J. Mar. Biol. Ass. U.K. (2003), **83**, 111–117
Printed in the United Kingdom

Biological effects of tributyltin exposure on the caprellid amphipod, *Caprella danilevskii*

Madoka Ohji*, Takaomi Arai and Nobuyuki Miyazaki

Otsuchi Marine Research Center, Ocean Research Institute, The University of Tokyo, 2-106-1, Akahama, Otsuchi, Iwate 028-1102, Japan. *Corresponding author, e-mail: ohji@wakame.ori.u-tokyo.ac.jp

To examine the biological effects of tributyltin (TBT) exposure, the caprellid amphipod, *Caprella danilevskii*, was exposed to five levels (0, 10, 100, 1000 and 10 000 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 10 000 ng TBTCl l^{-1} within five days after spawning due to the acute toxic concentration for the species. 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 seawater with no TBT added. 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.

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 TBT (tributyltin) 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), although the use of TBT will be banned completely at the beginning of 2003. 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 became clear about 20 years ago that antifouling chemicals with biocide properties such as those of TBT exert adverse effects on environmental components of the marine ecosystem. Tributyltin 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 (Snoeij et al., 1987; Zelikoff et al., 1988; Vos et al., 1989; Aschner & Aschner, 1992). Recently, the relationship between the metabolic capacity, accumulation, and toxicity of BTs (butyltin compounds) in marine organisms at various trophic levels in the food chain has been investigated (Fent, 1996; Takahashi et al., 1999; Ohji et al., 2002). Tributyltin accumulation in the marine ecosystem along the food chain is different from that of organochlorines (Tanabe & Tatsukawa, 1991), with TBT accumulating in most organisms at levels up to ~70 000 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 because of their lower metabolic capacity to degrade TBT, causing them to accumulate BTs at elevated concentrations (78–180 ng g^{-1} wet wt) (Takahashi et al., 1999; Ohji et al., 2002). 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. 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 & Steinberg, 1970). Caprellids are an important trophic link as one of the dominant secondary producers between unicellular algae and fish in the coastal water ecosystem.

Furthermore, these organisms are important prey resources for small fish in the coastal ecosystem (Fuse, 1962; Caine, 1989; Holbrook & Schmitt, 1992). The generation length and life span of *Caprella* have been well-investigated (Takeuchi & 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 & 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 the 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., 2002). 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. 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 study were to examine the biological effects of TBT exposure during the embryonic stage of the caprellid amphipod, *Caprella danilevskii* Czerniavski. 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

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 (Figure 1). Female maturation was divided into three stages: immature, premature and mature based on the morphology of oostegites on pereonites 3 and 4.

Seawater and TBT solution

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

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. Tributyltin concentrations of seawater samples were determined according to our previous method (Ohji et al., 2002), and were confirmed to be less than the detection limit. Detection limit of TBT was 2.0 ng l^{-1}.

A TBT-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 10 000 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 10 000 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., 2002). The concentrations remained the same between pre- and post-experiments.

