

IMPOSEX AND POPULATION CHARACTERISTICS OF *THAIS DISTINGUENDA* AS AN INDICATOR OF ORGANOTIN CONTAMINATION ALONG THE SOUTH EAST COAST OF PHUKET ISLAND, THAILAND

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ABSTRACT

Imposex, the development of a penis and/or vas deferens of female gastropods, has been linked to the presence of the biocide tributyltin (TBT) used in antifouling paints. The muricid *Thais distinguenda* was chosen as an indicator organism and imposex was examined at 21 stations as transects from different centres of boating activity off Phuket Island on the west coast of Thailand. Significant correlations (Spearman Rank) were obtained between distance from presumed source of TBT, and percentage of females with imposex and relative penis size index. Distance from source was positively correlated with density of *T. distinguenda*, but no significant correlations were found for sex ratio or size distribution of snails. The factor of correlation and probability varied with boating activity and the degree of exposure of each site to the open sea. Transplantation of *T. distinguenda* from a station without imposex, to a station with imposex, revealed an increase in imposex after 80 days. The areas with the highest incidence of imposex were considered moderately contaminated, but localised within 3.5 km from the presumed sources of TBT.

INTRODUCTION

Imposex, where male sex characteristics are imposed on female gastropods, was first observed by Blaber (1970) in *Nucella lapillus* (Linnaeus, 1758) and years later linked to the presence of organotin compounds used in antifouling paints for boat hulls (Smith 1981). Tributyltin (TBT) has been described as the most toxic compound ever deliberately introduced into the aquatic environment (Goldberg 1986). TBT has been linked causally to imposex after experiments exposing female *Nucella lapillus* to TBT showed increased testosterone levels (Spooner *et al.* 1991). It is established that imposex is a unique sensitive and specific response to organotin contamination in *Nucella lapillus* (Foale 1993).

Because of its abundant distribution and sensitivity to TBT, *Nucella lapillus* has been used in numerous temperate area studies (Bryan *et al.* 1986; Gibbs *et al.* 1987; Douglas *et al.* 1993). In subtropical and tropical areas several species belonging to the sub-order Neogastropoda have been chosen as

indicator species (Ellis & Pattisina 1990; Stewart *et al.* 1992; Wilson *et al.* 1993; Evans *et al.* 1995a).

Among the muricids, *Thais* spp. are often chosen in spite of taxonomic problems due to pronounced geographical variation, because they are sensitive to TBT, abundant, and easily collected on rocky shores (Spence *et al.* 1990). Ellis & Pattisina (1990) stated that TBT has been reported to affect at least 45 species of gastropods, and within a few years the number increased to 72 species (Evans *et al.* 1996). The number is probably higher, because only a fraction of the number of species of gastropods has been investigated for imposex.

In general, gastropods with imposex have been observed in areas with high boating activity (eg Smith & McVeagh 1991), mariculture facilities using antifouling paints (Davies & Bailey 1987) or shipyards (Stewart *et al.* 1992). The presence of imposex in the open ocean was first noticed by Ten Hallers-Tjabbes *et al.* (1994) in

*Buccinum undatum* (Linnaeus, 1758) along the deep sea routes in the North Sea. The effect of TBT has also been linked to shell malformation and reduced production of oysters in commercial farms in France and Great Britain (Dyrynda 1992). Based on this relation between degradation of oyster cultures and TBT a legislation to limit the use of TBT based antifouling paints to vessels of a minimum length of 25 meters was implemented in several countries during the eighties (Wilson *et al.* 1993; Evans *et al.* 1995a). The effect of this restriction has been decreasing levels of TBT measured in the water column, sediment and tissue of molluscs and several populations of *Nucella lapillus* have recovered (Evans *et al.* 1991, 1994).

In Thailand, however, and in most of the South East Asian region, the use of TBT is unregulated, so the marine environment continues to be exposed to TBT.

Only one investigation on imposex has been published from the Gulf of Thailand (Swennen *et al.* 1996). The present investigation is the first study using data from the Andaman Sea at Phuket Island.

The intentions of this investigation were to measure the level of imposex and population characteristics of *Thais distinguenda* (Röding, 1798) along the south east coast of Phuket and nearby islands, to establish the distribution of imposex, its level and its effect on the populations of *T. distinguenda*.

#### MATERIALS AND METHODS

After an initial survey along the south east coast of Phuket Island (7°44'-7°56' N, 98°20'-98°30' E) the muricid *T. distinguenda* was chosen as an indicator species, being the most abundant neogastropod along the rocky coastline.

