



## TBT pollution and effects in molluscs at US Virgin Islands, Caribbean Sea

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### ABSTRACT

An almost ubiquitous occurrence of imposex and butyltins in the molluscs from US Virgin Islands gives evidence to a widespread contamination with the antifouling agent tributyltin (TBT), which most likely is related to a relatively intense ship traffic. Three different muricid neogastropod species *Thais deltoidea*, *Thais rustica* and *Purpura patula* all seem to have potential as suitable and sensitive bioindicators for assessing levels and effects of TBT pollution in coastal areas including coral reefs in the Caribbean Sea. However, considerable interspecies differences in especially accumulation potential of butyltins were seen in this study. Furthermore, a high accumulation potential of TBT in the edible gastropod West Indian topshell (*Cittarium pica*) was found, despite that no signs of imposex were observed in this archaeogastropod species.

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### 1. Introduction

The coastal waters in the Caribbean Sea have relatively high densities of marine traffic. The region is an increasingly popular cruise ship destination with 14.5 million passengers visiting Caribbean ports in 2000, which is a 47% increase compared to 1995 (UNEP, 2004). In addition, larger pleasure boats, commercial cargo and oil transport add to the amount of ship traffic. This makes the region vulnerable to pollutants related to these activities, for example toxic antifouling agents used on ship hulls. However, research and monitoring of distribution, fate and ecotoxicology of anthropogenic contaminants have focused almost exclusively on countries and ecosystems in temperate zones of the world. Subsequently, tropical ecosystems including the Caribbean Sea, which combined include as much as 75% of the global biodiversity, have largely been neglected in this context (Lacher and Goldstein, 1997; Fernandez et al., 2007). Few studies have shown that antifouling agents, like tributyltin (TBT) and newer booster biocides like Irgarol 1051, can pose a significant threat to sensitive tropical ecosystems like coral reefs (Smith et al., 2003; Owen et al., 2002; Negri et al., 2002). Some studies suggest that TBT pollution has the potential to cause major mortality of resident coral communities and might also have a negative impact on the recruitment and recovery of adult populations (Owen et al., 2002; Negri et al., 2002; Smith et al., 2003). Furthermore, findings of significant contamination of the North-eastern part of the Caribbean Sea by Irgarol 1051 (Carbery et al., 2006) underscore the importance of increasing the understanding of local and regional exposure of tropical reef and sea

grass habitats to the antifouling agents in general. A ban of TBT has been enforced in most part of the world for pleasure boats (<25 m). Moreover, larger ships including the commercial shipping have in recent years begun to phase out TBT in line with the International Maritime Organization (IMO) antifouling convention, which was finally ratified in late 2007 and will enter into force by September 2008 (IMO, 2008).

TBT pollution in the marine environment has been related specifically to the development of imposex, i.e. a masculinisation of prosobranch gastropod females by the development of a vas deferens and/or a penis in addition to their female sex characteristics. Imposex can be induced by TBT at ambient concentration as low as 1 ng/l (Gibbs et al., 1987), although high interspecies differences can occur in the sensitivity and potential to develop imposex (Stroben et al., 1995). The mechanism by which TBT induces imposex in marine snails is not completely elucidated, although TBT causes elevated levels of androgens, like testosterone, in female snails. One of the potential mechanisms is inhibition of aromatase, which converts androgens to estrogens (Oehlmann et al., 2007). Recent studies suggest that retinoid X receptor (RXR) ligand binding can also be important for the mechanism of TBT induced imposex (Castro et al., 2007).

Imposex has been described in more than 240 gastropod species almost worldwide (Strand, unpubl.) and has been recognised as a valuable biomarker for baseline surveys and monitoring of biological effects of contaminants in the environment. However, despite recommendations, for instance given by Linton and Warner (2003), to use imposex as a bioindicator for TBT effects in integrated monitoring and management of Caribbean coastal zones, only a few studies investigating TBT levels or effects in Caribbean gastropods have previously been conducted in the region, i.e. only in some coastal areas of Columbia and

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Venezuela (Cantillo et al., 1999; Hernandez and Stotz, 2004; Miloslavich et al., 2007). To our knowledge, no previous studies have been conducted on the Caribbean Islands. In addition, marine gastropods, like queen conch (*Strombus gigas*) and West Indian topshell (*Cittarium pica*), are important marine resources for human consumption at the Caribbean Islands (Robertson, 2003). Subsequently, it is important to provide information on the effects of TBT exposure and accumulation in marine gastropods in the Caribbean region with regard to management and maintaining sustainable populations.

