Imposex in the intertidal snail *Thais brevidentata* (Gastropoda: Muricidae) from the Pacific coast of Costa Rica

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Abstract: The presence of imposex (female snails with male accessory organs) was studied in Costa Rica in the snail *Thais brevidentata*. Imposex is induced by organotins found in anti-foulant paints, especially tributyltin (TBT). *T. brevidentata* is commonly found in high densities in the mid-littoral zone of rocky shores. Snails from three Pacific coast sites in Costa Rica were collected for analyses. Two of the sites were located in the same general area in one of the main ports in Costa Rica, Caldera Port. A third site in Culebra Bay is a pristine area in the northern part of Costa Rica with little shipping traffic, and served as the reference site. Imposex was found only in Caldera females (28-30 %), and no imposex was found in snails from Culebra Bay. This is the first report of imposex in Costa Rica, and the first report of imposex in *T. brevidentata*. Although 30 % imposex was found in the Port area, this is relatively low compared to some harbors in the United States of America and Europe, where 100 % imposex has been reported in other gastropod species. Rev. Biol. Trop. 54 (Suppl. 1): 21-26. Epub 2006 Sept. 30.

Key words: imposex, organotins, bioindicator, TBT, coastal pollution, Gastropoda, *Thais brevidentata*, Costa Rica.

Since its discovery in the 1960's tributyltin (TBT) has been used in marine applications for its effective antifouling properties. However, its detrimental effects on non-target gastropods, namely the induction of imposex, have generated considerable public concern (Oberdörster and Cheek 2000). Imposex is defined as the imposition of male sexual characteristics on female gastropods, where normally gonochoristic female snails will grow both a penis and vas deferens (normally male accessory sex organs). Although the exact mechanism of imposex induction is still unknown, via peptide hormones that mimic a Penis Morphogenic Factor (Oberdörster and McClellan-Green 2000, Oberdörster *et al.* 2005); or via alteration of testosterone metabolism (Gooding *et al.* 2003, LeBlanc *et al.* 2005), imposex has been long used as a bioindicator of organotin pollution (Wilson *et al.* 1993, Axiak *et al.* 1995, Stroben *et al.* 1995, Tester and Ellis 1995, Tester *et al.* 1996, Pessoa *et al.* 2001, Sousa *et al.* 2005). Due to concerns about ecosystem and mariculture health, governments around the world have passed legislation restricting the use or organotins. In 2000, the International Maritime Organization (IMO) recommended a global ban on all organotin compounds including TBT, and the prohibition of new marine applications by 2003 (Champ 2000). Given that over one hundred fifty species of gastropods are affected by TBT (deFur *et al.* 1999), and since imposex has been found in both North and South America (Oberdörster *et al.* 1998, Gooding *et al.* 1999,

Evans *et al.* 2001, Gooding and LeBlanc 2001, Penchaszadeh *et al.* 2001, Fernandez *et al.* 2002), we hypothesized that imposex may be present in Costa Rican waters as well in areas that have heavy shipping and therefore the potential for organotin pollution.

The Pacific marine gastropod *Thais brevidentata* (Wood, 1828) had it genus changed from *Acanthina* and is reported for the Panamic Province (Skoglund 1992). This species is known as the short-toothed thorn drupe and is a common snail found in the mid-littoral zone of rocky shores in the Pacific coast of Costa Rica (Bakus 1968, Sibaja- Cordero and Vargas-Zamora 2006). This species has been extensively studied in terms of population dynamics in Culebra Bay (Spight 1978), and the life-history and feeding strategies have also been studied, such as in the Gulf of Nicoya estuary where *T. brevidentata* is known to forage at low tides during cloudy days and in the late afternoon while the substrate is damp. In addition, this species is an important food item for fish at high tide (Ortega 1986). This species appears in great abundance in certain regions along the Pacific coast from Mazatlán, Mexico to Paita, Peru, and is distributed in the intertidal zone of coastal areas where few other species are found along with *T. brevidentata* (Sibaja-Cordero and Vargas-Zamora 2006). Only very few reports of imposex have been made from Latin America (Gooding *et al.* 1999, Penchaszadeh *et al.* 2001, Fernández *et al.* 2002), mainly due to lack of resources and trained personnel to detect this phenomenon. Because of the widespread abundance, well-studied life-history, and ecological importance, *T. brevidentata* was selected as an appropriate species for biomonitoring of imposex levels. The purpose of this study was to identify a common gastropod species that is relatively small, abundant, easy to collect, and could serve as a biomonitor for organotin pollution in Costa Rica.

