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IMPOSEX AND ORGANOTIN COMPOUNDS IN *THAIS CLAVIGERA* AND *T. BRONNI* IN JAPAN

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Rates of occurrence and degrees of imposex, and tissue concentrations of organotin compounds (tributyltin, TBT; dibutyltin, DBT; butyltin, MBT; triphenyltin, TPT; diphenyltin, DPT; and phenyltin, MPT) in the rock shell, *Thais clavigera* and *T. bronni* (Mollusca: Gastropoda) were investigated at 32 sites in Japan from May 1990 to October 1992. The rate of occurrence of imposex was 100% in both species, at almost all sites surveyed. Degrees of imposex indicated by relative penis length (RPL) index reflected the pollution levels not only of TBT, but also TPT. In heavily polluted areas, many individuals were found with oviducts which were blocked by vas deferens development, and capsule glands which were filled with aborted egg masses. These organisms were thought to be sterile.

Tributyltin and TPT concentrations in both species of molluscs were higher than those previously reported in fish and shellfish. Tributyltin and TPT concentrations were generally higher in females than in males, for both species. This suggests that female reproductive organs may have high concentrations of TBT and TPT.

Estimated levels of TBT inducing imposex were 10–20 ng g^{-1} wet tissue for both species. As strong positive correlations between TBT and TPT concentrations were found in both species (r=0·857–0·966), the relationship between TPT concentration in tissue and RPL index was very similar to that for TBT. It is possible that TPT as well as TBT is related to induction or development of imposex in these species.

INTRODUCTION

Marine pollution by organotin compounds, such as tributyltin (TBT) and triphenyltin (TPT), which have been used in antifouling paints for ships, boats and fishing nets, has become increasingly serious in the coastal areas around Japan (Environment Agency of Japan, 1989, 1990, 1991, 1992). There are many reports on organotin concentrations in water, sediment, and fish and shellfish in Japan (*e.g.* Takeuchi *et al.*, 1987, 1989; Mizuishi *et al.*, 1989; Takahashi & Ohyagi, 1988; Morita, 1989; Takami *et al.*, 1988; Environment Agency of Japan, 1989, 1990, 1991, 1992).

The use of TBT in antifouling paints for ships and boats began in about 1965, and then TPT use began in Japan (Takahashi, K., personal communication). The use of TBT and TPT for fishing nets was started in around 1967 (Takahashi, K., personal communication). Thereafter, the use of these compounds spread throughout Japan, and usage levels are thought to have been increasing since. For example, the amount of TBT manufactured and imported increased from 3897 tons in 1984 to 6340 tons in 1989 in Japan (Ministry of International Trade & Industry, 1990). Thus it is thought that organotin pollution in Japan has progressed with the increase in use of TBT and TPT in antifouling paints.

The regulations for organotin compounds in Japan as of August 1993 are summarized as follows: manufacture, import and use of TBTO (bis (tributyltin) oxide) has been completely prohibited by law since 1990. Other TBT compounds (13 substances including TBT-copolymer) and TPT compounds (7 substances) may be used, manufactured or imported if their amounts are reported to the Ministry of International Trade & Industry (MITI). Although it is possible to use TBT or TPT formulated antifouling paints for any kind of ships and boats, including smaller than 25 m, the sale of TPT products to the Japanese domestic market ceased in June 1989, as a result of the administrative guidance of MITI, and TPT pollution seems to have decreased recently (Shiraishi & Soma, 1992). The amount of TBT antifouling paint used has not changed after regulation (Sugita, 1992; MITI, 1990), and TBT levels in the environment have not decreased yet (Environment Agency of Japan, 1992).

Imposex (the development of male sex organs in females) in neogastropods is a global problem, and has been described in many species (*e.g.* Bryan *et al.*, 1986; Gibbs *et al.*, 1990, 1991; Bright & Ellis, 1990; Spence *et al.*, 1990; Stewart *et al.*, 1992; Stroben *et al.*, 1992). It is likely that the number of species reported to exhibit imposex will increase following additional surveys. In the case of *Nucella lapillus* (L.), imposex was initiated at a level as low as 1 ng l^{-1} of TBT in sea-water (Gibbs *et al.*, 1987). As a result of oviduct blockage, oogenesis suppression and testes development, females of *N. lapillus* fail to reproduce (Gibbs & Bryan, 1986; Gibbs *et al.*, 1988), and consequently natural populations have declined in England (Bryan *et al.*, 1986).

Since pollution levels of organotin compounds in sea-water around Japan have already exceeded the level at which imposex is induced in the laboratory in *N. lapillus* (Environment Agency of Japan, 1989, 1990, 1991, 1992), imposex and population decline in gastropods might also be occurring in Japan. However, there are few detailed surveys on imposex in Japan. In this study the authors investigated the occurrence and development of imposex, and the tissue concentrations of organotin compounds in the rock shell, *Thais clavigera* (Küster) and *T. bronni* (Dunker) (Prosobranchia: Muricidae) at 32 sites in Japan. The relationships between organotin compounds such as TBT and TPT, and imposex were also examined. The results are given below.

MATERIALS AND METHODS

Sampling and imposex identification

Specimens were collected at 32 sites in Honshu and Kyushu, Japan (Figure 1) from May 1990 to October 1992. These sites were selected considering the severity of their organotin pollution: some sites were representative of heavily polluted areas and others of less polluted areas. Where possible, 20 or more specimens each of *Thais clavigera* and

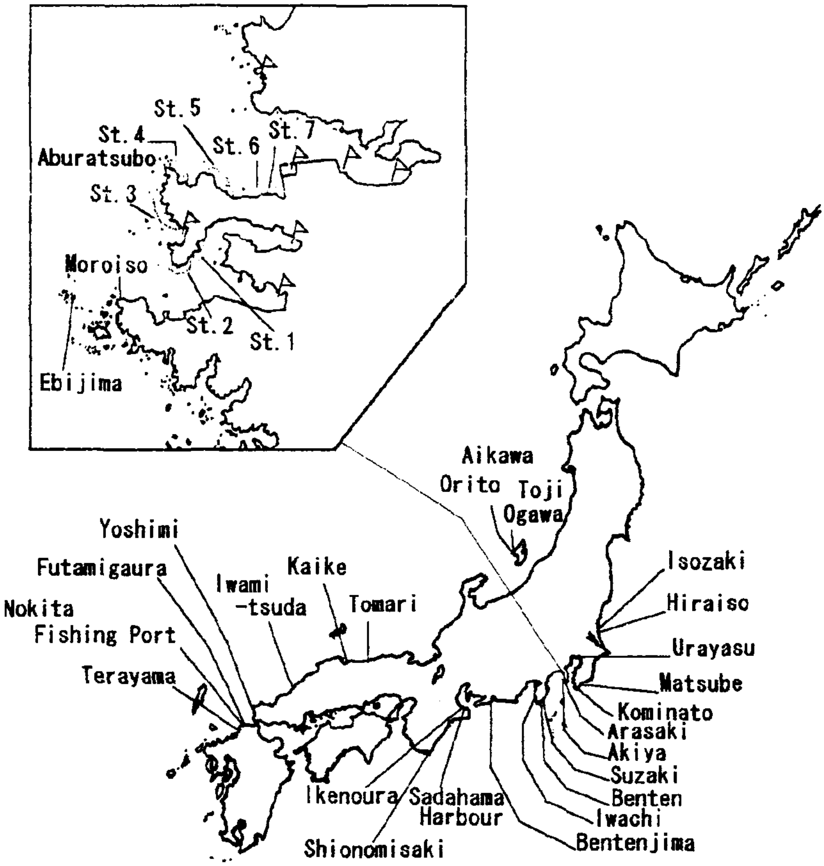

Figure 1. Sampling sites for the specimens of *Thais clavigera* and *T. bronni*. The flag symbols indicate marinas, harbours and piers around Aburatsubo in Miura Peninsula.

