5	<d.l.	0.0	16.9	4.5
6	Wong Mau Chau	71.8	4.3	12.2	3.6	1.8	0.2	85.8	8.0
7	Kong Tau Pai	32.9	0.2	6.3	3.0	1.8	0.2	41.0	3.4
8	Sai Kung Pier	44.4	1.1	55.0	4.9	14.8	0.3	114.3	6.3
9	UST	16.2	0.8	18.6	4.1	5.0	0.6	39.9	5.5
10	Clear Water Bay	<d.l.	0.0	<d.l.	0.0	<d.l.	0.0	<d.l.	0.0
11	Shek Mei Tao	22.5	3.0	19.9	6.1	<d.l.	0.0	42.4	9.1
12	Tung Lung Island	41.2	3.5	29.0	0.4	5.8	1.4	76.0	5.3
13	Waglan Island	12.0	0.3	5.6	0.5	2.4	0.0	20.0	0.8
14	Po Toi	37.8	0.8	17.6	3.0	4.0	0.0	59.4	3.8
15	Shek O	<d.l.	0.0	<d.l.	0.0	4.3	2.5	4.3	2.5
16	Turtle Cove	27.3	4.3	6.4	1.3	6.0	0.8	39.7	6.3
17	Chung Hum Kok	21.3	1.9	13.2	1.8	3.2	0.3	37.7	4.1
18	Repulse Bay	336.6	3.8	33.0	1.8	9.9	0.6	379.5	6.1
19	Deep Water Bay	28.1	1.3	33.6	2.6	13.1	3.5	74.8	7.4
20	Aberdeen	700.7	35.2	197.3	29.3	16.7	0.3	914.7	64.7
21	Sok Kwu Wan	38.8	5.9	49.0	9.1	12.4	0.8	100.1	15.8
22	Ha Mei Wan	108.4	1.5	39.4	5.9	18.3	1.8	166.2	9.3
23	Mui Wo	27.2	4.2	144.2	1.0	10.9	2.0	182.3	7.2
24	Cheung sha	14.2	0.9	35.7	1.9	5.8	0.2	55.7	3.0
25	Tai O	21.0	0.3	56.8	4.8	15.8	2.7	93.6	7.8
26	Butterfly Beach	42.6	5.1	131.7	2.1	18.3	4.4	192.6	11.6
27	Kadoorie Beach	41.1	2.2	58.5	5.4	15.4	1.7	115.0	9.3
<i>Thais luteostoma</i>									
4	Wu Kwai Sha	20.1	2.6	3.8	0.3	2.7	0.3	26.6	3.1
13	Waglan Island	<d.l.	0.0	<d.l.	0.0	3.8	0.2	3.8	0.2
14	Po Toi	23.2	0.2	9.4	0.0	4.4	0.7	36.9	1.0
21	Sok Kwu Wan	22.9	1.4	30.1	3.8	16.5	2.0	69.5	7.2
22	Ha Mei Wan	31.5	2.0	<d.l.	0.0	6.6	0.6	38.0	2.6
28	Central Pier	45.7	3.6	69.9	4.6	27.6	2.8	143.2	11.1
29	Tsim Sha Tsui	51.2	0.8	85.4	17.6	33.5	4.0	170.1	22.4

MBT: monobutyltin; DBT: dibutyltin; TBT: tributyltin; <d.l.: below detection limit.

recorded in 10 of the 27 sampling sites for *T. clavigera* and 3 of the 7 sites for *T. luteostoma*. High percentage sterilization occurred in Aberdeen (83.3%), Sok Kwu Wan (71.4%), Kadoorie Beach (53.3%) for *T. clavigera*; and Tsim Sha Tsui (84.6%), Central Pier (76.9%), Sok Kwu Wan (54.5%) for *T. luteostoma* (Table 1).

