

**ENDOCRINE DISRUPTION RESULTING IN POPULATIONS DECLINE OF
JAPANESE GASTROPOD MOLLUSKS, CAUSED BY TRIBUTYLTIN
AND TRIPHENYLTIN FROM ANTIFOULING PAINTS**

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Introduction

Imposex, the superimposition of male genital organs (penis and vas deferens) on female gastropods, is cause-specific and occurs at low concentrations of certain organotins such as tributyltin (TBT) and triphenyltin (TPHT) ¹⁻⁶. Reproductive failure is known to occur in severely affected stages ^{7,8}. Since the first report from Plymouth, England in 1969, imposex has been reported all over the world for more than 140 species ⁹⁻¹¹. The main cause of population decline in at least seven of these species is considered to be reproductive failure related to imposex ¹². Here, we will report the present status of gastropod imposex and organotin pollution in Japan, together with endocrine disruption observed in abalone.

Materials and Methods

Gastropod specimens were obtained through either collection by authors themselves or purchase from fishermen and/or fishermen's unions. Sex was determined based on the presence of female accessory sex organs, such as albumen, sperm-ingesting and capsule glands, except for abalone. Regarding abalone specimens, sex determination was performed based on histological observation of gonad. A masculinized female was defined as a female with male sexual characteristics: Imposex was recognized to be the individual that had penis and/or vas deferens as well as female accessory sex organs. Anatomical and histopathological characteristics of penis, vas deferens, accessory sex organs and gonad of each individual were observed. After the biological observation, such as imposex examination, tissue concentrations of organotin compounds, such as TBT, TPHT and their metabolites were quantitatively measured by gas chromatography with a flame photometric detection (GC-FPD), as described in Horiguchi *et al.* (1994)¹³.

Results and Discussion

1. Imposex in the rock shell (*Thais clavigera*) and organotin pollution in Japan

Among rock shell samples collected between September 1996 and January 1999 from 93 locations along the Japan coast, imposex was observed in 86 locations whereas no or rare cases were found in the remaining seven locations. The percentage occurrence of imposex was as high as or close to 100% in 73 locations. It is expected that spawning obstruction occurs in more than half the population of females when the relative penis length (RPL) index exceeds 40, based on the relationships among the RPL index, the vas deferens sequence (VDS) index and the percentage occurrence of oviduct (vulva) blockage in females. Among the 93 locations, RPL index values exceeding 40 were found in 40 locations (Fig. 1). High values of RPL and VDS indices were observed in Shizugawa, the southern part of the Miura Peninsula, Shimoda, Hamana Lake, Mikawa Bay, the head of Ise Bay, Osaka Port, the Seto Inland Sea, in the vicinity of Kanmon-kaikyo, Sasebo, Nagasaki Port, Uchinoura, and in the vicinity of Miho Bay. Compared to the results of the survey conducted last time, the index values were slightly decreased or remained almost unchanged in some locations.

TPhT concentrations in the tissue of the rock shell showed a decrease over time but varied distinctly among locations; relatively high pollution levels remaining in a few locations were detected. Decreases in TBT concentrations were not so distinct and the degree of decrease was low compared to that of TPhT (Fig. 2). Changes over time were not observed in some locations near marinas and dockyards.

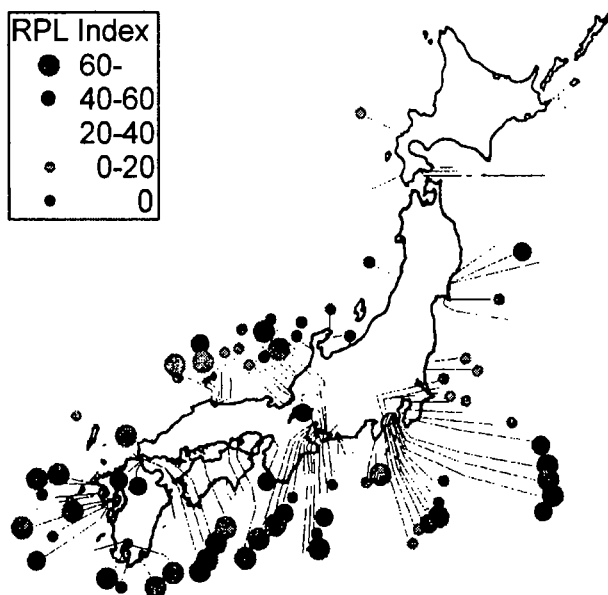


Fig. 1 The geographical distribution of observed values of the relative penis length (RPL) index in the rock shell (*Thais clavigera*) collected in 93 locations along the coast of Japan between September 1996 and January 1999.

