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Effects of Testosterone on the Calanoid Copepod, *Acartia omorii* Bradford

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Abstract : One of the most abundant calanoid copepod in Japanese coastal water, *Acartia omorii* was used as a test organism for the endocrine disrupting agent, testosterone. Acute toxicity of testosterone to adult female of *A. omorii* was ascertained and the effect was time dependent as ordinary toxic substances. Testosterone adversely affects on adult female survivability at above 1 μM, giving 96h-LC_{50} of 8 μM. The effects of testosterone on egg production and egg hatch success occur in concentrations higher than 0.1 and 2 μM, respectively, and the effects were not exposure-time dependent. Maternal exposure seems to give no effect on egg hutching success. The test using the effect on egg production of *A. omorii* could be used as highly sensitive method for monitoring the adverse effect of endocrine disruptor on the growth of crustaceans.

Keywords : *copepod, testosterone, survival, egg production, egg hatch*

1. Introduction

Marine environmental contamination by endocrine disruptor has been widely studied. Tributyltin (TBT) is the most commonly used anti-biofouling agent which works as an inhibitor of androgenic metabolism resulting increase of testosterone levels in neogastropods to cause imposex (BETTIN *et al.*, 1996). It also affects on various invertebrates in marine environment (see DEPLEDGE and BILLINGHURST, 1999). Recently, livestock wastes and municipal wastewater are recognized as a source of estrogens and testosterone (TASHIRO *et al*, 2003 ; FINLAY-MOORE *et al.*, 2000) , thus the adverse effects of natural endocrine disruptor on marine organisms are also concerned.

Copepods are the dominant constituent of the plankton in every sea area usually comprise at least 70% of the plankton fauna (RAYMONT, 1983) and thought as important primary consumer in marine food chain. Therefore, endocrine disruptors might cause the catastrophic damage to coastal ecosystem if they adversely affect copepod production. *Acartia* spp. are the most abundant calanoid copepods along the cost of Japan (UEDA, 1986 ; YAMAJI, 1956) , and *A. omorii* is strictly confined in brackish waters and embayed water bodies (UEDA, 1987) where pollutant materials could easily accumulate.

In this study, we tested the effects of testosterone on the adult survival, egg production and egg hutch of *Acartia omorii* .

2. Materials and Methods

Test animal *A. omorii* was obtained from the dock basin in Shinagawa campus of Tokyo University of Marine Science and Technology, which is besides the canal connected to the inner most part of Tokyo Bay. Zooplankton was collected by a plankton net (200- μm mesh size) and a single gravid female of *A. omorii* was immediately sorted and cultured in an egg-collection tank filled with filtered (Whatman GF/F glass fiber filter) and diluted seawater

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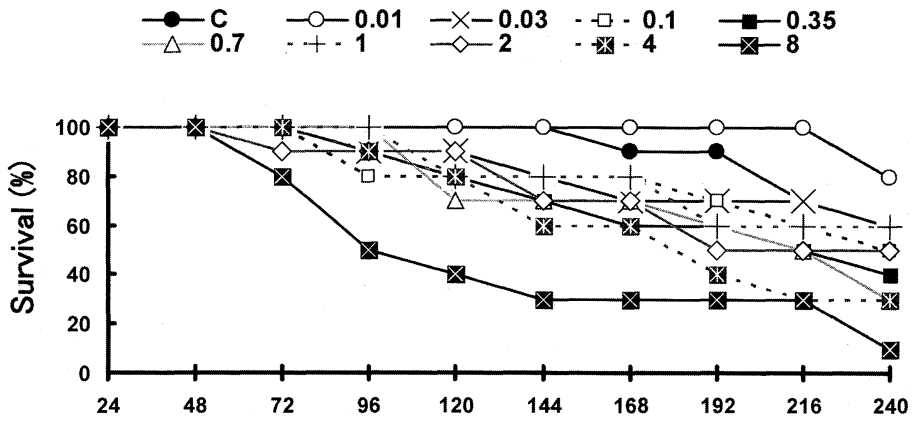

Fig. 1. Time course of survival percentages of adult females of *Acartia omorii* exposed to various concentrations of testosterone.

