Chapter 10
Biological Effects of Tributyltin on the Caprellidea (Crustacea: Amphipoda)

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10.1 Introduction

During the past several decades, butyltin compounds (BTs), one of the representative groups of organotin compounds (OTs), have been widely used as an antifouling agent in paints for boats, ships, and aquaculture nets (Fent 1996, Champ and Seligman 1996), thus these compounds have been found in a variety of marine organisms, often at concentrations exceeding acute or chronic toxicity levels (Bryan and Gibbs 1991; Alzieu 1996). The hazardous effects of antifouling paints containing BTs in marine ecosystem have become a significant environmental issue all over the world (Champ and Wade 1996; Bosselmann 1996). To prevent the destruction of marine ecosystems, BT application to small boats and fish farming equipment has been banned or regulated in developed countries since the late 1980s (Champ and Wade 1996; Bosselmann 1996). Nevertheless, significant accumulation of BTs has been noted at various trophic levels in the marine food chain including plankton, algae, crustaceans, fishes and cetaceans, indicating that BTs impact continues to be felt in marine ecosystems.

Tri-organotins, tributyltin (TBT) are reported to be the most toxic compounds, and at nanogram-per-liter levels, TBT has adverse effects on many aquatic organisms, for example, producing retardation of regenerative growth, delayed molting, reduction in burrowing activity and deformities in limbs in the fiddler crab (Weis and Perlmutter 1987; Weis et al. 1987; Weis and Kim 1988), impairment of egg

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production in the calanoid copepod (Johansen and Møhlenberg 1987), reduction in larval growth in the silverside (Hall et al. 1988) and avoidance reactions in the Baltic amphipod (Laughlin et al. 1984). Recently, a relationship between metabolic capacity, accumulation and toxicity of BTs in marine organisms has been reported in terms of comparisons of BT residue levels in organisms at various trophic levels in the food chain (Fent 1996; Takahashi et al. 1999; Ohji et al. 2002a). The results indicate that though BTs accumulated in most organisms at levels up to 70,000 times higher than those in seawater, no significant biomagnification was 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. 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 (Ohji et al. 2002a). The BTs seem to be accumulated in a species specific manner. Thus, studying the implications of species-specific accumulation and the biological effects of BTs on caprellids may provide some clues to understanding the accumulation mechanisms in the coastal ecosystem as well as the mode of action of BTs in organisms.

Caprellid amphipods are small crustaceans (1–3 cm in body length), and are distributed worldwide, living especially in algae beds, on 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 fish in coastal water ecosystem and form an important prey resources for small fish in coastal water ecosystems (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 duration of 25.6 days which includes the incubation time of embryos and maturation time of hatched juveniles, and has a shortened life-span of 1–3 months. Therefore, caprellids may prove to be a convenient and important model for the study of the biological effects of TBT in coastal ecosystems. Recently, use of caprellids to monitor small temporal and spatial changes in baseline concentrations of BTs was proposed – '*Caprella* watch' (Ohji et al. 2002a). However, little information is presently available regarding TBT's biological effects on such characteristics as sex ratio, survival, growth and reproduction. The determination of such effects is a prerequisite to reliable biomonitoring of the state of coastal ecosystem using *C. danilevskii* as a model.

TBT has had marked effects on the development of imposex in female dog whelks during exposure experiments after hatching (Gibbs et al. 1988). Several hypotheses have been proposed concerning the imposex induction mechanisms, such as those involving cytochrome P450–mediated aromatase inhibition, testosterone excretion–inhibition, functional disorder of female cerebropleural ganglia, and involvement of a neuropeptide–APGWamide (Bettin et al. 1996, Ronis and Mason 1996, Féral and Le Gall 1983, Oberdörster and McClellan-Green 2002, 2003).

Recently, it has been reported that TBT and TPT are high affinity ligands for the human retinoid X receptors (hRXRs) and that the natural ligand of RXR significantly caused the development of imposex in female rock shells (Nishikawa et al. 2004). It is reported that intersex individuals in the caprellid were observed in coastal waters (Takeuchi 1990). Therefore, sex disturbance might also occur in response to TBT exposure after hatching in the caprellids. However, the action mechanism of TBT may differ among organisms and the effects of TBT exposure might also differ according to developmental stage. Therefore, in the present study, two periods of TBT exposure were investigated, including post-hatching and during the embryonic stage in order to examine the biological effects of TBT on the caprellid.

This chapter summarizes experiments to examine: the sensitivity to TBT, capacity to metabolize TBT, and biological effects, on the caprellid amphipods. Compared to the caprellids, similar acute toxicity experiments were conducted on the gammarids, 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; Holbrook and Schmitt 1992; Horinouchi and Sano 2000). Population-level effects of chemical pollutants are evaluated in terms of decrement 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 on the caprellid amphipod, *Caprella danilevskii* Czerniavski were examined after hatching and during the embryonic stage. The results form the basis of discussions on the biological impact of TBT on caprellids and the fluctuation of abundance of this species in the coastal ecosystems.

10.2 Acute Toxicity

10.2.1 Materials and Methods

10.2.1.1 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 (Fig. 10.1). Specimens were used for the experiment within 2 h after collection.

In order to clarify the metabolic capacity to degrade TBT, BT 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 at -80°C until chemical analysis.

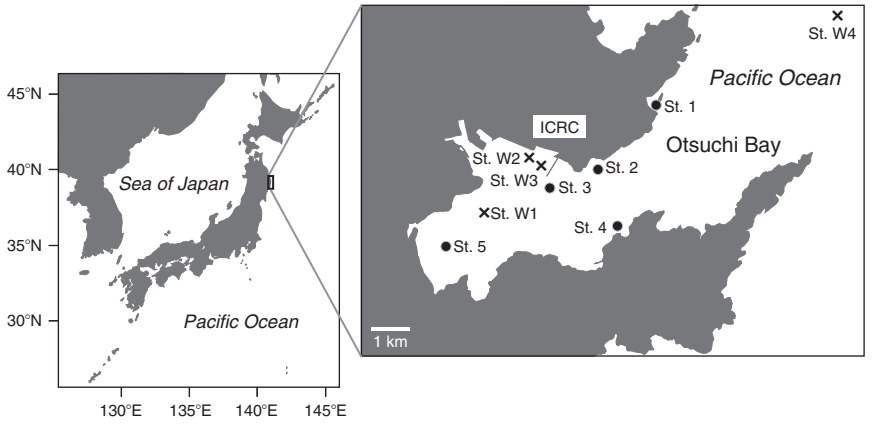

Fig. 10.1 Map showing the sampling locations in Otsuchi Bay, Japan. ● and X indicate the sampling sites of specimens and seawater, respectively. ICRC indicates the location of International Coastal Research Center, Ocean Research Institute, The University of Tokyo

10.2.1.2 Seawater and Tributyltin Solution

The seawater for control and dilution was collected from St. W4 10 m below the surface where TBT was anticipated to be low (Fig. 10.1).

Test solutions of tributyltin chloride (TBTCl) were made as follows. Prior to TBT exposure experiments, the seawater was filtered through a 0.47-μm Millipore filter. A primary solution of 500 μg TBTCl l^{-1} was made by adding 0.5 ml of 2,000 mg TBTCl l^{-1} in 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 highest concentration test solution (100 μg TBTCl l^{-1}) was prepared from the primary 500 μg TBTCl l^{-1} solution, which was diluted with filtered seawater. The other five test concentrations (0.001, 0.01, 0.1, 1 and 10 TBTCl μg l^{-1}) were prepared by diluting the 100 μg TBTCl l^{-1} solution with 0.05 ml l^{-1} acetone solution.

To determine the concentrations and proportions of BT residues in the seawater of Otsuchi Bay, seawater samples were collected at a depth of 0.5 m at Sts. W 1–3 with 1 l polycarbonate bottles (Fig. 10.1). 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 was also analyzed and stored in a 20 l poly tank.