Along the south east coast of Phuket three different sites of high boating activity were identified and used to investigate the differences in gradients of imposex. The first site chosen was the deep sea port of Phuket, which is the only port on the west coast of

Thailand where large cruise ships and oil tankers can go alongside the quay. The second site was Chalong Marina which is the largest marina on the west coast of Thailand. The third site was Phuket Harbour which is used by a large fleet of fishing boats and ferries. In addition several shipyards discharge their waste directly into the harbour.

Twenty-one stations including 8 islands were selected to represent gradients of contamination from these presumed sources of TBT. The remote islands were chosen as "controls" where TBT contamination was presumed to be absent (Fig. 1).

Specimens were collected at low tide, from February to April 1996.

A minimum of 50 individuals of *T. distinguenda* were collected at each station, except at station 15 in Chalong Bay where only 16 specimens were found. Samples were preserved in 70 % ethanol.

*T. distinguenda* is distributed mainly in a narrow band across the intertidal zone. A transect line was placed parallel with the shore and in the middle of the distribution of this species. The collections were made repeatedly along a transect line with marks for every meter to enable calculations of density and size distribution of snails. Twenty-one m<sup>2</sup> quadrates were randomly placed along the transect line to estimate the density, and all specimens were collected for determination of size distribution. Additional specimens to complete the sample size were collected haphazardly. Size distribution was determined by counting the number of snails in each of the following length intervals: 0-20; 20-25; 25-30; 30-35 and 35-40 mm. Sex ratio was calculated as the proportion of males in the sample.

The total length of each *T. distinguenda* was measured from the apex to the distal end of the siphonal canal to the nearest 0.5 mm using sliding callipers. The shells of the snails were crushed with a hammer, soft parts were carefully removed and placed in a Petri dish and examined using a binocu-