T. bronnni were collected, and transported to the laboratory on ice. In the laboratory, shell height and body weights with and without shell were measured, sex was determined and the specimens examined for imposex. Individuals having capsule, albumen and sperm-ingesting glands were identified as female (Figure 2B). Females with male sex organs were identified as individuals exhibiting imposex, namely having a penis and vas deferens together with capsule, albumen and sperm-ingesting glands (Figure 2C). Penis length was measured, and the relative penis length (RPL) index and relative penis size (RPS) index (Gibbs *et al.*, 1987) were calculated. Relative penis length index (%) = {(mean penis length in females) / (mean penis length in males)} *100. Relative penis size index (%) = {(mean penis length in females)^3 / (mean penis length in males)^3 } *100 (Gibbs *et al.*, 1987). According to the developmental stage of imposex, imposex specimens were classified into grade I to V. Criteria for this classification are as follows: grade I, penis length <1/4 of shell height; grade II, penis length >1/4 of shell height; grade III, end of oviduct blocked (Figure 2C); grade IV, end of oviduct blocked, and darkened mass in capsule gland; grade V, other deformity, such as adhesion of penis to end of oviduct, split of oviduct and sex change (testis development into ovary).

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Table 1. *Specimens of* Thais clavigera *and* T. bronni *used in this study.*

Locality	Position	Date	Species	N	(m:f)	Shell height in mm (min - max mean) Male		Female	
Isozaki	N36°22'	910516	A	30	(18:12)	19.8 - 37.4	25.8	21.7 - 31.5	26.9
	E140°37'		B	15	(7: 8)	19.1 - 38.1	26.3	18.3 - 41.0	26.9
Hiraiso	N36°21'	911121	A	20	(11: 9)	16.1 - 21.5	19.0	17.7 - 20.0	19.1
	E140°37'	920803	A	10	(5: 5)	16.9 - 25.0	21.1	21.2 - 29.4	23.7
Matsube	N35°8'	900629	A	25	(11:14)	19.2 - 31.6	25.5	20.6 - 32.6	24.8
	E140°18'								
Kominato	N35°7'	910801	A	35	(16:19)	23.5 - 31.3	27.7	24.1 - 37.6	28.6
	E140°11'		B	4	(1:3)	-	41.9	26.6 - 30.6	28.7
Urayasu	N35°39'	910715	A	5	(4:1)	22.3 - 38.7	29.5	-	30.7
	E139°57'								
Moroiso	N35°9.0'	910808	A	27	(15:12)	22.1 - 37.2	27.4	23.1 - 32.0	27.3
	E139°36.9'		B	17	(9:8)	21.5 - 32.8	29.6	26.9 - 36.3	30.6
Aburatsubo	N35°9.4'								
	E139°37.5'								
St.1		900711	A	8	(4:4)	35.9 - 42.0	39.4	31.0 - 39.0	35.0
		900905	B	5	(0:5)	-	-	39.0 - 65.0	49.9
St.2		900522	A	11	(6:5)	27.0 - 35.0	32.0	29.0 - 30.0	29.8
		900711	B	20	(10:10)	36.0 - 59.0	53.3	39.0 - 65.0	49.9
St.3		900626	A	20	(7:13)	25.8 - 32.3	29.1	25.0 - 36.6	29.5
			B	2	(1:0)*	-	48.1	-	-
St.4		900525	A	20	(15:5)	21.2 - 36.4	25.5	25.0 - 29.7	27.1
			B	20	(10:10)	32.7 - 43.8	37.3	33.0 - 44.2	38.3
St.5		900525	A	20	(5:15)	28.8 - 30.9	30.0	27.2 - 35.1	30.1
			B	20	(15:5)	41.8 - 50.6	45.4	42.3 - 48.1	46.0
St.6		900626	A	17	(10:7)	21.6 - 30.8	24.6	21.3 - 30.1	25.4
			B	5	(3:2)	27.5 - 35.5	30.4	31.8 - 36.1	33.9
St.7		900626	B	2	(2:0)	32.6 - 40.8	36.7	-	-
Ebijima		900725	A	22	(5:17)	27.2 - 36.9	31.7	22.2 - 38.1	29.8
			B	7	(1:6)	-	39.0	36.1 - 50.0	41.4
Arasaki	N35°12'	910809	A	24	(9:15)	18.3 - 27.4	20.6	16.2 - 28.4	21.6
	E139°37'		B	22	(21:1)	15.4 - 31.8	21.0	-	18.3
Akiya	N35°14'	920803	A	20	(12:8)	17.7 - 22.9	20.1	16.9 - 20.2	18.9
	E139°36'								
Suzaki	N34°39'	920320	A	22	(6:16)	26.0 - 30.0	28.0	27.1 - 35.3	29.1
	E138°59'								
Benten	N34°40'	920320	A	11	(8:3)	21.3 - 34.7	28.1	25.4 - 29.0	27.0
	E138°58'		B	14	(14:0)	25.4 - 40.3	33.1	-	-
Iwachi	N34°44'	920821	A	14	(6:8)	17.2 - 20.0	18.5	15.7 - 24.3	18.7
	E138°46'								
Bentenjima	N34°41'	910819	A	21	(14:7)	18.2 - 31.1	24.7	21.0 - 25.7	23.5
	E137°36'								
Ikenoura	N34°29'	910813	A	10	(5:5)	28.1 - 38.6	32.4	29.4 - 34.6	31.4
	E136°49'								
Sadahama Harbour	N34°29' E136°51'	910813	A	24	(16:8)	17.8 - 27.6	23.8	20.7 - 26.4	23.8
Shionomisaki	N33°26'	910814	A	15	(6:9)	23.3 - 34.1	28.3	26.4 - 33.8	28.8
	E135°45'		B	6	(4:2)	25.6 - 43.1	33.1	30.0 - 35.3	32.7
Futamigaura	N33°39'	921006	A	22	(8:14)	18.7 - 20.5	19.6	18.6 - 22.3	20.3
	E130°14'								
Nokita Fishing Port	N33°37' E130°10'	921006	A	22	(5:17)	20.5 - 21.3	21.0	19.2 - 22.6	21.1
Terayama	N33°32'	921006	A	16	(4:12)	17.1 - 24.0	19.8	17.1 - 22.2	19.1
	E130°9'								

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Table 1 *(continued)*.