3.2. Body burden of organotin compounds

The mean concentrations of organotin (OT) compounds (MBT, DBT and TBT) in the soft-body tissue ranged from <0.05 (i.e. the detection limit) to 914.7 ng Sn g⁻¹ dw (i.e., <0.05 to 1458.1 ng OT g⁻¹ dw) for *T. clavigera* and 3.8 to 170.1 ng Sn g⁻¹ (i.e. 9.4–324.4 ng g⁻¹) for *T. luteostoma* (Table 2). In *T. clavigera*, the mean tissue concentrations of TBT, DBT and MBT ranged from <0.05 to 18.33 ng Sn g⁻¹, <0.05 to 197.31 ng Sn g⁻¹ and <0.05 to 700.66 ng Sn g⁻¹, respectively (Table 2). These OT compounds, how-

ever, were not detected in the populations from Chek Chau and Clear Water Bay, although imposex was observed in these two sites. For *T. luteostoma*, mean tissue TBT ranged from 2.7 to 33.5 ng Sn g⁻¹, DBT ranged from <0.05 to 85.4 ng Sn g⁻¹ and MBT ranged from <0.05 to 51.2 ng Sn g⁻¹ (Table 2).

Fig. 2. Relative penis size index (RPSI; a) and vas deferens sequence index (VDSI; b) versus tissue organotin concentration in *Thais clavigera* (closed circle) and *Thais luteostoma* (opened circle). Results of regression analysis are also shown.

Fig. 3. Percentage of females versus relative penis size index (RPSI; a) and vas deferens sequence index (VDSI; b) and tissue organotin concentration (c) for *Thais clavigera*. Results of regression analysis are also presented.

3.3. Correlations between imposex level and organotin burden

Relationships between imposex indices and organotin concentrations were tested using non-parametric Spearman's rank correlation due to the patchiness of part of the dataset. RPSI and VDSI were positively correlated with the tissue concentrations of all three OTs in both *T. clavigera* and *T. luteostoma* (Fig. 2). Similar positive correlations were also identified between TBT and RPSI and VDSI in both species (statistical results shown in Appendix 2). Both imposex indices also increased significantly with increasing DBT concentrations in the tissues of *T. clavigera* but not in *T. luteostoma* (Appendix 2). Likewise, the percentage of sterilized females (i.e. VDSI ≥ 5) also significantly increased with increasing tissue concentration of DBT, TBT or total OTs (Appendix 2). Tissue concentrations of MBT, however, did not show any significant correlation with the imposex indices or percentage of sterilized females in both species.

3.4. Correlations between sex ratio, imposex level and organotin burden

Across all sites, the percentage of *T. clavigera* females decreased significantly with increasing RPSI (Regression analysis: $r^2 = 0.58$, $p < 0.0001$), VDSI ($r^2 = 0.59$, $p < 0.0001$) and total tissue concentrations of the three OTs ($r^2 = 0.40$, $p < 0.001$) (Fig. 3). Similar relationships were not statistically significant for *T. luteostoma*, probably due to small sample size.

3.5. Relationships between shipping activity and organotin contamination

The level of shipping activity for each site was estimated based on the shortest distance between the site and existing shipping facility/activity, e.g. public pier, marina, typhoon shelter, container terminal/cargo working area and shipping channel (Table 1). The level of imposex showed a decreasing trend with distance from the potential source of the contaminant in *T. clavigera* (Fig. 4, 2004), but such trends were less prominent for *T. luteostoma* (Table 1; figure is not shown). The level of RPSI of *T. clavigera* generally dropped to < 5 in sites 10 km or more away from the major shipping activities (Fig. 4, 2004). For VDSI of *T. clavigera*, a gentler decline of value to below 4 was observed beyond 10 km. Similar trends were also observed for tissue concentrations of the three OTs and TBT, respectively (figures are not shown).

3.6. Historical trends of organotin contamination

The present results of the imposex levels were also compared with past data (available for *T. clavigera* only) which were collected in 1990 (2 years before the restriction of use of TBT paint) (Mak, 1992), 1996 (Blackmore, 2000) and 1997 (Li, 2003), respectively (Fig. 4; Appendix 3). The 1990 study (Mak, 1992) recorded RPSI and percentage sterile female in 11 studies sites, 6 of which occurred in the present study. The 1990s level of RPSI was generally higher than the post-1992 studies. Such elevated levels were probably related to the higher application rate of TBT based antifoulants before the implementation of

Fig. 4. Relative penis size index (RPSI; upper panel) and vas deferens sequence index (VPSI; lower panel) versus distance from shipping facility/activity for *Thais clavigera* in 1990, 1996, 1997 and 2004. Data of 1990 are from Mak (1990), 1996 data are from Blackmore (2000) and 1997 data are from Li (2003).

