Bioavailability and effects of tributyltin in the caprellid amphipod, *Caprella danilevskii*: A review

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In order to examine the biological effects of tributyltin (TBT), both acute and chronic tests were conducted using amphipod crustaceans. In acute test, five species of caprellids and three species of gammarids collected from Otsuchi Bay, Japan, which belong to a closely related ecological niche, were used for the exposure experiments at seven test concentrations (0, 0.001, 0.01, 0.1, 1, 10 and 100 μg TBTCl l^{-1}) for 48 h at 20°C. The 48-h LC_{50} values of the caprellids were 1.2–6.6 μg l^{-1}, and these were significantly lower than those of the gammarids (17.8–23.1 μg l^{-1}). This suggests that caprellids are more sensitive to TBT than gammarids. Furthermore, in the comparison of the 48-h LC_{50} values for TBT among the various trophic level organisms determined in the previous studies, the caprellids belong to a sensitive group of organisms. The proportions of TBT and its derivatives, dibutyltin (DBT) and monobutyltin (MBT), were measured in the amphipods collected from the bay. In the caprellids, TBT was the predominant compound, accounting for 72% of the total butyltin which reflected the butyltin ratio in seawater, while in the gammarids, TBT's breakdown products (DBT and MBT) predominated, accounting for 75% of the total butyltin. This difference suggests that caprellids may have lower metabolic capacity to degrade TBT than gammarids. Therefore, the difference in sensitivity to TBT among the amphipods is might to be related to the species-specific capacity to metabolize TBT. In chronic test, TBT exposure at ambient water levels, the caprellid amphipod, *Caprella danilevskii*, was exposed to five levels (0, 10, 100, 1000 and 10000 ng l^{-1}) of TBT during the embryonic stage (five days). Although the female proportion was 36% of the total in the control, the female proportion changed dramatically in the hatched juvenile, i.e. the proportion of females was found to increase to 55.6% at 10 ng l^{-1}, 85.7% at 100 ng l^{-1}, and 81.8% at 1000 ng l^{-1}. All specimens died in 10000 ng TBTCl l^{-1} within five days after spawning due to the acute toxic concentration for the species. No significant differences were observed to occur in the sex proportion in response to the exposure after hatching (50 days) in a previous study. Sex disturbance might therefore be induced during the embryonic stage in the caprellid. Reproductive inhibitions such as brood loss and oogenesis inhibition occurred even at 10–100 ng TBTCl l^{-1} exposures in the short-term period in both parental females and their offspring females. The embryo survival rate in the offspring decreased drastically as the TBT concentrations increased, with the decrease being observed at TBT concentrations as low as 10 ng l^{-1} (69%) during the five days. In parental females, the survival rate also decreased at more than 100 ng TBTCl l^{-1}, despite movement after five days into the no TBT-added seawater. Therefore, our data suggest that nanogram concentrations of TBT similar to those encountered in coastal waters around the developed countries can directly affect sex proportion, reproduction, and survival in the caprellid.

Key words: tributyltin, amphipod, caprellid, acute toxicity, chlonic toxicity, metabolic capacity, sensitivity, sex ratio, survival rate, reproduction

INTRODUCTION

It is widely accepted that antifouling paints are the most important contributors of organotin compounds to the marine environment, where they have been responsible for many deleterious effects on nontarget aquatic life (Alzieu 1986, Hall et al. 1988). Accordingly, several countries have already restricted their application, particularly to larger vessels. However, the use of tributyltin (TBT) in antifouling paints is still important for its applications on large sea-going vessels, resulting in environmentally significant TBT water concentrations in the open sea (Rivaro et al. 1999) with approximately 69% of shipping vessels still being painted with paints containing TBT as an antifouling agent (Ambrose 1994). Therefore, despite efforts to reduce its use, TBT levels in the marine environment are still high. Consequently, research on its occurrence and fate in the aquatic environment is needed in order to recognize potential sources and to assess the effectiveness of corresponding environmental management policies.

In organotin compounds, it has become clear about twenty years ago that antifouling chemicals with biocide properties such as those of TBT exert adverse effects on environmental components of the marine ecosystem. TBT is not only the most common derivative in antifouling paints, but also the most toxic organotin species for marine organisms (Alzieu 1989). Numerous investigations have been carried out regarding TBT contamination and its toxic effects in organisms, and several in vivo studies have shown that organometals, including TBT, are immunotoxic, neurotoxic, genotoxic, and hepatotoxic (Aschner and Aschner 1992, Snoeij et al. 1987, Vos et al. 1989, Zelikoff et al. 1988). Recently, accumulation of butyltin compounds (BTs) in marine organisms at various trophic levels in the food chain has been investigated. (Fent 1996, Takahashi et al. 1999). TBT accumulation in the marine ecosystem along the food

chain is different from that of organochlorines (Tanabe and Tatsukawa 1991), with TBT accumulating in most organisms at levels up to ~ 70000 times higher than those in seawater, but with no significant biomagnification being observed in the higher levels of the food chain (Takahashi et al. 1999). High concentrations have, however, been found in lower trophic animals such as caprellids ($78-180 \, \text{ng g}^{-1}$ wet wt). It seems that TBT accumulates specifically for the caprellids in the marine ecosystem regardless of the trophic level in the food chain, and it can be a break point for the disturbance in the natural food chain structure. It is considered causing them to accumulate BTs at elevated concentrations because of their lower metabolic capacity to degrade TBT. Thus, studying the implications of species-specific accumulation and the biological effects of BTs may provide some clues to understanding accumulation mechanisms in the coastal ecosystem and its possible relation to TBT.

The caprellid amphipods are small crustaceans usually 1–3 cm in body length, and are distributed worldwide, especially in algae beds, buoys, and on aquaculture nets of the subtidal zone in temperate regions (McCain and Steinberg 1970). Caprellids are an important trophic link as one of the dominant secondary producers between unicellular algae and fishes in coastal water ecosystem. Furthermore, these organisms are important prey resources for small fishes in coastal ecosystem (Fuse 1962, Caine 1989, Holbrook and Schmitt 1992). The generation length and life span of Caprella have been well-investigated (Takeuchi and Hirano 1991). Caprella danilevskii has a short generation time of 25.6 d, which includes the incubation time of embryos and the maturation time of hatched juveniles, and a life span of only 1–3 months (Takeuchi and Hirano 1991). Therefore, studies on the effects of TBT on caprellids can be convenient, making them an important organism for increasing our understanding of the biological effects of TBT in coastal ecosystem. Recently, the use of caprellids in monitoring temporal and spatial changes in baseline concentrations of BTs has been proposed (Takeuchi et al. 2001, Ohji et al. 2002b). However, there is little information presently available regarding the biological effects in relation to sex proportion, survival rate, growth rate, and reproduction as a function of BTs exposure (Ohji et al. 2002a, b). Such verification is likely a prerequisite to understanding the biological impacts of chemical toxicants as well as the interpretation of BTs accumulation process in the coastal ecosystem.

The objectives of the present review were to examine the sensitivity to TBT, metabolic capacity of TBT and biological effects on the caprellids. Compared to the caprellids, similar experiments conducted on the gammarid, which have a similar ecological niche, habitat, body size and life history (Fuse 1962, Myers 1971, Dahl 1977, Imada et al. 1981, Hiwatari and Kajihara 1988, Hong 1988, Sedberry 1988, Takeuchi and Hirano 1991, 1992a, b, 1995, Holbrook and Schmitt 1992, Horinouchi and Sano 2000). Population-level effects of chemical pollutants are evaluated in terms of decrements of mean extinction time of populations based on LC_{50} values, and estimating extinction risk of populations is utilized for the conservation of wildlife (Tanaka and Nakanishi 2000). Furthermore, the biological effects of TBT exposure at ambient water levels during the embryon-

ic stage of the caprellid amphipod, Caprella danilevskii Czerniavski were examined. The results form the basis of discussions on the fluctuation of abundance of this species in the coastal ecosystem as well as the biological impact of TBT on it.

MATERIALS AND METHODS
Acute toxicity experiments
Specimens

Five species of caprellids, Caprella equilibra, C. penantis R-type, C. verrucosa, C. subinermis and C. danilevskii, and three species of gammarids, Jassa slatteryi, Cerapus erae and Eohaustorioides sp. were collected by SCUBA and dredging from Otsuchi Bay, northeastern Japan, August–December, 1998 and August–September, 1999 (Table 1, Fig. 1). Specimens were used for the experiment within 2 h after collection.

In order to clarify the metabolic capacity to degrade TBT, butyltin accumulation and the proportions of TBT and its derivatives (DBT and MBT) were analyzed in each species collected at the same time and from the same location as the samples used in the acute toxicity tests. Immediately after collection, the samples were placed in polyethylene bags and frozen in a deep-freezer at $-80°C$ until chemical analysis.

Preliminary experiments concerning the solvent effect

Because of the high hydrophobicity of TBT, an organic solvent is required to make TBT dissolve in seawater. In the present experiment, acetone was used as an organic solvent, and a preliminary experiment on the toxic effect of acetone was conducted for Caprella danilevskii. Amphipods were exposed to five test concentrations of acetone (0, 0.0625, 0.125, 0.25 and $0.5 \, \text{ml l}^{-1}$ in seawater). Ten individuals of Caprella danilevskii collected from Otsuchi Bay were kept in deep Petri dishes (9 cm in diameter, 6 cm in height) containing 250 ml of each test solution at 20°C without food for 48 h. Three glass rods (0.1 cm in diameter, 3 cm in length) were set in each Petri dish as substrates. The preliminary experiment revealed that the death rate of caprellids after 48 h was 0% at acetone concentrations below $0.125 \, \text{ml l}^{-1}$. Thus, the acetone concentration in the test solution for the acute toxicity tests was set at $0.05 \, \text{ml l}^{-1}$.

Seawater and TBT solution

The seawater for control and dilution was collected from St. W4 at 10 m below the surface where TBT was expected to be low in August and October, 1998 (Fig. 1).