Locality	Position	Date	Species	N (m:f)	Shell height in mm (min - max mean) Male		Female	
Yoshimi	N34°4' E130°55'	921005	A	20 (11:9)	18.3 - 22.9	20.1	17.9 - 27.0	21.0
Iwami-tsuda	N34°43'	910717	A	20 (14:6)	15.7 - 23.4	19.3	12.5 - 28.8	20.4
	E131°52'		B	7 (3:4)	34.3 - 48.3	40.0	33.9 - 45.3	41.4
Kaike	N35°28'	910716	A	22 (13:9)	14.0 - 27.5	19.0	14.3 - 26.0	19.3
	E133°22'		B	2 (1:1)	-	32.0	-	38.7
Tomari	N35°31'	910715	A	31 (16:15)	17.1 - 26.6	22.3	16.1 - 28.5	21.7
	E133°58'		B	9 (2:7)	35.0 - 35.4	35.2	21.7 - 37.3	28.5
Aikawa								
Orito	N38°1'	910816	A	20 (9:11)	17.8 - 33.4	21.7	16.8 - 26.4	21.8
	E138°14'	920421	A	603(315:283)**	11.6 - 29.1	19.0	8.3 - 29.5	19.3
			B	34 (11:23)	17.8 - 26.4	20.1	9.8 - 26.5	19.8
Ogawa/Toji	N38°4'	911029						
	E138°15'	911030	A	8 (3:5)	29.7 - 42.7	35.2	31.6 - 40.8	35.0

Species A, *T. clavigera;* B, *T. bronni.* *, Sex determination for the other individual was not conducted; **, Sex determination for 5 individuals was not conducted.

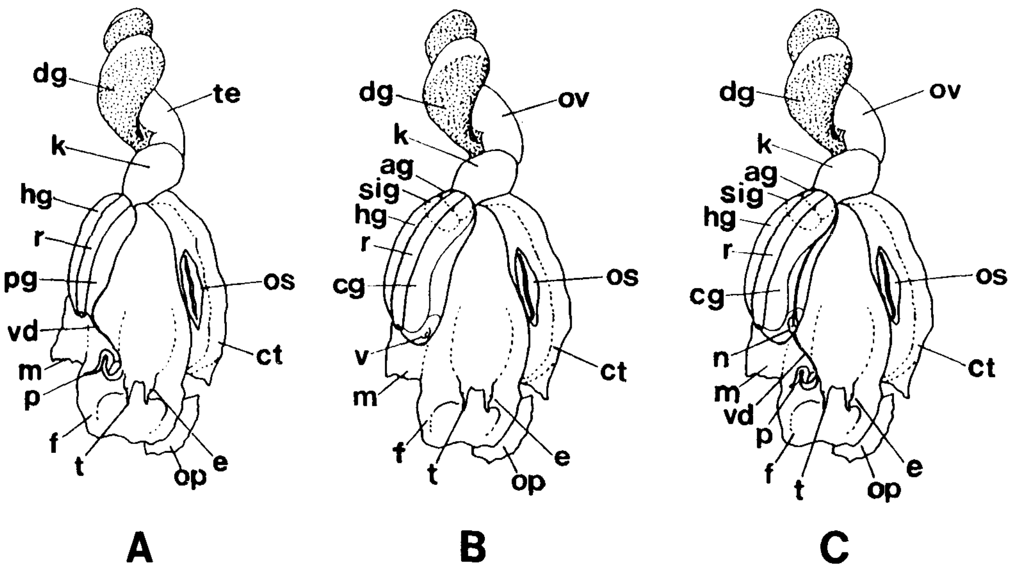

Figure 2. Anatomical features of *Thais clavigera.* (A) Male; (B) female; (C) imposex grade III/IV. ag, albumen gland; cg, capsule gland; ct, ctenidium; dg, digestive gland; e, eye; f, foot; hg, hypobranchial gland; k, kidney; m, mantle; n, nodule; op, operculum; os, osphradium; ov, ovary; p, penis; pg, prostate gland; r, rectum; sig, sperm-ingesting gland; t, tentacle; te, testis; v, vulva; vd, vas deferens.

In this study it was determined that penis length was about half the shell height in males (Tables 1 & 3). So, for convenience, grade II was distinguished from grade I as a penis length greater than a quarter of shell height. In the specimens of grade III and IV, the end of the oviduct was clearly blocked and the vas deferens was well-developed (Figure 2C). The darkened mass was regarded as compressed and aborted egg capsules. The specimens of grades III, IV and V are thought to be sterile. These conditions are similar to those observed in *Nucella lapillus* (Gibbs & Bryan, 1986; Gibbs *et al.*, 1987, 1988).

After examination for imposex, specimens were used in chemical analysis for tissue concentrations of organotin compounds. Specimens of each species were grouped by size, sex, and imposex grade, with each group having one to several individuals. After biological examination, specimens were kept frozen below –20°C until chemical analysis. Details on the specimens used are shown in Table 1.

Determination of tissue concentrations of organotin

Homogenized tissue samples (approximately 4 g) of *T. clavigera* and *T. bronni*, were extracted twice, using 1N HBr/ethanol (10 ml) and 0·1% tropolone/benzene (10 ml) under ultrasonication, spiked with surrogate standard (tripentyltin chloride). Extracted solutions were washed with 2M $NaBr/H_2O$ (50 ml), and the benzene phase was concentrated until almost dry by a rotary evaporator. The residue was partitioned with methanol/hexane, and the solvent (methanol) was exchanged with benzene. Organotins were derivatized with propylmagnesium bromide, and cleaned up by silica gel column chromatography. Organotin compounds in the sample were determined by gas chromatography with a flame photometric detector (GC–FPD) after addition of tetrapentyltin as an internal standard. Tissue concentrations of tributyltin (TBT), dibutyltin (DBT), butyltin (MBT), triphenyltin (TPT), diphenyltin (DPT), and phenyltin (MPT) were determined as the chloride, based on tripentyltin, and expressed on a wet tissue basis for the homogenate. Gas chromatography (GC) conditions for the determination of organotin are shown in Table 2. The detection limit was 50 pg; corresponding to 7·9–12·5 ng g^{-1} wet tissue sample. The mean recovery of the surrogate standard was 78·4% (63·3–102·8%), for both species.

This analytical procedure was confirmed, using the certified reference material of Japanese sea bass, *Lateolabrax japonicus*, for TBT and TPT analysis (NIES CRM no. 11). Determined values of TBT and TPT in this material, by this analytical procedure, were within the certified value for TBT 1·3 ±0·1SD μg g^{-1} dry wt, and the reference value for TPT 6·3 μg g^{-1} dry wt, respectively (Okamoto, 1991). The recovery of the surrogate standard, tripentyltin chloride, was 74·7 ±4·5% (N=5).

Table 2. *Measurement conditions during gas chromatography (GC) for determination of organotin compounds in tissue of* Thais clavigera *and* T. bronni.

Instrument	HEWLETT PACKARD 5890A with autoinjector 7673A (1 μl splitless injection)
Detector	FPD (with a filter for tin)
Column	capillary column, 30 m x ϕ0.25 mm (GL Science Inc.)
Column head pressure	115 kPa
Liquid phase	TC-5 (5% phenyl/methyl silicon)
Liquid phase thickness	0.25μm
Carrier gas	He
Initial Temperature	70°C (initial time 0.00 min)
Rate	10.0°C min^{-1}
Final temperature	120°C (final time 0.00 min)
Rate A	4.0°C min^{-1}
Final Temperature A	220°C (final time A 0.00 min)
Rate B	8.0°C min^{-1}
Final Temperature B	260°C (final time B 3.00 min)

RESULTS

Imposex: its occurrence and grade

Occurrence rates of imposex, according to sampling site, are shown in Table 3. The rate of occurrence of imposex was 100% at almost all sites for both *Thais clavigera* and *T. bronni*. The only exception was Aikawa-Orito, Sado Island, where no or few individuals exhibiting imposex were observed in either species surveyed. There were a few females without a penis at a few sites: for *T. clavigera*, two among 15 females and 16 males at Tomari, for *T. bronni*, two among eight females and seven males at Isozaki, and one among three females and one male at Kominato.