Fig. 5. Results of the probabilistic ecological risk assessment: (a) species sensitivity distribution to TBT contrasted from chronic toxicity data from literature (filled circle), i.e. distribution of PNETC and distribution of tissue TBT burden in *Thais clavigera* from various sites in Hong Kong (open circle), i.e. distribution of MTC and (b) the distribution of the computed risk quotient generated from Monte Carlo simulation.

TBT use restriction in 1992. From 1996 to 2004, both imposex indices remained at a similar level in areas within 10 km from the sites with high marine traffic or adjacent to large harbours/ports (Fig. 4). In contrast, there was a considerable reduction of RPSI between 1997 and 2004 at the areas 10 km or more away from the major shipping activities (Fig. 4). This was probably due to the implementation of restriction of TBT use on smaller vessels. In terms of percentage of sterilization (Appendix 3), it was noticed that there was some improvement at Sai Kung Pier, UST, and Chung Hum Kok sites where the marine

traffic is dominated with small vessels. But the percentage of sterilization remained high at Deep Water Bay, Kadoorie Beach, Sok Kwu Wan and Aberdeen (Appendix 3).

3.7. Risk assessment

Studies on chronic toxicity of TBT on gastropod and bivalve molluscs were limited (Appendix 4). At low aqueous TBT levels 1–2 ng l⁻¹, female neogastropods can develop imposex. The corresponding tissue concentration

for imposex induction in *T. clavigera* was estimated to be about 33 ng TBTCI g⁻¹ dry wt (Appendix 4; Horiguchi et al., 1994). As low levels of imposex might not cause detrimental effect on individual lifetime fitness and population sustainability, such PNETCs were excluded from the construction of species sensitivity distribution (SSD). The final SSD included six PNETC values, covering endpoints such as inhibition of growth, reproduction and immunological suppression (Fig. 5a; Appendix 4). Based on the probabilistic risk assessment, 5.4% of all populations of *T. clavigera* are at risk (i.e. likely to suffer at least reduction in body weight) due to exposure to TBT (Fig. 5b). Based on the overlap of the MTC and PNETC distributions (Fig. 5a), seriously impacted areas included Aberdeen, Repulse Bay, Butterfly Beach, Mui Wo and Ha Mei Wan.

4. Discussion

There were great spatial variations in organotin (OT) contamination levels in Hong Kong waters, as shown by the tissue OT concentrations in the neogastropods. These large variations were strongly associated with marine traffic and shipping activities (Fig. 4). High OT body burden and imposex indices were observed in areas such as Aberdeen, Central Pier, and Tsim Sha Tsui where high frequencies of marine traffic or great number of large vessels are often encountered.

All female gastropods developed imposex characters from a total of 29 sampling sites for both species. Similar results were also obtained in past studies since 1990, 100% imposex rate was observed in *T. clavigera* in all 11 sampling sites in 1990 (Mak, 1992), in 10 of the 11 sampling sites in 1996 (Blackmore, 2000) and 26 of the 28 sites in 1997 (Li, 2003). Sites with <100% imposex rate in past studies were Wu Kwai Sha (1996), Ma Shi Chau (1997) (in close proximity to Wu Kwai Sha), and Clear Water Bay (1997). All these sites are relatively far away from major shipping activities.

In general, eastern to northeast waters of Hong Kong (site no. 1–14) are less influenced by TBT pollution, as reflected by lower RPSI and VDSI values. These sites are remote from the commercial shipping activities and channels, and are more open to the prevailing oceanic current. However, the effect of OT contamination from Yantian Port appeared to have become more apparent overtime. Considering the imposex indices of five sites, i.e. 1, 2, 3, 6 and 7 (Fig. 1), which form a line away from Yantian Port, RPSI drop from 1.59 to 0.06 and VDSI from 3.92 to 2.38. Tissue TBT concentration also showed a similar trend, indicating the Yantian Port as a potential source of TBT contaminant. Site 7 (Kong Tau Pai), which is located farthest from the shipping activities in mid-south waters and far away from Yantian Port, exhibited the lowest RPSI and VDSI recorded in this study. Continuous development of port activities in Hong Kong and Yantian may be expected to increase the threat of OT pollution to Hong Kong waters, especially in the northeastern region.