Test solutions of tributyltin chloride (TBTCl) were made as follows. Prior to the TBT-exposure experiments, the seawater was filtered through a 0.47-μm Millipore filter. A solution of $500 \, \mu\text{g TBTCl l}^{-1}$ was made by adding 0.5 ml of $2000 \, \text{mg TBTCl l}^{-1}$ acetone solution to 2 l of seawater and was then stirred for 12 h by a magnetic stirrer. A solution of 0.05 ml acetone l^{-1} was used as the control, and dilution was also made by adding 0.1 ml of acetone to 2 l of seawater. After stirring, the bottle was plugged and stored at 4°C. The most dense solution of $100 \, \mu\text{g TBTCl l}^{-1}$ was made from $500 \, \mu\text{g TBTCl l}^{-1}$ solution, which was diluted by filtered seawater, and the other five test concentrations (0.001, 0.01, 0.1, 1 and $10 \, \mu\text{g TBTCl l}^{-1}$) were prepared by diluting

Table 1. Sample details of caprellid and gammarid amphipods (Crustacea) collected from Otsuchi Bay. Sampling locations of organisms refer to Fig. 1. Numerical data indicate mean and standard deviation.

| Species	Month and year	Location	Depth (m)	Body length (mm)
Caprellidea				
Caprella equilibra	Oct 1998	St. 3 (Buoy)	3.0	8.0±2.3
Caprella penantis R-type	Aug 1999	St. 2 (Nanamodori)	3.0	5.5±1.0
Caprella verrucosa	Sep 1998	St. 2 (Nanamodori)	2.0	4.9±1.1
Caprella subinermis	Sep 1998	St. 1 (Nagane)	3.0	6.3±1.4
Caprella danilevskii	Sep 1999	St. 2 (Nanamodori)	3.0	5.7±1.0
Gammaridea				
Jassa slatteryi	Sep 1999	St. 3 (Buoy)	3.0	5.1±0.7
Cerapus erae	Nov 1998	St. 5 (Nebama)	10	3.2±0.5
Eohaustorioides sp.	Nov 1998	St. 4 (Kojirahama)	1.0	6.2±0.7
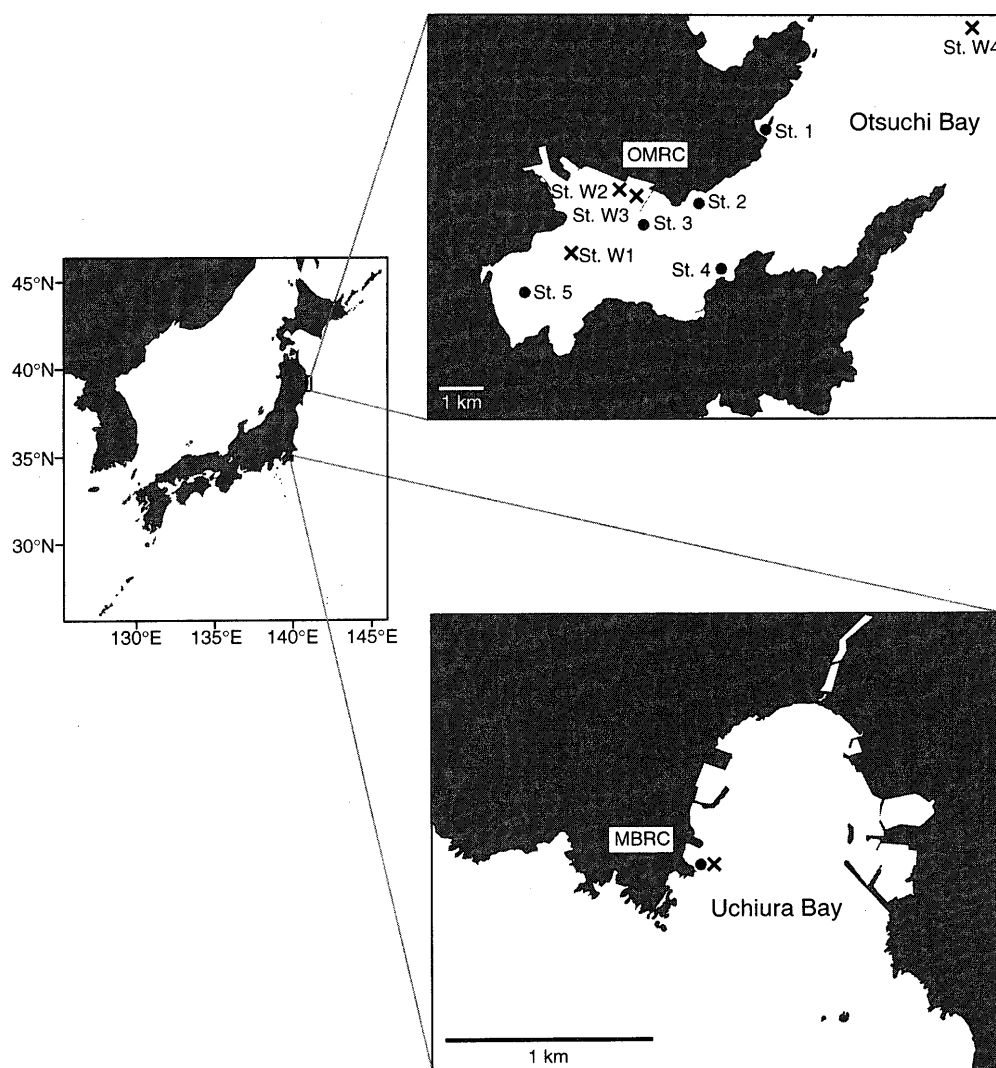
Fig. 1. Map showing the sampling locations of organisms (●) and seawater (×) in Otsuchi Bay (top) and Uchiura Bay (bottom), Japan. OMRC indicates the location of Otsuchi Marine Research Center, Ocean Research Institute, The University of Tokyo. MBRC indicates the location of Marine Biosystems Research Center, Chiba University.

$100 \mu g$ TBTCl l^{-1} solution with 0.05 ml acetone l^{-1} solution.

In order to reveal the concentrations and proportions of butyltin residues in the seawater of Otsuchi Bay, seawater samples were collected at the depth of 0.5 m at Sts. W 1–3 with 1 l polycarbonate bottles in December 1998 (Fig. 1). The seawater collected was immediately acidified with 1 ml of 12 M HCl and stored at 4°C in the dark until chemical analysis. The seawater for control and dilution collected at a depth of 10 m at St. W4 outside the bay in August and October, 1998, was also analyzed and stored in a 20 l poly tank.
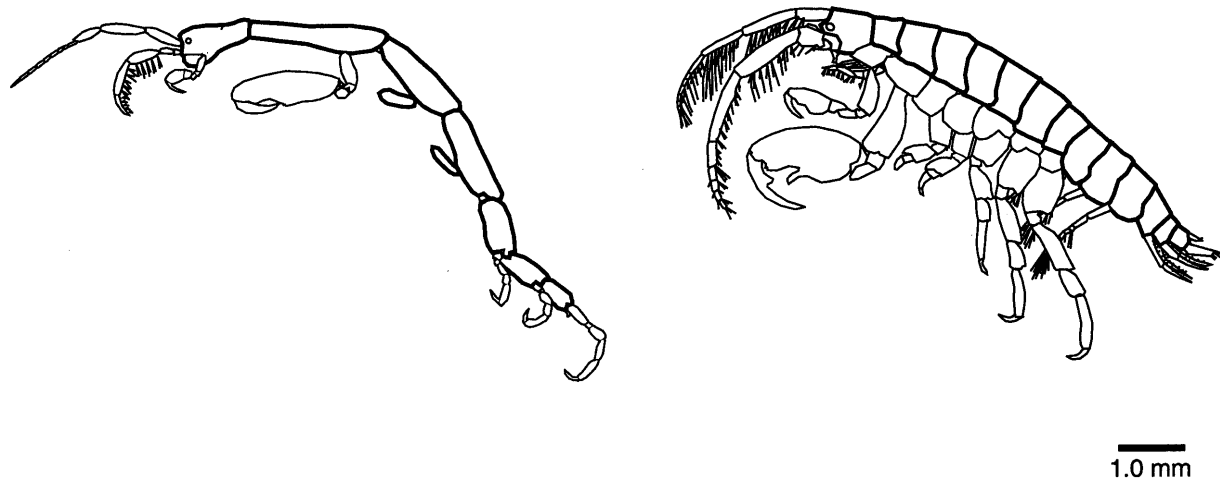
1.0 mm

Fig. 2. Caprellid and gammarid amphipods. Body lengths were measured from the basal part of antenna I on the head to the posterior end of pereonite VII in caprellids and urosome III in gammarids, respectively.

Toxicity experiments

The acute toxicity test was modified from the ecological effect testing method in the risk assessment program of the Organization for Economic Cooperation and Development (OECD) (OECD 1998).

Two deep Petri dishes (9 cm in diameter, 6 cm in height) containing 250 ml of each test solution were prepared 6 h prior to the experiments. Three glass rods (0.1 cm in diameter, 3 cm in length) in each Petri dish were used as substrates. Caprellids and gammarids collected from Otsuchi Bay were immediately brought back to the laboratory, and seven or eight individuals were maintained in each Petri dish at 20°C without food. Survival rates at each test concentration were observed for 48 h. After the experiment, organisms were fixed in 10% formalin. Body lengths were measured from the basal part of antenna I on the head to the posterior end of pereonite VII in caprellids and urosome III in gammarids, respectively (Fig. 2).

TBT concentration in the test solution

Before the experiments, 100 μg TBTCl l^{-1} of the test solution were analyzed to confirm the accuracy of TBTCl present in the test solution by the same method mentioned in the next section. In addition, four other test concentrations (0.1, 1, 10 and 100 μg TBTCl l^{-1}) were also analyzed after the experiments to confirm whether the concentrations remained the same even after 48 h.

Chemical analysis

The analytical procedure for BTs was conducted following a method by means of which organotin compounds were determined by GC-FPD after derivatization using a Grignard reagent "*n*-propyl magnesium bromide" (Takahashi et al. 1999), and this method was slightly modified from previously reported methods (Harino and Fukushima 1992, Iwata et al. 1994, Environment Agency Japan 1990). Briefly, for seawater samples, acidification with HCl as extraction with 0.1% tropolone-benzene was performed. The moisture in the solvent was removed with anhydrous Na$_2$SO$_4$. BTs in the extract were then propylated by adding *n*-propyl magnesium bromide as a Grignard reagent. After decomposition of the excess Grignard reagent with 1 M

H$_2$SO$_4$, the derivatized extract was transferred to 10% benzene-hexane. The extract was then passed through a Florisil packed glass column (eluting with hexane). The final hexane elute from the column was concentrated to 5 ml and subjected to GC quantification. For biological samples, 1–2 g (wet wt) of the whole bodies of crustaceans were homogenized with 0.1% tropolone-acetone and 2 M HCl. The homogenate was centrifuged at 3000 rpm, and BTs in the supernatant were transferred to 0.1% tropolone-benzene. The other steps were similar to those for seawater.