MATERIALS AND METHODS

Study sites: Two sampling sites were selected in the Gulf of Nicoya estuary, and one in Culebra Bay, all on the Pacific coast of Costa Rica (Fig. 1A). Sites 1 and 2 were both in Caldera Port, which experiences heavy ship traffic. Caldera Port can have, at times, more than 20 cargo, fishing, and cruise vessels anchored approximately 500 m from either site 1 or site 2. Site 1 is located on a beach inside the harbor, while site 2 is located approximately 1 km away from site 1, on the same beach but separated by a channel. Site 1 is located approx 600 m from the main dock of the Port of Caldera and site 2 is located at approx 300 m from the same dock, on the other side**.** The Caldera port is located in the mid upper Gulf of Nicoya. Coastal development has increased significantly in this estuary over the past decades (Vargas 1995). Site 3 is in northern Costa Rica near Panama Beach, in Culebra Bay within the Gulf of Papagayo. Culebra Bay includes many beaches with several hotel complexes being built in recent years. Marine activities include diving and leisure boating, but no main ports are in the vicinity and the whole bay is still relatively pristine (Jiménez 2001a). Therefore, this area served as a reference site even though there are some slight differences between Culebra Bay and the Gulf of Nicoya, both of which remain within the range of living conditions for *T. brevidentata*. Culebra Bay has more oceanic conditions than the Gulf estuary, and the Bay includes areas with significant coral growth (Jiménez 2001b).

Collection of snails: At each site, 100 to 150 specimens of *T. brevidentata* (Fig. 1B, C) were randomly sampled by hand at low tide and transported to the laboratory in plastic bags containing seawater from each site. Once in the laboratory, the specimens were transferred to three 12-quart plastic buckets filled with seawater and aerated. Clean seawater was exchanged as necessary until the animals were analyzed for imposex (not more than five days hold time). Before determining imposex, each specimen was relaxed on ice for 15 min. Once relaxed, shell length and width were measured using a vernier caliper. Some shells from sandier areas had more severe erosion of