There was a significant pattern of sex ratio with respect to the imposex indexes and tissue organotin concentration in *T. clavigera* (Fig. 3), represented by male-biased population in sites with severe organotin contamination. The TBT related sex ratio alternation has been reported in *T. clavigera* (Tan, 1997; Blackmore, 2000; Shim et al., 2000; Li, 2003) and other gastropods, such as *N. lapillus* (Bryan et al., 1986), *T. biserialis* (Ramasamy and Murugan, 2002), *Ocenebrina aciculate* (Oehlmann et al., 1996b). The affected population may suffer from reproductive failure and even local extinction (Gibbs and Bryan, 1996; Evans et al., 1996). This phenomenon has been suggested to be caused by selective mortality on female (Bryan et al., 1986; Tan, 1997; Nicholson and Evans, 1997) or sex change of female to male (Oehlmann et al., 1996b; Rilov et al., 2000). Functional sex change of gastropod induced by organotins, however, has not been directly demonstrated at any developmental stage. Juvenile *Buccinum undatum* exposed to TBT, just after hatching, showed a dose-dependent proportion of the young developing male sexual characters (Mensink et al., 1996), but no follow up study has been reported to ascertain whether the functional sex ratio is altered. Gibbs et al. (1988) recorded suppression of oogenesis and production of sperm in female *N. lapillus* raised from hatched juveniles under the influence of TBT. The reproductive function of masculinization was not determined, and was believed to be hypothetical due to the absence of sterile female in the study population. Moreover, an anatomical study on imposex females of *T. clavigera* showed a lack of testis and fully developed prostate gland that could prevent functional sex change (Yu et al., 2004). The drifted sex ratio in the present study is probably associated with an unequal mortality rate between the sexes that may be operating at different life stages.

Tissue TBT concentrations ranged from <0.05 to 18.33 ng Sn g⁻¹ dw for *T. clavigera* and 2.68–33.48 ng Sn g⁻¹ dw for *T. luteostoma* in the present study. Higher values have been obtained in other studies of conspecifics, body TBT burden of 43–74 ng Sn g⁻¹ dw has been recorded in *T. clavigera* around mariculture areas in Taiwan (Hung et al., 2001). TBT concentrations of 5–508 ng Sn g⁻¹ dw had been recorded in *T. clavigera* along the Korean coast, with generally >200 ng Sn g⁻¹ in animals near harbours and shipyards (Shim et al., 2000). Horiguchi et al. (1994) examined imposex and organotin levels in *T. clavigera* in Japan, TBT levels, measured in ng g⁻¹ as chloride in wet weight, were up to 420 (≈185.3 ng Sn g⁻¹ wet weight). In spite of different ranges of measured TBT concentrations, the correlation between imposex level and tissue burden of TBT as well as shipping activities have commonly been observed in all these studies. Although there were insufficient data on imposex level and TBT tissue burden of *T. luteostoma*, the general patterns of imposex development and organotin accumulation revealed in the current study were similar to those of *T. clavigera*, suggesting their potential use as a good biomonitor.

Restriction of TBT usage in 1992 seemed to have been somewhat effective in Hong Kong, as represented by a drop of RPSI from 1990 to 1996 in all areas and from 1996/1997 to 2004 in areas at 10 km or more away from major shipping activities. However, imposex levels did not show any further reduction from 1996/97 to 2004 in areas within 10 km of major shipping activities. A similar conclusion was reached in a comparable study conducted in Iceland using *N. lapillus* (Jörundsdóttir et al., 2005). Recovery or re-colonization of gastropod populations has been reported in different taxa and locations after the restriction of TBT-based antifoulant application (e.g. Evans et al., 1995; references cited in Jörundsdóttir et al., 2005); the problem persisted, however, in other cases (e.g. Oehlmann et al., 1998; Horiguchi, 2000; Santos et al., 2002; Gibson and Wilson, 2003). The persistence of organotin pollution showed that the contaminant has not been effectively, or only partially, suppressed by the existing regulation. In fact, many studies have reported that levels of organotin remained high near commercial ports even though significant decline occurred in the peripheral areas (Evans et al., 2001; Reitsema et al., 2003). The majority of the vessels utilizing Hong Kong waters were commercial vessels of over 25 m that were not regulated in their application of TBT-based antifoulant. Additionally, weak enforcement of regulation (Ko et al., 1995) and poor dock practice (Birchenough et al., 2002) in Hong Kong concomitantly amplify the problem of OT contamination.

