Marine Pollution Bulletin xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

# Population dynamics of the rock shell *Reishia clavigera* associated with different degrees of organotin contamination in Hong Kong

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ARTICLE INFO

*Article history:*
Received 25 August 2016
Received in revised form 7 February 2017
Accepted 14 February 2017
Available online xxxx

*Keywords:*
Biomonitoring
Recovery
Recruitment
ELEFAN
Gastropod
*Thais clavigera*

ABSTRACT

Application of organotins in antifouling systems on ship hulls has been globally prohibited by the International Maritime Organization since September 2008. It is, therefore, anticipated that there is a recovery of imposex-affected gastropod populations worldwide. We studied the population dynamics of the rock shell *Reishia clavigera* in six locations around Hong Kong's coastal waters, covering different degrees of organotin contamination for 25 months (2011 − 2013). Abundance and density of *R. clavigera* were higher in clean sites, while they tended to grow faster in polluted sites. Over time, we observed recruitments in clean sites while recruitments in polluted sites were limited. The results suggested that the lack of apparent recovery of the local *R. clavigera* populations was probably due to the prevalence of organotin contamination, especially triphenyltin, in this region. This study, therefore, calls for mitigation and long-term monitoring of organotin contamination in marine environments of Hong Kong and South China.



## 1. Introduction

Superimposition of male sexual organs (i.e., penis and vas deferens) on female gastropods was first reported by Blaber (1970) who discovered that a penis-like outgrowth appeared behind the right cephalic tentacle of a female dogwhelk *Nucella lapillus*, occupying a similar position of male's penis. Such a phenomenon was later termed as "imposex" (Smith, 1971) and was found to be associated with endocrine disrupting organotin compounds (OTs), such as tributyltin (TBT) and triphenyltin (TPT), which had been widely used as antifouling agents and pesticides since the 1960s (Hoch, 2001). The OT-induced development of vas deferens in female gastropods can eventually block the oviduct through which females release their egg capsules, causing sterilization of the females in severe cases (Gibbs et al., 1987). Hence, such an abnormality within individuals can have population-level effects; for example, it can cause a change of the functional sex ratio through a partial or complete loss of fertile females. These changes ultimately lead to population declines, as noted for wild populations of *N. lapillus* in England (Bryan et al., 1986; Gibbs and Bryan, 1986; Spence et al., 1990a), and further alter the shore community (USEPA, The United States Environmental Protection Agency, 2003).

Management actions of OT pollution in the marine environment first started in the late 1980s when most European countries prohibited the use of OT-based antifouling paints on vessels <25 m in length (Sonak et al., 2009). Hong Kong implemented a similar restriction in 1992 (Leung et al., 2006). A global complete ban on the application of OTs in antifouling systems on ship hulls was ultimately implemented by the International Maritime Organization (IMO) in September 2008 (Sonak et al., 2009). In view of these measures, OT contamination should, in theory, be alleviated and the gastropod populations should be able to recover worldwide.

This anticipated recovery has been documented worldwide since the ban of application of OT-based antifouling paints, in particular in Europe (e.g., Evans et al., 1994; Birchenough et al., 2002; Guðmundsdóttir et al., 2011). Recently, Morton (2009) demonstrated a clear and progressive recovery of a population of *N. lapillus* on the south coast of the UK, which was attributed to the complete ban on OTs in antifouling systems since 2003. However, in Hong Kong, levels of OTs remained high in marine water and freshwater (Kueh and Lam, 2008; Ho et al., 2016), and also in marine biota (Nakayama et al., 2009; Ho and Leung, 2014a).

The rock shell *Reishia clavigera* (previously known as *Thais clavigera*; see Claremont et al., 2013) commonly inhabits sheltered rocky and boulder shores in Hong Kong (Morton and Morton, 1983) and it has been widely used as a biomonitor of OT contamination in the Asia-Pacific region since the 1990s (e.g., Mak, 1992; Horiguchi et al., 1995; Leung et al., 2006; Tang et al., 2008). While most studies in this region investigated the induction of imposex on individuals (e.g., Shim et al., 2000; Leung et al., 2006; Qiu et al., 2011), little is known regarding the effects of OT contamination on this species at population level. This study,

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http://dx.doi.org/10.1016/j.marpolbul.2017.02.032

therefore, aimed to investigate the population dynamics of *R. clavigera* in six selected locations along a gradient of OT contamination in Hong Kong's coastal marine waters. The population structure, recruitment pattern, growth and mortality were investigated through an extensive field-based study. It was hypothesized that (1) there would be more individuals and more recruitments of *R. clavigera* in sites with less OT contamination than in those with higher OT contamination; and (2) such improvements should be observed in all sites over time as a result of the global ban on application of OT-based antifouling paints on ship hulls since September 2008, assuming that this international convention is an effective means for controlling the release of these compounds.

## 2. Materials and methods

### 2.1. Preliminary survey

During June to December 2011, at least one initial visit was made to each of the five selected rocky shores around Hong Kong waters during low tide (Table S1 in Appendix A). These sites were chosen based on the results of previous studies (i.e., Leung et al., 2006; Ho et al., 2016), covering different degrees of OT contamination based on imposex levels across the western, southern and eastern waters of Hong Kong (Table 1). Two heavily polluted sites (Sok Kwu Wan and Kadoorie Beach), two moderately polluted sites (Turtle Cove and Deep Water Bay) and a relatively clean site (Clear Water Bay) were selected. At each site, a 20-m stretch of rocky shore was examined to determine the abundance and density of *R. clavigera*. The number of *R. clavigera* was counted using 20–25 randomly placed double-strung quadrats (length × width = 50 cm × 50 cm) at two tidal heights (0.75 m and 1.25 m above the chart datum, C.D., respectively) at which *R. clavigera* were abundant (Tong, 1986; personal observation).