10.2.1.3 Acute 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.

10.2.1.4 Chemical Analysis

Analysis of BTs was conducted by GC-FPD after derivatization using a Grignard reagent "*n*-propyl magnesium bromide" (Ohji et al. 2002a). 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 and extraction with 0.1% tropolone-benzene was performed. The moisture in the solvent was removed with anhydrous Na_2SO_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_2SO_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 3,000 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 flame photometric detection (GC-FPD: Hewlett-Packard 5890 Series II gas chromatograph with a DB-1 capillary column). Tributyltin chloride, dibutyltin dichloride and monobutyltin trichloride of known amounts (0.1 µg each) spiked into uncontaminated seawater and krill containing undetectable levels of BT 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.

10.2.2 Results

10.2.2.1 Tributyltin Concentration in the Test Seawater Solution

The average TBT concentration which was produced for 100 µg TBTCl l^{-1} before the experiments was 104 ± 8.7 µg 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 µg TBTCl l^{-1}) were also analyzed after the experiments, and average TBT concentrations were 0.079 ± 0.01, 0.90 ± 0.10, 8.6 ± 0.78 and 95 ± 4.9 µg TBTCl l^{-1} (n = 2), respectively. This confirmed that the concentrations remained approximately the same even after 48 h. Therefore, the possibility of TBT adsorption on the surface of the glass containers and any evaporation during preparation of solutions could be eliminated.

10.2.2.2 Acute Toxicity in Amphipods

The 48-h LC_{50} values determined for five species of caprellids ranged from 1.2 µg TBTCl l^{-1} in *Caprella penantis* R-type to 6.6 µg TBTCl l^{-1} in *C. equilibra* (n = 5) (Fig. 10.2). These values for caprellids were significantly lower than those for the three species of gammarids which had LC_{50} values ranging from 17.8 µg TBTCl l^{-1} in *Jassa slatteryi* to 23.1 µg 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.

10.2.2.3 Residue Profile of Butyltins in Seawater

Butyltins were detected in seawater collected from Sts. W1 to 3 (Table 10.1). At St. W3, TBT was the predominant compound at a concentration of 19 ng l^{-1}, accounting for 63.1% of the total BTs (ΣBTs = TBT + DBT + MBT), followed by MBT, 5.8 ng l^{-1}

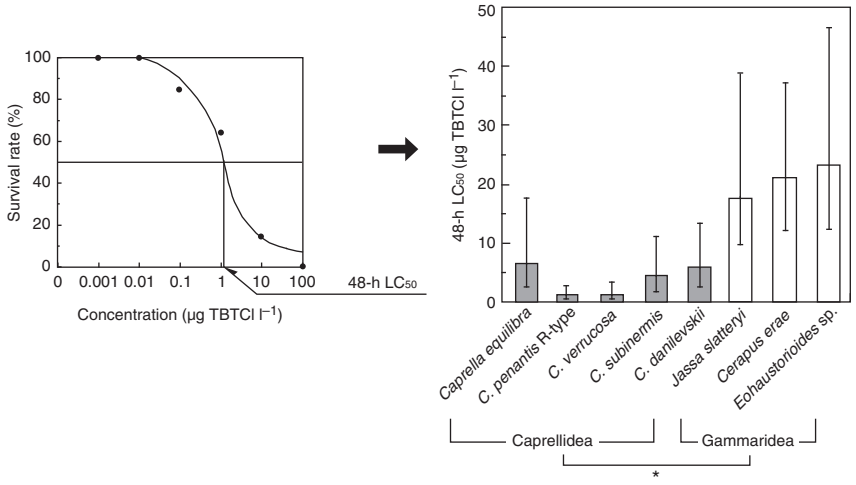

Fig. 10.2 Comparison of 48h-LC_{50} values for TBTCl in caprellid and gammarid amphipods (Crustacea). Bars indicate 95% confidence intervals. The left figure shows the dose-response curve for 48h-LC_{50} of *Caprella verrucosa* as a representative. Mann-Whitney *U*-test, $^{*}p < 0.05$

Table 10.1 Butyltin concentrations of the seawater (ng l^{-1}) and caprellid and gammarid amphipods (Crustacea) (ng g^{-1} wet wt) collected from Otsuchi Bay, Japan

Medium	TBT	DBT	MBT	ΣBTs
Seawater				
St. W1	<2.0	<3.0	6.2	6.2
St. W2	<2.0	<3.0	<5.0	–
St. W3	19	5.3	5.8	24
St. W4	<2.0	<3.0	<5.0	–
Caprellidea				
Caprella equilibra	55	8.6	7.4	64
Caprella penantis R-type	38	<1.0	9.9	38
Caprella verrucosa	81	12	11	93
Caprella subinermis	29	7.2	10	36
Caprella danilevskii	29	<1.0	16	29
Gammaridea				
Jassa slatteryi	6.8	4.9	14	12
Cerapus erae	9	11	29	20
Eohaustorioides sp.	30	24	46	54

ΣBTs = TBT + DBT + MBT

(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.

10.2.2.4 Accumulation Profile of Butyltins in Amphipods

Concentrations of ΣBTs in caprellids collected from 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 10.1). In caprellids, TBT was the predominant compound and accounted for 72% of the ΣBT concentrations (n = 5) (Fig. 10.3). 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).

10.3 Chronic Toxicity – Exposure After Hatching

10.3.1 Materials and Methods

10.3.1.1 Specimens

Caprella danilevskii was collected by SCUBA from the rocky shore in Ostuchi Bay, northeastern Japan, and the specimens were brought back to the laboratory and kept in an aquarium provided with running seawater. Premature females and mature

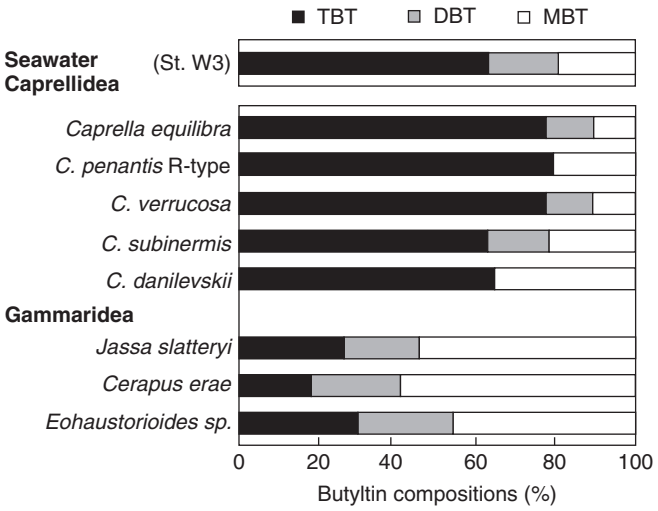

Fig. 10.3 Butyltin speciation in the seawater of St. W3 and the whole body of caprellid and gammarid amphipods (Crustacea) collected from Otsuchi Bay, Japan

males were sorted and provided for the experiments (Fig. 10.4). These specimens were kept in deep Petri dishes (6 cm in diameter, 6 cm in height) which contained filtered seawater with a Teflon mesh piece (2 × 2 cm) as substrate, and maintained at 20°C under a 12:12 h light: dark photoperiod. One ovigerous mature female was allocated per dish, and a total of four females were prepared for an exposure experiment (20 females in five concentration-exposure experiments). Diatom colonies *Chaetoceros calcitrans* (Paulsen) Takano were added once a day to each Petri dish; this amount was more than sufficient to meet 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 2 days. After the confirmation that premature females had reached the mature stage, mature females were allowed to copulate with males, thus stimulating the release of eggs in the brood pouch. After releasing eggs into the brood pouch, mature males were transferred to other Petri dishes, and ovigerous mature females were held within the filtered seawater. The condition of mature females such as hatching and the emergence of juveniles was observed under a binocular microscope at 12 h intervals each day.