To understand the suitability of using *Thais* spp. as bio-indicators for assessing the possible change in the ambient organotin levels in the future, especially when the IMO Antifouling Convention becomes effective, the sensitivity of the species to temporal OT variation is important. *T. clavigera* has been shown to be sensitive to TBT (Horiguchi et al., 1994, 1995, 1997). Imposex was induced in adult female *T. clavigera* at an environmental concentration of approximately 1 ng l^{-1} of TBT in 85-day flow-through exposure experiments, and imposex was promoted 30 days post either TBT or TPT (triphenyltin) injection (Horiguchi et al., 1995, 1997). There was a good correlation between imposex level and TBT concentration for *T. clavigera* (Horiguchi et al., 1994; present study). However, as the development of imposex is an irreversible process (Gibbs et al., 1987), the degree of imposex in an animal can only reflect the elevation of OTs during its life span. The irreversible nature of imposex and the relative long life cycle of these gastropods may limit the responsiveness of the animal to a decrease of TBT level (Foale, 1993; Gibbs and Bryan, 1996). *T. clavigera* is a perennial species with a life span of 7 years or more and maturity is attained after about 2 years (Tong, 1986). On this basis, a rapid drop in aqueous TBT concentration may take more than 2 years to be reflected by the imposex status in the new cohorts.

It is believed that tissue TBT concentration is a reliable alternative for monitoring short-term change. The reaction (response) time of tissue concentration is shorter, half-life

of TBT in mollusc tissue has been reported to be 22 days in *T. clavigera* (Horiguchi et al., 1995), 11–36 days in the clam, *Venerupis decussata* (Gomez-Ariza et al., 1999), 26 days in the bivalve *Dreissena polymorpha* (Van Slooten and Tarradellas, 1994), and about 2–3 months in the gastropod *Nassarius reticulatus* (Sousa et al., 2005). An OT injection experiment showed that tissue TBT concentration of *T. clavigera* declined from 221.6 and 900.8 ng g^{-1} wet weight in day 2 of injection to 20.3 and 116.6 ng g^{-1} wet weight in 30 days, respectively (Horiguchi et al., 1997). Thus, half-life of TBT in mollusc tissue is relatively short, and tissue concentration may be useful in reflecting the recent changes in ambient OT levels.

Nowadays, many pollutant measurements, particularly those for lipophilic compounds, are carried out using sediment or biota samples rather than water samples, because of the large variations and tiresome procedures for seawater chemical analysis due to low water solubility of these chemicals (Leung and Lam, 2004). As a result, tissue burden data of these contaminants are often more available than those in other environmental media. There are a number of issues in regard to how these data can be used in ecological risk assessment (ERA). Can we directly apply these tissue burden data in ERA? What is the threshold tissue level of a particular substance? At present, the most commonly used method in ERA is based on two major parameters, namely predicted environmental concentrations (PECs) and predicted no effect concentrations (PNECs). The former is usually obtained by quantifying the concentrations of a particular pollutant in water or sediment samples which are randomly collected in the environment of interest. A PNEC of a substance is based on its toxic effects on single, individual-level endpoints (e.g., survival, fecundity, and growth; LCx, ECx or NOEC) and derived using either fixed application factor approach or one of the species sensitivity distribution (SSD) models (Wheeler et al., 2002). Subsequently, a statistical distribution of the risk quotient (RQ) can be computed as a ratio of PECs/PNECs using Monte Carlo simulation. If $\text{RQ} > 1$, the ecosystem is at risk. However, a number of problems have been identified when using this model. Most available toxicity data for ERA are derived from toxicity tests using seawater and, to a lesser extent, sediment as a test medium. The situation is that we often have PECs in terms of pollutant concentrations in biota whereas most PNECs are water-based. In the present study, an attempt has been made to utilize appropriate bioconcentration factors (BCFs) to estimate the tissue body burden of TBT in the gastropod and bivalve molluscs used in chronic or sub-chronic toxicity tests. In so doing, PEC and PNEC can be converted/standardized to MTC and PNETC for probabilistic ERA. The present study demonstrates that this approach is workable and can provide an effective solution to make use of body burden data in direct ERA.

