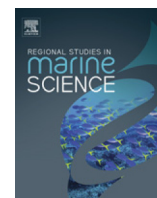




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# Imposex status associated with organotin contamination in *Reishia clavigera* after reciprocal transplantation between clean and polluted sites in Hong Kong

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## HIGHLIGHTS

- *Reishia clavigera* was transplanted reciprocally between clean and polluted sites.
- Tissue TPT concentrations increased in *R. clavigera* transplanted to polluted site.
- There was no significant change in imposex level in any transplantation treatment.
- They had lower tissue organotin concentration in winter than that in summer.
- This study calls for tightened management controls on these prevalent chemicals.

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## ABSTRACT

Recent biomonitoring studies have showed that contaminations of triphenyltin (TPT), relative to levels of tributyltin, have become more serious in Hong Kong and other South China coasts. However, there is a lack of field-based evidence to demonstrate the casual relationship between TPT and imposex development in the rock shell *Reishia clavigera* in this region. This study aimed to investigate the temporal changes of imposex development and tissue concentration of six organotins (OTs) in tissues of *R. clavigera* after their reciprocal transplantation between relatively clean and polluted sites in Hong Kong for six months. The results revealed a significant increase of tissue concentration of total OTs, in particular TPT, in the rock shells transplanted from a clean site to a polluted site, while a decrease of tissue OT concentrations was observed in those treated conversely. Vas Deferens Sequence Index, one of the imposex indices, only increased slightly for rock shells transplanted from clean to polluted sites, while Relative Penis Size Index elevated remarkably for the same treatments. In general, the rock shells had lower tissue concentrations of OTs in winter than that in summer, though the exact mechanism is still unknown. This study has further confirmed that OT contamination is still prevalent in Hong Kong's coastal marine waters, which necessitates tightened management controls on this group of chemicals especially on TPT compounds.

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## 1. Introduction

The endocrine disrupting organotin compounds (OTs), including tributyltins (TBT) and triphenyltin (TPT), are able to induce imposex (i.e., growth of penis and vas deferens) in female marine gastropods (Tittley-O'Neal et al., 2011; Yi et al., 2012). Since the 1990s,

the intertidal rock shell *Reishia clavigera* (= *Thais clavigera*; see Claremont et al., 2013) has been widely used as a biomonitor for OT contamination in coastal marine environments in the Asia-Pacific region (e.g., Shim et al., 2000; Wang et al., 2010) including those in Hong Kong (e.g., Mak, 1992; Blackmore, 2000; Leung et al., 2006; Qiu et al., 2011; Ho et al., 2016).

Unlike many locations around the globe which have shown declines of OT contamination in recent years (e.g., Galante-Oliveira et al., 2006; Oliveira et al., 2009; Guðmundsdóttir et al., 2011; Bray et al., 2012), the marine environment of Hong Kong is still threatened by OTs as reflected by high concentrations of OTs, in particular TPT, in the tissue of *R. clavigera* (Ho et al., 2016) and other

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marine organisms (Nakayama et al., 2009; Ho and Leung, 2014a). Likewise, heavy contaminations of TBT and TPT in the coastal areas of South China, for example, in Shenzhen (Ho and Leung, 2014b) and in Xiamen (Wang et al., 2008), were also observed in recent years. In northern China and Japan, high concentrations of TPT were found in organisms occupying different trophic levels in marine food webs, and TPT was found to be biomagnified along the marine food chain (Hu et al., 2006; Murai et al., 2008). Being a predatory gastropod on rocky shore, *R. clavigera* can uptake and accumulate TPT from its food sources, including barnacles and oysters (Blackmore, 1998), and thus high concentrations of this compound were found in this species.

However, all previous biomonitoring studies in Hong Kong have not incorporated measurements of phenyltins (PTs) in the tissue of *R. clavigera* except our recent study conducted in 2010 and 2015 (Ho et al., 2016), which detected high concentrations of PTs in their tissues. As Horiguchi et al. (1997) demonstrated that TPT was able to trigger imposex in *R. clavigera*, recent detections of high incidence of imposex in Hong Kong's populations of this species could be attributed to high levels of PTs in the marine environment. Nevertheless, there is a lack of field-based evidence to support this causal relationship between TPT and imposex development in *R. clavigera* in Hong Kong.

This study attempted to investigate the relationship between the degree of imposex and OT contamination (as reflected by tissue concentrations of both butyltins and phenyltins) in *R. clavigera* along the coast of Hong Kong, through a field-based transplantation experiment. By reciprocal transplantation of the rock shells between relatively clean and polluted sites, this study aimed to test two hypotheses: (1) individuals transplanted from a clean site to a polluted site will show a significant increase in tissue concentration of OTs and exhibit a more severe imposex status; and (2) individuals transplanted from a polluted site to a clean site will show a significant reduction in tissue concentration of OTs but their imposex status will remain constant since the imposex development is irreversible. The results of this study will also provide better understanding of the temporal dynamics of imposex development and uptake of OTs in this marine neogastropod species (Davies, 2000; Shim et al., 2000; Bech et al., 2002).