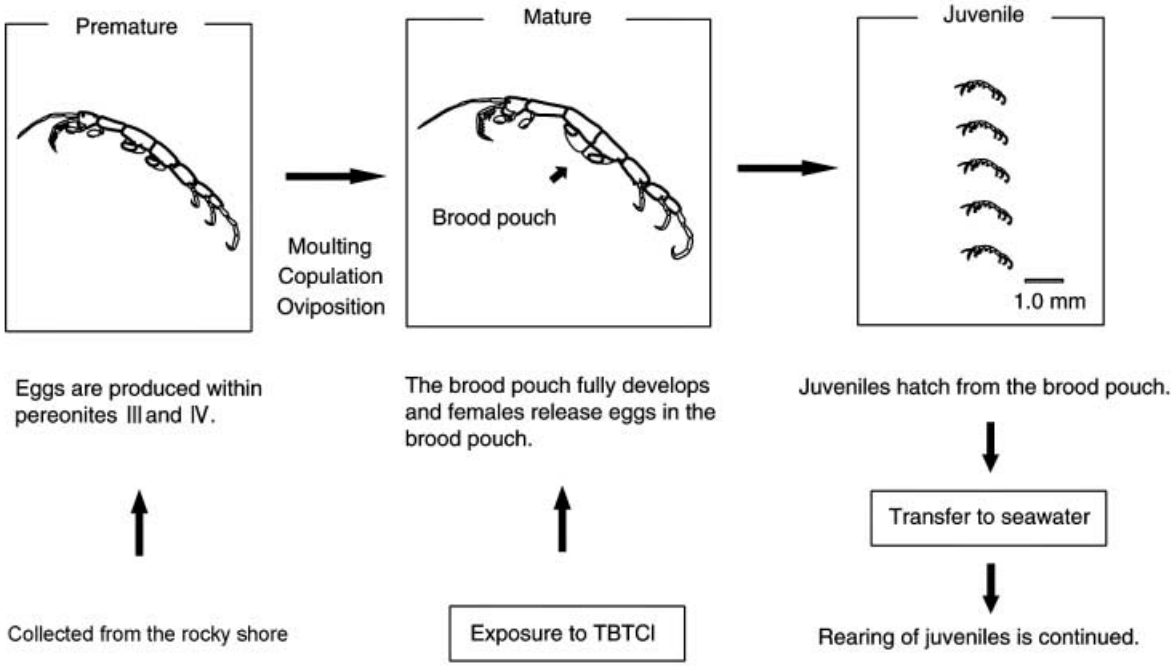

Figure 1. Schematic view of the experimental methodology used to investigate the biological effects of TBTCl.

https://doi.org/10.1017/S0025315403006878h Published online by Cambridge University Press

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 (first mature stage in parent) (Figure 1). 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 L:D 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 10 000 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 1 on the head to the posterior end of pereonite 7 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 2 and the presence of abdominal appendages in males.

Furthermore, parental females were also transferred to the filtered seawater. After moulting, 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