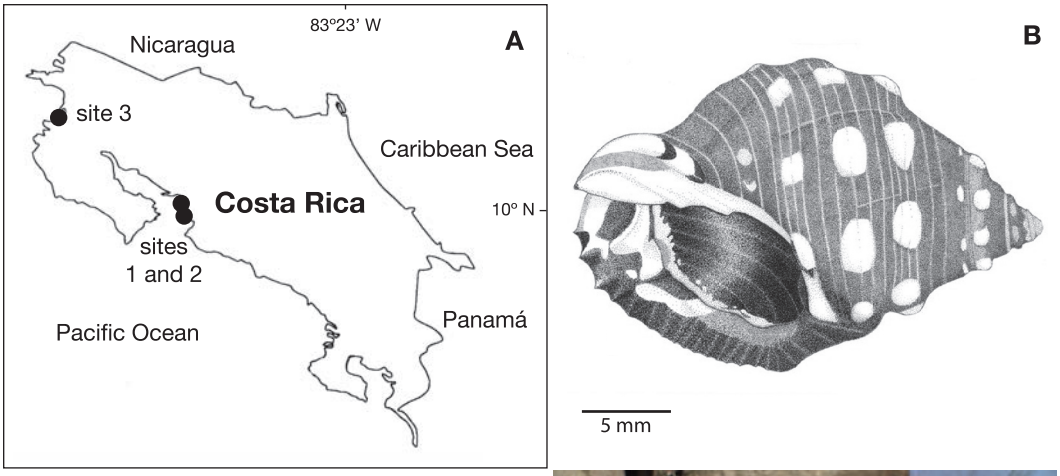

Fig. 1 A. Map of Costa Rica with sampling locations indicated by dots. Sites 1 and 2 are approximately 1 km apart in the Port of Caldera (09º55' N - 84º43' W), while site 3 (Culebra Bay) is in northern Costa Rica (10º35' N - 85º40' W). B. Shell of the gastropod *Thais brevidendata* drawn from a live specimen from the port of Caldera. C. Live specimens of *T. brevidentata* from the port of Caldera.

the shell lengthwise (eg, the tips were eroded away), and therefore width is a more accurate measure of animal size in this case. After removing the soft parts from the shell after gently cracking them open, sex was determined based on the presence or absence of an egg capsule gland and ovaries for females, and testis for males. Penis length was measured for males and females exhibiting imposex, although oftentimes imposex females had only a small bud. This is similar to what has been seen in many other species (Fioroni *et al.* 1991, Stroben *et al.* 1992a, b, Schulte Oehlmann *et al.* 1995, Stroben *et al.* 1995, Oehlmann *et al.* 1998), and which has been carefully characterized into two different indexes by Huet *et al.* (1995). These indexes are the Relative Penis Size Index (RPSI) and the Vas Deferens Sequence Index (VDSI), and although there is variation in the exact location and sizes of the penis length and vas deferens, the overall idea of indexing these to body size has been widely adopted for biomonitoring. The paper by Huet *et al.* (1995) has an excellent generalized scheme that can be adapted for many species.

Percent imposex differences were assessed by χ^2-test using the reference site as the expected % imposex. Shell width and normalized penis lengths were analyzed using ANOVA followed by a post-hoc Tukey test using SYSTAT v. 8.0 software. Normalized penis length data was not normally distributed, and therefore data was log transformed (Zar 1984) using the equation $X_{norm} = \log (X+1)$.

RESULTS

Comparable incidences of imposex were found at site 1 and site 2 of Caldera Port, 30 % and 28 % respectively (Table 1), with penis lengths from a small bud to 1.5 mm in length. Imposex was not discovered in Culebra Bay. Males had similar penis lengths between the sites, although the Culebra Bay males and females were smaller overall (Table 1). Although data is presented for absolute male penis lengths, the more relevant measure is normalized penis lengths, where (mm penis length/mm shell width)= normalized penis length, which is similar to the RPSI developed

by Huet *et al.* (1995). When normalizing penis length for size, males from Culebra Bay had significantly longer penises/mm shell width than males from Caldera Port.

DISCUSSION

Imposex were discovered in the Caldera Port area but not at the reference site. This port is one of the largest ports in Costa Rica, and experiences heavy shipping traffic. Within the port, the two sites studied differed in current and sedimentation rates since they were approximately 1 km apart and separated by a deep water channel and dock. However, the incidence of imposex between the two sites was similar. The differences in shell size between the reference site and the Port is similar to what has been found near Beaufort, NC, where mud snails from the Port areas tend to be larger than snails from cleaner sites (pers. obs.). It is hypothesized that port areas tend to have more runoff from sewage, and that the nutrient loading increases food sources for the snails. To date no evaluation of tin has been conducted in the Gulf of Nicoya estuary. However, other trace metals have been analyzed from sediment samples and found at concentrations characteristic of non-industrialized estuaries (Dean *et al.* 1986, Fuller *et al.* 1990, García-Céspedes *et al.* 2004). However, imposex is a much more sensitive indicator of organotin contamination than can be easily done by Atomic Absorption (AA) chromatography since snails will often develop imposex in as low as 1 ng TBT/L (deFur *et al.* 1999), which is far below the detection limit of most AA's.