10.3.1.2 Seawater and Tributyltin Solution

The seawater used for these experiments was collected at 10 m depth outside Otsuchi Bay, where TBT concentrations at 0.5 and 10 m deep were confirmed to be less than the detection limit (Ohji et al. 2002a), and stored in a 20 l polyethylene tank. The tributyltin-seawater solution and the control seawater that contained only

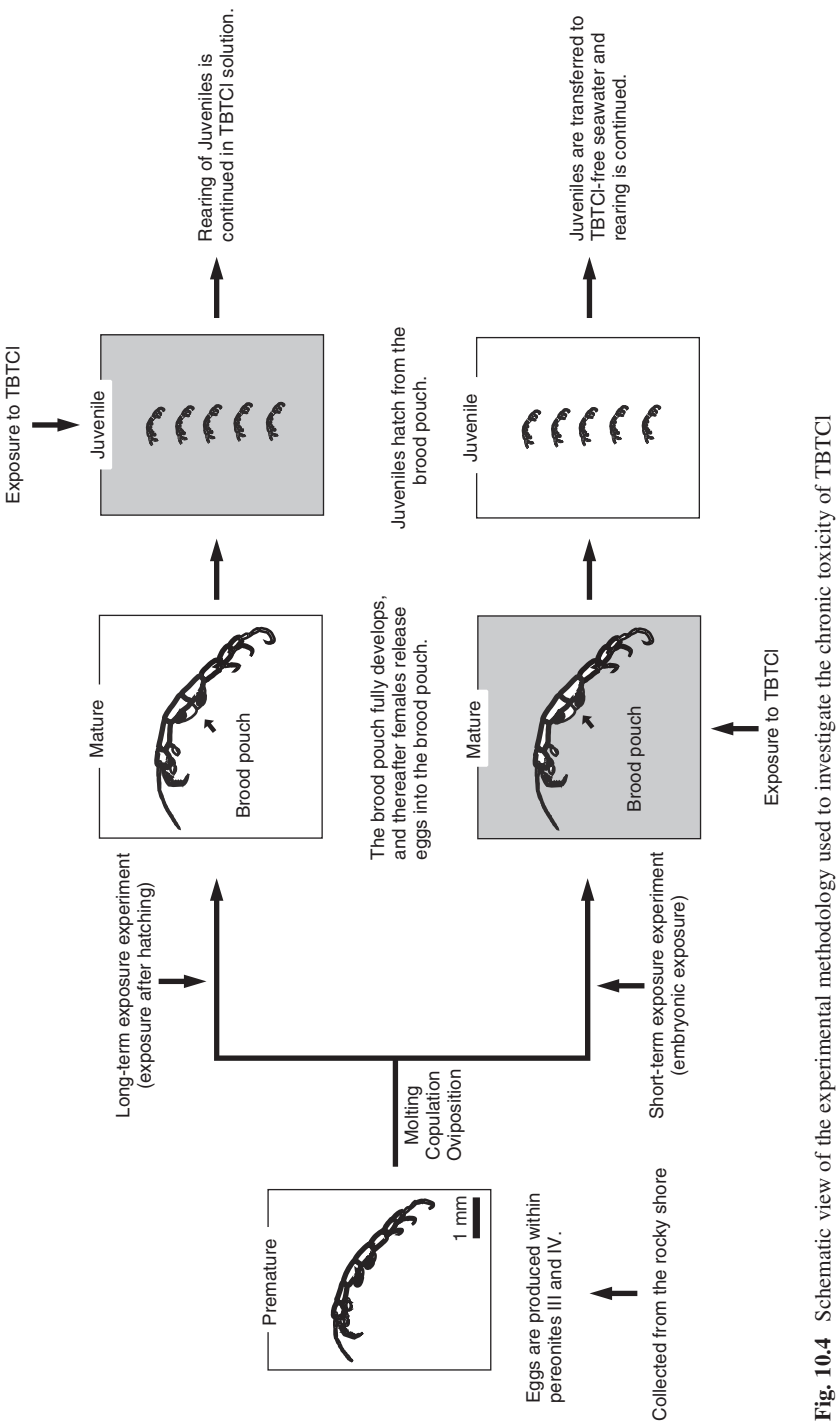

Fig. 10.4 Schematic view of the experimental methodology used to investigate the chronic toxicity of TBTCl

acetone were made in the following procedure. Prior to the TBT exposure experiments, the seawater was filtered through a 0.47 µm Millipore filter. A solution of 10,000 ng TBTCl l^{-1} was made by adding 5 µl of 2,000 mg TBTCl l^{-1} acetone solution to 1 l of seawater, and thereafter the solution was stirred for 12 h. Control and diluent solutions were prepared using 5 µl acetone l^{-1} seawater. In the present study, five test concentrations of TBTCl (0, 10, 100, 1,000 and 10,000 ng l^{-1}) were prepared by dilution of the stock solution. These solutions were freshly prepared each week. The five test concentrations of TBTCl were measured to confirm the accuracy of TBTCl present in the test solutions during the experiment in the previous report (Ohji et al. 2002a). The concentrations remained the same between pre- and post-experiments.

10.3.1.3 Exposure Experiments After Hatching

After hatching from the brood pouch, juveniles were transferred into Petri dishes containing the TBTCl solution at each concentration with Teflon mesh pieces set as a substrate, and were continued to rear. Two juveniles were allocated per dish, and a total of 20–27 specimens were used for the exposure experiment (122 juveniles in five exposure experiments). The juveniles that emerged from the brood pouch were classified as instar I. Their body lengths were measured from the basal part of the antenna I on the head to the posterior end of pereonite VII. The sex was determined from instar II. The maturity of the females was divided into three stages: immature, premature and mature based on the morphology of the oostegites on pereonites III and IV.

Mature females were allowed to copulate with mature males collected from the field and to release eggs in the brood pouch. The number of eggs in the brood pouch was counted at the same time each day. 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. Males and females that survived over 50 days were fixed with10% formalin, as were the animals that died during the experiment period. The sex of the hatched juveniles was determined from the presence of oostegites in females and the development of gnathopod II and the presence of abdominal appendages in males.

10.3.2 Results

10.3.2.1 Sex Ratio

Sex ratio of male to female was 55.0% and 45.0%, respectively in the control (0 ng TBTCl l^{-1}) (Fig. 10.5). The ratio was almost constant in spite of increasing of the TBTCl concentrations ranging from 50.0% (1,000 ng TBTCl l^{-1}) to 55.6% (100 ng TBTCl l^{-1}) for males and ranging from 44.4% (100 ng TBTCl l^{-1}) to

50.0% (1,000 ng TBTCl l^{-1}) for females, although all specimens died in the 10,000 ng TBTCl l^{-1} experiment because of acute toxic concentration for this species (Ohji et al. 2002a). No significant differences were found in the sex ratio between the control and other three concentrations of TBTCl (chi-squared test, $p > 0.5$).

10.3.2.2 Survival

As the TBTCl concentrations were increased, survival rates within 50 days after hatching decreased, 25.0% in 10 ng l^{-1}, 11.1% in 100 ng l^{-1} and 8.3% in 1,000 ng l^{-1} (Fig. 10.6). All specimens died in 10,000 ng TBTCl l^{-1} within 4 days after hatching, while all control specimens survived (100%). Significant differences were found in the survival rate between the control and the other four concentrations of TBTCl (log-rank test, $p < 0.0001$).

10.3.2.3 Growth

In each concentration of TBTCl except for 10,000 ng l^{-1}, body length increased as the organism became older (Fig. 10.7). However, significant differences were seen in body length between the control and 100 ng TBTCl l^{-1} and between the control and 1,000 ng TBTCl l^{-1} in each instar after instar II of either males or females (Mann-Whitney *U*-test, $p < 0.05$). No significant difference was found in the body length between the control and 10 ng TBTCl l^{-1} in each instar of either males or females (Mann-Whitney *U*-test, $p > 0.05$). This indicates that a decrease in growth rate results after exposure to 100 and 1,000 ng TBTCl l^{-1} in spite of the organism's sex.