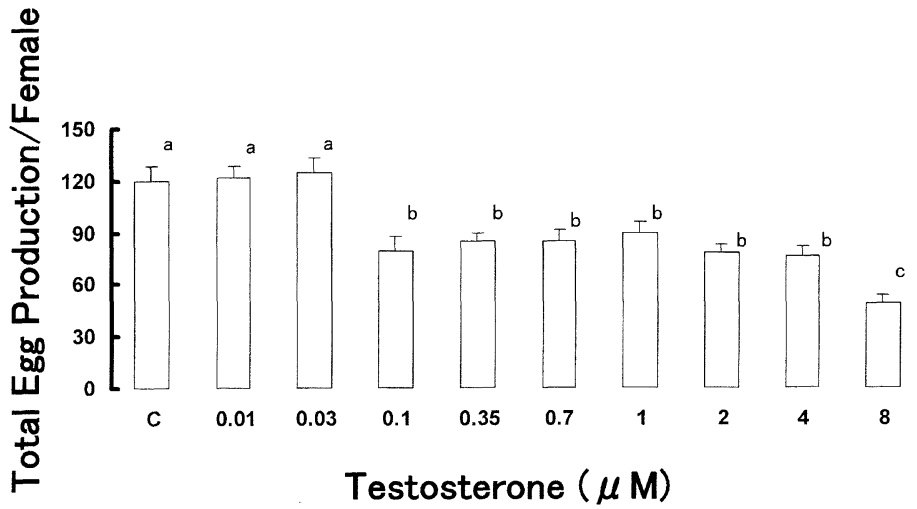

Fig. 2. Total egg production by a female of *Acartia omorii* exposed to various concentrations of testosterone during 168 h exposure. a, b and c denotes significant difference from each other ($p \leq 0.05$) and no significance in each symbol (ANOVA, Fisher's LSD test)

by ultra pure water (milli Q). The tank was composed by an outer chamber of a drinking glass and an inner chamber of 50-mm outer diameter acrylic tube with 200-μm mesh nylon netting bottom. As Inner diameter of the tapered drinking glass at 3 cm from bottom is 50 mm, thus copepod eggs sink the space between the two chambers. Collected eggs were then moved into 1.5 L glass jar with seawater as above. Experimental conditions were the same in all observation including mass culture where temperature was 20±1 ℃ ; salinity 25 ; light dark cycle 12L : 12D with light intensity of 80–90 μ mole quanta $m^{-2}s^{-1}$. Copepods were fed *Tetraselmis* sp. and *Isochrysis* sp. (ca. 10,000 cells/ml, each) after 3 days and 7 days of the initiation of the mass culture, and every 24 h for adult survival and egg production experiment.

Adult copepod survival observation

Bath administration of adult copepods were conducted in exposure media with testosterone (17β-hydroxy-4-androsten-3-one, Wako Pure Chemicals, Ltd) concentrations of 0.01, 0.03, 0.1, 0.35, 0.7, 1, 2, 4 and 8 μM in seawater. Each

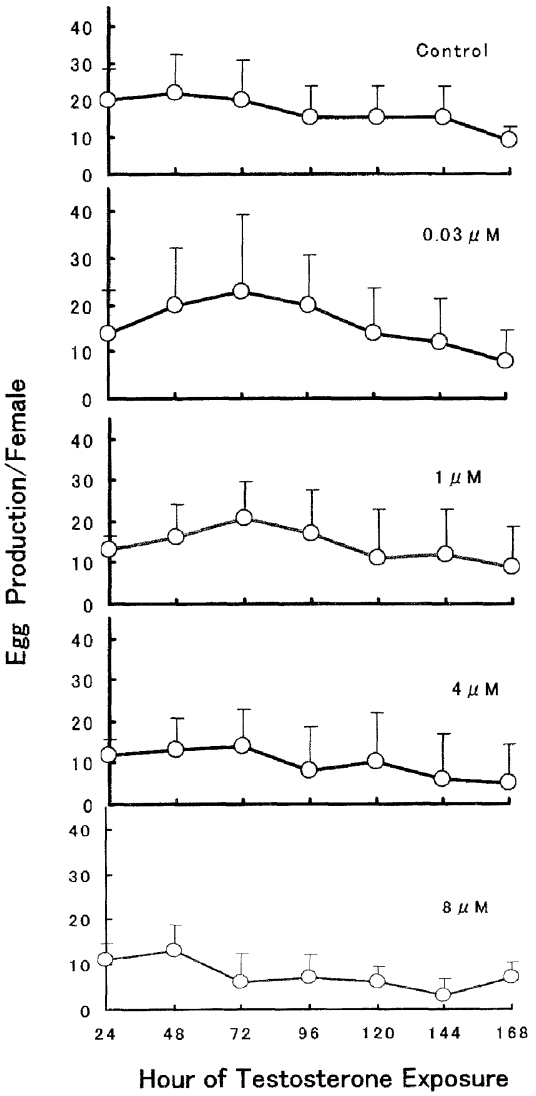

Fig. 3. Daily egg production by *Acartia omorii* exposed to various concentrations of testosterone. Data are presented as means and standard deviations in 10 replications.

concentration was prepared from initial solution of testosterone dissolved in ethanol. A control (seawater) and a solvent control (seawater containing highest ethanol concentration used in the test) experiment were also conducted.

One adult copepod was incubated in a 150 ml glass jar filled with 100 ml exposure medium and ten individuals were tested. Survivability was checked at every 24-h interval for 2 weeks using a stereoscopic microscope (Olympus, SZ40). Mortalities were recorded when no movement were observed for the period of 60 seconds.