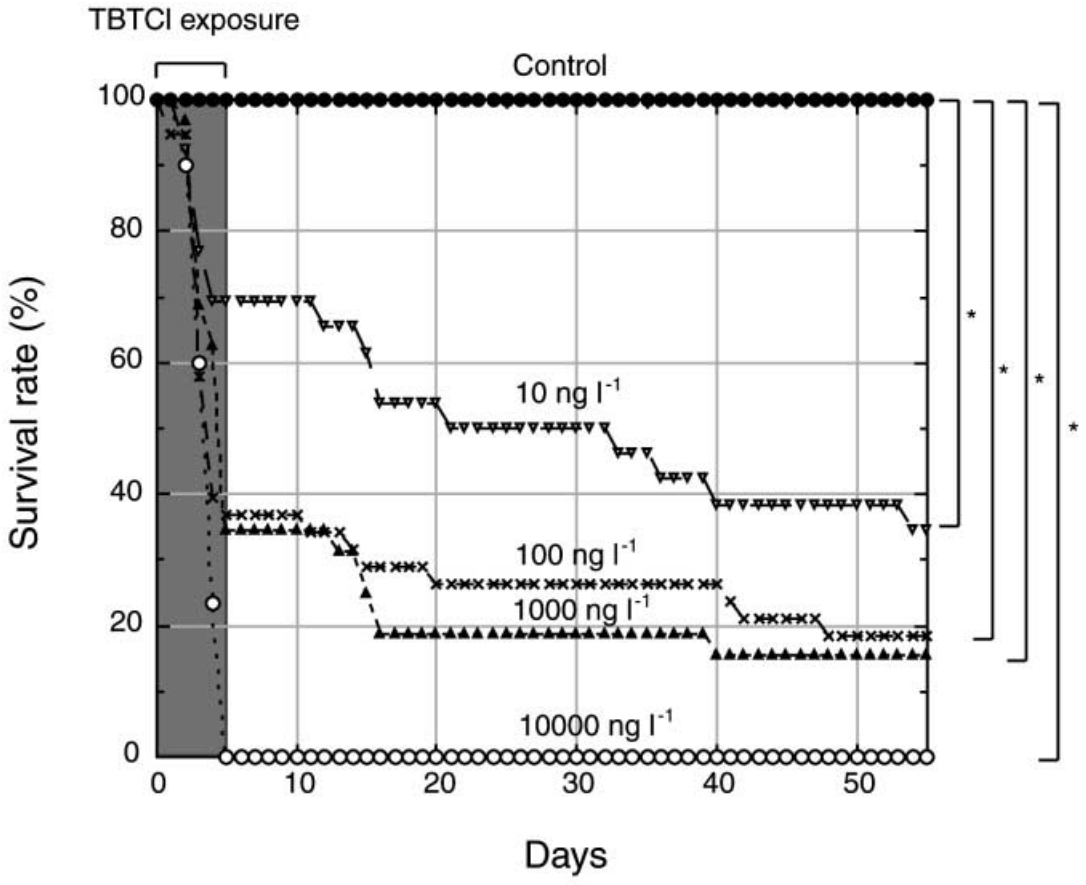

Figure 2. Changes in the survival rate during spawning and sacrifice in offspring exposed to TBTCl during the embryonic stage and thereafter reared in seawater with no TBTCl added. Log-rank test; $*P<0.0001$.

https://doi.org/10.1017/S0025315403006878h Published online by Cambridge University Press

Table 1. *Reproductive conditions of parental female exposed to TBTCl during the five days which corresponds to the first mature stage. Numerical data, ND and bar, indicate mean and standard deviation, no data because of death of all specimens, and no obervation, 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	–

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 10 000 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 χ^2-test. Differences in both reproduction and growth between the control and each concentration of TBTCl were tested by the Mann–Whitney *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

Condition of parental females

Eleven ovigerous females were allocated to each concentration compartment of TBTCl (0, 10, 100, 1000 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 1). 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 10 000 ng TBTCl l^{-1} due to the acute toxic concentration for the species (Ohji et al., 2002). 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 1). 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 1).

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 ng l^{-1}, 36.8% at 100 ng l^{-1}, 34.4% at 1000 ng l^{-1} and 0% at 10 000 ng l^{-1}. 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 at 10 000 ng l^{-1}. Significant differences were found between control and 1000 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 ng TBTCl l^{-1}, 1000 ng TBTCl l^{-1} and 10 000 ng TBTCl l^{-1} (Wilcoxon's signed-rank test, $P < 0.05$–0.01) (Table 1).

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 (Figure 2). 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 1000 ng TBTCl l^{-1} and 0% at 10 000 ng TBTCl l^{-1}, although the survival rate in the control was 100% (Figure 2). The drastic change in survival rate was observed twice, at 10–15 days and during 35–45 days after spawning (Figure 2).

Sex proportion in the first generation of offspring

The female proportions were 36% in the control, corresponding to previous field observations (Takeuchi & 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 1000 ng l^{-1}. Significant differences occurred in the sex proportion between the control and 100 ng TBTCl l^{-1} and between the control and 1000 ng TBTCl l^{-1} (χ^2 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 in each instar and in the

Table 2. *First 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
10 000	ND	ND

achievement days to each instar 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 moulting inhibition occurs after hatching in response to exposure to TBTCl in the embryonic period.

Achievement instar and achievement day to maturity after hatching in the female caprellid ranged from VIII to IX and from 37 days to 45 days, respectively (Table 2). Significant differences were seen in the achievement instar between the control and 10 ng TBTCl l^{-1}, between the control and 100 ng TBTCl l^{-1} and between the control and 1000 ng 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, $P > 0.05$).

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. No significant differences in the sex proportion between control and other concentrations of TBTCl were observed (χ^2-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

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 ng l^{-1}, 85.7% at 100 ng l^{-1} and 81.8% at 1000 ng 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 (M. Ohji, unpublished data). 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 & Gibbs, 1998), feminization in rainbow trout by alkylphenolic chemicals (Jobling et al., 1996), intersex in harpacticoid copepods (Moore & Stevenson, 1991) and American lobsters (Sangalang & 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. 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 (M. Ohji, unpublished data). Tributyltin caused the development of imposex in many gastropods (Gibbs et al., 1988). Tributylin 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 & Mason, 1996) in gastropods. These *in vivo* increases in androgens may result in an androgenization of organisms. Jobling et al. (1996) have shown that some of the breakdown products of alkylphenol polyethoxylate surfactants induce vitellogenesis in male fish as an oestrogen receptor-mediated effect. In the caprellid, the mechanism of sex disturbance seems to differ from fish and gastropod mentioned above, although no study has revealed such mechanism in the crustacean. Further experiments are needed to clarify the action of TBT as a hormone disrupter in the endocrine system of caprellids.