In this study, the TBT concentrations and the incidence of imposex in three sensitive muricid neogastropod species *Purpura patula*, *Thais rustica* and *Thais deltoidea* are investigated, and TBT levels in the edible *C. pica* and the bivalve *Isoognomon alatus* from US Virgin Islands are also included. The study was carried out as a part of the world-wide Danish research expedition during 2006 and 2007, Galathea3, which was established both as a platform for several scientific research projects and as an on-going platform for education and dissemination of research and natural sciences to the public in Denmark (<http://www.galathea3.dk/uk>).

## 2. Materials and methods

### 2.1. Sampling and storage

The gastropods and bivalves were sampled during the visit of the Galathea3 expedition at the US Virgin Islands St. Thomas, St. Croix and St. John, March 15–25, 2007. The molluscs were collected manually or by snorkeling at 11 sites along the coastlines from depths of 0–5 m, both in the close vicinities of harbours and marinas and at so-called coastal reference sites located a few kilometres away from harbours and marinas (Fig. 1, Table 1). The sampled mollusc species included the muricid neogastropods *P. patula* (widemouth rocksnail), *T. deltoidea* (deltoid rocksnail) and *T. rustica* (rustic rocksnail), the archaeogastropoda *C. pica* (West Indian topshell) and the bivalve *I. alatus* (flat tree oyster). *P. patula* and *C. pica* were collected on rocky shores in the tidal zone, whereas *T. deltoidea* and *T. rustica* were mainly collected in sublittoral coral reef habitats. *I. alatus* was collected from hard substrate structures or mangrove roots. After sampling the gastropods were frozen at  $-20^{\circ}\text{C}$ , however, the bivalves were first allowed to depurate for 12–24 h before they were dissected and frozen. All biological examinations for imposex and organotin analyses were conducted in laboratories, after the Galathea3 expedition had returned to Denmark, April 2007.

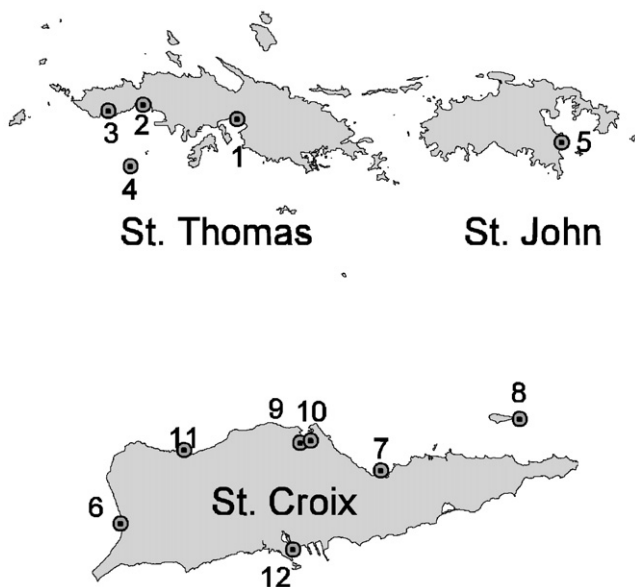


Fig. 1. Locations of sampling sites at the US Virgin Islands St. Thomas, St. Croix and St. John in the Caribbean Sea, March 2007.

Table 1

Sampling site information for <sup>CR</sup>coastal reference sites and <sup>H</sup>harbours at US Virgin Islands, March 2007.