## 2. Materials and methods

### 2.1. Transplantation experiment

A maximum of 300 adult *R. clavigera* (i.e., shell length  $\geq 17$  mm; see Tong, 1986) were randomly collected from the sheltered shore of each of the four sampling sites, namely Clear Water Bay, Po Toi Island, Sai Kung Pier and Sok Kwu Wan, during low tide in the summer of 2012 (Fig. 1). According to Leung et al. (2006) and Ho et al. (2016), Clear Water Bay and Po Toi Island were identified as relatively clean areas, having relatively low imposex indices and low tissue OT concentrations in *R. clavigera*. On the other hand, Sai Kung Pier and Sok Kwu Wan were regarded as heavily OT-polluted areas that were indicated by high imposex indices and tissue OT concentrations in *R. clavigera*. Two sets of pairwise reciprocal transplantation of *R. clavigera* were conducted: one set for locations in eastern waters (i.e., between Clear Water Bay and Sai Kung Pier) and another set for locations in southern waters (i.e., between Po Toi Island and Sok Kwu Wan).

After collection, the rock shells were tagged by coloured bee tags with numbers (Opalithplättchen, Germany), and further marked by non-toxic, white correction fluid on both apices of the shells. The rock shells were then transplanted live to the corresponding paired site within 24 h. After six months, a comprehensive search in the open area of each transplantation site was conducted to recapture the tagged animals (Table S1

in Supplementary data (Appendix A)). Equal effort was made to search for the tagged rock shells and to collect up to 30 individuals randomly during each visit (4.5 man-hours; usually 1.5 h by 3 persons). During the sampling (both before and after transplantation), another 30 local (i.e., non-transplanted) adult rock shells were randomly collected in each site for imposex and OT analyses. Identification of the tagged rock shells was based on the white correction fluid marking and/or the coloured tag. The individuals were immediately transferred back to the laboratory where they were analysed for imposex status and tissue OT concentrations.

### 2.2. Imposex determination

Shell length of *R. clavigera* was measured by vernier callipers (to the nearest 0.1 mm) and its wet weight was measured after blotted dry with tissue paper by an electronic balance (to the nearest 0.001 g; Libror EB-430HU, Shimadzu, Japan). The individuals were then de-shelled with a bench vice and the soft tissues were taken out for dissection under a stereomicroscope (Olympus SZH10, Japan). The animals were sexed (Cheung et al., 2010), and the penis length of each male or imposex-female was quantified (to the nearest 1 mm). Levels of imposex development were assessed by Vas Deferens Sequence Index (VDSI) and Relative Penis Size Index (RPSI) (Gibbs et al., 1987, 1991; Cheung et al., 2010).

### 2.3. Tissue OT concentration

OTs were analysed based on the method described by Guðmundsdóttir et al. (2011). Quantification of mono-, di-, and tri-butyltin (MBT, DBT and TBT) and mono-, di-, and tri-phenyltin (MPT, DPT and TPT) was performed using a gas chromatograph (GC; Bruker 450-GC, Bruker Inc., Billerica, MA, USA) equipped with a mass-selective detector (MS; Bruker 320-MS, Bruker Inc., Billerica, MA, USA), following the same settings as described in Ho and Leung (2014b). Limits of detection ranged from 0.2 to 1.5  $\mu\text{g kg}^{-1}$  dw for the six compounds. An average recovery rate of surrogate standard (tri-*n*-propyltin chloride) was 27.7% (range: 8.6%–74.6%). No correction was made for the recoveries of surrogate standard to the concentrations reported.

All standards were bought from Sigma-Aldrich (St. Louis, MO, USA) or Chiron (Trondheim, Norway). All solvents in HPLC Grade were purchased from Tedia (Fairfield, OH, USA).

### 2.4. Statistical analysis

Differences in shell length, tissue weight, male and female penises length, VDSI and tissue OT concentrations (i.e., MBT, DBT, TBT, MPT, DPT, TPT and total OTs) of the transplanted rock shells before and after the 6-month transplantation were determined by non-parametric Mann-Whitney tests. Significant changes of VDSI, tissue total OTs concentrations, male and female penises length of local (i.e., non-transplanted) rock shells before and after the experimental period were also inferred by Mann-Whitney tests. All statistical tests were conducted in IBM SPSS Statistics 20.0 (SPSS Inc., Chicago, IL, USA, 2011). Significance levels were set as  $P \leq 0.05$ .

## 3. Results

### 3.1. Retrieval rate and growth

Of the 1093 transplanted *R. clavigera* individuals, only 82 of them were retrieved across the four sites after six months (Table S1), which was fewer than the expectation that 30 individuals could be retrieved in each site (sum = 120). In particular,

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