Sample extracts were analyzed by capillary gas chromatography with a flame photometric detection (GC-FPD: Hewlett-Packard 5890 Series II gas chromatograph with a DB-1 capillary column). Monobutyltin trichloride, dibutyltin dichloride and tributyltin chloride of known amounts (0.1 μg each) spiked into uncontaminated seawater and krill containing undetectable levels of butyltin residues were concurrently run with samples through the whole analytical procedure as external standards for seawater and biological samples, respectively. Procedural blanks were included with every batch of samples to check for interfering compounds. The concentrations refer to TBT, DBT and MBT as corresponding ion. The concentration of TBT$^+$ corresponds to 0.89 times that of TBTCl.

Statistics

Median lethal concentration (LC$_{50}$) for 48 h was calculated from a dose-response curve by means of probit methods using EcoTox-Statistics.1.1 (Yoshioka 1998). For the comparison of LC$_{50}$ values between caprellid and gammarid amphipods, statistical analysis was performed by means of a Mann-Whitney U-test using StatView 5.0 (SAS Institute Inc. 1998).

Chronic toxicity experiments

Specimens

Caprella danilevskii was collected by SCUBA from the rocky shore in Uchiura Bay, Japan (Fig. 1), after which specimens were immediately brought to the laboratory and kept in an aquarium provided with running seawater. Premature females and mature males were sorted and provided for the experiments (Fig. 3). Female maturation was
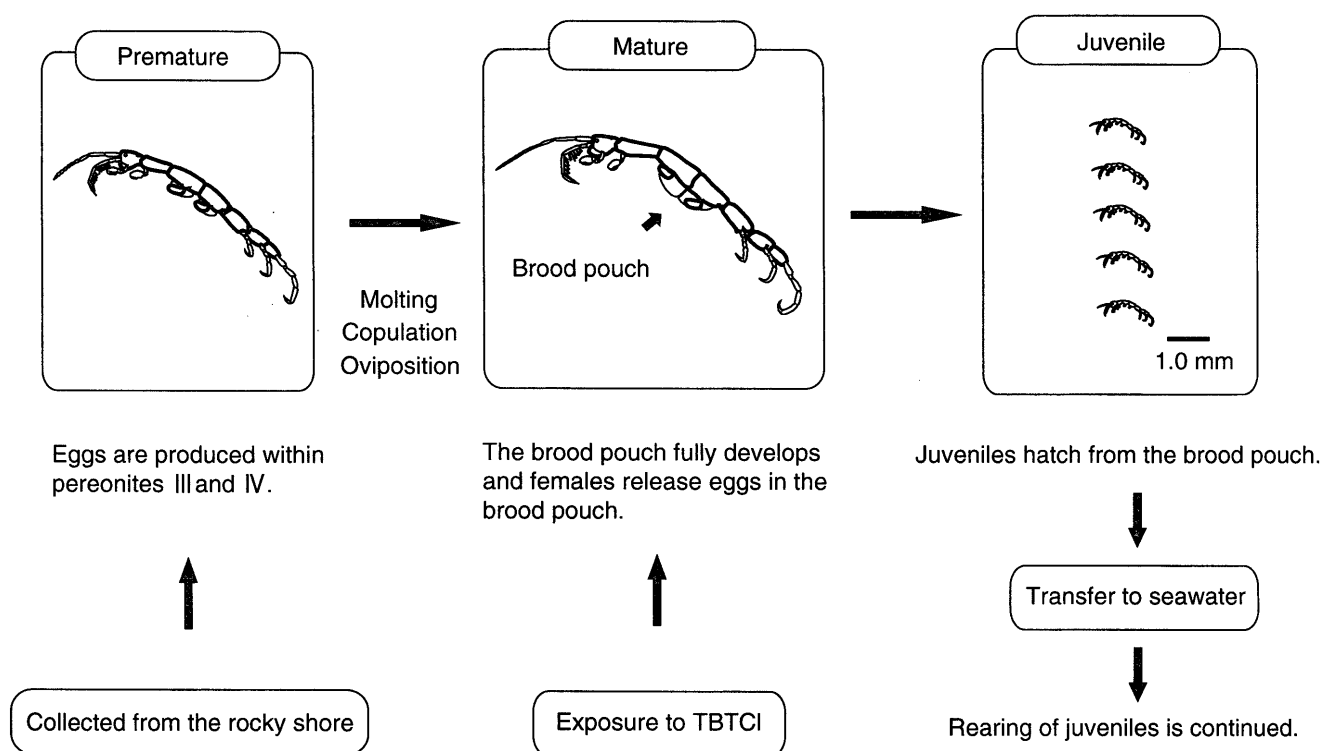
Fig. 3. Schematic view of the experimental methodology used to investigate the biological effects of TBTCl.

divided into three stages: immature, premature and mature based on the morphology of oostegites on pereonites III and IV.

Seawater and TBT solution

The seawater used for the present experiments was collected from a depth of 10 m outside Otsuchi Bay where TBT concentrations at 10 m deep were confirmed to be less than the detection limit (Ohji et al. 2002b).

A tributyltin-seawater solution and the control seawater that contained only acetone were made as follows. Prior to the TBT-exposure experiments, the seawater was filtered through a 0.47-μm Millipore filter. A solution of 10000 ng TBTCl l^{-1} was made by adding 5 μl of 2000 mg TBTCl l^{-1} acetone solution to 1 l of seawater, after which the solution was stirred for 12 hours. Control and dilute solutions were made, adjusting to 5 μl acetone l^{-1} seawater. In the present study, five test concentrations of TBTCl (0, 10, 100, 1000 and 10000 ng l^{-1}) were prepared using dilute solution. Those condensed and dilute solutions were made every week. The five test concentrations of TBTCl were measured to confirm the accuracy of TBTCl present in those test solutions during the experiment in the previous report (Ohji et al. 2002b). The concentrations remained the same between pre- and post-experiments.

To determine the TBT levels in the habitat of the specimens, seawater samples were collected at a depth of 0.5 m together with the specimens using a 1 l polycarbonate bottle. The seawater collected was immediately acidified with 1 ml of 12 M HCl and stored at 4°C in the dark until chemical analysis. TBT concentrations of seawater samples were determined according to our previously described method (Ohji et al. 2002b) and were confirmed to be less than the detection limit. Detection limit of TBT was 2.0 ng l^{-1}.

Toxicity experiments

After confirmation that premature females had reached the mature stage, these parental females were allowed to copulate with males, and spawning was stimulated (first mature stage in parent) (Fig. 3). After spawning in the brood pouch, ovigerous mature females were transferred to Petri dishes (6 cm in diameter, 6 cm in height) containing each concentration of TBTCl, respectively, with a Teflon mesh piece (2 cm×2 cm) as a substrate; specimens were then maintained at 20°C and a 12 : 12 hours light : dark photoperiod. One ovigerous mature female was allocated per dish, and a total of 11 females were used for the exposure experiment (55 females in five-concentration exposure experiments). Colonies of diatom, *Chaetoceros calcitrans* (Paulsen) Takano, were added to each Petri dish once a day; this amount was sufficient to supply the daily dietary demands of the caprellids. The seawater in each dish was changed every day, and Petri dishes and Teflon mesh pieces were replaced every two days. The conditions of ovigerous parental females and egg number in the brood pouch were observed each day at the same time under a binocular microscope.

Specimens were exposed to five concentrations (0, 10, 100, 1000 and 10000 ng l^{-1}) of TBTCl for five days, which corresponded to the period of embryonic development. After being released from the brood pouch, the juveniles were transferred into the filtered seawater containing neither TBTCl nor acetone. Two juveniles were allocated per dish, and a total of 11–25 specimens were used for the exposure experiment (68 juveniles in five exposure experiments). The juveniles released from the brood pouch were classified as instar I. At each instar, the body length of every juvenile was measured from the basal part of the antenna I on the head to the posterior end of pereonite VII

under a binocular microscope. The sex was determined from instar II. The sex of the hatched juveniles was determined based on the presence of oostegites in females and the development of gnathopod II and the presence of abdominal appendages in males.

Furthermore, parental females were also transferred to the filtered seawater. After molting, these females recopulated with a mature male that was collected from the field. After spawning (second mature stage in parent), the eggs were counted at each concentration of TBTCl to examine the effects of TBTCl on the oogenesis stage. In the present study, oogenesis in the premature stage, and embryo development and new oogenesis in the mature stage were distinguishable under the binocular microscope.

After reaching maturity, female juveniles exposed to TBTCl during the embryonic period were allowed to copulate with mature males collected from the field, and spawning was stimulated (first generation of offspring). The eggs in the brood pouch were counted at the same time each day. After juveniles were released from the brood pouch, these juveniles continued to be reared until instar II, and the sex was determined under the light microscope (second generation of offspring). Males and females that survived over 50 days were fixed with 10% formalin. The animals that died during the experiment period were also fixed with 10% formalin.

Statistical analysis

Comparisons of life span between the control condition ($0\,ng\,TBTCl\,l^{-1}$) and each concentration (10, 100, 1000 and $10000\,ng\,l^{-1}$) of TBTCl were carried out by the log-rank test. A comparison of sex proportion between the control and each concentration of TBTCl was carried out by the

chi-squared test. Differences in both reproduction and growth between the control and each concentration of TBTCl were tested by the Mann-Whiney U-test. Comparisons between the number of eggs spawned and the number of juveniles hatched, and between the number of eggs spawned in the first mature stage and the number of eggs spawned in the second mature stage in the parental female were carried out by Wilcoxon's signed-rank test. All statistical analyses were carried out by Stat View 5.0 (SAS Institute Inc 1998).

RESULTS

Acute toxicity experiments

Acute toxicity in amphipods

The 48-h LC_{50} values determined for five species of caprellids ranged from $1.2\,\mu g\,TBTCl\,l^{-1}$ in *Caprella penantis* R-type to $6.6\,\mu g\,TBTCl\,l^{-1}$ in *C. equilibra* (n=5) (Fig. 4). These values in caprellids were significantly lower than those in the three species of gammarids which had LC_{50} values ranging from $17.8\,\mu g\,TBTC\,l^{-1}$ in *Jassa slatteryi* to $23.1\,\mu g\,TBTCl\,l^{-1}$ in *Eohaustorioides* sp. (n=3) (Mann-Whitney U-test, p<0.05). The body lengths of the five species of caprellids were 4.9–8.0 mm, while those of the three species of gammarids were 3.2–6.2 mm (Table 1).