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-litre 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 & Møhlenberg, 1987) and in the sea urchin *Paracentrotus 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 signalling. These processes consist primarily of a disruption of calcium homeostasis (Chow et al., 1992; Matsuoka & Igisu, 1996) or calcium signalling (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 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 (five days) during the embryonic stage. 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 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 ng l^{-1} despite movement of females into the no TBT-added seawater after the five-day exposure. These results suggest that TBT exposure even at present levels in ambient water and even for short-periods might influence the population in the coastal environment. A 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/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/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 changes, reproduction, and survival in the caprellid as well as the imposex found in gastropods. 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 (M. Ohji, unpublished data). It has been reported that organotin compounds degrade slowly in the environment, with the TBT half-life in the water being between one and three weeks (Seligman et al., 1986; 1988), and in sediment on the order of one to five years (Waldock et al., 1987; Adelman et al., 1990). Furthermore, it has been reported that caprellids have a lower metabolic capacity to degrade TBT and therefore accumulate TBT at higher concentrations (78–180 ng g^{-1} wet wt) than other organisms in the coastal ecosystem (Takahashi et al., 1999; Ohji et al., 2002). 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 & 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.

We are grateful to the staff of the Marine Biosystems Research Center, Chiba University, for their support of sample collection and their laboratory experience, and to the staff of the Otsuchi Marine Research Center, Ocean Research Institute, The University of Tokyo, for their support in the collection of seawater. We also wish to thank Dr S. Tanabe for assistance with the chemical analyses. The present study was partially supported by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology, Japan (no. 12NP0201).

REFERENCES

Adelman, D., Hinga, K.R. & Pilson, M.E.Q., 1990. Biochemistry of butyltins in an enclosed marine ecosystem. *Environmental Science and Technology*, **24**, 1027–1032.

Alzieu, C., 1986. TBT detrimental effects on oyster culture in France. Evolution since antifouling paint regulation. In *Proceedings of the organotin symposium, oceans '86 conference, Washington, 23–25 September 1986*, **4**, 1130–1134.

Alzieu, C., 1989. Rapport scientifique et technique de l'IFREMER l'étain et les organoétains en milieu marine. *Biogéóchimie et Écologie*, **17**, 1–94. [In French with English summary.]

Ambrose, P., 1994. Antifouling news. *Marine Pollution Bulletin*, **28**, 134.

Aschner, M. & Aschner, J.L., 1992. Cellular and molecular effects of trimethyltin and triethyltin: relevance to organotin neurotoxicity. *Neuroscience Behaviour Review*, **16**, 427–435.

Batley, G., 1996. The distribution and fate of tributyltin in the marine environment. In *Tributyltin: case study of an environmental contaminant* (ed. S.J. de Mora), pp. 139–166. Cambridge: Cambridge University Press.

Bettin, C., Oehlmann, J. & Stroben, E., 1996. TBT-induced imposex in marine neogastropods is mediated by an increasing androgen level. *Helgoländer Meeresuntersuchungen*, **50**, 299–317.

Bryan, G.W., Gibbs, P.E., Hummerstone, L.G. & Burt, G.R., 1986. The decline of the gastropod *Nucella lapillus* around south-west England: evidence for the effect of tributyltin from antifouling paints. *Journal of the Marine Biological Association of the United Kingdom*, **66**, 611–640.

Caine, E.A., 1989. Caprellid amphipod behavior and predatory strikes by fish. *Journal of Experimental Marine Biology and Ecology*, **126**, 173–180.

Campbell, P.M. & Hutchinson, T.H., 1998. Wildlife and endocrine disruptors: requirements for hazard identification. *Environmental Toxicology and Chemistry*, **17**, 127–135.