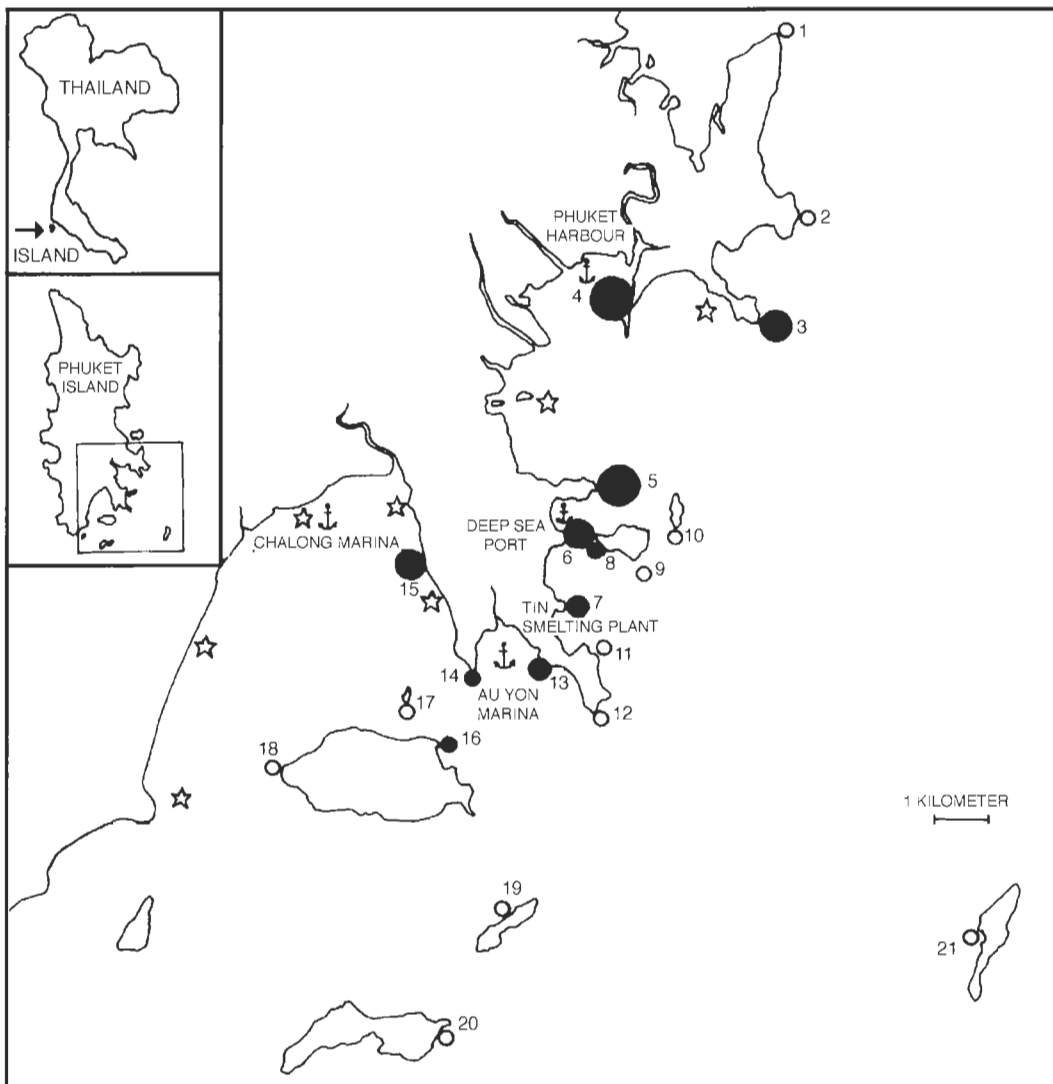


Figure 1. Sampling stations along the south east coast of Phuket Island: ☆ - *T. distinguenda* not found; ○ - no imposex recorded; ● - 1-25 % of the females had imposex; ● - 25-50 % had imposex; ● - 50-75 % had imposex; ● - more than 75 % had imposex.

lar microscope. Sex determination was done by identification of the sperm ingesting gland in females, a dark coloured area located between the capsule gland and the albumen gland. The capsule gland of the female is harder in texture and more pronounced than the prostate gland in the male. Because of different levels of maturation these glands can only be used as an aid in

sex determination. In most specimens ovaries could be separated from testis by colour. The ovaries are bright rust or orange in colour whereas the testis is dark green to brown. The contrast between colours of digestive gland and ovaries is more pronounced than between testis and digestive gland. In specimens from the heavily contaminated areas around the Deep Sea Port

Table 1. Name and number of sampling stations and corresponding imposex levels and densities of *T. distinguenda*.

Station no.	Station name	Sample size (number of snails)	Imposex frequency (% females)	Density $\pm$ St. Dev. (no./m <sup>2</sup> )
1	Laem Nga	58	0	2.4 $\pm$ 2.66
2	Laem Mai Phai	53	0	2.8 $\pm$ 2.53
3	Laem Phap Pha	51	57.4	2.9 $\pm$ 4.09
4	Laem Tukkae (port)	50	89.67	1.05 $\pm$ 4.47
5	Laem Nam Bor	50	84	3.25 $\pm$ 2.34
6	Deep Sea Port	88	65.79	2 $\pm$ 1.86
7	Tin smelting plant	54	34.38	0.8 $\pm$ 1.11
8	Koh Taphao Yai (West)	92	7.1	3.05 $\pm$ 3.33
9	Koh Taphao Yai (South)	95	0	6.45 $\pm$ 3.94
10	Koh Taphao Noi	50	0	6.2 $\pm$ 5
11	Hotel Cape Panwa	124	0	2.9 $\pm$ 3.14
12	Laem Panwa	111	0	5.0 $\pm$ 4.6
13	Laem Yam Yen	52	50	0.95 $\pm$ 1.23
14	Laem Wing	51	22.2	1.9 $\pm$ 1.86
15	Chalong Bay (East)	16	57	0.15 $\pm$ 0.49
16	Koh Lon (East)	52	5.56	2.15 $\pm$ 1.53
17	Koh Thanan	56	0	1.35 $\pm$ 1.23
18	Koh Lon (West)	58	0	3.45 $\pm$ 2.24
19	Koh Aew	50	0	4.9 $\pm$ 4.41
20	Koh Hey	50	0	4.9 $\pm$ 2.36
21	Koh Maiton	50	0	7.8 $\pm$ 4.31

the colour of the gonads was an unreliable tool for sex determination. In cases of doubt, a gonadal smear was examined under a compound microscope to identify sex of the individual.

The shape of the penis of *T. distinguenda* is curved, tapered and slightly flattened, ending in a thin threadlike structure, the penial flagellum, which is approximately one third the total length of the penis. The lengths of both male penis and female pseudo-penis were measured to the nearest 0.1 mm using an eyepiece ocular micrometer mounted on a binocular microscope. The total length of the penis was measured from the beginning of the penial flagellum to the base of the penis.