Table 3. *Summary of results of country-wide survey on imposex in* Thais clavigera *and* T. bronni.

Site	Species	Imposex occurrence (%)	Mean penis length (mm) male	Mean penis length (mm) female	RPL (%)	RPS (%)	Relative occurrence ratios of imposex grade (%) 0*	I	II	III	IV	V
Isozaki	A	100	19.8	4.8	24.2	1.4	0	100	0		0	**
	B	75	10.3	3.0	29.1	2.5	25	75	0		0	**
Hiraiso	A+	100	7.6	2.2	28.9	2.4	0	100	0		0	**
	A++	100	5.3	3.3	60.4	22.0	0	100	0	0	0	**
Matsube	A	100	15.8	4.2	26.6	1.9	0	100	0		0	**
Kominato	A	100	17.0	4.1	24.1	1.4	0	100	0		0	**
	B	67	13.0	1.2	9.2	0.1	33	67	0		0	**
Urayasu	A	100	16.5	7.0	42.4	7.6	0	100	0		0	**
Moroiso	A	100	14.9	11.2	75.2	42.5	0	0	83		17	**
	B	100	13.5	9.4	69.6	33.8	0	25	50		25	**
Aburatsubo												
St.1	A	100	18.8#	11.5##	61.2	22.9	0	0	0		100	**
	B	100	###	23.0	###	###	0	0	100		0	**
St.2	A	100	14.3	13.2	92.3	78.7	0	0	100		0	**
	B	100	20.3	18.4	90.6	74.5	0	10	50		40	**
St.3	A	100	15.9	13.6	85.5	62.6	0	0	69		31	**
	B	###	19.0	###	###	###						
St.4	A	100	13.2	11.2	84.8	61.1	0	0	80		20	**
	B	100	16.8	14.4	85.7	63.0	0	10	80		10	**
St.5	A	100	14.0	14.0	100	100	0	0	60		40	**
	B	100	23.2	18.6	80.2	51.5	0	0	100		0	**
St.6	A	100	11.7	12.0	102.6	107.9	0	0	100		0	**
	B	100	11.0	14.0	127.3	206.2	0	0	100		0	**
St.7	B	###	19.0	###	###	###						
Ebijima	A	100	18.0	13.7	76.1	44.1	0	0	71		29	**
	B	100	17.0	13.2	77.6	46.8	0	0	83		17	**
Arasaki	A	100	13.8	9.4	68.1	31.6	0	0	93		7	**
	B	100	9.0	9.0	100	100	0	0	100		0	**
Akiya	A	100	9.6	6.0	62.5	24.4	0	25	50	0	25	0
Suzaki	A	100	17.1	4.2	24.6	1.5	0	88	0	0	13	0
Benten	A	100	11.5	8.0	69.6	33.7	0	0	33	0	67	0
	B	###	14.6	###	###	###						
Iwachi	A	100	9.9	8.6	86.9	65.6	0	0	63	13	25	0
Bentenjima	A	100	12.0	9.1	75.8	43.6	0	0	57		43	**
Ikenoura	A	100	13.6	10.9	80.1	51.5	0	0	40		60	**
Sadahama H.	A	100	11.9	10.8	90.8	74.8	0	0	25		75	**

Table 3 *(continued).*

Site	Species	Imposex occurrence (%)	Mean penis length (mm) male	female	RPL (%)	RPS (%)	Relative occurrence ratios of imposex grade (%) 0*	I	II	III	IV	V
Shionomisaki	A	100	10.1	6.2	61.4	23.1	0	67	33		0	**
	B	100	8.6	3.5	40.7	6.7	0	100	0		0	**
Futamigaura	A	100	8.7	6.5	74.7	41.7	0	7	64	0	27	0
Nokita F.P.	A	100	9.9	7.3	73.7	40.1	0	0	35	12	53	0
Terayama	A	100	8.5	7.5	88.2	68.7	0	0	50	25	17	8
Yoshimi	A	100	10.1	8.8	87.1	66.1	0	0	67	11	22	0
Iwami-tsuda	A	100	9.0	2.8	31.1	3.0	0	100	0		0	**
	B	100	22.3	7.0	31.4	3.1	0	100	0		0	**
Kaike	A	100	11.2	9.6	85.7	63.0	0	0	100		0	**
	B	100	19.0	10.0	52.6	14.6	0	0	100		0	**
Tomari	A	87	16.8	3.3	19.6	0.8	13	87	0		0	**
	B	100	20.0	4.6	23.0	1.2	0	86	14		0	**
Aikawa												
Orito	A	0	7.8	0	0	0	100	0	0		0	**
	A+++	7	nm	nm	~0	~0	93	7	0	0@	0@	0@
	B+++	0	nm	0	0	0	100	0	0	0	0	0
Ogawa/Toji	A	100	14.7	1.5	10.2	0.001	0	100	0	0	0	0

Species A, *T. clavigera*; B, *T. bronni*. *, normal female; **, detailed examination for imposex grade V not conducted, so occurrence unclear; +, samples collected 21 Nov 1991; ++, samples collected 3 Aug 1992; +++, samples collected 21 Apr 1992; #, unpreserved individuals measured; ##, measured after preservation in formalin; ###, no females collected for the species, or value not calculated because only male or female specimens were collected; @, conjecture based on minor symptoms of imposex; nm, not measured.

Mean penis lengths for female and male, RPL indices and RPS indices are shown for both species in Table 3. No standardization based on shell height was made in the calculation of the values of mean penis length, RPL index and RPS index, because there is not a clear positive correlation between shell height and penis length in females, although there is a weak positive correlation in males. It may be noted that there are similar trends in the values of the RPL and RPS indices. The values were low at Isozaki, Hiraiso, the sites on the Boso Peninsula (Kominato and Matsube), Suzaki, Iwamitsuda, Tomari and Aikawa-Ogawa. Although the values for *T. clavigera* at Urayasu, located at the innermost part of Tokyo Bay, were relatively low, those for the same species at Shionomisaki, located at the tip of the Kii Peninsula and directly facing the Pacific Ocean, were relatively high. For the other sites, the values were high; the values of the RPL index were in a range of about 60% to over 100%. It can be clearly seen that the indices increase gradually as the sites approach marinas or piers, the sources of organotin pollution, on the Miura Peninsula (Table 3).