Chow, S.C., Kass, G.E.N., McCave, M.J. & Orrenius, S., 1992. Tributyltin increases cytosolic free Ca^{2+} concentration in thymocytes by mobilizing intracellular Ca^{2+}, activating a Ca^{2+} entry pathway, and inhibiting Ca^{2+} efflux. *Archives of Biochemistry and Biophysiology*, **298**, 143–149.

Corsini, E., Viviani, B., Marinovich, M. & Galli, C.L., 1997. Role of mitochondria and calcium ions in tributyltin-induced gene regulatory pathways. *Toxicology Applied Pharmacology*, **353**, 136–143.

Fent, K., 1996. Ecotoxicology of organotin compounds. *Critical Review of Toxicology*, **26**, 1–117.

Fuse, S., 1962. The animal community in the *Sargassum* belt. *Physiology and Ecology*, **11**, 23–45. [In Japanese with English summary.]

Gibbs, P.E., Pascoe, P.L. & Burt, G.R., 1988. Sex change in the female dogwhelk, *Nucella lapillus*, induced by tributyltin from antifouling paints. *Journal of the Marine Biological Association of the United Kingdom*, **68**, 715–731.

Girard, J.P., Ferrua, C. & Pesando, D., 1997. Effects of tributyltin on Ca^{2+} homeostasis and mechanisms controlling cell cycling in sea urchin eggs. *Aquatic Toxicology*, **38**, 225–239.

Girard, J.P., Szpunar, J., Pedrotti, M.L. & Pesando, D., 2000. Toxicity of tri-*n*-butyltin to sea urchin eggs and larvae: relation to bioaccumulation at the nanomolar level. *Environmental Toxicology and Chemistry*, **19**, 1272–1277.

Hall, L.W., Bushong, S.J., Ziegenfuss, M.C., Johnson, W.E., Herman, R.L. & Wright, D.A., 1988. Acute and chronic effects of tributyltin on a Chesapeake Bay copepod. *Environmental Toxicology and Chemistry*, **7**, 41–46.

Holbrook, S.J. & Schmitt, R.J., 1992. Cause and consequences of dietary specialization in surfperches: patch choice and intraspecific competition. *Ecology*, **73**, 402–412.

Jobling, S., Sheahan, D.A., Osborne, J.A., Matthiessen, P., Sumpter, J.P., Tylor, T. & Zaman, N., 1996. Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. *Environmental Toxicology and Chemistry*, **15**, 194–202.

Johansen, K. & Møhlenberg, F., 1987. Impairment of egg production in *Acartia tonsa* exposed to tributyltin oxide. *Ophelia*, **27**, 137–141.

Matsuoka, M. & Igisu, H., 1996. Induction of c-*fos* expression by tributyltin in PC12 cells: involvement of intracellular Ca^{2+}. *Environmental Toxicology and Pharmacology*, **2**, 373–380.

Matthiessen, P. & Gibbs, P.E., 1998. Critical appraisal of the evidence for tributyltin-mediated endocrine disruption in molluscs. *Environmental Toxicology and Chemistry*, **17**, 37–43.

McCain, J.C. & Steingerg, J.E., 1970. Amphipoda I. I. Fam. Caprellidae. In *Crustaceorum Catalogus* (ed. H.E. Gruner and L.B. Holthuis), Pars **2**, pp. 1–78. Amsterdam: SPB Academic Publishing.

Moore, C.G. & Stevenson, J.M., 1991. The occurrence of intersexuality in harpacticoid copepods and its relationship with pollution. *Marine Pollution Bulletin*, **22**, 72–74.

Ohji, M., Takeuchi, I., Takahashi, S., Tanabe, S. & Miyazaki, N., 2002. Differences in the acute toxicities of tributyltin between the Caprellidea and the Gammaridea (Crustacea: Amphipoda). *Marine Pollution Bulletin*, **44**, 16–24.

Omori, M., 1980. Feeding habit of yearling red sea bream (*Pagrus major*) in Yuya Bay. *Bulletin of the Seikai Regional Fisheries Research Labolatory*, **54**, 93–109. [In Japanese with English summary.]

Rivaro, P., Pensiero, G. & Frache, R., 1999. Occurrence of butyltin compounds in water and mussel samples collected in an oil port. *Applied Organometallic Chemistry*, **13**, 727–732.