The following methods were used to measure imposex:

The imposex frequency was calculated as the proportion of females with imposex compared to the total number of females in the sample. A relative penis size index (RPSI) was calculated as  $(\text{mean length of female penes} \cdot 100) \cdot (\text{mean length of male penes})^{-1}$  (Stewart *et al.* 1992). The Relative Penis Size Index cubed (RPS<sup>3</sup>I) was calculated as  $((\text{mean length of female penes})^3 \cdot 100) \cdot$

$(\text{mean length of male penes})^{-3}$  (Bryan *et al.* 1986). The RPS<sup>3</sup>I value has been used extensively in imposex investigations especially on *Nucella lapillus*, because the length of the barrel shaped penis is related to the cube of its length (Stewart *et al.* 1992). RPSI was preferred in this investigation, both because *T. distinguenda* has a differently shaped penis, and because the RPS<sup>3</sup>I values were very low, when the differences in length between female and male penes were high.

After dissection all snails were dried for 24 hours at 80 °C and weighed to obtain dry weight.

#### Transplantation

The population at station 12 at Laem Panwa was considered healthy, because 111 adult *T. distinguenda* were investigated with no sign of imposex. Therefore 200 specimens were collected from this station on the 3 April and tagged by cutting a mark in the shell with a hacksaw. The marked snails were released at station 6 (Deep Sea Port) where the highest RPSI value occurred within a natural population of *T. distinguenda*. Isolated rocks surrounded by sand

were chosen as the site to minimise emigration. Fifty days later, 22 June, 32 marked *T. distinguenda* were recaptured. Eighty days later (23 July) another 60 snails were recaptured. The recaptured snails were preserved in 70 % alcohol, and examined for imposex in the laboratory.

### RESULTS

The highest RPSI value was found at the Deep Sea Port and the second highest RPSI value and the highest imposex frequency was found at Phuket Harbour (Tab. 1). The level of imposex at these two stations could be a combined effect, because the distance between the Deep Sea Port and Phuket Harbour is only 5 km. The RPSI value in between these two stations at Laem Nam Bor (station 5) was considerably lower. However, it had the second highest level of imposex. In Chalong Bay, 57 % of the females had imposex. Another contaminated source may be at Ao Yun Marina, because 50 % imposex was found at Laem Yam Yen (station 13) (Tab. 1).

Imposex was very localised around the three hotspots. At none of the stations was imposex found at a distance of more than 3.5 km from the presumed source of TBT.

Based on the distribution of imposex along the coast of Phuket Island (Fig. 1), Phuket Harbour, the Deep Sea Port and Chalong Marina were used as presumed sources of TBT. A Spearman Rank Correlation was determined between the distance from these hotspots to the nearest of each of the 21 stations and the following factors: sex ratio, density, imposex and RPSI (Tab. 2). Angular transformation was performed on percentage data before analysis (Sokal & Rohlf 1981).

The proportion of adults to juveniles was not significantly different between stations with imposex and stations without. The size distributions of snails from the pooled data from the four most remote stations and the four stations with high levels of imposex were not significantly different (t-test). The

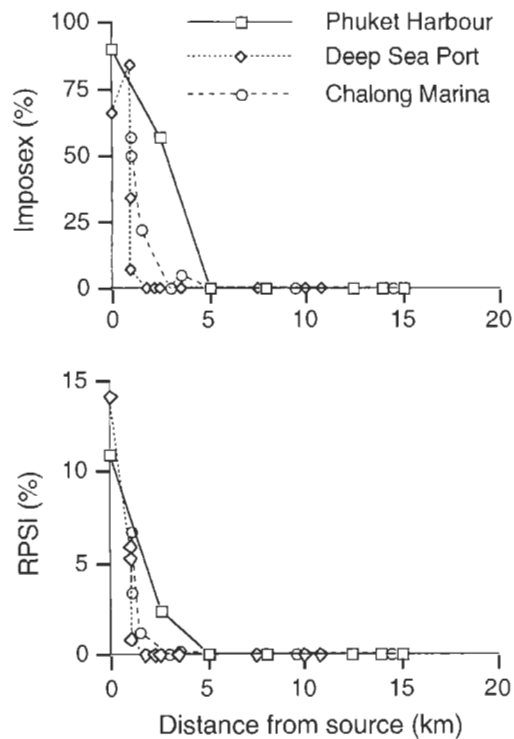


Figure 2. Gradients of imposex frequency and RPSI levels in relation to distance from three different types of boating activities.

average weight of all snails was divided by their average length to estimate their feed condition (weight per unit length). There was no significant difference in snails from populations with or without imposex.