When the relationship between RPL index or RPS index and the development of imposex is examined, it can be seen that with an increase in these indices, the relative occurrence of grade II and grade III imposex increased, and grade IV imposex occurred more frequently at higher indices (Table 3). The percentage occurrence of grade IV seemed to reflect the distance from the site to the pollution source (marina, harbour or pier), and in *T. clavigera* was as high as 60–75% at sites close to piers (Ikenoura and Sadahama Harbour). At the sites in Miura Peninsula, the percentage occurrence of

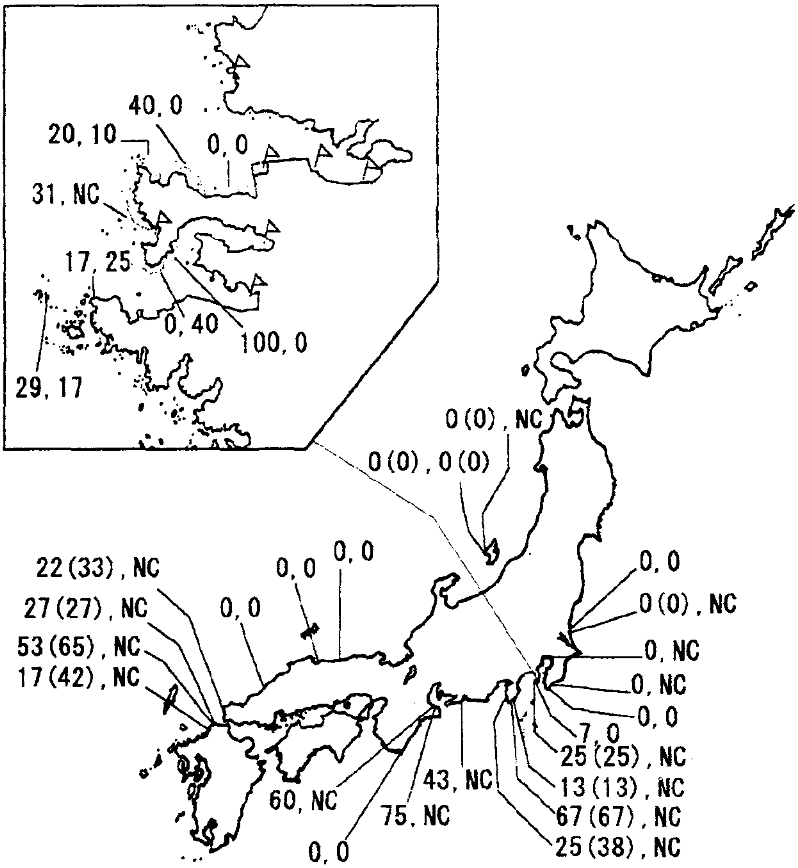

Figure 3. Occurrence of imposex grade IV in the specimens of *Thais clavigera* and *T. bronni*. The first numeral is for *T. clavigera*, and the second for *T. bronni* at every site. NC means that no specimens were collected for the species, or that the value is still unknown because there were no females in the specimens collected. The numeral within a parenthesis indicates the sum of occurrence percentages for imposex grades III and IV. Grades III and IV imposex organisms are thought to be sterile. The flag symbols indicate marinas, harbours and piers around Aburatsubo in Miura Peninsula.

grade IV imposex increased as the site approached the marina: 6·7% at Arasaki, 16·7% at Moroiso, 29·4% at Ebijima, and 100% at station 1 in Aburatsubo (Figure 3). The cases in Shimoda (Suzaki and Benten) and Fukuoka (Futamigaura and Nokita Fishing Port) were also quite similar.

Organotin concentrations in tissue

Organotin concentrations in *T. clavigera* and *T. bronni* are shown in Figure 4. There are similar trends in the concentrations and compositions of organotin compounds in both species. Concentrations of TBT and TPT in both species collected at Aburatsubo, Bentenjima and Sadahama Harbour were high, where imposex indices of the specimens were also high. The maximum concentrations of TBT and TPT for *T. clavigera* were 430 ng g^{-1} (wet) and 1700 ng g^{-1}, respectively. Those for *T. bronni* were 750 ng g^{-1} and 1770 ng

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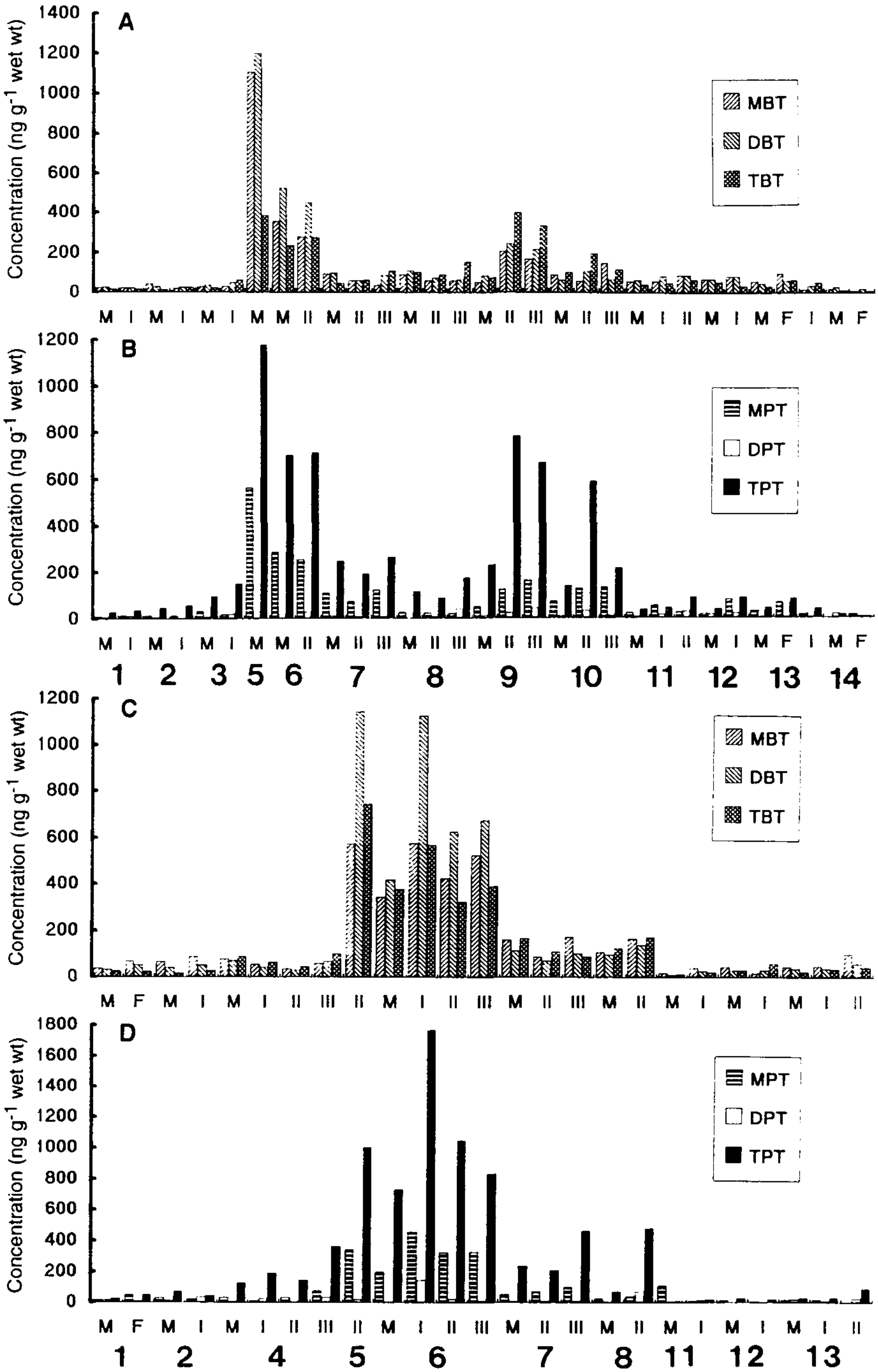

Figure 4. Organotin concentrations in tissues of *Thais clavigera* and *T. bronni*. (A) Butyltin compounds in *T. clavigera*; (B) phenyltin compounds in *T. clavigera*; (C) butyltin compounds in *T. bronni*; (D) phenyltin compounds in *T. bronni*. M, male; F, female; I, imposex grade I; II, imposex grade II or III; III, imposex grade IV. The numbers (1–14) under the abbreviations represent the collection sites for the specimens as follows: 1, Isozaki; 2, Kominato; 3, Urayasu; 4, Moroiso; 5, Station 1 in Aburatsubo; 6, Station 2 in Aburatsubo; 7, Ebijima; 8, Arasaki; 9, Bentenjima; 10, Sadahama Harbour; 11, Shionomisaki; 12, Iwami-tsuda; 13, Tomari; 14, Orito.

g^{-1}, respectively. Tributyltin and TPT concentrations in both species were higher than concentrations previously reported in fish and shellfish (Takeuchi *et al.*, 1987, 1989; Mizuishi *et al.*, 1989; Morita, 1989; Takami *et al.*, 1988; Ishizaka *et al.*, 1989; Higashiyama *et al.*, 1991; Environment Agency of Japan, 1989, 1990, 1991, 1992).