The number of days required between hatching to the instar X, which corresponds to the experimental period in the control, 10 and 100 ng TBTCl l^{-1}, was approximately

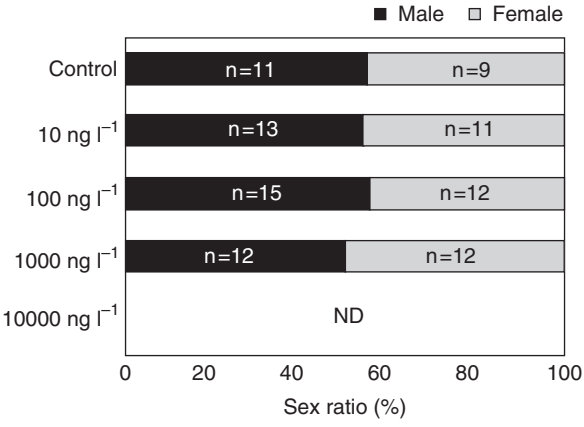

Fig. 10.5 Sex ratio in juveniles exposed to TBTCl after hatching. ND indicates no data because of the death of all specimens

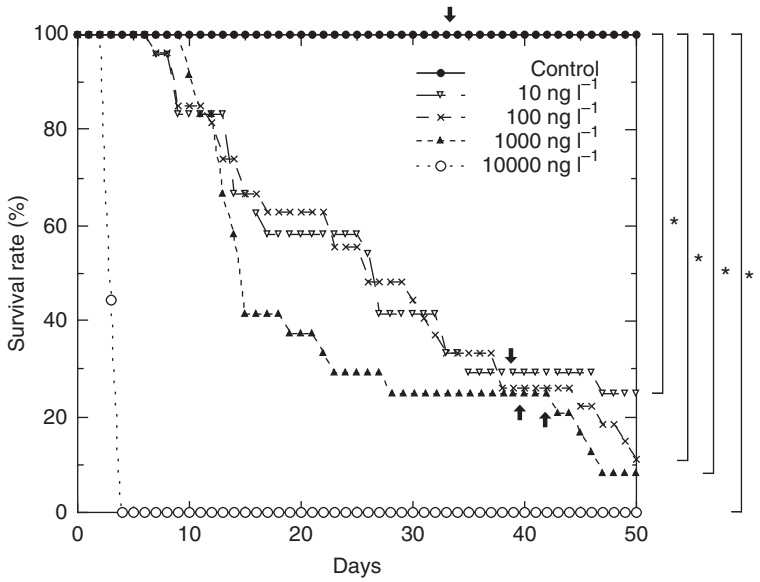

Fig. 10.6 Survival rate of specimens exposed to TBTCl after hatching. The number of hatched juveniles was calculated as 100%. Arrows indicate the days required from hatching to maturation in females. Log-rank test, $^{*}p < 0.0001$

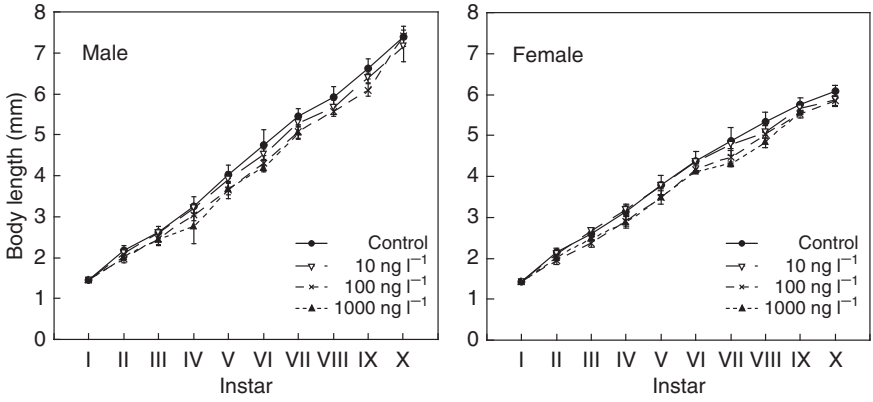

Fig. 10.7 Body length at each instar of specimens exposed to TBTCl after hatching

50 days for both males and females. Although all male specimens died after instar VII and females died after instar IX in 1,000 ng TBTCl l^{-1} (Fig. 10.8), a significant difference was found in the day required from hatching to each instar between the control and 1,000 ng TBTCl l^{-1} in both males and females (Mann-Whitney *U*-test, $p < 0.05$). However, no significant differences were found between other combinations (Mann-Whitney *U*-test, $p > 0.05$).

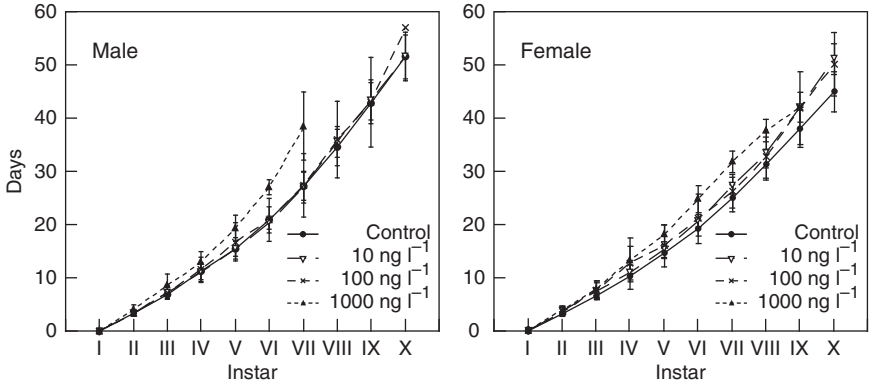

Fig. 10.8 Days required from hatching to each instar of specimens exposed to TBTCl after hatching

Table 10.2 Instar and the days required from hatching to maturation of juveniles exposed to TBTCl after hatching

Concentration (ng TBTCl l^{-1})	Instar	Day
Control	VIII ± 0.5	33 ± 1.6
10	VIII ± 1.0	39 ± 1.4
100	VIII ± 0.6	39 ± 2.3
1,000	IX	42
10,000	ND	ND

Numerical data and ND indicate mean and standard deviation and no data because of death of all specimens, respectively

10.3.2.4 Maturation and Reproduction

The instar and day required from hatching to maturity in the female caprellid ranged from VIII to IX and from 33 to 42 days, respectively (Table 10.2). Significant differences were seen in the day required from hatching to maturity between the control and 10 ng TBTCl l^{-1} and between the control and 100 ng TBTCl l^{-1} (Mann-Whitney *U*-test, $p < 0.05$), while no significant differences were seen in the instar required from hatching to maturity for all other combinations (Mann-Whitney *U*-test, $p > 0.05$). Though all specimens were observed to mature completely during instar VIII and instar IX in the control, several specimens died at premature and immature stages during those instars and instar X in other TBTCl concentrations. This suggests that a delay in the day required from hatching to maturity is caused by exposure in TBTCl.

After maturation, the number of eggs in the brood pouch, the number of juveniles hatched and the period from spawning to juvenile hatching ranged from 2.0 to 2.7, from 0.3 to 2.7 and from 5.0 to 6.0 days in the increasing TBTCl concentration (Table 10.3). A significant difference was found in the number of juveniles hatched between control and 100 ng TBTCl l^{-1} (Mann-Whitney *U*-test, $p < 0.05$).