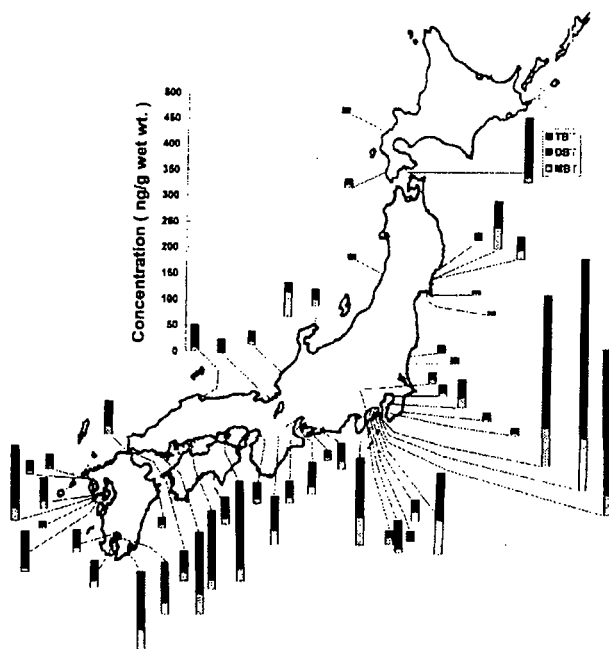


Fig. 2 The geographical distribution of tissue concentrations of TBT and its metabolites in the rock shell (*Thais clavigera*) collected in 93 locations along the coast of Japan between September 1996 and January 1999.

2. Ovarian dysmaturity accompanied by imposex may have brought about decreased catches in the ivory shell (*Babylonia japonica*)

The occurrence of imposex, and decreases in catches and in the amount of spawning from parent snails at seed-producing facilities have been recognized in the ivory shell (Fig. 3). In contrast to the rock shell, however, oviduct blockage due to vas deferens formation is not observed in the ivory shell; thus, physical suppression of spawning is unlikely. It was suspected that decreases in catches were caused by ovarian insufficiency associated with imposex. Gonad tissue preparations were obtained by a conventional method from a total of 135 ivory shell individuals sampled every month between December 1988 and November 1989 in Prefecture A. The results of microscopy revealed a distinct reproductive cycle (peak maturity in summer) in males (43 specimens) but not in females (92 specimens). Females did not show prominent maturity and reproductive cycles similar to males even in summer. In addition to the suppressed ovarian maturity, spermatogenesis in the ovaries was observed in six female specimens (five imposex and one normal). It can be inferred that suppressed ovarian maturity including spermatogenesis in some cases appeared as dysfunction of ovaries and this might be the cause of the reduction in the spawning volume. Interestingly, significant accumulation of TPhT was observed in the ovaries, and the penis length was positively correlated with triorganotin concentration (total concentration of TBT and TPhT) in the ovaries of imposex females (Fig. 4).

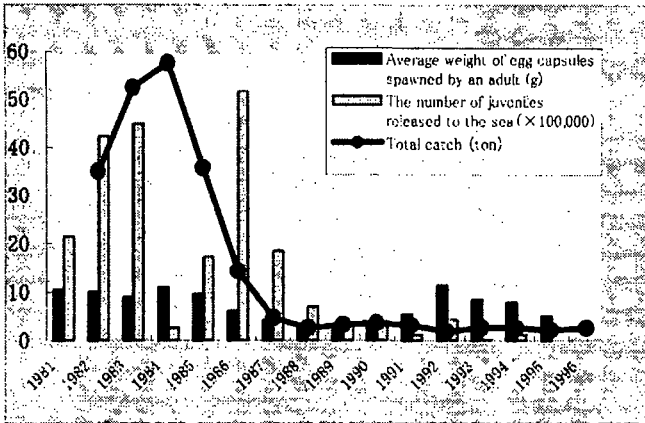


Fig. 3 Temporal trends of total catch and the amount of spawning from parent ivory shell s at seed-producing facilities in Prefecture A.

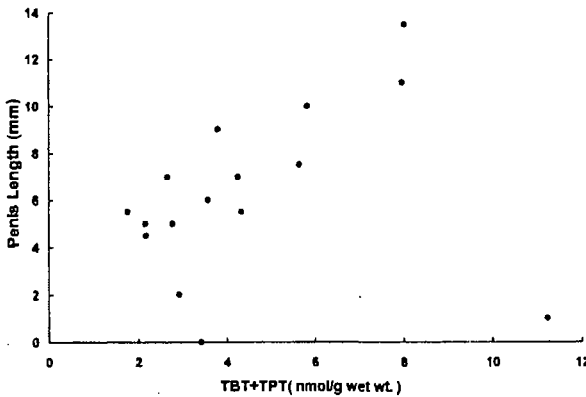


Fig. 4 Positive correlation between triorganotin concentration (total concentration of TBT and TPhT) in the ovaries and the penis length of imposex-exhibited females.

3. Occurrence of imposex in other marine mesogastropods and neogastropods in Japan

Imposex was also observed in Alabaster False Tun, *Galeocorys leucodoma*, (Order Mesogastropoda, Family Oocorythidae) trawled from a depth of 200-250 m off the Atsumi Peninsula in 1999¹². Among the 69 species of Japanese marine gastropods studied including the rock shell and the ivory shell, a total of 39 species (seven from Mesogastropoda and 32 from Neogastropoda) were observed to be affected by imposex (Table 1). Although imposex has been observed mostly in shallow-water species in previous surveys, we need to conduct detailed studies on species living at depths of 200 m or more because of our latest finding of imposex in Alabaster False Tun.

