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A bioassay using sea urchin egg development to identify organotin pollution in sea water

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Abstract— Recording the effects of organotin compounds on sea urchin and mollusk egg development is valuable for marine pollution studies. The results of sea urchin egg bioassays to assess organotin pollution in the surface and bottom waters and sediment pore water at sites around Hatakejima Island in Tanabe Bay are reported. Completely normal developmental rates occurred at a tributyltin (TBT) concentration of $0.01\,\mu g\,L^{-1}$. When clean mud was stored at cool temperatures for one month with water containing TBT, the TBT concentration that did not affect fertilization and development of eggs was 10,000 times higher than the TBT concentration in seawater samples that had no effect on sea urchin egg development. Thus, the mud may absorb and help degrade TBT.

Key words: pollution, bioassay, organotin, sea urchin egg, mollusk egg, developmental abnormality, NOEC (no observed effect concentration), seawater, sediments

Introduction

In 1970, polluted water was found around Hatakejima Island in Tanabe Bay (Figure 1), Central Japan, using sea urchin egg development bioassays. The pollution, thought to be organic pollutants, gradually decreased until 1981 as reported by Kobayashi (1991). From 1982 to 1990 and sometimes in 2006, polluted water was detected around the island and in Tsunashirazu Cove in autumn and winter (Figure 2). The highly polluted water mass was reported by Kobayashi (1991) to be due to tributyltin (TBT) compounds in the water. The contamination was likely caused by antifoulant used because One of the authors (Kobayashi) had found used cans of antifoulant containing TBT at the fish culture base in Tanabe Bay in 1985. In the early 1980s, organotins were found to induce deleterious effects on mollusks such as imposex in dog whelks (Gibbs and Bryan 1986), shell thickening in oysters (Waldock and Thain 1983) and population collapse in mussels (Beaumont and Budd 1984). Regulations controlling the use of organotin compounds have completely prohibited their use in Japan since 1990. Chemical analysis of organotin compounds in water and sediment was reported by Harino et al. (2005a), and its degradation in water was also reported by Harino et al. (2005b). The effects of organotin compounds on the development of sea urchin eggs have been investigated by many researchers, e.g., Cima et al. (1998), Ozretic et al. (1998), His et al. (1999), Marin et al. (2000), Carr et al. (2001), Arizzi Noveli et al. (2002), Moschino and Marin (2002), and Bellas et al. (2005). The effects of organotin compounds on mollusk egg development have been observed by many researchers, i.e., Roberts (1987), His et al. (1999).

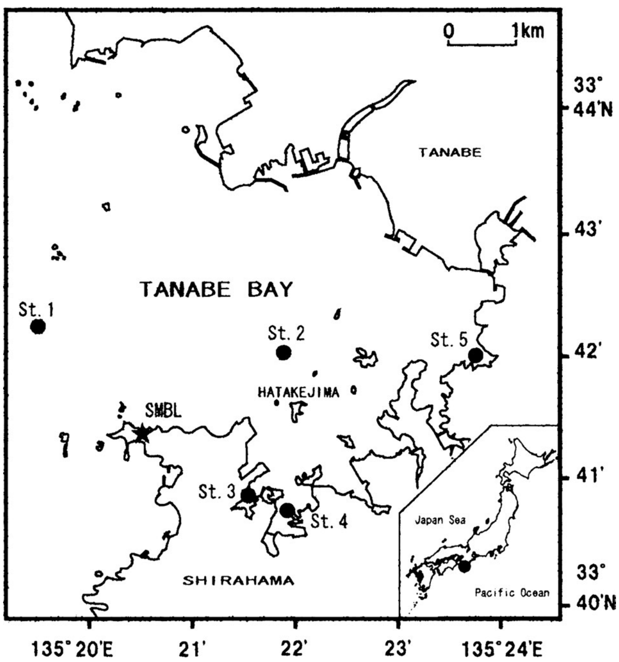

Figure. 1. Tanabe Bay and the sampling stations. St. 1: Open sea, St. 2: Open-sea side of Hatakejima, St. 3: Tsunashirazu ★ SMBL: Seto Marine Biological Laboratory.