Table 10.3 Reproductive conditions of mature females exposed to TBTCl after hatching

Concentration (ng TBTCl l^{-1})	Number of embryos spawned	Number of juveniles hatched	Incubation period of embryos	Duration of instar
Control	2.7 ± 1.7	2.7 ± 1.7	5.0 ± 0.0	7.0 ± 0.9
10	2.0 ± 2.8	0.5 ± 0.7	6.0	7.5 ± 2.1
100	2.7 ± 3.1	0.3 ± 0.6	6.0	9.7 ± 2.1
1,000	ND	ND	ND	ND
10,000	ND	ND	ND	ND

Numerical data and ND indicate mean and standard deviation and no data because of death of all specimens, respectively

Molting interval after maturation ranged from 7.0 to 9.7 days in each TBTCl concentration. A significant difference was seen between the control and 100 ng TBTCl l^{-1} (Mann-Whitney *U*-test, $p < 0.05$). This indicates that a delay in the molting interval is caused by exposure to TBTCl.

The decrease in the number of eggs because of eggs dropping from the brood pouch (brood loss) was found in one of two females that reached the mature stage, while the other did not succeed in egg formation in 10 ng TBTCl l^{-1}. In 100 ng TBTCl l^{-1}, brood loss was found in two of three females that reached the mature stage and the other one did not succeed in egg formation. The percentages of hatching success after spawning were decreased as the TBTCl concentration increased except for 1,000 and 10,000 ng l^{-1}, i.e. 100% in the control, 25.0% in 10 ng l^{-1} and 12.5% in 100 ng l^{-1}. In 1,000 and 10,000 ng l^{-1}, all specimens died before or after reaching maturation.

10.3.2.5 Morphological Alterations

During the experiment period, morphological alterations such as loss of gill, contraction of gill, necrosis and loss of pereopod, cramp of pereopod (mis-clinging to the substrate with pereopod), molting disorder, and substances attached on the surface of the body were observed, and their occurrence increased as the TBTCl concentration increased (Fig. 10.9).

10.4 Chronic Toxicity – Embryonic Exposure

10.4.1 Materials and Methods

10.4.1.1 Specimens

Caprella danilevskii was collected by SCUBA from the rocky shore in Uchiura Bay, Japan, 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. 10.4).

10.4.1.2 Seawater and Tributyltin Solution

The seawater used for the present experiments was collected from a depth of 10 m outside Otsuchi Bay.

A tributyltin-seawater solution and the control seawater were made according to our previously described method in the section of TBT exposure after hatching. In the present study, five test concentrations of TBTCl (0, 10, 100, 1,000 and 10,000 ng l^{-1}) were prepared using dilute solution. These 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. 2002a). The concentrations remained the same between pre- and post-experiments.

10.4.1.3 Embryonic Exposure Experiments

After confirmation that premature females had reached the mature stage, these parental females were allowed to copulate with males, and spawning was stimulated

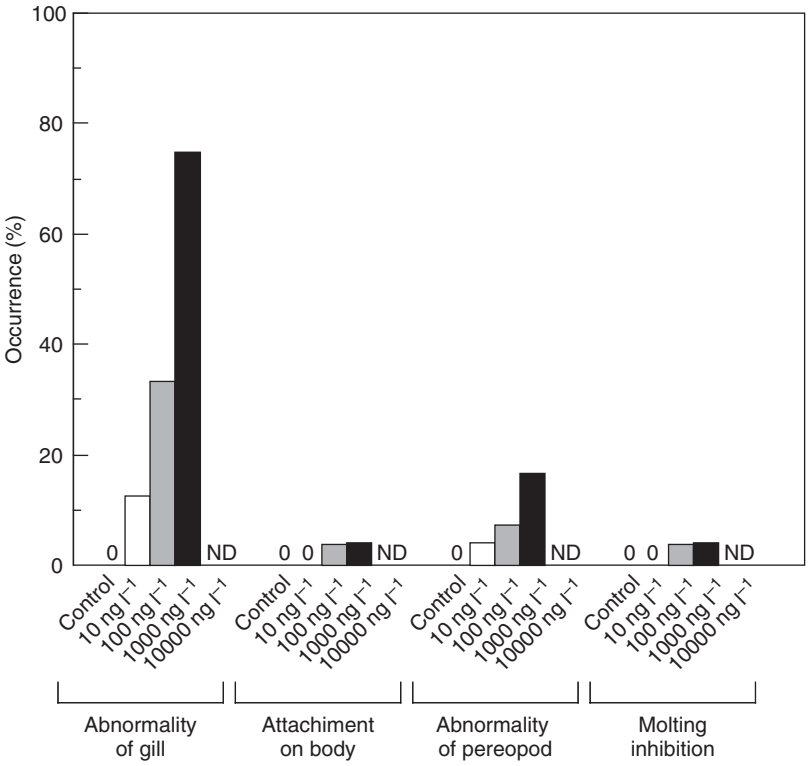

Fig. 10.9 Occurrence of morphological alterations of specimens exposed to TBTCl. Numerical data and ND indicate number of specimens and no data because of the death of all specimens, respectively

(first mature stage in parent) (Fig. 10.4). 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 × 2 cm) as a substrate; specimens were then maintained at 20°C and a 12:12 h 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 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 2 days. The condition 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, 1,000 and 10,000 ng l^{-1}) of TBTCl for 5 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. The sex was determined from instar II.

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.

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 were continued to rear 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.

10.4.2 Results

10.4.2.1 Condition of Parental Females

Eleven ovigerous females were allocated to each concentration compartment of TBTCl (0, 10, 100, 1,000, and 10,000 ng l^{-1}). The number of eggs per female ranged from 2.3 ± 1.7 (mean ± SD) to 3.5 ± 2.2 in the brood pouch (Table 10.4). 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 5 days were observed at more

Table 10.4 Reproductive conditions of mature female exposed to TBTCl during the 5 days which corresponds to the first mature stage

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
1,000	2.9 ± 2.3	1.0 ± 1.3
10,000	2.7 ± 1.4	0.0
Second spawning		
Control	3.1 ± 1.8	–
10	1.4 ± 1.4	–
100	1.3 ± 1.9	–
1,000	1.0 ± 1.0	–
10,000	ND	–

Numerical data, ND and bar indicate mean and standard deviation, no data because of death of all specimens, and no observation, respectively

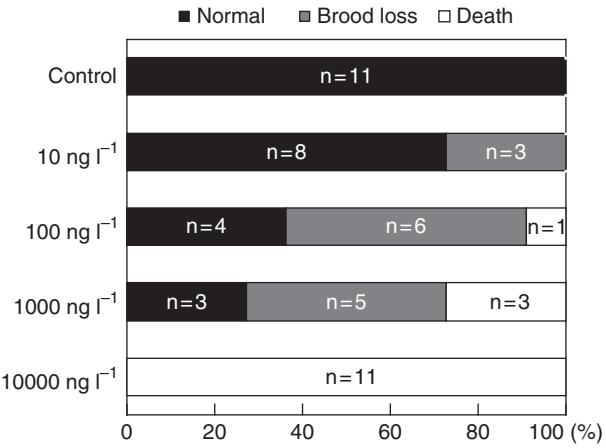

Fig. 10.10 Condition of the parental female in the first mature stage after 5-day exposure to TBTCl

than 100 ng TBTCl l^{-1} (Fig. 10.10) and all specimens died at 10,000 ng TBTCl l^{-1} due to the acute toxic concentration for the species (Ohji et al. 2002a). Brood loss of the females also occurred at concentrations higher than 10 ng TBTCl l^{-1}, ranging from three to six specimens, while no brood loss was observed in the control (0 ng TBTCl l^{-1}) (Fig. 10.10).