Table 1 Japanese marine gastropods which have been observed to be affected by imposex (up to July 1999)

Mesogastropoda	
Strombidae	<i>Strombus luhuanus</i>
Naticidae	<i>Neverita didyma</i>
Oocorythidae	<i>Galeocorys leucodoma</i>
Cymatiidae	<i>Fusitriton oregonensis</i> , <i>Monoplex echo</i> , <i>Charonia sauliae sauliae</i>
Tonnidae	<i>Tonna luteostoma</i>
Neogastropoda	
Muricidae	<i>Rapana venosa venosa</i> , <i>Ergalatax contractus</i> , <i>Ceratostoma burnetti</i> , <i>Cronia pothuauui</i> , <i>Morula musiva</i> , <i>Morula granulata</i> , <i>Morula marginatra</i> , <i>Drupella fragum</i> , <i>Thais clavigera</i> , <i>Thais bronni</i> , <i>Thais luteostoma</i> , <i>Nucella freycineti heyseana</i> , <i>Nucella freycineti alabaster</i> , <i>Nucella emarginata</i> , <i>Nucella lima</i>
Buccinidae	<i>Japeuthria ferra</i> , <i>Searlesia fuscolabiata</i> , <i>Pusiosstoma mendicaria</i> , <i>Kelletia lischkei</i> , <i>Babylonia japonica</i> , <i>Volutharpa ampullacea perryi</i> , <i>Buccinum middendorffi</i> , <i>Buccinum opisthoplectum</i> , <i>Neptunea arthritica arthritica</i>
Melongenidae	<i>Hemifusus tuba</i>
Nassariidae	<i>Reticunassa festiva</i>
Fasciolaridae	<i>Fusinus perplexus perplexus</i>
Cancellariidae	<i>Sydaphera spengleriana</i>
Conidae	<i>Virroconus ebraeus</i> , <i>Virroconus fulgetrum</i> , <i>Virgiconus lividus</i> , <i>Comus marmoreus bandanus</i>

4. Endocrine disruption in abalone

Abalone catches in Japan have been decreasing since the 1970s. Various studies have been conducted to determine the cause of the decrease but it still remains unresolved. With the hypothesis that organotins (TBT and TPhT) cause reproductive insufficiency in abalone, we have been studying this subject since 1994. Our findings thus far are outlined below¹⁴.

Based on the results of a preliminary survey that was started in 1994, the gonadal maturity of the giant abalone (*Haliotis madaka*) was histologically studied, with area B selected as reference area, which was considered to be normal in terms of catches and other characteristics, and area C selected as the affected area, where decreases in catches were prominent and the ratio of artificially raised and released individuals to the total catch was very high (i.e., reproducibility was very low in the natural abalone population). The results revealed that both female and male giant abalones in area B became sexually mature in the late fall or early winter whereas those in area C did not become sexually mature at this time. In area C, the males exhibited several peaks of sexual maturity in a year but the sexual maturity of females varied widely among individuals and the average level of sexual maturity in the female population was suppressed. While only a hermaphrodite (as evidenced by ovarian spermatogenesis) was found in area B, 11 (20.4%) hermaphrodites out of 54 specimens were observed in area C¹⁴.

Because abalones fertilize externally by releasing sperm and eggs to seawater, it is very important for both sexes to become sexually mature at the same time in order to improve fertility. Their fertility may decrease if there are many sexually immature individuals of either sex or if the peaks of sexual maturity do not coincide for both sexes. Because abalones are known to be dioecious, the observation of approximately 20% hermaphrodites (masculinization of females) in area C is abnormal. This is considered to be a masculinization phenomenon similar to that of imposex observed in the rock shell and the ivory shell¹⁴.

Because imposex in gastropods is known to be typically caused by certain organotins such as TBT and TPhT, organotins are also suspected as a cause of the masculinization observed in the giant abalone in area C. The determined concentrations of organotins in the tissue of the giant abalone from both areas were significantly higher in area C than in area B¹⁴.

An *in situ* exposure test was conducted using caged abalone (*Haliotis gigantea*) transplanted from area B to a location close to a dockyard in area C (giant abalones could not be used for the test because of difficulty in obtaining a sufficient number of individuals). The test was carried out for seven months starting in June 1998 and we collected about 40 specimens in January 1999. Gonad tissue preparations of individual abalones were observed under a microscope, and masculinization, such as ovarian spermatogenesis, was observed in 15 out of 17 female specimens (88.2%). Also, a significant accumulation of organotins during the test period was observed. No ovarian spermatogenesis was observed in the control specimen from area B¹⁴.

The microscopic features of the observed ovarian spermatogenesis are the same as those found in the ivory shell suffering from imposex. Ovarian spermatogenesis and testicular development associated with imposex have also been found in the rock shell and other Neogastropoda species. The findings of our study indicate that masculinization (ovarian spermatogenesis) that can be considered essentially equal to imposex

occurs in abalone as an endocrine disruption phenomenon, without the development of external genital organs such as a penis. We infer that some environmental factor, most probably organotins, caused ovarian spermatogenesis in abalone around the dockyard in area C¹⁴.

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