In conclusion, imposex was recorded in all female *T. clavigera* and *T. luteostoma* from 29 sites in Hong Kong. RPSI and VDSI were significantly correlated with tissue

organotin and TBT concentration. Imposex level and body burden organotin were highest in populations nearest to major shipping activities/facilities, and gradually decreased with distance away from these sources. A decline in imposex level was recorded after the enforcement of TBT regulation in 1992. Since then, there was some further reduction of RPSI in areas 10 km or more away from major shipping activities, but no apparent improvement in areas within 10 km of major shipping activities between 1996/97 and 2004. Yantian Port, a rapid development port facility in Mainland China since 1996, posed a new source of TBT contamination to the eastern waters of Hong Kong. The threat of TBT pollution has persisted over the last 7/8 years and may be expected to increase with the further port development activities. The IMO convention of TBT-anti-

fouling paint prohibition is considered necessary to reduce OT pollution in Hong Kong waters.

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Appendix 1

Port vessel activity of Hong Kong in 1994, 1999 and 2004. Percentage change over the past 5 years are presented in parenthesis (Source: Marine Department of Hong Kong SAR, 2005).

Port vessel activity	1994	1999	2004
Ocean Cargo Vessels	34 750	35 140 (+1.1%)	32 540 (−7.4%)
Ocean Passenger Vessels	2250	2430 (+8.0%)	3360 (+38.3%)
River Cargo Vessels	92 050	115 330 (+25.3%)	117 540 (+1.9%)
River Passenger Vessels	63 180	59 930 (−5.1%)	71 980 (+20.1%)
Total	192 230	212 840 (+10.7%)	225 430 (+5.9%)

Appendix 2

Results of Spearman's rank correlation between organotins (OT: total organotin; TBT: tributyltin; DBT: dibutyltin; MBT: monobutyltin) and imposex indexes (RPSI: relative penis size index; VDSI: vas deferens sequence index) and percentage of sterilized females for *T. clavigera* and *T. luteostoma*, respectively; n.s.: not significant.

Factor	Variance	<i>Thais clavigera</i>		<i>Thais luteostoma</i>	
		r_s	p	r_s	p
OT	RPSI	1	<0.001	1	<0.001
	VDSI	1	<0.001	1	<0.001
	% Sterilized female	0.421	0.029	0.906	0.005
TBT	RPSI	0.664	0.001	0.937	0.007
	VDSI	0.684	<0.001	0.893	0.012
	% Sterilized female	0.546	0.003	0.906	0.005
DBT	RPSI	0.606	<0.001	n.s.	n.s.
	VDSI	0.742	<0.001	n.s.	n.s.
	% Sterilized female	0.485	0.010	0.915	0.004
MBT	RPSI	n.s.	n.s.	n.s.	n.s.
	VDSI	n.s.	n.s.	n.s.	n.s.
	% Sterilized female	n.s.	n.s.	n.s.	n.s.

Appendix 3

Site name, vas deferens sequence index (VDSI), relative penis size index (RPSI) and percentage of sterilized females of *Thais clavigera* at all locations. Data of 1990 are from Mak (1990), 1996 data are from Blackmore (2000) and 1997 data are from Li (2003).

No.	Site	RPSI				VDSI			% Sterilization			
		1990	1996	1997	2004	1996	1997	2004	1990	1996	1997	2004
<i>T. clavigera</i>												
1	Kat O	5.7			1.6			3.92	0.0			0.0
2	Pak Sha Chau				0.4			3.57				0.0
3	Chek Chau				0.6			3.50				0.0
4	Wu Kwai Sha		0.1	0.8	0.2	2.00	2.89	2.67		0.0	0.0	0.0
5	Heng On Estate			6.0	0.8		3.36	3.64			0.0	0.0
6	Wong Mau Chau				0.1			3.03				0.0
7	Kong Tau Pai				0.1			2.38				0.0
8	Sai Kung Pier			45.3	34.4		4.97	4.70			63.0	40.0
9	UST		15.0		8.9	4.00		4.17		19.0		11.1
10	Clear Water Bay	47.3	0.6	0.4	0.5	2.00	3.08	3.04	0.0	0.0	0.0	0.0
11	Shek Mei Tao				0.1			3.22				0.0
12	Tung Lung Island			11.9	12.6		3.81	4.00			0.0	0.0
13	Waglan Island				0.2			3.00				0.0
14	Po Toi			4.4	2.3		3.48	3.52			0.0	0.0
15	Shek O			9.8	29.7		3.59	3.90			0.0	5.0
16	Turtle Cove			2.6	0.4		3.50	3.33			0.0	0.0
17	Chung Hum Kok			26.9	9.0		4.22	4.10			22.0	4.8
18	Repulse Bay			20.9	11.8		3.92	3.90			0.0	10.5
19	Deep Water Bay			32.9	7.7		4.25	4.35			25.0	29.4
20	Aberdeen	97.3	41.3	45.8	64.2	6.00	5.09	5.00	85.7	92.0	67.0	83.3
21	Sok Kwu Wan	71.4		49.8	12.1		5.13	5.36	81.3		71.0	71.4
22	Ha Mei Wan			9.2	1.9		3.50	3.65			0.0	0.0
23	Mui Wo	61.7		32.0	3.0		4.11	4.00	10.0		11.0	0.0
24	Cheung sha		3.9		1.3	3.00		4.00		0.0		0.0
25	Tai O	20.7	10.6	6.6	3.2	3.00	3.44	3.79	0.0	0.0	0.0	0.0
26	Butterfly Beach				15.6			4.11				33.3
27	Kadoorie Beach		21.8	53.4	38.4	4.00	4.85	4.87		21.0	64.0	53.3