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 10.4). Significant differences were

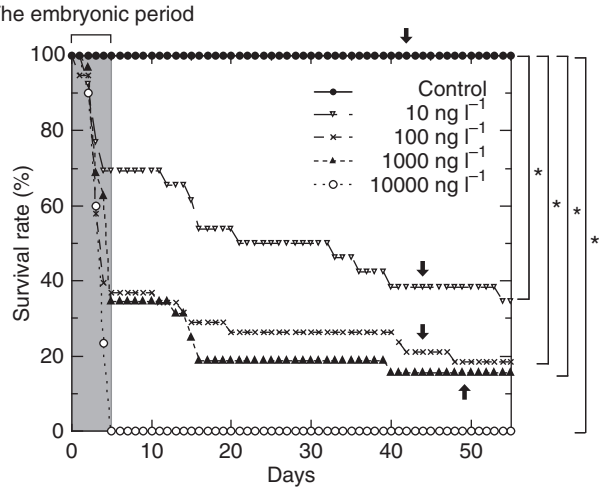

Fig. 10.11 Changes in the survival rate during spawning and sacrifice in offspring exposed to TBTCl during the embryonic stage and thereafter reared in seawater with no TBTCl added. Arrows indicate the days required from hatching to maturation in females. Log-rank test, $^{*}p < 0.0001$

found in the number of eggs between the control and three concentrations (10, 100 and 1,000 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 and 1,000 ng TBTCl l^{-1} (Wilcoxon's signed-rank test, $p < 0.05$) (Table 10.4).

10.4.2.2 Survival 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 ng l^{-1}, 36.8% at 100 ng l^{-1}, 34.4% at 1,000 ng l^{-1} and 0% at 10,000 ng l^{-1} (Fig. 10.11). 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 ± 1.7 in the control. However, it decreased as the TBTCl concentrations increased, ranged from 1.6 ± 1.6 at 10 ng l^{-1} to 0 at10,000 ng l^{-1}. Significant differences were found between control and 1,000 ng TBTCl l^{-1} and between the control and 10,000 ng TBTCl 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, 1,000 and 10,000 ng TBTCl l^{-1} (Wilcoxon's signed-rank test, $p < 0.05$–0.01) (Table 10.4).

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

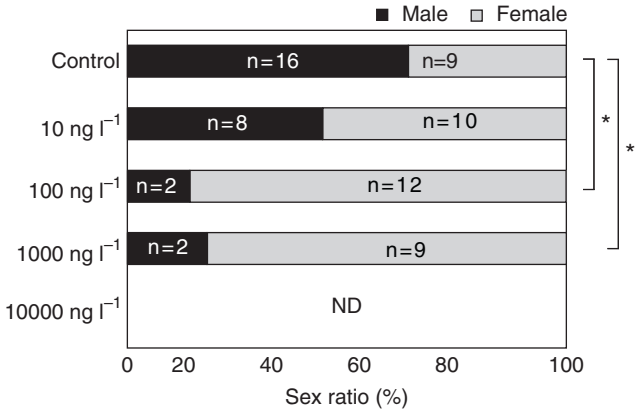

Fig. 10.12 Sex ratio in offspring of the first generation exposed to TBTCl during the embryonic stage. Chi-squared test, $^{*}p < 0.05$. ND indicates no data because of the death of all specimens

and acetone (Fig. 10.11). 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 ng TBTCl l^{-1}, 21.1% at 100 ng TBTCl l^{-1}, 15.6% at 1,000 ng TBTCl l^{-1} and 0% at 10,000 ng TBTCl l^{-1}, although the survival rate in the control was 100%. The drastic change in survival rate was observed twice, at 10–15 days and during 35–45 days after spawning.

10.4.2.3 Sex Ratio in the First Generation of Offspring

The female proportions were 36% in the control (Fig. 10.12), 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 ng l^{-1}, 85.7% at 100 ng l^{-1} and 81.8% at 1,000 ng l^{-1}. Significant differences occurred in the sex proportion between the control and 100 ng TBTCl l^{-1} and between the control and 1,000 ng TBTCl l^{-1} (chi-squared test, $p < 0.01$).

10.4.2.4 Growth, Maturation and Reproduction in the First Generation of Offspring

In the present study, no significant differences were found in the body length in 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.

The instar and day required from hatching to maturity in the female caprellid ranged from VIII to IX and from 37 to 45 days, respectively (Table 10.5). Significant

Table 10.5 First instar and the day required from hatching to maturation of juvenile exposed to TBTCl during the embryonic period

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
1,000	IX ± 0.8	45 ± 12.1
10,000	ND	ND

Numerical data and ND indicate mean and standard deviation and no data because of death of all specimens, respectively

differences were seen in the instar required from hatching to maturity between the control and 10 ng TBTCl l^{-1}, between the control and 100 ng TBTCl l^{-1} and between the control and 1,000 ng TBTCl l^{-1} (Mann-Whitney *U*-test, $p < 0.05$–0.01), while no significant differences were seen in the day required from hatching to maturity for all other combinations (Mann-Whitney *U*-test, $p > 0.05$).

In the first mature stage in offspring, oogenesis inhibition and brood loss were observed at 100 and 1,000 ng TBTCl l^{-1}. Three of six mature females exhibited apparent oogenesis inhibition at 100 ng TBTCl l^{-1} and three of five at 1,000 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 1,000 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 1,000 ng TBTCl l^{-1}.

10.4.2.5 Sex Ratio in the Second Generation of Offspring

The proportion of females in the control and at 10, 100 and 1,000 ng TBTCl l^{-1} were 28.6%, 28.6%, 22.2% and 33.3%, respectively. 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.

10.5 Discussion

10.5.1 Differences in Sensitivity and Metabolic Capacity to Degrade Tributyltin Between Caprellid and Gammarid Amphipod

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 μg

Table 10.6 Review of the 48-h LC_{50} for TBT in various marine organisms

Organism	Compound	Concentrations (μg l^{-1})	Temperature (°C)	Reference
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 equilibra	TBTCl	6.6	20	Ohji et al. (2002a)
Caprella penantis R-type	TBTCl	1.2	20	Ohji et al. (2002a)
Caprella verrucosa	TBTCl	1.3	20	Ohji et al. (2002a)
Caprella subinermis	TBTCl	4.6	20	Ohji et al. (2002a)
Caprella danilevskii	TBTCl	5.9	20	Ohji et al. (2002a)
Amphipoda: Gammaridea				
Jassa slatteryi	TBTCl	17.8	20	Ohji et al. (2002a)
Cerapus erae	TBTCl	21.2	20	Ohji et al. (2002a)
Eohaustorioides sp.	TBTCl	23.1	20	Ohji et al. (2002a)
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)

The concentrations were converted into TBTCl
ND indicates no data

TBTCl l^{-1}, were significantly lower than those in gammarids, 17.8–23.1 μg TBTCl l^{-1}. Moreover, in the comparison of the 48-h LC_{50} values for TBT among various trophic level organisms (Table 10.6), caprellids belong to a sensitive group of organisms. Hayakawa (1976) tested the acute toxicity of the antifouling paint for steel ship's bottoms, 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 for the chemical analysis of field-collected crustacean and seawater samples showed that TBT predominantly accumulated in caprellids and that the proportions of BTs in these organisms were similar to those found in seawater from St. W3 (Fig. 10.3). In contrast to caprellids, TBT's breakdown products, DBT and MBT, were predominant in gammarids (Fig. 10.3). 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) and life history (Myers 1971; Hiwatari and Kajihara 1988; Takeuchi and Hirano 1991; 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 known that differences in BT residue levels and the proportion of TBT in organisms are related to environmental and physiological factors. 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. 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, the crab *Callinectes sapidus*, the fish *Leistomus canthurus*, and the shrimp *Penaeus aztecus* are able to metabolize TBTO, while the oyster *Crassostrea virginica* show only a limited ability to metabolize TBTO (Lee 1986). TBT is known to metabolize 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). 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. 10.2) are related to 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.