Ronis, M.J.J. & Mason, A.Z., 1996. The metabolism of testosterone by the periwinkle (*Littorina littorea*) *in vitro* and *in vivo*: effects of tributyltin. *Marine Environmental Research*, **42**, 161–166.

Sangalang, G. & Jones, G., 1997. Oocytes in testis and intersex in lobsters (*Homarus americanus*) from Nova Scotian sites: natural or site-related phenomenon? *Canadian Technical Reports Fish Aquatic*, **2163**, 46.

SAS Institute Inc., 1998. Stat View 5.0. Mac version.

Seligman, P.F., Valkirs, A.O. & Lee, R.F., 1986. Degradation of tributyltin in San Diego Bay, California, waters. *Environmental Science and Technology*, **20**, 1229–1235.

Seligman, P.F., Valkirs, A.O., Stang, P.M. & Lee, R.F., 1988. Evidence for rapid degradation of tributyltin in a marina. *Marine Polluttion Bulletin*, **19**, 531–534.

Smith, B.S., 1981. Male characteristics on female mud snails caused by antifouling bottom paints. *Journal of Applied Toxicology*, **1**, 22–25.

Snoeij, N.J., Bol-Schoenmakers, M., Penninkis, A.H. & Seimen, W., 1988. Differential effects of tri-*n*-butyltin chloride on macromolecular synthesis and ATP levels of rat thymocyte subpopulations obtained by centrifugal elutriation. *International Journal of Immunopharmacology*, **10**, 29–37.

Snoeij, N.J., Penninkis, A.H. & Seimen, W., 1987. Biological activity of organotin compounds—an overview. *Environmental Research*, **44**, 335–353.

Spooner, N., Gibbs, P.E., Bryan, G.W. & Goad, L., 1991. The effect of tributyltin upon steroid titres in the female dogwhelk, *Nucella lapillus*, and the development of imposex. *Marine Environmental Research*, **32**, 37–49.

Stridh, H., Orrenius, S. & Hampton, M.B., 1999. Caspase involvement in the induction of apoptosis by environmental toxicants tributyltin and triphenyltin. *Toxicology and Applied Pharmacology*, **156**, 141–146.

Takahashi, S., Tanabe, S., Takeuchi, I. & Miyazaki, N., 1999. Distribution and specific bioaccumulation of butyltin compounds in a marine ecosystem. *Archives of Environmental Contamination and Toxicology*, **37**, 50–61.

Takeuchi, I., 1998. Dry weight, carbon and nitrogen components of the caprellid amphipods (Crustacea) inhabiting the *Sargassum yezoense* community of Otsuchi Bay, northeastern Japan. *Marine Biology*, **130**, 417–423.

Takeuchi, I. & Hirano, R., 1991. Growth and reproduction of *Caprella danilevskii* (Crustacea: Amphipoda) reared in the laboratory. *Marine Biology*, **110**, 391–397.

Takeuchi, I., Takahashi, S., Tanabe, S. & Miyazaki, N., 2001. *Caprella* watch; a new approach for monitoring butyltin residues in the ocean. *Marine Environmental Research*, **52**, 97–113.

Tanabe, S. & Tatsukawa, R., 1991. Persistent organochlorines in marine mammals. In *Organic contaminants in the environment* (ed. K.C. Jones), pp. 275–289. London: Elsevier Science Publishers.

Vos, J.V., Van Loveren, H., Wester, P. & Vethaak, D., 1989. Toxic effects of environmental chemicals on the immune system. *Trends of Pharmacology Science*, **10**, 289–292.

Waldock, M.J., Thain, J.E. & Waite, M.E., 1987. The distribution and potential toxic effects of TBT in UK estuaries during 1986. *Applied Organometallic Chemistry*, **1**, 287–301.

Zelikoff, J.T., Li, J.H., Hartwig, A., Wang, X.W., Costa, M. & Rossman, T.G., 1988. Genetic toxicicology of lead compounds. *Carcinogenesis*, **9**, 1727–1732.

Submitted 30 January 2002. Accepted 4 November 2002.