Imposex was not discovered at Panama Beach in Culebra Bay. This was expected due to the low level of shipping traffic and its relatively pristine environment. The absence of imposex in the Culebra Bay area is a useful bioindicator of a healthy ecosystem. In several locations in the USA and Europe, low imposex levels can be found at reference sites. For example, in Beaufort, NC (USA) mud snail imposex levels are usually around 5 % in the Rachel Carson Estuarine Reserve, which serves as a reference location for the Morehead City, NC (USA) Port (Oberdörster and McClellan-Green 2000). In Portugal, imposex in the snail *Nassarius reticulatus* ranged from 0.0 to 100 % in 23 sites (Sousa *et al.* 2005). Having no imposex in Culebra Bay, Costa Rica, is therefore an encouraging sign, given the current trend for continued ecotourism development.

The levels of imposex in the heavy shipping area of the Caldera Port are relatively low compared to areas in the USA. For example, in the Morehead City, NC Port imposex levels are near 100 % (Oberdörster and McClellan-Green 2000). Overall, the sites studied in Costa Rica have lower imposex levels than some working harbors in the USA (Oberdörster and McClellan-Green 2000). Imposex levels should

TABLE 1
Imposex status and male penis length of Thais brevidentata *snails from three sites in the Pacific coast of Costa Rica*

	# per site (n)	% Imposex	Female width (mm)	Male width (mm)	Male Penis Length (mm)
Caldera Port site 1	50 F 40 M	30	18.10 ± 1.92	16.81 ± 1.18	5.39 ± 1.00
Caldera Port site 2	50 F 36 M	28	17.95 ± 1.05	17.10 ± 1.18	6.40 ± 1.32
Culebra Bay site 3	50 F 16 M	0 [A]	15.44 ± 1.07 [A]	14.48 ± 1.05 [A]	6.03 ± 1.19 [B]

A significantly different from the other sites; $p<0.01$
B normalized penis lengths are different from the other sites, $p<0.001$

continue to be monitored, and, in the future, we recommend assessing incidence of imposex at other locations along the Pacific and Caribbean coasts of Costa Rica. TBT contamination is a major concern to the health of an aquatic environment as evidenced by the IMO proposed world-wide TBT ban. Although the sites chosen for this study show low levels of imposex, Costa Rica may need to develop a monitoring policy to continue to protect its coasts.

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RESUMEN

La presencia de imposexo (caracoles hembras con órganos sexuales masculinos accesorios) fue evaluada en Costa Rica en el caracol *Thais brevidentata*. El imposexo es inducido por organo-estaños utilizados en pinturas tipo anti-foulant, especialmente el tributilestaño (TBT). *T. brevidentata* es un gastrópodo marino abundante en la zona rocosa litoral media en tres sitos en la costa Pacífica de Costa Rica. Dos de los sitios están localizados en el área de influencia de uno de los principales puertos de Costa Rica, el puerto de Caldera. El tercer sitio, que sirvió de referencia, está en la Bahía Culebra en la costa norte de Costa Rica y es una bahía relativamente poco alterada y con poca actividad naviera. Se encontró caracoles hembra con imposexo (28-30 %) solamente en el área del puerto de Caldera. Éste es el primer informe de imposexo en Costa Rica y el primer informe de imposexo en *T. brevidentata*. No obstante, solo 30 % de caracoles hembra con imposexo fue encontrado en el área del puerto de Caldera, este porcentaje es relativamente bajo cuando se le compara con otros puertos en los Estados Unidos de América y Europa, donde se ha encontrado un 100 % de imposexo en otras especies de gastrópodos.

Palabras clave: imposexo, organo-estaño, bioindicador, TBT, contaminación costera, Gastropoda, *Thais brevidentata*, Costa Rica.

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