Relative occurrence ratios of butyltin compounds varied from site to site (Figure 4). For example, DBT concentrations were relatively high compared to TBT levels in both species at Aburatsubo, but TBT concentrations were higher than those of DBT, particularly in females, at Bentenjima, Sadahama Harbour, Arasaki and Moroiso. For phenyltin compounds, the concentrations were TPT > MPT > DPT at almost all sites (Figure 4). The average percentage compositions over all sites for butyltin and phenyltin compounds in both species are shown in Table 4.

Table 4. *Average percentage compositions over all sites for butyltin and phenyltin compounds in* Thais clavigera *and* T. bronni *(mean ±SD).*

			Percentage compositions (%)							
			TBT		:	DBT		:	MBT	
T. clavigera	male	(N=13)	25.5	±7.4	:	38.8	±6.2	:	35.7	±6.3
	female	(N=18)	37.0	±11.1	:	34.5	±7.8	:	28.5	±9.2
T. bronni	male	(N=9)	30.2	±8.0	:	30.6	±4.0	:	39.2	±7.0
	female	(N=17)	30.7	±11.0	:	33.6	±7.9	:	35.7	±10.3
			Percentage compositions (%)							
			TPT		:	DPT		:	MPT	
T. clavigera	male	(N=13)	64.2	±11.7	:	11.7	±14.6	:	24.0	±9.1
	female	(N=18)	63.1	±17.9	:	10.7	±9.0	:	26.2	±13.7
T. bronni	male	(N=9)	59.0	±24.0	:	9.7	±11.9	:	31.4	±22.0
	female	(N=17)	69.7	±14.0	:	10.4	±11.9	:	19.9	±10.4

The concentrations of TBT and TPT were different in males and females, with levels being generally higher in females in both species (Figure 4). The relationships between males and females can be described by the following regression equations. Units of all regressions are ng g^{-1} wet tissue.

Thais clavigera: TBT in female = 8·146+1·094*TBT in male (r=0·967) (N=11) (1)

TPT in female = 25·93+0·996*TPT in male (r=0·965) (2)

Thais bronni: TBT in female = 10·22+0·908*TBT in male (r=0·966) (N=9) (3)

TPT in female = 38·24+1·402*TPT in male (r=0·933) (4)

The correlation matrix (correlation coefficients between each pair of organotin compounds determined) is shown in Table 5; high positive correlation coefficients were generally observed. High correlation coefficients (r=0·857–0·966) were also found between TBT and TPT levels. This has never been reported before for fish or shellfish (Takeuchi *et al.*, 1989; Ishizaka *et al.*, 1989; Higashiyama *et al.*, 1991; Environment Agency of Japan, 1989, 1990, 1991, 1992).

Table 5. *Correlation coefficients between each pair of organotin compounds determined in* Thais clavigera *and* T. bronni.

Female *T. clavigera* (N=12)

	TBT	DBT	MBT	TPT	DPT	MPT
TBT		0.797**	0.874***	0.950***	0.663*	0.780**
DBT	0.797**		0.966***	0.869***	0.586*	0.914***
MBT	0.874***	0.966***		0.912***	0.679*	0.940***
TPT	0.950***	0.869***	0.912***		0.580*	0.906***
DPT	0.663*	0.586*	0.679*	0.580*		0.554
MPT	0.780**	0.914***	0.940***	0.906***	0.554	

Male *T. clavigera* (N=13)

	TBT	DBT	MBT	TPT	DPT	MPT
TBT		0.972***	0.957***	0.965***	0.801**	0.968***
DBT	0.972***		0.994***	0.989***	0.881***	0.991***
MBT	0.957***	0.994***		0.980***	0.900***	0.983***
TPT	0.965***	0.989***	0.980***		0.859***	0.996***
DPT	0.801**	0.881***	0.900***	0.859***		0.871***
MPT	0.968***	0.991***	0.983***	0.996***	0.871***	

Female *T. bronni* (N=10)

	TBT	DBT	MBT	TPT	DPT	MPT
TBT		0.984***	0.869**	0.857**	0.274	0.928***
DBT	0.984***		0.925***	0.883***	0.297	0.970***
MBT	0.869**	0.925***		0.964***	0.520	0.974***
TPT	0.857**	0.883***	0.964***		0.536	0.947***
DPT	0.274	0.297	0.520	0.536		0.351
MPT	0.928***	0.970***	0.974***	0.947***	0.351	

Male *T. bronni* (N=9)

	TBT	DBT	MBT	TPT	DPT	MPT
TBT		0.977***	0.988***	0.966***	-0.356	0.781*
DBT	0.977***		0.985***	0.965***	-0.281	0.821**
MBT	0.988***	0.985***		0.981***	-0.281	0.776*
TPT	0.966***	0.965***	0.981***		-0.251	0.819**
DPT	-0.356	-0.281	-0.281	-0.251		-0.296
MPT	0.781*	0.821**	0.776*	0.819**	-0.296	

*, significant at 5% level; **, significant at 1% level; ***, significant at 0·1% level.

Estimation of the level of TBT inducing imposex

As it is thought that induction of imposex in marine gastropods is a specific response to TBT pollution (Bryan *et al*., 1988), we examined the relationships between the tissue concentration of TBT and the degree of imposex in *T. clavigera* and *T. bronni*. The relationships appear to be described by sigmoid curves, in both species (Figure 5). The level of TBT inducing imposex was estimated to be 10–20 ng g^{-1} wet tissue for *T. clavigera* from this relationship (Figure 5A–C). The estimated level for *T. bronni* was the same (Figure 5D).

However, the relationships between the tissue concentration of TPT and the degree of imposex were also observed to fit sigmoid curves in both species (Figure 6). Thus it is possible that TPT is also related to induction or development of imposex in these species (Horiguchi, 1993).