10.5.2 Growth and Morphological Alterations

In the TBT exposure experiments after hatching, the marked delay in growth and molting during the early developmental and mature stages was found to occur regardless of gender in *Caprella danilevskii* (Ohji et al. 2003a) (Fig. 10.13). However, no significant difference was found in the growth and molting inhibition in the embryonic exposure experiments (Ohji et al. 2002b). These considerations suggest that effects on sensitive stages by exposure to TBT may extend over a long period after hatching in the caprellids. Similar growth delays induced by exposure to TBT have also been reported in various other organisms, e.g. the mysid *Acanthomysis sculpta* (Davidson et al. 1986), American oyster *Crassostrea virginica* (Thain 1986), blue mussel *Mytulis edulis* (Strømgren and Bongard 1987) and American lobster *Homarus americanus* (Laughlin and French 1980). Furthermore, several morphological alterations caused by TBT exposure after hatching were observed during the growth of *C. danilevskii*, though no morphological alterations were observed in response to TBT exposure during the embryonic period (Fig. 10.13). The morphological alterations resulting from exposure to TBT such as imposex in the gastropod *Nucella lapillus* (Bryan et al. 1986; Gibbs and Bryan 1986, 1987), shell thickening in the oyster *Crassostrea gigas* (Thain and Waldock 1986) and deformities in regenerated limbs of the crab *Uca Pugilator* (Weis and Kim 1988) have also been reported. Exposure to TBT after hatching may also have induced the cramp of the pereopod observed in this study, since TBT is known to be neurotoxic (Watanabe 1980). Collectively evidence suggests that TBT might act as a developmental toxicant or teratogen, affecting the processes of differentiation and morphogenesis during growth.

10.5.3 Maturation and Reproduction

Conspicuous inhibition of maturation and reproduction occurred in mature females even at nanogram-per-liter levels of TBT exposure (corresponding to present TBT levels in the coastal environment) both after hatching and during

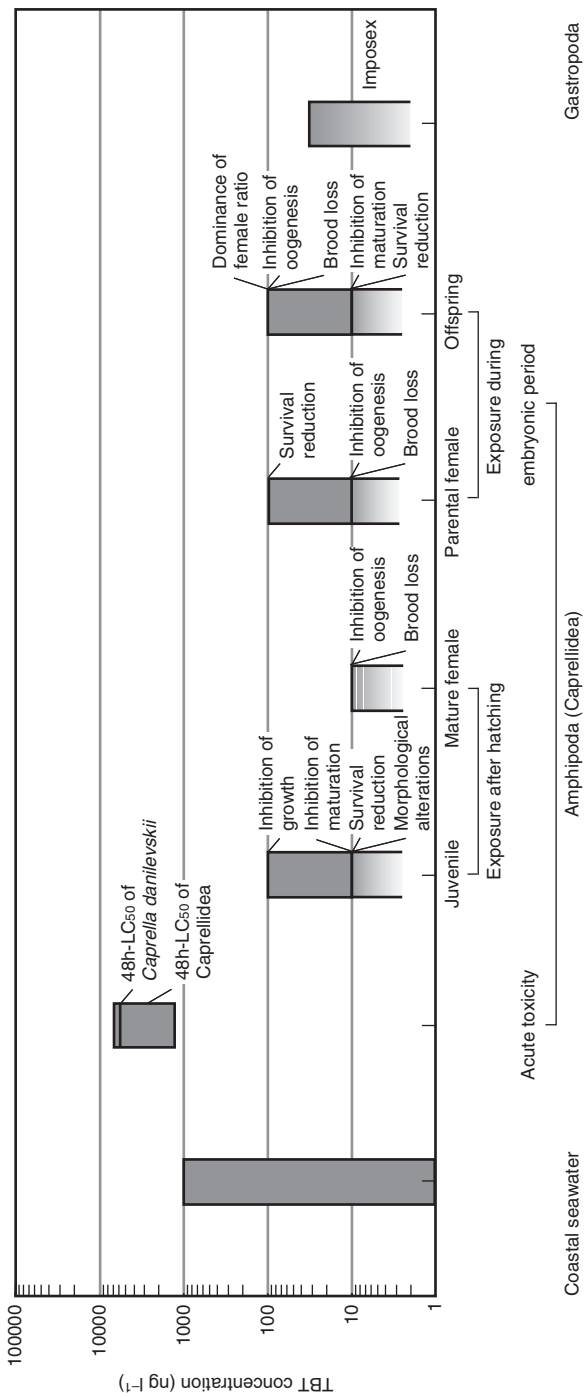

Fig. 10.13 Summary of biological effects of TBT on caprellids in the present studies, and on gastropods, and TBT concentration in coastal seawater. LC_{50} indicates median lethal concentration. LC_{50} values for caprellids are based on Ohji et al. (2002a). Results of experiments of TBT exposure after hatching and during embryonic period based on Ohji et al. (2003a) and Ohji et al. (2002b), respectively. 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)

the embryonic stage, although such inhibitions were not apparent in control treatments for *Caprella danilevskii* (Ohji et al. 2002b, 2003a) (Fig. 10.13). In gastropods, masculinization (imposex) by TBT exposure is the superimposition of male sex organs (development of a penis and vas deferens) on female individuals, with this condition leading to reproductive failure and consequently population decline (Bryan et al. 1986, Gibbs and Bryan 1986, 1987; Bettin et al. 1996; Matthiessen and Gibbs 1998) (Fig. 10.13). Therefore, TBT exposure might also affect the maturation and reproduction systems of the caprellids, although no external morphological alterations of reproductive organs were observed in the present study. In caprellids, TBT might not induce morphological alterations in the reproductive organs but cause disruptions in the internal physiological mechanisms concerning maturation and reproduction over the whole life stages. 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 macromolecular 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; 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 caprellids, resulting in brood loss and oogenesis inhibition in the species. In the present study, impaired reproductive success also occurred in both short- and long-term exposure to TBT. Therefore, our data suggest that nanogram concentrations of TBT similar to those encountered in coastal waters can directly affect reproduction in the caprellids, and that this phenomenon is an environmentally realistic scenario in the coastal ecosystem.

10.5.4 Sex Disturbance by Tributyltin Exposure in Caprellids

It is noteworthy that no significant differences were seen regarding change in the sex ratio by TBT exposure at all levels after hatching in the present study (Ohji et al. 2003a) (Fig. 10.13). However, an increase in the female ratio in hatched juveniles was found in embryonic exposure experiments with *Caprella danilevskii* (Ohji et al. 2002b). As TBT concentrations increase, the proportion of females were found to increase to 55.6% at 10 ng l^{-1}, 85.7% at 100 ng l^{-1} and 81.8% at 1,000 ng l^{-1}. Although the sex ratio in the present study was altered in response to TBT exposure, the number of females was almost constant (9–12) regardless of increases in TBT

concentrations. 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. 2004, 2005). These considerations suggest that sex disturbance may be induced during the embryonic stage in caprellids. This phenomenon contrasts with results for gastropod molluscs in which TBT exposure after hatching induced imposex in females (Matthiessen and Gibbs 1998). TBT had marked effects on the development of imposex in the dog whelk in exposure experiments after hatching (Gibbs et al. 1988). Several hypotheses have been proposed about the imposex induction mechanisms, such as those involving cytochrome P450 – mediated aromatase inhibition (Bettin et al. 1996), testosterone excretion – inhibition (Ronis and Mason 1996), functional disorder of female cerebropleural ganglia (Féral and Le Gall 1983), and involvement of a neuropeptide – APGWamide (Oberdörster and McClellan-Green 2002, 2003), although the exact physiological/biochemical pathway was still unclear. Recently, it is reported that TBT and TPT bind the hRXRs with high affinity and that injection of 9-cis retinoic acid (RA), the natural ligand of hRXRs, into females of the rock shell *Thais clavigera* induces the development of imposex (Nishikawa et al. 2004). Cloning of the RXR homologue from *T. clavigera* revealed that the ligand-binding domain of rock shell RXR was very similar to vertebrate RXR and bound to both 9-cis RA and to organotins. These results suggest that RXR plays an important role in the induction/differentiation and growth of male genital tracts in female gastropods. Since this phenomenon differs from our results, it is suggested that the mode of action of TBT may differ among organisms. Furthermore, as the factor of difference of phenomenon among organisms, it is also considered that the effects of TBT exposure might differ according to the developmental stage of the organism. In gastropods, TBT induces sex disturbance after sex determination, while, in contrast, TBT appears to induce sex disturbance prior to sex determination in caprellids. Sex differentiation in crustaceans, i.e. amphipods, isopods and decapods, is known to control by a hormone secreted from the androgenic gland (Charniaux-Cotton 1954; Katakura 1960; Taketomi et al. 1996). It is known that the androgenic gland is produced during the embryonic period. In our previous study, sex disturbance caused by TBT was induced in an earlier embryonic stage in the caprellid (Ohji et al. 2003b). Therefore, it is considered that TBT might affect the production of the androgenic gland or the secretion of androgenic hormone in the caprellid.