TBTCl concentration in the test seawater solution

The average TBTCl concentration which was produced for $100\,\mu g\,TBTCl\,l^{-1}$ before the experiments was $104\pm 8.7\,\mu g\,TBTCl\,l^{-1}$ (Mean±SD) (n=7). This confirmed the accuracy of the test concentration in the medium. In addition, four other test concentrations (0.1, 1, 10 and $100\,\mu g\,TBTCl\,l^{-1}$) were also analyzed after the experiments, and average TBTCl concentrations were 0.079 ± 0.01, $0.90\pm$
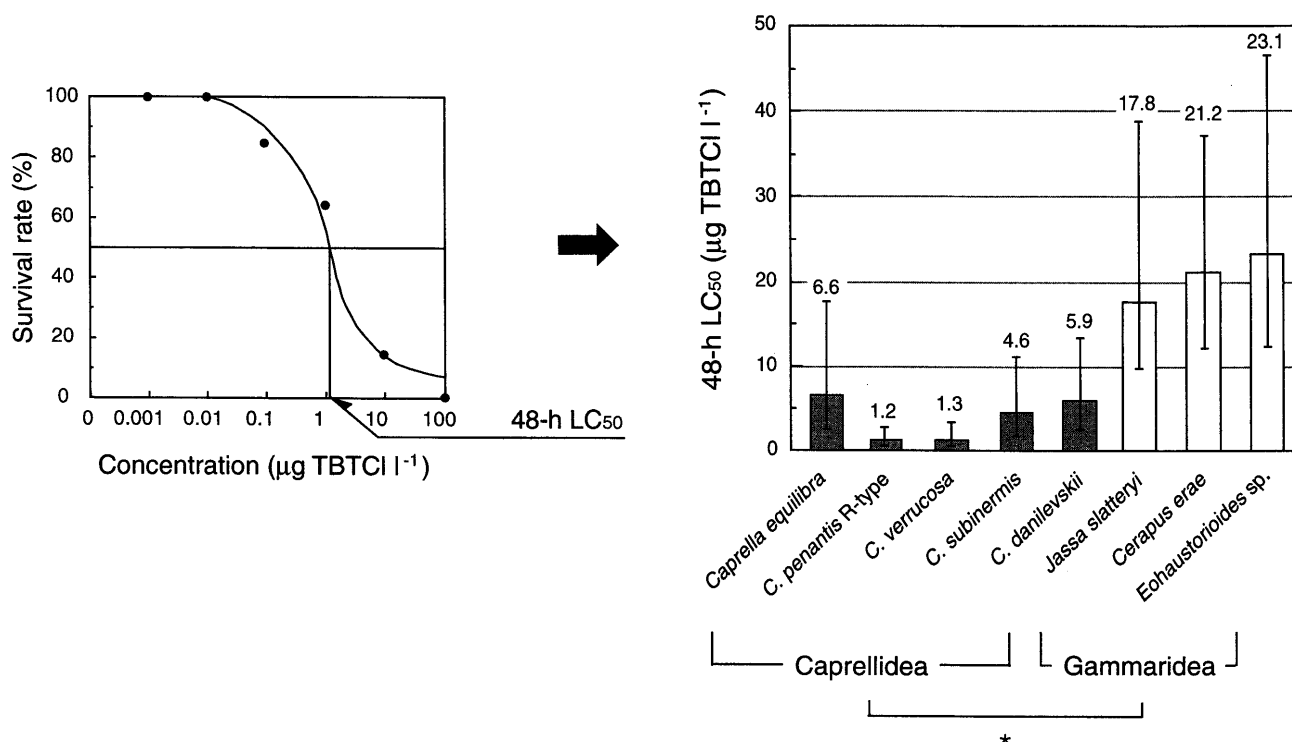

Fig. 4. Comparison of 48h-LC_{50} values for TBTCl in caprellid and gammarid amphipods (Crustacea). Vertical bars indicate 95% confidence intervals. The left figure shows the dose-response curve for 48h-LC_{50} of *Caprella verrucosa* as a representative. Mann-Whitney U-test, *p<0.05.

Table 2. Butyltin concentrations of seawater (ng l^{-1}) and caprellid and gammarid amphipods (Crustacea) (ng g^{-1} wet wt) collected from Otsuchi Bay. ΣBTs: MBT+DBT+TBT. ND indicates the concentration less than detection limit.

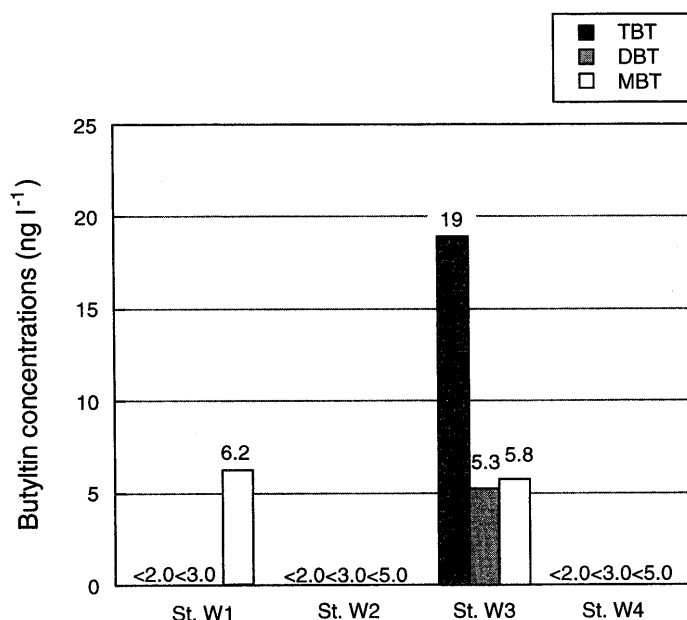| Samples	MBT	DBT	TBT	ΣBTs
Seawater				
St. W1	6.2	<3.0	<2.0	6.2
St. W2	<5.0	<3.0	<2.0	ND
St. W3	5.8	5.3	19	30
St. W4	<5.0	<3.0	<2.0	ND
Caprellidea				
Caprella equilibra	7.4	8.6	55	71
Caprella penantis R-type	9.9	<1.0	38	48
Caprella verrucosa	11	12	81	105
Caprella subinermis	10	7.2	29	46
Caprella danilevskii	16	<1.0	29	45
Gammaridea				
Jassa slatteryi	14	4.9	6.8	26
Cerapus erae	29	11	9	49
Eohaustorioides sp.	46	24	30	100

Fig. 5. Butyltin concentrations (ng l^{-1}) in seawater in Otsuchi Bay. Seawater of Sts. W1–3 was collected at depth of 0.5 m and that of St. W4 was from a depth of 10 m. Detection limits of TBT, DBT and MBT were 2.0, 3.0 and 5.0 ng l^{-1} for seawater samples, respectively.

0.10, 8.6±0.78 and 95±4.9 μg TBTCl l^{-1} (n=2), respectively. This confirmed that the concentrations remained the same even after 48 h. Therefore, the possibility of TBTCl absorption on the surface of the glass containers and evaporation during preparation of solution could be eliminated.

Residue profile of butyltins in seawater of Otsuchi Bay

Butyltins were detected in seawater collected from Sts. W1 to 3 (Table 2, Fig. 5). At St. W3, TBT was the predominant compound at a concentration of 19 ng l^{-1}, accounting for 63.1% of the total butyltin (ΣBTs=MBT+DBT+TBT), followed by MBT, 5.8 ng l^{-1} (19.3%) and DBT, 5.3 ng l^{-1} (17.6%). Concentrations of TBT, DBT and MBT in seawater from St. W4 were below the detectable levels.

Accumulation profile of butyltins in amphipods

Concentrations of ΣBTs in caprellids collected in Otsuchi Bay were 45–105 ng g^{-1} wet wt (n=5), which were comparable to those in gammarids (26–100 ng g^{-1} wet wt) (n=3) (Table 2). In caprellids, TBT was the predominant compound and accounted for 72% of the ΣBT concentrations (n=5) (Table 2, Fig. 7). In contrast, in gammarids, TBT was less than 25% and the breakdown products, DBT and MBT, were the predominant compounds contributing to 75% of the ΣBTs (n=3).

Chronic toxicity experiments

Condition of parental females

Eleven ovigerous females were allocated to each concentration compartment of TBTCl (0, 10, 100, 1000 and 10000 ng l^{-1}). The number of eggs per female ranged from
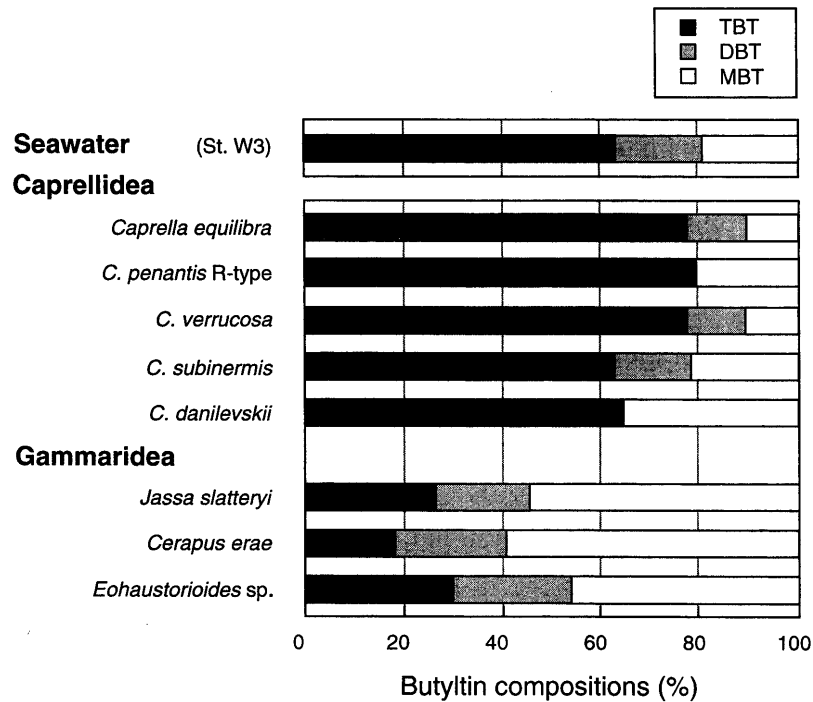
Fig. 6. Butyltin compositions in seawater of St. W3 and the whole body of caprellid and gammarid amphipods (Crustacea) collected from Otsuchi Bay.