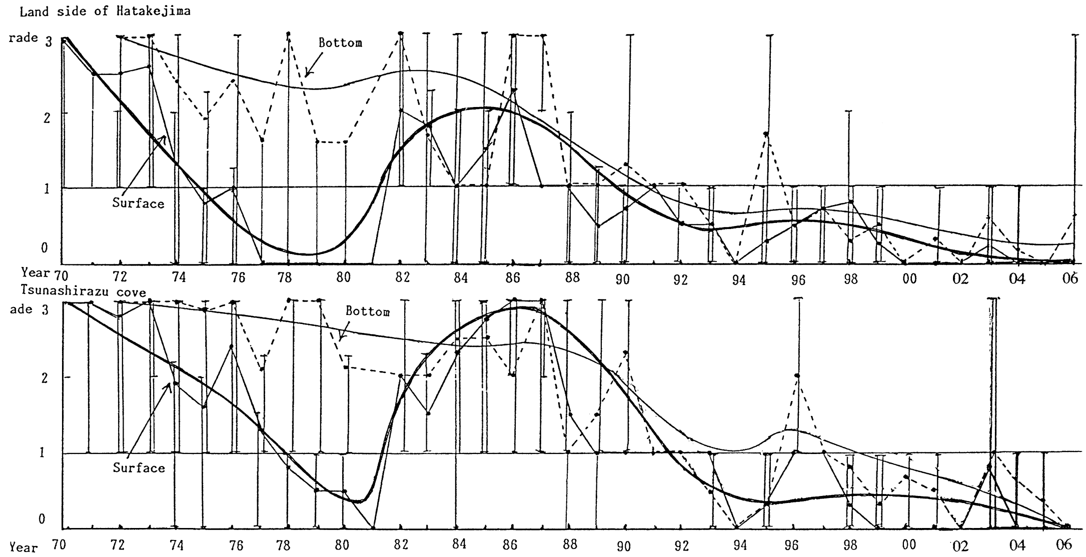

Figure 2. Changes in the average inhibition grade of Ranking IV around Hatakejima Island 1970–2006.

The aim of this paper is to describe the results of sea urchin egg bioassays to assess organotin pollution of the surface and bottom water, sediment pore water and in sediment samples, at sites around Hatakejima Island in Tanabe Bay between 2004 and 2006 (Figure 1). The authors determined if sea urchin bioassays could be used to detect organotin pollution in water and sediment samples taken from around Hatakejima Island. The authors also evaluated the effect of sea water containing known concentrations of TBT on sea urchin egg development and compared our results with those of reports on the effects of organotin compounds on the sea urchin and mollusk egg development. TBT samples were also stored in the refrigerator with clean mud to determine whether mud changes the effective TBT concentration in sea water samples. Finally, the results of the effects of organotins on some of the mollusk development parameters recorded by other researchers are compared with those of sea urchins.

Materials and Methods

Pollution bioassay using sea urchin eggs

Eggs and sperm came from the sea urchin, *Anthocidaris crassispina*, maintained at the Seto Marine Biological Laboratory. Eggs were obtained using the acetylcholine-injection method and sperm was obtained from the testes. About a thousand sea urchin eggs were inseminated and placed in unpolluted seawater or TBT solutions at a range of concentrations. The unpolluted sea water used for the experiments was collected from the open ocean and transported to the laboratory. Ten ml of water was used for each experiment. The timing of first cleavage and pluteus formation and the percentage of abnormalities observed in the developing eggs were determined in each experiment.

In most cases, the first cleavage occurred 55 min after fertilization at 27°C. The rate and state of the first cleavage, namely the proportion of undivided cells and normal divided cells in each experiment, were checked after 60 min. Developmental changes in the eggs were observed after successive divisions. Finally, the proportion of normal plutei, retarded, malformed and pre-pluteal embryos or larvae were recorded 42 h after fertilization.