### 2.2. Bimonthly survey

Bimonthly surveys were conducted over a 25-month period (January 2011 to January 2013) on the populations of *R. clavigera* at the five aforementioned sites in Hong Kong. All these sites were visited 12 times during the low tides over the survey period. Po Toi Island (another relatively clean site; see Table 1) was added as the sixth survey site starting from March 2012, thus it had only been visited six times by the end of the survey (Fig. 1). We selected six sites only due to limitations of manpower and logistics resources, as the field survey is highly labour-intensive. These six sites were chosen because (1) they are of similar habitat type, i.e., semi-exposed rocky or boulder shores; (2) they are of different degrees of OTs contamination, as suggested by previous studies (Leung et al., 2006; Ho et al., 2016); (3) *R. clavigera* is relatively abundant in those sites compared to other neighbouring field sites; and (4) the habitats are covered with similar food species for *R. clavigera*, mainly the rock oyster *Saccostrea cucullata* and the acorn barnacle *Amphibalanus amphitrite*. The survey period was long enough to cover the annual life cycle of *R. clavigera* (i.e., growth, reproduction and recruitment; see Tong, 1986). We used the same method as described in the preliminary survey, randomly placing 25 double-strung quadrats (length × width = 50 cm × 50 cm) along a 20-m transect at the two tidal heights (see Section 2.1). Thus, the total number of quadrats at each site was 50 (i.e., 25 quadrats × 2 tidal heights). The shell length (i.e., the distance between the apex and the tip of anterior siphonal canal) of each individual in a quadrat was measured by vernier calipers (to the nearest 0.1 mm; SPI 31-415-3, Swiss Precision Instruments, USA). All measurements were done *in situ* and *R. clavigera* were returned to their original position after measurement in order to avoid disturbance to the population and allow repeatable observations during the survey period (Tong, 1986; Chow, 2004; Morton, 2009). Incidences of reproductive aggregation, sheltering and appearances of egg capsules (including the colour and size) were also recorded.

### 2.3. Data analysis

To illustrate the population structure, shell length data from the two tidal heights were combined for each visit. The relative abundance of *R. clavigera* was plotted against their shell length (grouped into 1 mm size-classes) to generate bimonthly size-frequency histograms for each site. Relative abundance, instead of actual abundance, was used because it helped reduce bias from the fluctuations in the number of samples over the survey period (Chow, 2004).

To identify individual cohort and estimate the number of cohorts, the shell length data were analysed using the Bhattacharya's method of Electronic Length Frequency Analysis (ELEFAN; International Center for Living Aquatic Resources Management, the Philippines) implemented in FiSAT (The FAO-ICLARM Stock Assessment Tools; Version 1.2.2). Separation of cohorts was further confirmed by the separation index (SI). If SI is ≥2, the two neighbouring cohorts are properly resolved (Gayanilo et al., 2005). The mean and standard deviation of shell length, and the number of individuals in a cohort were obtained. Separation of cohorts was conducted by eye-fitting if ELEFAN failed to identify the cohorts.

The growth rate of an individual cohort of *R. clavigera* was estimated by the linear regression between shell length and time, and expressed as increase of shell length (in mm) per month. Using different cohorts as replicates, average growth rates among different sites were compared by one-way analysis of variance (ANOVA) test. The growth performance of *R. clavigera* was also estimated by the von Bertalanffy Growth Function (VBGF) parameters such as VBGF growth constant ($K$) and asymptotic length ($L_\infty$). Recruitment pattern was revealed by reconstructing the recruitment pulses from a time series of length-frequency data in ELEFAN. Mortality of a cohort was also documented by its disappearance on the length-frequency histogram.

Month-to-month comparisons of the number of *R. clavigera*, mean shell length, proportion of juveniles and number of cohorts in each site (except in Po Toi Island since the survey lasted for a year only) between 2011 and 2012 were conducted using Wilcoxon signed-rank test. Differences of the shell length (average, minimum and maximum), density, number of cohort, growth rate of *R. clavigera* were tested among sites using one-way ANOVA (if the datasets had homogeneous variances) followed by Tukey HSD test, or Kruskal-Wallis test (if the datasets had heterogeneous variances) followed by Dunn's test. The homogeneity of variances was tested using Levene's test.

**Table 1**
Imposex indices (VDSI = Vas Deferens Sequence Index and RPSI = Relative Penis Size Index) and tissue concentration of total organotins (OTs = sum of mono-, di- and tri-butyltin, and mono-, di- and tri-phenyltin) in *Reishia clavigera* collected in the six selected sites in 2010. Data were extracted from Ho et al. (2016).

| Pollution level | Site | VDSI | RPSI | Tissue concentration of total OTs (μg kg$^{-1}$ dry weight) |
|---|---|---|---|---|
| Polluted | Kadoorie Beach | 4.00 | 21.21 | 2905.4 |
| | Sok Kwu Wan | 5.73 | 37.30 | 7529.4 |
| Moderately polluted | Deep Water Bay | 3.45 | 7.10 | 1395.6 |
| | Turtle Cove | 3.30 | 3.96 | 3220.9 |
| Relatively clean | Clear Water Bay | 2.81 | 1.71 | 521.4 |
| | Po Toi Island | 2.76 | 2.19 | 1647.7 |