Appendix 4

Chronic and subchronic toxicity values of body burden tributyltin (ng g^{-1} dry weight; listed in an ascending order) for various molluscs. Bold values were used for constructing the species sensitivity distribution (see Fig. 5a).

Group	Species	Chronic value (ng/l)	Exposure duration (days)	Bioconcentration factor (BCF)	Estimated body burden concentration (ng/g)	Type of chronic value	End point of toxicity
Gastropod	Littorina littorea	2	Field study	34600 ^a	69.2	LOEC	Female developed intersex ^b

Appendix 4 (continued)

Group	Species	Chronic value (ng/l)	Exposure duration (days)	Bioconcentration factor (BCF)	Estimated body burden concentration (ng/g)	Type of chronic value	End point of toxicity
Gastropod	<i>Nucella lapillus</i>	1	Field study	100 000 ^c	100	LOEC	Females lost weight and developed imposex ^d
		2	8	100 000 ^c	200	LOEC	Females lost weight and developed imposex ^e
Gastropod	<i>Thais clavigera</i>	N.A.	Field study	N.A.	141 10–20 ng TBTCI/g wet tissue ^u	Geomean LOEC	Females developed imposex ^f
Gastropod	<i>Thais clavigera</i>	ca. 1	85	5000–10 000 ^g	ca. 20 ng TBTCI/g wet tissue ^u	LOEC	Females developed imposex ^g
Gastropod	<i>Hinia incrassata</i>	1.5	Field study	122 000 ^h	183	LOEC	Development of imposex ^h
Bivalve	<i>Scrobicularia plana</i>	14	30	50 000 ⁱ	700	LOEC	Reduce larval shell growth and survivorship ^j
Bivalve	<i>Ruditapes decussata</i>	90	7	12 000 ^k	1080	LOEC	Elevation in the NADPH cytochrome (P450) reductases and decrease in NADH cytochrome b ₅ reductases ^l
Bivalve	<i>Crassostrea virginica</i>	30	63	49 000 ^m	1470	LOEC	Decrease resistance against bacterial challenge ⁿ
Bivalve	<i>Mytilus edulis</i>	5	32	400 000 ^o	2000	NOEC	Activity of hemocyte and membrane injury ^p
		5.4	15	400 000 ^o	2160	EC10	Growth rate reduction ^q
		10	60	400 000 ^o	4000	LOEC	Reduced shell growth ^r
Bivalve	<i>Crassostrea gigas</i>	48	Field study	310 000 ^s	2585 14880	Geomean LOEC	Growth inhibition ^t

^a Kure and Depledge (1994).^b Oehlmann (2004).^c Bryan et al. (1993).^d Gibbs et al. (1987).^e Davies et al. (1997).^f Horiguchi et al. (1994).^g Horiguchi et al. (1995).^h Oehlmann et al. (1998).ⁱ Bryan and Gibbs (1991).^j Ruiz et al. (2005).^k Morcillo and Porte (2000).^l Sole (2000).^m Roberts et al. (1987).ⁿ Fisher et al. (1999).^o Salazar and Salazar (1991).^p St-Jean et al. (2002).^q Stenalt et al. (1998).^r Huang and Yong (1995).^s Roberts et al. (1987).^t Batley et al. (1989).^u Supposing that moisture content of the whole soft tissue of *Thais clavigera* is 70%, the values can be translated into approximately 33–67 and 67 ng TBTCI g⁻¹ dry tissue, respectively.

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