Table 3. Reproductive conditions of parental female exposed to TBTCl during the 5 days which corresponds to the first mature stage. Numerical data, ND and dash, indicate mean and standard deviation, no data because of death of all specimens and no observation, respectively.

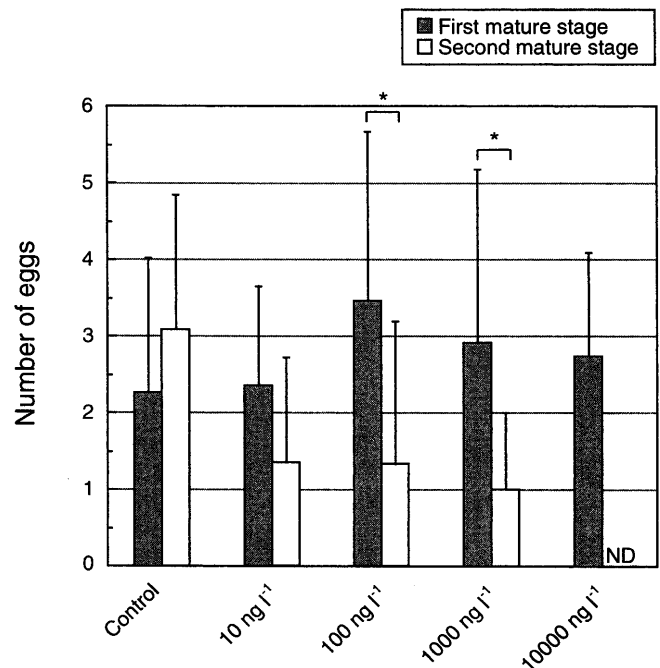| Concentration (ng TBTCl l^{-1})	Number of embryos spawned	Number of juveniles hatched
First spawning		
Control	2.3±1.7	2.3±1.7
10	2.4±1.3	1.6±1.6
100	3.5±2.2	1.3±1.9
1000	2.9±2.3	1.0±1.3
10000	2.7±1.4	0.0
Second spawning		
Control	3.1±1.8	—
10	1.4±1.4	—
100	1.3±1.9	—
1000	1.0±1.0	—
10000	ND	—

Fig. 7. Comparison of the number of eggs released into the brood pouch between the first and second mature stages in the parental female. Vertical bars indicate standard deviation. ND indicates no data because of death of all specimens. Wilcoxon's signed-rank test, *p<0.05.

2.3±1.7 to 3.5±2.2 in the brood pouch (Table 3, Fig. 7). No significant differences were found in the number of eggs spawned between the control and the other four concentrations of TBTCl (Mann-Whitney U-test, p>0.1). A number of deaths of ovigerous females exposed for five days was observed at more than 100 ng TBTCl l^{-1}, and all specimens died at 10000 ng TBTCl l^{-1} due to the acute toxic concentration for the species (Ohji et al. 2002b) (Fig. 8). Brood loss (drop of eggs from the brood pouch) of the females also occurred at concentrations higher than 10 ng TBTCl l^{-1}, ranging from 3 to 6 specimens, while no brood loss was observed in the control (0 ng TBTCl l^{-1}).

The number of eggs per female spawned in the brood pouch in the second mature stage ranged from 1.0±1.0 to

3.1±1.8 (Table 3, Fig. 7). Significant differences were found in the number of eggs between the control and three concentrations (10, 100 and 1000 ng l^{-1}) of TBTCl (Mann-Whitney U-test, p<0.05–0.01). Furthermore, significant differences in the number of eggs were found between the first and second mature stages at 100 ng TBTCl l^{-1} and 1000 ng TBTCl l^{-1} (Wilcoxon's signed-rank test, p<0.05) (Table 3, Fig. 7).
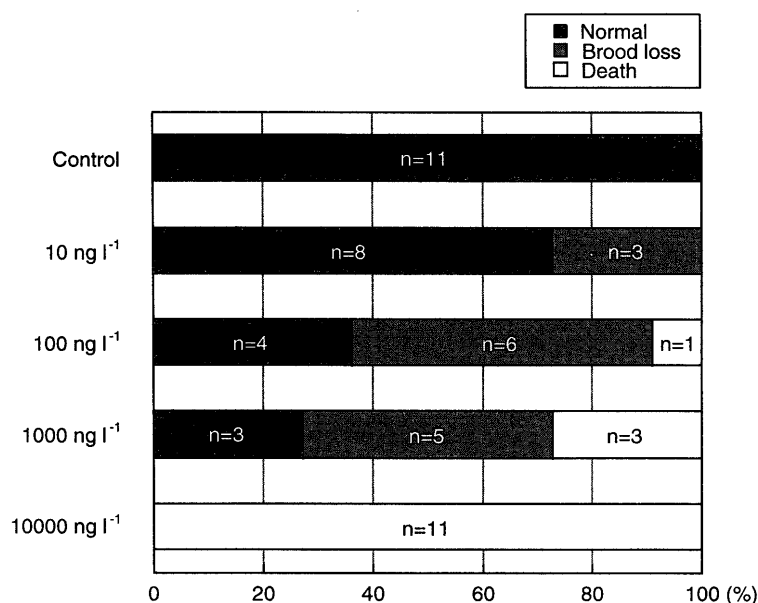
Fig. 8. Condition of the parental female in the first mature stage after 5-day exposure to TBTCl.

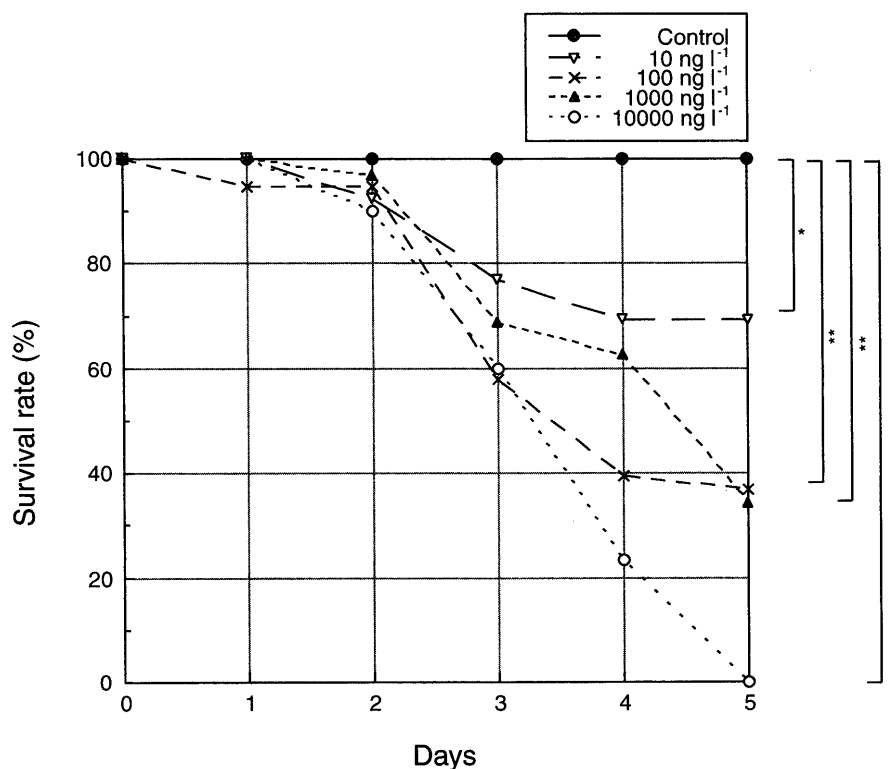**Fig. 9.** Changes in the survival rate in embryos exposed to TBTCl during the embryonic stage (5 days). Log-rank test, *p<0.05, **p<0.0001.

Survival rate in the first generation of offspring

The embryo survival rate (estimated from the amount of brood loss, the number of eggs in the brood pouch in dead specimens, and the total number of eggs) during the TBTCl exposure period decreased as the TBTCl concentrations increased, i.e. 69.2% at $10 \, \text{ng} \, \text{l}^{-1}$, 36.8% at $100 \, \text{ng} \, \text{l}^{-1}$, 34.4% at $1000 \, \text{ng} \, \text{l}^{-1}$ and 0% at $10000 \, \text{ng} \, \text{l}^{-1}$ (Fig. 9). Significant differences were found in the embryo survival rates between the control and the other four concentrations (log-rank test, p<0.05–0.0001).

The number of juveniles hatched per female was $2.3 \pm$

1.7 in the control. However, it decreased as the TBTCl concentrations increased, ranged from 1.6 ± 1.6 at $10 \, \text{ng} \, \text{l}^{-1}$ to 0 at $10000 \, \text{ng} \, \text{l}^{-1}$ (Fig. 10). Significant differences were found between control and $1000 \, \text{ng} \, \text{TBTCl} \, \text{l}^{-1}$ and between the control and $10000 \, \text{ng} \, \text{TBTCl} \, \text{l}^{-1}$ (Mann-Whitney U-test, p<0.05–0.0001). Furthermore, significant differences were found between the number of eggs spawned in the brood pouch and the number of juveniles hatched at $100 \, \text{ng} \, \text{TBTCl} \, \text{l}^{-1}$, $1000 \, \text{ng} \, \text{TBTCl} \, \text{l}^{-1}$ and $10000 \, \text{ng} \, \text{TBTCl}$ l^{-1} (Wilcoxon's signed-rank test, p<0.05–0.01) (Table 3, Fig. 10).