Two hundred eggs or embryos were fixed with 5% formaldehyde at the completion of the experiments for observe under the microscope precisely. The tests were repeated three times on three different batches of eggs. Stock solution of TBT was prepared in dimethyl sulfoxide). The authors had obtained the TBT from Tokyo kasei kogyo co. Japan. The concentrations were prepared by 1:10 serial dilutions of stock solution with clean seawater.

Test waters

Seawater samples collected from the open ocean were used as controls. Surface and/or bottom water samples and pore water from the mud around Hatakejima Island (Figure 1) were collected. To assess the effect of mud on the TBT concentration, various concentrations of TBT added to unpolluted mud and kept cool for one month were also used. The weight of mud were 50 g each, the TBT concentration range was$10^{-4\sim-8}$ µg L^{-1} and with 25 ml of water respectively. The storage condition was 5°C in refrigerator 1month.

Results

Sea urchin eggs developed normally in the open ocean seawater samples and in all the surface water samples from around Hatakejima Island (Figure 1, Table 1-1). In bottom

Table 1. Supposed effects of organotin compounds on egg development of sea urchin.

Water or Chemicals	Site or Concentration	I st cleavage (60 min) Normal %	One cell %	Pluteus formation (42 h) Normal %	Retard %	Malform %	Prepluteus %	Ultimate state
CONTROL	Laboratory	98.5+0.3	1.5+0.3	96.0+0.6	1.2+1.0	1.0+0.3	1.8+0.3	normal
-1	Open sea	98.7+0.2	1.3+0.2	98.0+0.3	0.8+0.2	0.5+0	0.7+0.2	normal
SURFACE	Open sea side of Hatakejima	98.5+0.3	1.5+0.3	97.5+0.3	1.0+0.3	0.5+0	1.0+0.3	normal
WATER	Tsunashirazu	98.2+0.3	1.8+0.3	97.3+1.0	1.0+0	0.7+0.2	1.0+0.3	normal
-2	Open sea	98.5+0.3	1.5+0.3	97.2+0.6	0.7+0.2	1.3+0.2	0.7+0.2	normal
BOTTOM	Open sea side of Hatakejima	94.2+0.8	5.8+0.8	94.8+1.1	1.8+0.6	1.5+0.3	1.8+0.6	normal ?
WATER	Tsunashirazu	97.2+0.4	2.8+0.4	94.0+1.2	1.8+0.9	2.3+0.2	1.7+0.2	normal ?
-3	Open sea	97.2+0.6	2.7+0.6	97.2+0.4	0.7+0.2	0.8+0.2	1.3+0.3	normal
PORE WATER	Open sea side of Hatakejima	95.2+0.4	4.8+0.4	93.6+0.5	1.8+0.3	1.0+0.3	3.5+0.8	normal ?
FROM MUD	Tsunashirazu	94.0+1.0	6.0+1.0	85.0+2.5	4.3+2.9	3.8+1.3	6.8+1.0	retard
-4	10×−4	0	100					cytolysis
CLEAN MUD	−5	41.2+11.1	58.8+11.1				100	cytolysis
50 g+TBT	−6	85.0+3.3	15.0+3.3	75.2+3.0	7.2+1.9	8.8+2.4	8.8+1.4	retard
WATER 25 cc	−7	96.3+0.4	3.7+0.4	96.5+0.4	1.5+0.3	1.2+0.2	0.8+0.2	normal
1 month in cool								
-5	10×−6	45.2+2.7	54.8+2.7				100	cytolysis
	−7	65.2+3.0	34.8+3.0	18.0+1.0	6.5+1.0	5.0+1.8	70.5+1.8	retard
TBT	−8	74.2+2.0	25.8+2.0	47.0+2.9	10.8+1.4	16.5+9.2	25.7+3.2	retard
	−9	85.7+1.0	14.3+1.0	75.2+2.9	6.3+2.0	10.8+0.6	7.8+1.2	retard
water	−10	92.3+0.4	7.7+0.4	94.8+3.7	1.9+0.1	1.5+0.4	1.8+0.2	normal ?
	−11	97.3+0.4	2.7+0.4	97.2+0.6	0.8+0.2	0.7+0.2	1.2+0.4	normal