The sex ratio of the transplanted population at the Deep Sea Port at recapture was 1:1, and a RPSI value of 0.3 was found in one snail out of 16 females 50 days after release. On the second sampling occasion, 80 days after release, 18.5 % had imposex and the RPSI value was 0.65.

### DISCUSSION

The presence of imposex in *T. distinguenda* was clearly localized around the three hotspots along the south east coast of Phuket Island, and the distribution was limited to a maximum of 3.5 km from these presumed sources (Fig. 1). Considerable differences in

Table 2. Spearman Rank Correlation between distance from each presumed source of TBT and imposex frequency, RPSI, density of indicator organism, and sex ratio (proportion of males). ns = not significant.

	Phuket Harbour		Deep Sea Port		Chalong Marina	
	Correlation	Probability	Correlation	Probability	Correlation	Probability
Imposex	-0.46	0.05	-0.57	0.009	-0.68	0.02
RPSI	-0.46	0.05	-0.59	0.007	-0.68	0.02
Density	0.71	0.09	0.625	0.05	0.97	0.006
Sex ratio	0.13	0.76 ns	-0.36	0.25 ns	-0.51	0.14 ns

the distribution of imposex were found between the three sites (Fig. 2). Phuket Harbour with several ship yards is located in a narrow channel with only one opening at station 4 with high levels of both imposex and RPSI, and fairly high levels 2.5 km away at station 3. But imposex was not present 5 km from the opening at station 2, probably because this station is exposed to the open sea. The semi diurnal tide of Phuket ranging from 2.15-2.27 m at spring tide to 0.85-1.15 m at neap tide measured at station 12 (Limpsaichol 1981) exchanges the water efficiently in the shallow bay between the Deep Sea Port and Phuket Harbour. At each falling tide station 3, 4 and 5 will be exposed to the water from this semi enclosed bay and the combined effect of TBT contamination from both Phuket Harbour and the Deep Sea Port. The gradient of imposex from the Deep Sea Port towards east, the open sea, declines rapidly to zero after only one km, despite the high levels of imposex and RPSI in the harbour. Imposex was not found 1.75-2.5 km south of the Deep Sea Port at station 9, 10 and 11, presumably because of the near presence of deeper water and the exposed location of the sites. The main deep water shipping lane to the Deep Sea Port passes station 11 and 12 within 0.5 km, but the strong current around the cape and rapid water exchange is the most likely reason why no imposex was found at these stations. The level of imposex at the tin smelting plant one km south of the Deep Sea Port was halved and not present 1.2 km further south of the tin smelting plant, indicating that the plant itself does not discharge organotin compounds with an effect similar to TBT.

The actual centre of Chalong Marina is diffuse because hundreds of yachts, big game boats and small ferries are moored within an area of one km<sup>2</sup>. The distribution of imposex from Chalong Marina reaches 3.5 km at station 14 and 16, with a possible source from Au Yon Marina. Au Yon Marina only harbours about 10 yachts, so the widespread distribution of imposex is probably caused by reduced water exchange of the semi-enclosed Chalong Bay.

The Spearman Rank Correlation between distance from presumed source and level of imposex and RPSI revealed a significant correlation for all three sites (Tab. 2).

A significant Spearman Rank Correlation between density of snails and distance from source were found for all sites, but the highest level of significance was found at Chalong Bay. Evans *et al.* (1996) found a significantly higher density of dog whelks at open coast-sites compared to semi-enclosed sites. *T. distinguenda* was more abundant on exposed stations with clear oceanic water compared to stations with muddy water close to mangrove areas, indicating that water quality in terms of amount of suspended solids may be the most dominant factor in the regulation of density.

In March 1995 water samples were measured for butyltin concentrations at the Deep Sea Port at Phuket Island and 90 ng l<sup>-1</sup> MBT, 50 ng l<sup>-1</sup> TBT and no detectable DBT was found (Kan-atireklap 1996). According to Dowson *et al.* (1993), sea water containing 20-100 ng l<sup>-1</sup> TBT is considered moderately contaminated. Phuket Harbour is the major site for shipyards on the island. TBT con-

taminated water from high pressure hosing of the boat hulls is discharged directly into the harbour. Such activities would cause fluctuating TBT concentrations in the nearby areas, making it difficult to obtain reliable estimates of the actual level of TBT contamination from water samples. According to the data of the present investigation and the TBT levels from 1995, the areas close to the Deep Sea Port and Phuket Harbour can be characterised as moderately contaminated.