At all concentrations, the survival rate in offspring continued to decrease despite the movement of hatched juveniles into seawater that did not contain both TBTCl and acetone (Fig. 11). Significant differences were found in the survival rate between the control and the other four concentrations (log-rank test, $p<0.0001$). The survival rate of females at maturity decreased to 38.5% at $10 \, \text{ng} \, \text{TBTCl} \, l^{-1}$, 21.1% at $100 \, \text{ng} \, \text{TBTCl} \, l^{-1}$, 15.6% at $1000 \, \text{ng} \, \text{TBTCl} \, l^{-1}$
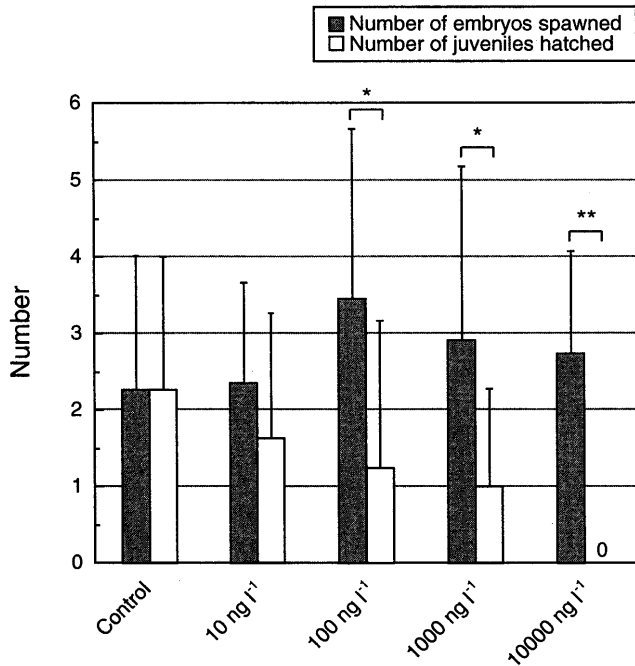

Fig. 10. Comparison between the number of eggs released to the brood pouch and the number of juveniles hatched. Wilcoxon's signed-rank test, $*p<0.05$, $**p<0.01$.

and 0% at $10000 \, \text{ng} \, \text{TBTCl} \, l^{-1}$, although the survival rate in the control was 100% (Fig. 11). The drastic change in survival rate was observed twice, at 10–15 days and during 35–45 days after spawning (Fig. 11).

Sex proportion in the first generation of offspring

The female proportions were 36% in the control (Fig. 12), corresponding to previous field observations (Takeuchi and Hirano 1991). However, as the TBTCl concentrations increased, the proportion of females increased, i.e. 55.6% at $10 \, \text{ng} \, l^{-1}$, 85.7% at $100 \, \text{ng} \, l^{-1}$ and 81.8% at $1000 \, \text{ng} \, l^{-1}$ (Fig. 12). Significant differences occurred in the sex proportion between the control and $100 \, \text{ng} \, \text{TBTCl} \, l^{-1}$ and between the control and $1000 \, \text{ng} \, \text{TBTCl} \, l^{-1}$ (chi-squared test, $p<0.01$).

Growth, maturation and reproduction in the first generation of offspring

In the present study, no significant differences were found in the body length of each instar and in the time taken for each instar from hatching between the control and each concentration of TBTCl in either males or females (Mann-Whitney U-test, $p>0.05$). These results suggest that no growth or molting inhibition occurs after hatching in response to exposure to TBTCl in the embryonic period.

Achievement instar and the day to maturity after hatching in the female caprellid ranged from VIII to IX and from 37 days to 45 days, respectively (Table 4). Significant differences were seen in the achievement instar between the control and $10 \, \text{ng} \, \text{TBTCl} \, l^{-1}$, between the control and $100 \, \text{ng} \, \text{TBTCl} \, l^{-1}$ and between the control and $1000 \, \text{ng} \, \text{TBTCl} \, l^{-1}$ (Mann-Whitney U-test, $p<0.05$–0.01), while no significant differences were seen in the achievement day for all other combinations (Mann-Whitney U-test,
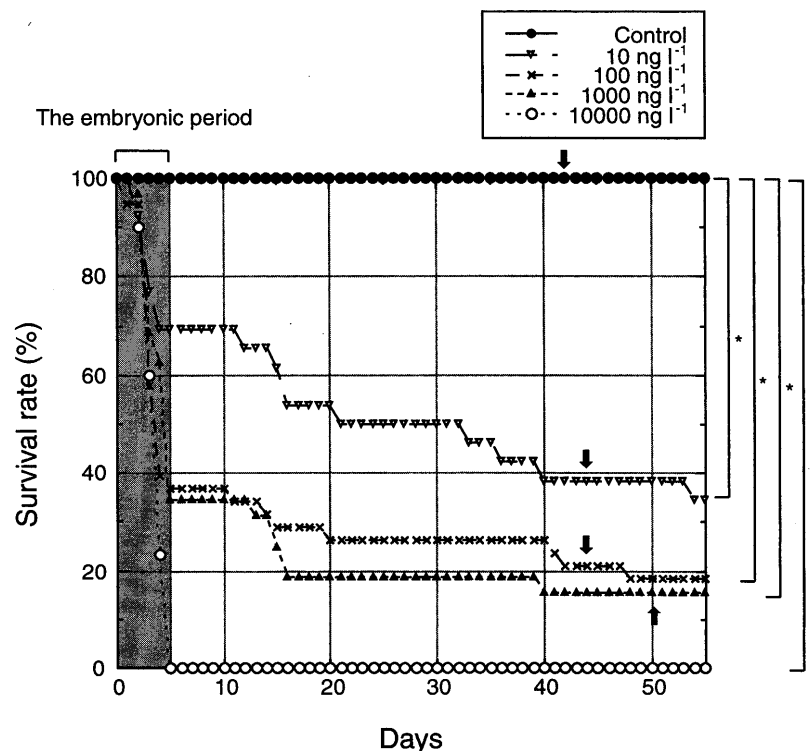

Fig. 11. Changes in the survival rate during spawning and sacrifice in offspring exposed to TBTCl during the embryonic stage and thereafter reared in seawater with no TBTCl added. Arrows indicate the days to mature. Log-rank test, $*p<0.0001$.
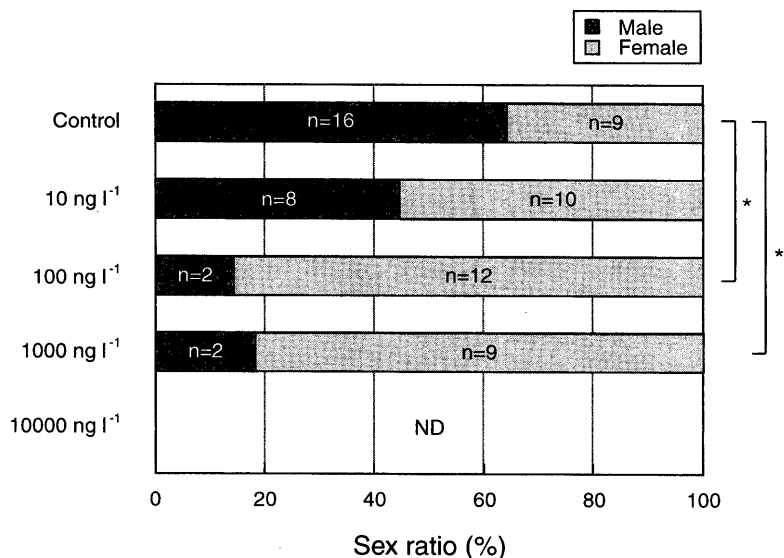
Fig. 12. Sex ratio in offspring of the first generation exposed to TBTCl during the embryonic stage. Chi-squared test, $*p<0.05$.

Table 4. Instar and the day required from hatching to maturation of offspring exposed to TBTCl during the embryonic period. Numerical data and ND indicate mean and standard deviation and no data because of death of all specimens, respectively.

Concentration $(ng\,TBTCl\,l^{-1})$	Instar	Day
Control	VIII±0.4	37±2.6
10	IX±0.5	39±4.3
100	IX±0.0	39±2.5
1000	IX±0.8	45±12.1
10000	ND	ND

$p>0.05$).

In the first mature stage of offspring, oogenesis inhibition and brood loss were observed at $100\,ng\,TBTCl\,l^{-1}$ and $1000\,ng\,TBTCl\,l^{-1}$. Three of six mature females exhibited apparent oogenesis inhibition at $100\,ng\,TBTCl\,l^{-1}$ and three of five at $1000\,ng\,TBTCl\,l^{-1}$. Brood loss was apparent in one of six mature females at $100\,ng\,TBTCl\,l^{-1}$ and in two of five at $1000\,ng\,TBTCl\,l^{-1}$. These abnormal ratios during the mature stage increased as the TBTCl concentrations increased, i.e. 0% at the control and at $10\,ng\,TBTCl\,l^{-1}$, 66.7% at $100\,ng\,TBTCl\,l^{-1}$ and 100% at $1000\,ng\,TBTCl\,l^{-1}$.

Sex proportion in the second generation of offspring

The proportion of females in the control and at 10, 100 and $1000\,ng\,TBTCl\,l^{-1}$ were 28.6%, 28.6%, 22.2% and 33.3%, respectively (Fig. 13). No significant differences in the sex proportion between control and other concentrations of TBTCl were observed (chi-squared test, $p>0.5$). These results suggest that TBTCl exposure in the embryonic period does not affect the sex proportion in the second generation.

DISCUSSION

Acute toxicity experiments

Accumulation of chemical substances in aquatic organisms can occur by water entering through the gills and from food entering through the mouth. Lee (1986) and Lee et al. (1989) carried out TBT exposure experiments involving these two pathways in the blue crab, *Callinectes sapidus*, and their results indicated that the tendency of TBT accumulation depends on organs. In oral exposures, TBT accumulated in the digestive gland (stomach) or hepatopancreas, while in water-borne exposures, the accumulation of TBT was significant in the gills. Thus, the route of uptake of TBT in gill-breathing organisms might be through the gills. In this study, using filtered seawater and no supply of prey, the pathway of uptake of TBT occurred only through the gills. Therefore, the results in this study are believed to reflect the toxic effect of TBT via the gills.

In the present study, the 48-h LC_{50} values in caprellids and gammarids, which belong to the same order, Amphipoda Crustacea, were compared in order to elucidate the acute toxicity of TBT. The 48-h LC_{50} values in caprellids, $1.2–6.6\,\mu g\,TBTCl\,l^{-1}$, were significantly lower than those in gammarids, $17.8–23.1\,\mu g\,TBTCl\,l^{-1}$. Moreover, in the comparison of the 48-h LC_{50} values for TBT among the various trophic level organisms (Table 5), caprellids belong to a sensitive group of organisms. Hayakawa (1976) tested the acute toxicity of the antifouling paint for steel ship's bottom, which contained TBTO as a dominant component, and reported that *Caprella penantis* was more sensitive than fish, *Atherion elymus*, and shrimp, *Leander serrifer*. These facts indicate that caprellids have low resistance to the acute toxicity of TBT. The ecological risk assessment evaluated in terms of LC_{50} values may present a possibility for interpreting the ecological risk of chemical pollutants in the context of population vulnerability (Tanaka and Nakanishi 2000). The concentration at which the intrinsic rate of natural increase corresponds to zero has a highly significant relationship to that of LC_{50} values (Tanaka and Nakanishi 1998). The extinction of a keystone species such as caprellid occupying an influential ecological niche in the food web may induce instability in the coastal ecosystem.