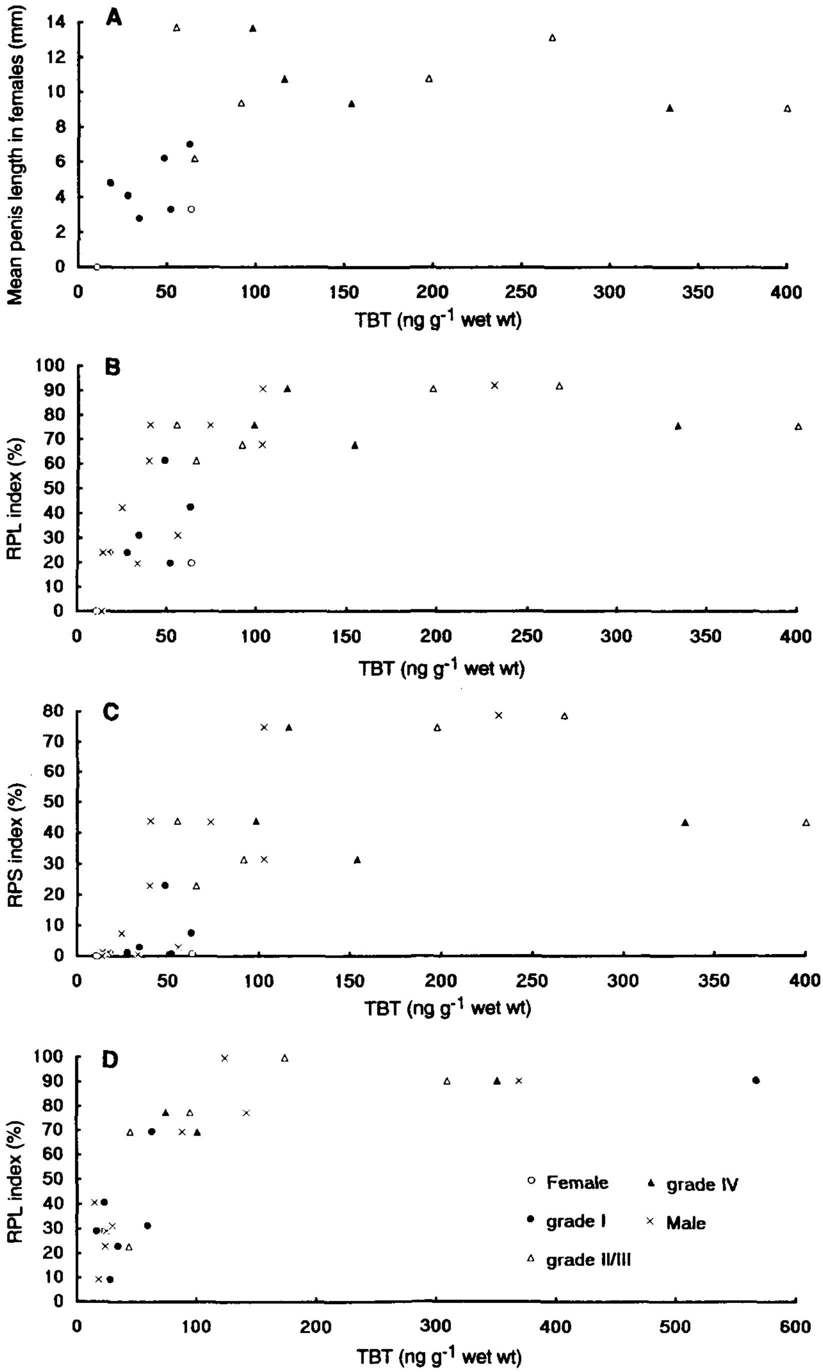

Figure 5. Relationships between TBT concentration in tissue and degree of imposex in *Thais clavigera* and *T. bronni*. (A) TBT concentration in tissue *vs* mean penis length in females of *T. clavigera*; (B) TBT concentration in tissue *vs* RPL index in *T. clavigera*; (C) TBT concentration in tissue *vs* RPS index in *T. clavigera*; (D) TBT concentration in tissue *vs* RPL index in *T. bronni*. Tributyltin concentrations for males as well as for females and every imposex grade are plotted together over all sites in B, C and D.

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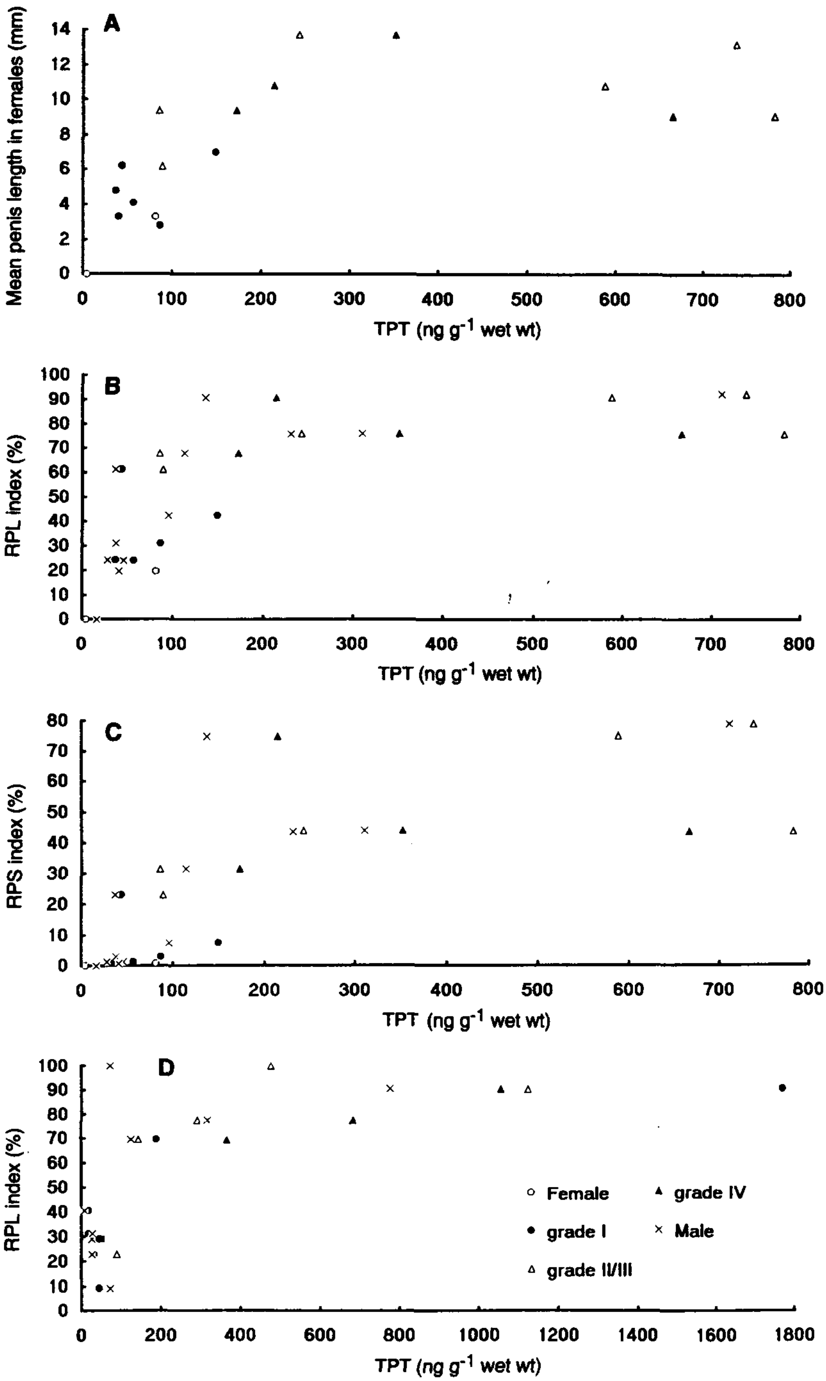

Figure 6. Relationships between TPT concentration in tissue and degree of imposex in *Thais clavigera* and *T. bronni*. (A) TPT concentration in tissue *vs* mean penis length in females of *T. clavigera*; (B) TPT concentration in tissue *vs* RPL index in *T. clavigera*; (C) TPT concentration in tissue *vs* RPS index in *T. clavigera*; (D) TPT concentration in tissue *vs* RPL index in *T. bronni*. Triphenyltin concentrations for males as well as for females and every imposex grade are plotted together over all sites in B, C and D.