Only one snail transplanted to the Deep Sea Port showed imposex 50 days after release. Eighty days after release 5 out of 28 females had imposex, but with a low RPSI value. The concentrations of TBT measured at the Deep Sea Port in 1995 and the findings that imposex developed in *Thais orbita* within two weeks of exposure to 10 ng TBT l<sup>-1</sup> sea water (Wilson & Ahsanullah 1991), indicate that *T. distinguenda* is not very sensitive to TBT contamination, or that the TBT concentration was low during the transplantation period. Further transplantation of *T. distinguenda* over longer periods and investigations of TBT concentrations in the same period of time are essential to obtain reliable data on the sensitivity of this species to TBT contamination.

Because the development of imposex is irreversible, it should be expected that the probability of developing this condition is higher among species with a long life span compared to species with a short one. The question of probabilities, however, is complicated by the fact that different species have different levels of sensitivity to TBT (Ellis & Pattisina 1990).

Muricids have been used extensively as indicators of TBT, because they are especially abundant in intertidal ecosystems world-wide and sensitive to TBT. Iwata *et al.* (1994) measured butyltin compounds (BTC) in marine mammals and found that BTC generally accumulated in the food chain presumably in a similar manner to DDT and PCB. Filter-feeding organisms such as

bivalves, barnacles, *etc* are among the preferred food items of many muricids, and the sensitivity to TBT of the muricids examined could be caused by their diet of organisms which accumulate TBT. If TBT is accumulated in filter-feeding organisms, high probabilities of developing imposex should be found among long living, carnivorous gastropods feeding on filter-feeding organisms.

The effects of severe TBT contamination on populations of *N. lapillus* encompass reduced recruitment and declining population size, because this species has direct larval development. Comparison of the effects on populations of *N. lapillus* and *Thais* spp. is difficult, because larvae of *Thais* spp. have a relatively long pelagic phase. Although a significant negative correlation of imposex on density of snails was observed in this investigation (Tab. 2), populations of *Thais* spp. affected by TBT contamination can be replenished by recruitment from healthy populations far from the contaminated area. The abundance of *T. distinguenda* may be closely related to food supply with an additional lesser effect of TBT contamination. The snails feed on sedentary organisms such as barnacles and bivalve spat, and the anti-fouling effect of TBT might also reduce the amount of food. From the present investigation it was impossible to establish whether this effect on abundance was caused by post settlement mortality or reduced settlement in the contaminated areas. The presence of a similar proportion of juveniles at both stations with and without imposex indicates that settlement is not limited by TBT. Evans *et al.* (1996) found predominantly old individuals and male biased populations of *N. lapillus* in severely contaminated areas. In the late stage of imposex the oviduct is blocked by the growth of vas deferens, preventing the release of egg capsules. This blockage causes a build-up of egg-capsules in the capsule gland which may eventually rupture and kill the animal (Wilson *et al.* 1993). Gibbs *et al.* (1987) made a classification of imposex for *Nucella lapillus* based

on 6 stages of the vas deferens sequence index (VDSI) where sterilisation and blockage of the oviduct belong to stages 5 and 6. However, VDSI observed in the present investigation did not exceed stage 3, hence premature death of females should not occur, resulting in TBT induced male biased sex ratios. In this investigation the sex ratio was not correlated with imposex. Similarly, Spence *et al.* (1990) did not find any indication of male biased sex ratios even in areas with considerably higher levels of imposex in an investigation of *Thais haemastoma* (Linnaeus, 1767) at the Azores. Even though *Thais distinguenda* showed less sensitivity to TBT compared to similar investigations on *Nucella lapillus*, they are useful as an indicator species, because of the strong correlation between the level of imposex and boat traffic.

The use of population characteristics in relation to TBT-contamination of species with pelagic larvae should be applied with some caution, because of the possible recruitment from healthy populations which may disguise the actual effects of TBT contamination in the target area.

However, the fact that *Thais* spp. have pelagic larvae could be an advantage as an indicator organism, because it would prevent this species from becoming extinct even in heavily TBT contaminated areas.

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