Results of the chemical analysis of the field-collected crustacean and seawater samples showed that TBT predominantly accumulated in caprellids and that the proportions
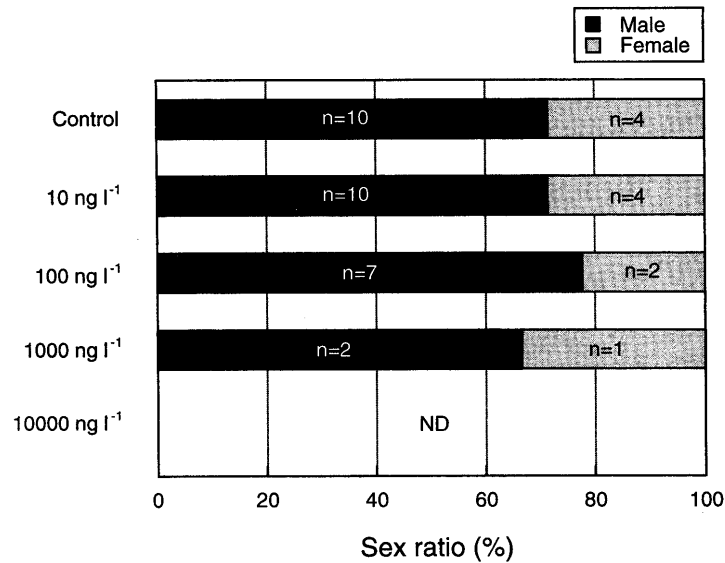
Fig. 13. Sex ratio in offspring of the second generation exposed to TBTCl during the embryonic stage.

Table 5. Review of the 48-h LC_{50} for TBT in various marine organisms. The concentrations were converted into TBTCl. ND indicates no data available.

Organisms	Butyltin	Concentrations ($\mu g\,l^{-1}$)	Temperature (°C)	References
Bacillariophyceae				
Skeletonema costatum	TBTO	15.6	ND	Walsh et al. (1985)
Mollusca				
Crassostrea gigas (Adults)	TBTO	1874	ND	Thain (1983)
Crassostrea gigas (Larvae)	TBTO	1.6	ND	Thain (1983)
Ostrea edulis	TBTO	>312	ND	Thain (1983)
Mytilus edulis (Adults)	TBTO	312	ND	Thain (1983)
Mytilus edulis (Larvae)	TBTO	2.5	ND	Thain (1983)
Copepoda				
Acartia tonsa	TBTO	1.2	20	Bushong et al. (1987)
Eurytemora affinis	TBT	2.5	20	Hall et al. (1988)
Amphipoda: Caprellidea				
Caprella equilibras	TBTCl	6.6	20	This study
Caprella penantis R-type	TBTCl	1.2	20	This study
Caprella verrucosa	TBTCl	1.3	20	This study
Caprella subinermis	TBTCl	4.6	20	This study
Caprella danilevskii	TBTCl	5.9	20	This study
Amphipoda: Gammaridea				
Jassa slatteryi	TBTCl	17.8	20	This study
Cerapus erae	TBTCl	21.2	20	This study
Eohaustorioides sp.	TBTCl	23.1	20	This study
Decapoda				
Crangon crangon (Adults)	TBTO	7.4	ND	Thain (1983)
Crangon crangon (Larvae)	TBTO	6.9	ND	Thain (1983)
Fish				
Agonus cataphractus	TBTO	27.1	ND	Thain (1983)
Oncorhyunchus mykiss	TBT	23.0	20	Alabaster (1969)
Solea solea (Adults)	TBTO	91.5	ND	Thain (1983)
Solea solea (Larvae)	TBTO	8.8	ND	Thain (1983)

of BTs in these organisms were similar to those found in seawater from St. W3 (Table 2, Fig. 5, Fig. 6). In contrast to caprellids, TBT's breakdown products, DBT and MBT, were predominant in gammarids (Table 2, Fig. 6). Thus, as with the above acute toxicity, there was a difference in the proportion of TBT among caprellids and gammarids, nevertheless both these groups of amphipods belong to similar

trophic levels (Imada et al. 1981, Sedberry 1988, Holbrook and Schmitt 1992, Horinouchi and Sano 2000) and share a similar habitat (Imada et al. 1981, Hong 1988) and are similar body size (Myers 1971, Dahl 1977, Hiwatari and Kajihara 1988, Takeuchi and Hirano 1991, 1992a) and life history (Myers 1971, Hiwatari and Kajihara 1988, Takeuchi and Hirano 1991, 1992a, b, 1995). Takahashi et al. (1999)

also reported that caprellids accumulated BTs with a significantly high proportion of TBT compared to gammarids. These results suggest that the metabolic capacity of caprellids to degrade TBT is lower than that of gammarids.

It has been reported that the differences in BT residue levels and the proportion of TBT in organisms are related to environmental and physiological factors (Takahashi et al. 1999). It seems that physiological and ecological characteristics, such as metabolic capacity and trophic levels of each organism, are important factors which influence the pattern of TBT accumulation (Takahashi et al. 1999, Takeuchi et al. 2001). Therefore, the results in the present study suggest that the difference in sensitivity to TBT among the amphipods is related to the species-specific capacity to metabolize TBT.

Generally, it is known that several groups of aquatic organisms, e.g. the Annelida, Arthropoda and Mollusca, have the metabolic capacity to degrade TBT (Maguire et al. 1984, Maguire and Tkacz 1985, Lee et al. 1987, 1989, Francois et al. 1989, Thain et al. 1990) and that metabolic capacity varies in different organism groups (Langston 1990, Laughlin et al. 1986, Lee 1986). For example, crab, *Callinectes sapidus*, fish, *Leistomus canthurus*, and shrimp, *Penaeus aztecus*, are able to metabolize TBTO, while oyster, *Crassostrea virginica*, show only a limited ability to metabolize TBTO (Lee 1986). TBT is metabolized by a detoxifying system involving two phases in vivo. The phase-one reactions involve the cytochrome P-450 dependent mixed-function oxygenase (MFO) system which hydroxylates TBT to alpha-, beta-, gamma-, and delta-hydroxydibutyltin derivatives (Fish et al. 1976). The phase-two reactions conjugate sugars or sulfate to hydroxybutyldibutyltin, and these highly polar conjugates are then rapidly eliminated from the organism. The MFO system of vertebrates and invertebrates is associated with the endoplasmic reticulum of the cell and is a multicomponent enzyme system composed of phospholipid, cytochrome p-450, and NADPH cytochrome P-450 reductase (Lu 1976, Lee 1981, Stegeman 1981). Thus, metabolism of a compound generally reduces persistence, increases elimination, and reduces toxicity (Lee 1996). The Mollusca have low cytochrome P-450 content and mixed function oxygenase activity (Lee 1981, Anderson 1985, Livingstone and Farrar 1985). In addition, it is also considered that differences between organisms in terms of metabolic capacity occur due to the inhibition of the cytochrome system by TBT. The binding of TBT to glutathione S-transferase and cytochrome P-450 results in the inhibition of these two detoxifying enzyme systems (Henry and Byington 1976, Rosenberg and Drummond 1983). Cytochrome P-450 systems control the conversion of cholesterol into a variety of hormones. Inhibition or stimulation of cytochrome P-450 systems can result in changes in hormone production or clearance (Levin et al. 1974, Kupfer and Bugler 1976). Therefore, it is believed that the cause of the different levels of susceptibility to the acute toxicity of TBT in the two groups of amphipods in the present study (Fig. 4) is differences in metabolic capacity. Further study is necessary to provide evidence of the linkage of TBT metabolites and TBT metabolizing enzyme systems to the observed effects.

Chronic toxicity experiments

The present study first demonstrates that the sex proportion in the crustacean changes dramatically even with short exposure to TBT in the embryonic period (five days). Although the female proportion was 36% of the total in the control, the proportion of females was found to increase to 55.6% at $10\,\mathrm{ng}\,\mathrm{l}^{-1}$, 85.7% at $100\,\mathrm{ng}\,\mathrm{l}^{-1}$ and 81.8% at $1000\,\mathrm{ng}\,\mathrm{l}^{-1}$. However, no significant difference was observed in the sex proportion in response to long-term exposure to TBT at these levels after hatching (50 days) in a previous study (Ohji et al. in press). These findings suggest that sex disturbance might be induced during the embryonic stage in the caprellid. The occurrence of sexual abnormality due to chemical pollution, including TBT, has been reported in various marine organisms based on field and laboratory experiments, i.e. masculinization (imposex) in female gastropods by TBT (Smith 1981, Matthiessen and Gibbs 1998), feminization in rainbow trout by alkylphenolic chemicals (Jobling et al. 1996), intersex in harpacticoid copepods (Moore and Stevenson 1991) and American lobsters (Sangalang and Jones 1997).

Though the sex proportion in the present study was changed in response to exposure to TBT, the number of females was almost constant (9–12) regardless of increases in TBT concentrations (Fig. 12). Accordingly, males seem to have a higher sensitivity to TBT than females. However, the survival rate in response to exposure to TBT has been found to be similar regardless of sex in the juvenile stage (Ohji et al. in press). TBT cause the development of imposex in many gastropod (Gibbs et al. 1988). TBT acts as a competitive inhibitor of cytochrome P450-mediated aromatase, resulting in an increase in androgens (Spooner et al. 1991, Bettin et al. 1996) and inhibition of androgen elimination (Ronis and Mason 1996) in gastropods. Therefore, the increase of androgen in vivo may result in androgenization of organisms. Since this phenomenon differs from our results, it is suggested that the action mechanism of TBT might differ among organisms and that the effects of TBT exposure might differ according to the developmental stage. Sex differentiation in crustaceans, i.e. amphipods, isopods and decapods, is known to be controlled by a hormone secreted from the androgenic gland (Charniaux-Cotton 1954, Katakura 1960, Taketomi et al. 1996). Therefore, it is considered that TBT might affect the production of the androgenic gland or the secretary of androgenic hormone in the caprellid. Further experiments are needed to clarify TBT action in the endocrine systems in the caprellid.