DISCUSSION

The occurrence of imposex in Japanese gastropods

Although the correlation between organotin pollution and imposex was not known, especially in Japan, ten or fifteen years ago, there were some reports on the occurrence of imposex in Japanese gastropods at that time. These reports were for *Thais clavigera*, the rapa whelk, *Rapana venosa* (Valenciennes) and the ivory shell, *Babylonia japonica* Reeve (Nakano & Nagoshi, 1980; Kajikawa *et al.*, 1983; Kajikawa, 1984). At that time, the rate of occurrence of imposex (females with penis-like outgrowth) in *T. clavigera* was already described as almost 100% at ten sites in Japan (Nakano & Nagoshi, 1980). Conversely, Matsuo & Habe (1969) examined samples for the presence of a penis for sex determination of *T. clavigera* specimens which were collected at Hayama-Shibazaki coast in Kanagawa Prefecture. These authors described the sex ratio for the species as 1:1 with a slight preponderance of females. Kon *et al.* (1966) described the histological maturity of *T. clavigera* specimens, which were collected on the Yoriihama coast at Niigata Prefecture. In their study, they recognized neither any extraordinary aspect to the gonads nor the presence of a penis in females of the species. Organotin use in antifouling paints began in approximately 1965 in Japan (Takahashi, K., personal communication) and imposex in Japanese gastropods seems to have been occurring locally in the late 1960s. In fact it is known that there were intermediates between male and female *B. japonica* occurring at Kagoshima Prefecture in approximately 1965 (M. Tawara, personal communication).

Organotin pollution and the symptoms of imposex

Serious symptoms of imposex, indicated by RPL index or imposex grade, were observed in specimens collected at the sites near marinas and harbours with heavy shipping traffic (*e.g.* Aburatsubo, Shimoda and Fukuoka), and less severe symptoms of imposex were observed at sites with fewer marine vessels, or where the effects of water currents from the open sea or rivers were strong (*e.g.* Aikawa, Isozaki, Kominato and Urayasu) (Table 3 and Figure 3). It is thus suggested that the symptoms of imposex in *T. clavigera* and *T. bronni* reflect the degree of organotin pollution. This is supported by the relationships between the tissue concentrations of organotin compounds and the degree of imposex in these species (Figures 5 & 6). It was also found that the symptoms of imposex in the specimens were different, even at relatively close sites (*e.g.* between the specimens at Suzaki and Benten on the Izu Peninsula, and between those at Aikawa-Orito and Aikawa-Ogawa on Sado Island, Table 3). Thus it is thought that organotin pollution is intensified in some local areas.

Chemical species of organotin compounds related to imposex

The estimated levels of TBT inducing imposex in *T. clavigera* and *T. bronni* are concluded to be approximately 10–20 ng g^{-1} wet tissue (Figure 5) if only TBT contributes to induction and development of imposex as reported for *Nucella lapillus* by Bryan *et al.*

(1988). However, we found an apparent positive correlation between tissue concentration of TPT and degree of imposex in *Thais* (Figure 6). Horiguchi (1993) showed that TPT induced and promoted the development of imposex in *T. clavigera*, from injection experiments. Also, it is reported that MPT can cause an increase in penis length in *Ocenebra erinacea* (L.) (Hawkins & Hutchinson, 1990). It will be necessary to confirm the effects of phenyltin compounds on induction and development of imposex in *T. clavigera* and *T. bronni*. After the confirmation of the effects of phenyltin compounds, the threshold levels of organotin compounds inducing imposex will again have to be examined in these species.

The symptoms of imposex and their effects on the Thais *population*

We identified many individuals exhibiting grade III and grade IV imposex, which were thought to be sterile, at the sites near marinas and harbours. An increase in the number of such individuals may cause a decline in the local populations of these species. However, it is also possible that these species do not exhibit a population decline because they have a planktonic stage as a veliger larvae for about two months after hatching (Nakano & Nagoshi, 1980), which is different from *N. lapillus* (Bryan *et al.*, 1986). The successful recruitment of larvae from unpolluted or slightly polluted areas to polluted areas may be possible. For this reason, even the populations with high percentages of imposex grades III and IV individuals might be maintained by less affected populations in unpolluted or slightly polluted areas. A long-term field study will be necessary to examine the effects of organotin pollution on the population dynamics in *T. clavigera* and *T. bronni*.

Recommendation of imposex index in Thais clavigera *and* T. bronni

Relative penis length index is thought to be the most appropriate imposex index among mean penis length in females, RPL index and RPS index in these species. The difference in the values of these indices by sites is expected to indicate the difference in degree of organotin pollution. However, when using female mean penis length, it is possible to underestimate the difference in pollution levels, and using the RPS index, it is possible to overestimate this difference. One of the reasons may be the form of the penis in these species. It is longer and thinner than that in *N. lapillus* and the degree of imposex in these species is therefore not expressed accurately by female mean penis length nor RPS index. Also, the variance of the data was the lowest, and the sigmoid curve-fitting was the best, using the relationship between TBT concentration in tissues and RPL index, in both species. Thus the index is recommended as the best expression of the degree of imposex in *T. clavigera* and *T. bronni*. It may also be noted that a change in male mean penis length will change the values of RPL index, even if there is no change in female mean penis length.

The relationships between tissue concentrations of organotin compounds such as TBT and imposex grades I through V were not as well-defined as expected. Several factors may have contributed to this. Firstly, in the earliest stages of the study, grade II and grade III imposex may not have been distinguished clearly by the authors. Secondly, there were occasionally differences in size of the specimens by site, which may reflect differences in tissue concentration due to age. Thirdly, there is the effect of the temporal changes of organotin concentrations in the marine environment by sites, following creation of regulations for the use of TBT and TPT in antifouling paints. Further examination of the relationship between tissue concentrations of organotin compounds and imposex grade would be useful. Although it is known that vas deferens sequence (VDS) is a good index for imposex in *N. lapillus* and *Hinia reticulata* (Gibbs *et al.*, 1987, 1988; Stroben *et al.*, 1992), in this study the authors were unable to examine the VDS for imposex in *T. clavigera* and *T. bronni*, because the vasa deferentia were already well-developed.

Metabolism of organotin compounds in these species

As TBT and TPT concentrations in *T. clavigera* and *T. bronni* were higher than those previously reported in other fish and shellfish, it is thought that TBT and TPT may be readily accumulated in these species. Furthermore, TPT appears to be more difficult to metabolize than TBT, as indicated by the relative occurrence ratios of butyltin and phenyltin compounds in both species. However, it is possible that TBT is also difficult to metabolize, because high positive correlations between TBT and TPT were observed in both species. High concentrations of DBT in both species at some polluted sites may have been accumulated through the consumption of prey, such as mussels, because it is known that DBT concentrations are the highest of the butyltin compounds in mussels (Higashiyama *et al.*, 1991; Shiraishi *et al.*, 1992). Further studies should include laboratory experiments to confirm bioconcentration factors and metabolic rates for TBT and TPT in these species, as the results of this study are based only on data from field studies.

The fact that tissue concentrations of TBT and TPT in females were generally higher than those in males suggests that the reproductive organs of female *Thais* may have high concentrations of TBT and TPT. The distributions of TBT and TPT in the organs of these species should also be examined in future studies.

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