waters around Hatakejima Island, normal developmental rates occurred for samples from the open sea, whereas reduced developmental rates occurred in water samples from sites at the open-sea side of Hatakejima Island and Tsunashirazu Cove (Table 1-2).

In the pore-water samples extracted from mud collected at sites around Hatakejima island, developmental rates were normal for eggs in pore water from the open-sea side. Developmental rates for the majority of pore water samples from the other sites were almost normal, except for samples from Tsunashirazu Cove, where the developmental rate was reduced (Table 1-3).

In samples of mud plus TBT water made with clean mud and higher concentrations of TBT ($10^{-4\sim-5}$ $\mu g\,L^{-1}$) cooled for one month, no or only low rates of fertilization and cleavage of eggs occurred, followed by cytolysis. At medium TBT concentration (10^{-6} $\mu g\,L^{-1}$), the cleavage rate and the proportion of eggs with normal development increased. However, retarded and abnormal development of eggs was observed. At low TBT concentration 10^{-7}($\mu g\,L^{-1}$), the frequency of normal developmental rates increased. Completely normal developmental rates occurred in mud containing water at a TBT concentration of 0.1 $\mu g\,L^{-1}$ (Table 1-4).

At higher concentrations of organotin (TBT) in water, eggs had low or no fertilization and cleavage, followed by induced cytolysis (10^{-6} $\mu g\,L^{-1}$ TBT). At medium concentrations, low rates of cleavage and retarded development were evident depending on the concentrations tested ($10^{-9}\sim10^{-7}$ $\mu g\,L^{-1}$ TBT). At lower concentrations, the rate of normal development increased ($10^{-11}\sim10^{-10}$ $\mu g\,L^{-1}$ TBT). The no observed effect concentration (NOEC) for each compound was 0.01 $\mu g\,L^{-1}$ TBT (Tables 1-5 and 2) and 0.001 $\mu g\,L^{-1}$ TPT (Table 2) .

Discussion

Occasionally, during 1990 to 2006, highly polluted water was measured in autumn and winter around Hatakejima Island and Tsunashirazu Cove in Tanabe Bay. Kobayashi (1991) suggested that the polluted water was due to TBT compounds. One of the authors (Kobayashi) had found the used cans of antifoulant containing TBT at the fishery base of Tanabe Bay in 1985.

At that time, the identification of TBT pollution at the fish culture facilities was somewhat secret. The sea urchin bioassay results in our study showed that normal egg developmental rates occurred in surface water samples from sites around Hatakejima Island. Normal developmental rates also

Table 2. NOEC values for embryonic development in literatures cited TBT and TPT.

Animals	Sea urchin					TPT remarks
Genus	Species	Concentration		Literatures		
Anthocidaris	*crassispina*	μg-1	0.01	Kobayashi	1991	
A.	*c.*		0.01	Kobayashi & Okamura	2002	
A.	*c.*		0.01	Kobayashi et al. unpublished data		
			0.001			TPT
Paracentrotus	*lividus*		0.001	Cima et al.	1998	
P.	*l.*		0.8	Ozretic et al.	1998	
P.	*l.*		0.02	His et al.	1999	
P.	*l.*		0.05	Marin et al	2000	
Arbacia	*puncutulata*		1.4	Carr et al.	2001	
P.	*l.*		1.82	Arizzi Novelli et al.	2002	
			0.48			TPT
P.	*l.*		1.5	Moschino & Mariin	2002	TPT
P.	*l.*		0.1	Bellas et al.	2005	
Animals	*Shells*					
Genus	Species	Concentration		Literatures		
Oyster						
Crassostrea	*virginica*		3.96	Roberts	1987	
C.	*gigas*		0.02	His et al.	1999	

Table 3. Concentrations of organotin compounds in waters and muds (Personal communication, Kobayashi et al.).