Conspicuous reproductive inhibitions such as brood loss and oogenesis inhibition occurred in both parental ovigerous females and ovigerous females of offspring in the first generation, even at nanogram-per-liter levels of TBT exposure (corresponding to present TBT levels in the coastal environment) during the embryonic stage, although such inhibitions were not apparent in the control in *Caprella danilevskii*. A similar phenomenon of impairment of egg production has been reported in the copepod, *Acarita tonsa* (Johansen and Møhlenberg 1987), and in the sea urchin, *Paracentrouts lividus* (Girard et al. 1997, 2000), in response to TBT exposure. The cytotoxicity of TBT often results in an arrest of cellular dynamics, leading to apoptosis (Stridh et al. 1999) or a blocking of cell division (Girard et al.

1997) primarily occurring through an alteration of macro-molecular syntheses (Snoeij et al. 1988, Girard et al. 1997) or membrane-mediated processes controlling cell signaling. These processes consist primarily of a disruption of calcium homeostasis (Chow et al. 1992, Matsuoka and Igisu 1996) or calcium signaling (Corsini et al. 1997 and Girard et al. 1997). Girard et al. (1997, 2000) have found that TBT inhibits sea urchin egg cleavage by altering many of the cellular events related to cell division. Furthermore, Girard et al. (2000) have suggested that the inhibition occurs in response to a few hours of TBT exposure and is sufficient to damage the organism during its embryonic life. A similar inhibition related to egg cleavage might occur in the caprellid, resulting in brood loss and oogenesis inhibition in the species. In the present study, impaired reproductive success also occurred in the short-term exposure to TBT during the embryonic stage (five days). Therefore, our data suggest that nanogram concentrations of TBT similar to those encountered in coastal waters can directly affect reproduction in the caprellid, and that this phenomenon is an environmentally realistic scenario in the coastal ecosystem.

The survival rate decreased drastically as TBT concentrations increased in the present study, with the decrease being found even at $10\,\mathrm{ng\,l^{-1}}$ (69%) despite the short exposure period corresponding to the embryonic period in offspring of the first generation. In parental females, the survival rate also decreased at TBT concentrations more than $100\,\mathrm{ng\,l^{-1}}$ despite movement of females into the no TBT-added seawater after the five-days exposure. These results suggest that TBT exposure even at present levels in ambient water and even for short-period might influence the population in the coastal environment. It is reported that the TBT affect the community of the caprellid at present. The biomass of the caprellid inhabiting sea grasses inner of the

Otsuchi Bay (49.8–125.0 individuals $\mathrm{m^{-2}}$) were a tenth as many as that of the mouth of the bay (1112.5 individuals $\mathrm{m^{-2}}$) (Takeuchi and Hino 1997). The significant difference of the caprellid biomass between inner and mouth of the Otsuchi Bay might be induced by the difference in TBT concentrations at each site because TBT concentrations were higher (3.9–19 $\mathrm{ng\,l^{-1}}$) at inner of the bay than that of the mouth (less than the detection limit) (Takahashi et al. 1999, Ohji et al. 2002b). Furthermore high biomass for caprellids has been reported in Japan since the 1960s (Fuse 1962). Seasonal fluctuations of the epifaunal animals living in the *Sargassum* zone of Kasaoka Bay, Japan from 1956 to 1958 have been studied, with the biomass of the caprellid being reported as 1.3 kg wet wt $\mathrm{m^{-2}}$ (Takeuchi 1998). Recently, such a high biomass and density of caprellid amphipods has not been reported for the coastal waters of Japan or in other developed countries. The caprellid biomass inhabiting the *Sargassum* zone in Otsuchi Bay, Japan, from 1993 to 1995 has been estimated as 100 g wet wt $\mathrm{m^{-2}}$. The present study seems to support the decrease in the caprellid biomass in the coastal ecosystem.

Even at ambient water levels, exposure to TBT during the embryonic stage influences sex proportion, reproduction, and survival in the caprellid as well as the imposex found in gastropods (Fig. 14). Adverse effects on survival, growth, maturation, and reproduction have also been observed in a long-term TBT exposure experiment with exposure occurring after hatching (50 days) at ambient water levels (Ohji et al. in press). It has been reported that organotin compounds degrade slowly in the environment, with the TBT half-life in the water being between 1 and 3 weeks (Seligman et al. 1986, 1988), and in sediment on the order of 1 to 5 years (Waldock et al. 1987, Adelman et al. 1990). Furthermore, it has been reported that caprellids have a
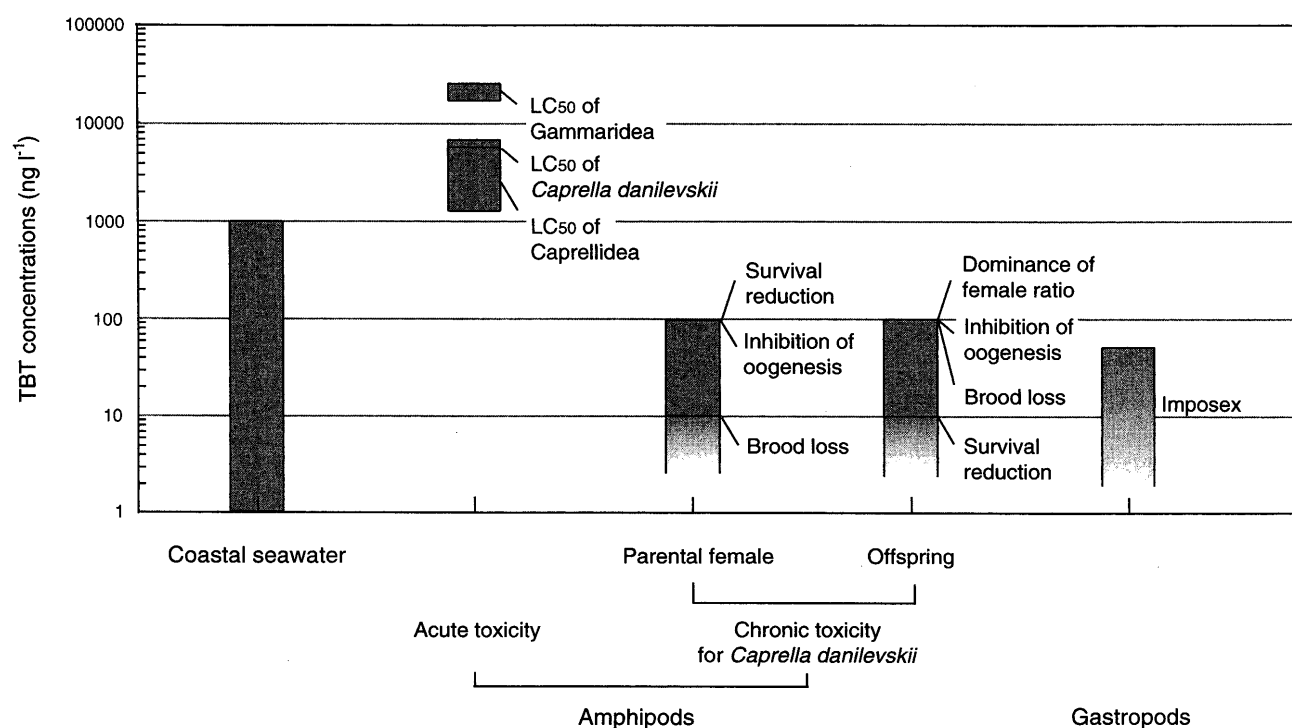

Fig. 14. Biological effects by exposing various levels of TBT in the caprellids and gammarids in the present studies, and in the gastropods. LC_{50} indicates median lethal concentration. TBT concentrations in coastal seawater are cited from Batley (1996). The occurrence levels of imposex in gastropods are cited from Bryan et al. (1986), Gibbs et al. (1988) and Bettin et al. (1996).

lower metabolic capacity to degrade TBT and therefore accumulate TBT at higher concentrations (78–$180\,\mathrm{ng\,g^{-1}}$ wet wt) than other organisms in the coastal ecosystem (Takahashi et al. 1999, Ohji et al. 2002b). Accordingly, TBT exposure, both short- and long-term, in the coastal environment might critically damage the life history characters of caprellids. The impaired reproductive success of a keystone species affects the entire population of species due to drops in the reproductive output below the critical level required for maintaining the population's survival, thus leading to changes in the ecosystem around keystone species (Campbell and Hutchinson 1998). Because caprellids link primary producers to higher consumers in the coastal water ecosystem (Fuse 1962, Omori 1980), the high ecological risk to caprellids due to their high sensitivity to TBT over their life history may result in a disturbance in the coastal water ecosystem.

CONCLUSION

The 48-h LC_{50} values of the caprellids were 1.2–$6.6\,\mu\mathrm{g\,l^{-1}}$, and these were significantly lower than those of the gammarids (17.8–$23.1\,\mu\mathrm{g\,l^{-1}}$). This suggests that caprellids are more sensitive to TBT than gammarids. Furthermore, in the caprellids, TBT was the predominant compound, accounting for 72% of the total butyltin which reflected the butyltin ratio in seawater, while in the gammarids, TBT's breakdown products (DBT and MBT) predominated, accounting for 75% of the total butyltin. This difference suggests that caprellids may have lower metabolic capacity to degrade TBT than gammarids. Therefore, the difference in sensitivity to TBT among the amphipods is might to be related to the species-specific capacity to metabolize TBT. Moreover, in the comparison of the 48-h LC_{50} values for TBT among the various trophic level organisms, the caprellids belong to a sensitive group of organisms.

In chronic test of TBT exposure at ambient water levels, the caprellid amphipod, *Caprella danilevskii*, was exposed to five levels (0, 10, 100, 1000 and $10000\,\mathrm{ng\,l^{-1}}$) of TBT during the embryonic stage (five days). Our data suggest that nanogram concentrations of TBT similar to those encountered in coastal waters around the developed countries can directly affect sex proportion, reproduction, and survival in the caprellid. The present study strongly revealed that the female proportion changed dramatically in the hatched juvenile, i.e. the proportion of females was found to increase to 55.6% at $10\,\mathrm{ng\,l^{-1}}$, 85.7% at $100\,\mathrm{ng\,l^{-1}}$, and 81.8% at $1000\,\mathrm{ng\,l^{-1}}$. Accordingly, even the short-term of TBT exposure, in the coastal environment might critically damage the life history characters of caprellids. Because caprellids link primary producers to higher consumers in the coastal water ecosystem, the high ecological risk to caprellids due to their high sensitivity to TBT over their life history may result in a disturbance in the coastal water ecosystem.

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