Furthermore, as mentioned above, TBT is known to be metabolized by the two phases detoxifying system involving the cytochrome P-450 dependent MFO system. The binding of TBT to glutathione *S*-transferase and cytochrome P-450 results in the inhibition of two detoxifying enzyme systems. Furthermore, 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, TBT may conceivably affect androgenic hormone production in the caprellids. Further experiments are needed to clarify TBT action in the endocrine systems in this genus.

10.5.5 Survival and Biomass of Caprellids in the Coastal Ecosystem

It is reported here that TBT affects the caprellid community at present. The biomass of the caprellids inhabiting sea grasses communities in the inner of the Otsuchi Bay (49.8–125.0 individuals m^{-2}) were a tenth of that near the mouth of the bay (1112.5 individuals m^{-2}) (Takeuchi and Hino 1997). This significant difference in caprellid biomass between inner and mouth of the Otsuchi Bay might be induced by the difference in TBT concentrations at each site, since TBT concentrations were higher in the inner bay (3.9–19 ng l^{-1}) than at the mouth (less than the detection limit) (Takahashi et al. 1999; Ohji et al. 2002a). Furthermore, prior to 1960, an extremely high biomass of the caprellids was reported from Japan (Fuse 1962). Seasonal fluctuation of the epifaunal animals living in the *Sargassum* zone in Kasaoka Bay, Japan, from 1956 to 1958 were studied, and it was reported that the biomass of the caprellids was 1.3 kg wet wt m^{-2}. In the past decade, such a high biomass and density of caprellid amphipods has not been reported in the coastal waters of Japan or of those of other developed countries. The biomass of caprellids inhabiting the *Sargassum* zone in Otsuchi Bay, Japan, from1993 to 1995 was estimated at 100 g wet wt m^{-2} (Takeuchi 1998). Although a reduction in TBT contamination was recorded after the ban (Environment Agency Japan 1995), TBT concentrations in Japanese coastal waters still persist, ranging from below the detectable level to 160 ng l^{-1} (180 ng l^{-1} as TBTCl) (Takeuchi et al. 2001) and averaging 10 ng l^{-1} (11 ng l^{-1} as TBTCl). Furthermore, the high TBT contamination in marine organisms and seawater continues in the coastal waters of Spain, France and Canada (Chau et al. 1997; Morcillo et al. 1997; Michel and Averty 1999), as well as in Asian and Oceanian countries (Kannan et al. 1995; Kan-antireklap et al. 1997) where no restrictions have been imposed. In small estuaries, marinas and moorings contribute significantly to TBT load, ranging from 24 ng l^{-1} (27 ng l^{-1} as TBTCl) to 2,440 ng l^{-1} (2,740 ng l^{-1} as TBTCl) (Lau 1991; Batley 1996). In the present study, a drastic decrease in the survival rate was observed in response to TBT exposure in both short- and long period even at 10 ng TBTCl l^{-1}, which corresponds to mean concentrations in the coastal waters (Ohji et al. 2002b, 2003a) (Fig. 10.13). Regarding survival rate throughout the whole life history, based on the results of the TBT exposure during the embryonic period (Ohji et al. 2002b) and after hatching (Ohji et al. 2003a), significant differences were also seen in the survival rate between the control and the other four concentrations of TBTCl (Fig. 10.14). The survival rate over 55 days (5 days in the embryonic period and 50 days from juvenile stage to mature stage after hatching) after spawning decreased in a range from 0% (10,000 ng TBTCl l^{-1}) to 17.3% (10 ng TBTCl l^{-1}) (2.9% in 1,000 ng TBTCl l^{-1} and 4.1% in 100 ng TBTCl l^{-1}) except for the control (100%). These considerations all lead to the conclusion that TBT exposure threatens the survival of caprellids through their whole life history, and may have contribute to the decrease in the caprellid biomass in the coastal ecosystems.

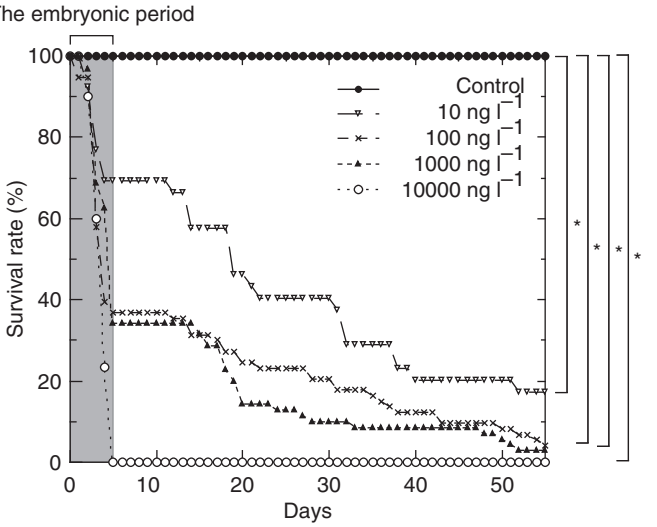

Fig. 10.14 Survival rate throughout whole life history in specimens based on the results of the TBT exposure after hatching (Ohji et al. 2003a) and during the embryonic period (Ohji et al. 2002a). The number of eggs at the beginning of the experiment was calculated as 100%. Log-rank test, $^{*}p < 0.0001$

10.6 Conclusions

In acute toxicity tests, the 48-h LC_{50} values for caprellids were significantly lower than those for gammarids. This suggests that caprellids are more sensitive to TBT than gammarids. Furthermore, in the caprellids, TBT was the predominant compound accumulated, which reflected the BT ratio in seawater, while in the gammarids, TBT's breakdown products (DBT and MBT) predominated. This difference suggests that caprellids have a lower metabolic capacity to degrade TBT than gammarids. Therefore, the difference in sensitivity to TBT among these amphipods could be related to the species-specific capacity to metabolize TBT. Moreover, in the comparison of 48-h LC_{50} values for TBT among various trophic level organisms, the caprellids belong to one of the more sensitive groups of organisms. In chronic toxicity tests, even at ambient water concentrations, exposure to TBT after hatching (50 days) influences survival, growth, maturation, reproduction and morphological alterations in the caprellids. Adverse effects on sex ratio, reproduction, and survival of caprellids have also been observed after TBT exposure only with exposure during the embryonic stage (5 days) at ambient water concentrations. Remarkably, the proportion of females increased dramatically in response to exposure to TBT in the embryonic period, though no significant difference was observed in the sex ratio in response to long-term exposure to TBT at these levels after hatching. These findings suggest that sex disturbance might therefore be induced

during the embryonic stage in caprellids. It has been reported that caprellids have a lower metabolic capacity to degrade TBT and therefore accumulate BTs at higher concentrations than other organisms in the coastal ecosystem. 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. Since caprellids link primary producers to higher consumers in coastal waters, 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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