Sampling site	Date	Depth	Position	Species
St. Thomas				
1 Charlotte Amalie Bay <sup>H</sup>	15–20/3–07	0–1 m	64.92°6W; 18.34°0N	<i>P. patula</i> , <i>T. deltoidea</i> , <i>C. pica</i>
2 Perverserance Bay <sup>CR</sup>	20/3–07	0 m	64.99°2W; 18.35°0N	<i>P. patula</i>
3 Fortuna Bay <sup>CR</sup>	17/3–07	0–5 m	65.01°6W; 18.34°5N	<i>P. patula</i> , <i>T. deltoidea</i> , <i>T. rustica</i>
4 Saba Island <sup>CR</sup>	19/3–07	0–3 m	65.00°0W; 18.30°7N	<i>T. deltoidea</i> , <i>T. rustica</i> , <i>C. pica</i>
St. John				
5 Coral Bay <sup>CR</sup>	18/3–07	0–2 m	64.70°0W; 18.32°4N	<i>T. deltoidea</i>
St. Croix				
6 Frederiksted pier <sup>H</sup>	23/3–07	0–2 m	64.88°4W; 17.71°4N	<i>T. deltoidea</i> , <i>T. rustica</i>
7 Christiansted Bay <sup>H</sup>	22/3–07	0 m	64.70°3W; 17.75°1N	<i>P. patula</i>
8 Buck Island Reef <sup>CR</sup>	24/3–07	0–1 m	64.60°6W; 17.78°6N	<i>T. rustica</i>
9 Salt River Bay marina <sup>H</sup>	25/3–07	0–1 m	64.75°9W; 17.70°0N	<i>I. alatus</i>
10 Salt River Bay mangrove <sup>CR</sup>	25/3–07	0–1 m	64.75°2W; 17.77°2N	<i>I. alatus</i>
11 Annaly Bay <sup>CR</sup>	26/3–07	0 m	64.84°0W; 17.76°5N	<i>P. patula</i>
12 St. Croix south coast <sup>CR</sup>	27/3–07	0–1 m	64.76°4W; 17.69°6N	<i>I. alatus</i>

### 2.2. Imposex examination

After species identification and measurement of shell height, the shells were gently cracked with a pair of pliers. The gastropods were sexed based on a well-developed pallial oviduct including coloured sperm-ingesting gland and/or a strait oviduct connecting the ovary with the pallial oviduct in females and a curled seminal vesicle in males. Afterwards the mantle cavity was cut open. The severity of imposex development was characterised by using a dissection microscope according to the stages 0–6 and the variants a, b and c in the scheme for the vas deferens sequence index (VDSI) (Fioroni et al., 1991; Stroben et al., 1992; Shi et al., 2005). This VDSI scheme has the

Table 2

Organotin levels (ng Sn/g dw) in marine gastropods and bivalves from <sup>CR</sup>coastal reference sites and <sup>H</sup>harbours at US Virgin Islands.

	Total number	Number of females	Frequency of imposex	VDSI <sup>a</sup>
<i>Purpura patula</i>				
1 Charlotte Amalie Bay <sup>H</sup>	10	4	(100%) <sup>b</sup>	(2.3) <sup>b</sup>
2 Perverserance Bay <sup>CR</sup>	42	15	7%	0.07
3 Fortuna Bay <sup>CR</sup>	9	5	(20%) <sup>b</sup>	(0.2) <sup>b</sup>
7 Christiansted Bay <sup>H</sup>	30	13	92%	2.7
11 Annaly Bay <sup>CR</sup>	35	15	20%	0.3
<i>Thais rustica</i>				
3 Fortuna Bay <sup>CR</sup>	40	26	19%	0.2
4 Saba Island <sup>CR</sup>	41	23	4%	0.04
6 Frederiksted pier <sup>H</sup>	42	21	71%	1.5
8 Buck Island Reef <sup>CR</sup>	38	22	0%	0.00
<i>Thais deltoidea</i>				
1 Charlotte Amalie Bay <sup>H</sup>	9	4	(100%) <sup>b</sup>	(5.8) <sup>b</sup>
3 Fortuna Bay <sup>CR</sup>	33	16	7%	0.4
4 Saba Island <sup>CR</sup>	41	20	75%	1.4
5 Coral Bay <sup>CR</sup>	33	21	57%	0.8
6 Frederiksted pier <sup>H</sup>	19	4	(75%) <sup>b</sup>	(2.0) <sup>b</sup>

<sup>a</sup> VDSI (vas deferens sequence index) according to Stroben et al. (1992).

<sup>b</sup> Data for frequencies of imposex and VDSI are listed in brackets, because of the limited number ( $n < 10$ ) of females examined.

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