		Waters		Muds	
Station	Year	TBT	TPT	TBT	TPT
St. 1	2004	<0.001	0.025	13	3.7
	2005	0.002	0.001	3	3
	2006	0.004	<0.001	3	1
St. 2	2004	0.008	0.073	1315	1844
	2005	0.002	0.005	228	531
	2006	0.002	<0.001		
St. 3	2004	0.008	0.032	140	67
	2005	0.014	0.004	85	38
	2006	0.008	0.001	114	95
			(mg/l)		(mg/kg dry)

__: Higher concentration (ca.>0.02), (threshold concentration)

__: Higher concentration (ca.>20), (threshold concentrtion)

occurred in bottom water samples from the open sea water around Hatakejima Island. However, developmental rates were reduced in bottom water samples from the open-sea side of Hatakejima Island and Tsunashirazu Cove. For mud pore-water samples from the open sea, developmental rates were normal and at other sites, they were nearly normal, except for Tsunashirazu Cove, where the developmental rate was retarded. These results indicate that the bottom and mud pore-waters were polluted on the open-sea side of Hatakejima Island and Tsunashirazu Cove, especially the bottom and mud pore water at Tsunashirazu Cove.

Many researchers have reported the effects of various organotin compounds on sea urchin development (as shown in Table 2). These researchers' data are described; the NOEC value for TBT ranges from 1.8~0.001 μg L^{-1}. The NOEC value of TBT is higher than TPT: 3.8 times from the work of Arizzi Novelli et al. (2002), and 10 times the values of Kobayashi et al. (unpublished data). Each study reported NOECs for both TBT and TPT. The TBT and TPT NOECs reflect the relative toxicity of TBT and TPT.

On the other hand, The NOEC value for oysters ranges between 4~0.02 μg L^{-1} TBT according to Roberts (1987) and His et al. (1999). Thus, sea urchin embryos may be more sensitive than mollusk embryos to TBT. In contrast, flow-through exposure experiments carried out over three months induced imposex in adult females of the mollusks *Thais clavigera* (Muricidae: Neogastropoda) at an environmental concentration of approximately 0.001 μg L^{-1} (TBT) according to Horiguchi et al. (1995). This is in the same range as the TBT NOEC for sea urchin embryo development (1.8~0.001 μg L^{-1}). These concentrations may indicate the TBT concentration that induces imposex.

The result that a 10,000-fold higher TBT concentration is required to affect sea urchin egg development when TBT-containing water is stored in cold with mud for one month seems to indicate the absorption of the compounds by the mud which then breaks them down. The mud reduced the effective concentration of TBT in water by 10,000-fold. In this study the TBT concentration range that induced cytolysis, delayed development and allowed normal development was narrower compared with the results of other experiments. The unpublished results of the sea urchin bioassay and chemical analysis by Kobayashi et al. (Personal communication) showed (Fig. 3) that the concentrations of TBT in waters around Hatakejima Island were similar to or less than the NOEC for sea urchins in my present results. In this Table, we have underlined some of the values that exceed a certain concentrations threshold, that is on waters and muds in both otganotin.

However, the concentrations of TPT in waters around Hatakejima Island were higher (25–73 times) than the sea urchin NOEC. Highly polluted water is occasionally observed around Hatakejima Island and Tsunashirazu Cove. The occurrence of polluted water is probably caused by the use of TPT in the area during these times. The recent use of TPT is indicated by the results of the chemical analysis of the mud samples (Kobayashi et al., personal communication). Although this use is, in principle, controlled by the officials, however, the use will contribute an ongoing effect of TPT on marine organisms, even if the organotins already in the local environment are dispersed, diluted, and/or absorbed by bottom mud and then broken down over time.

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