Egg production observation

Because *A. omorii* eat its own eggs (Liang *et al.*, 1994), usage of above mentioned egg collection tank is necessary for the separation of female from laid eggs. Randomly selected single gravid female from mass culture was moved into an egg collection tank with 150 ml exposure medium. Laid eggs were collected in following procedure for every 24 h for a week. First, the inner chamber was shut the top by hand to leave 100 ml exposure medium, and then shifted to another outer chamber with 50 ml new exposure medium. The eggs in the outer chamber was collected and counted under a stereoscopic microscope. Ten replication experiments were conducted.

Egg hatching observation

10 gravid females randomly selected from the mass culture stock were cultured in an egg collection tank with exposure medium, and eggs were collected at exposed periods of 1st, 3rd, 5th, 7th, 9th and 12th day. 20 eggs were moved into each of 10 ml chamber of 6-well micro plate filled with medium containing the same concentration of testosterone in which the female copepod was exposed. Hatched nauplii were sorted and counted under a stereoscopic microscope in every 24 h for 10 days.

3. Results and Discussion

Adult survivability is shown in Fig. 1. All animals survived to 5th, 6th and 9th day in control, solvent control (not shown) and 0.01 μM testosterone concentration, and clear toxic effect was observed above 1 μM. 50% lethal concentration (LC_{50}) after 96 hours was 8 μM and 144-h LC_{50} 4 μM. ANDERSON *et al.*, (2001) estimated 48-h LC_{10} and LC_{50} of *Acartia tonsa* as 9 and 19 μM, respectively. Our result on *A. omorii* is close to their results.

Total egg production per female decreased in higher testosterone concentration than 0.1 μM, however the effect was not significant among 0.1 and 4 μM level (ca. 25% decrease; Fig. 2). Relationship between the effects of testoster-

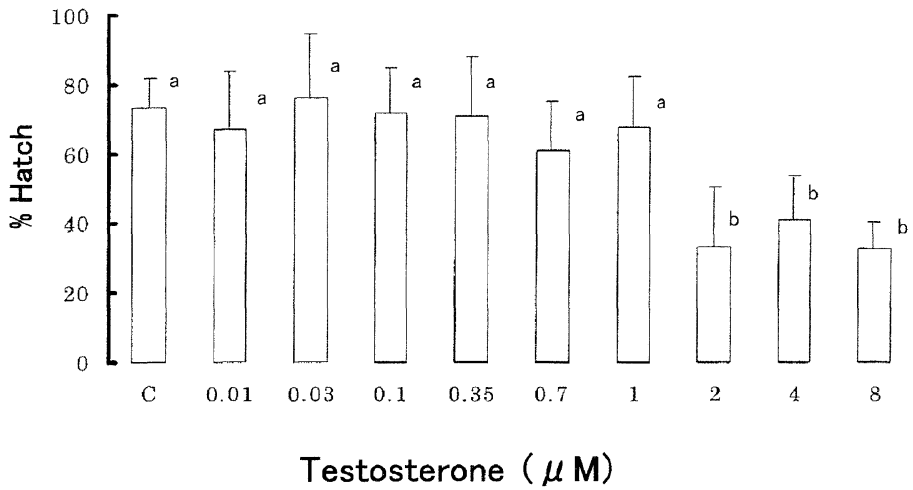

Fig. 4. Hutching success of eggs from 1 to 12day exposed *Acartia omorii* to different concentrations of testosterone. Mean and standard deviation are shown. a and b denotes significant difference from each other ($p \leq 0.05$) and no significance in each symbol (ANOVA, Fisher's LSD test).

one to exposed periods is not clear (Fig. 3.) The maximum lifetime of adult female of *A. omorii* is 30 days at 20℃ under culture condition and field collected females kept laying eggs for 22 and 28 days at 21.7 and 19.7℃, respectively (UYE, 1981). Daily egg production in each adult female fluctuated, though, tendency was as follows ; low egg production in the most recently ovigerous female, increased within a few days, then kept fairy constant until half of the adult stage, and then decreased with time and ceased a few days before their death (see UYE, 1981). 10 gravid females used in our experiment were randomly selected from mass culture, therefore they can be ranged from the most recently ovigerous one to old one close to their life-time. Thus, insignificant decrease in egg production for 7 days test period in control is reasonable. Whereas, not clear decrease of egg production with exposure period in higher testosterone concentration (4 and 8 μM) suggested that the adverse effect on egg production is not time - dependent.

Averages of hatching success of eggs from 1 to 12 day treated feamales of *Acartia omorii* in various concentrations of testosterone are shown in Fig. 4. Decreased egg hatch success was observed from 1 day treatment in higher concentrations of testosterone than 2 μM, however the relationship between exposed concentration and egg hatch success was not clear (data not shown). Moreover, the effect was not time-dependent as observed in egg production. Because, egg hatch test was conducted by exposing eggs in the same concentration of testosterone to which gravid female was exposed, it is suggested that testosterone only affects egg hatch process and does not affect egg itself through maternal exposure. Larval development of *A. tonsa* by testosterone occurred at concentrations (10% and 50% effective concentr ation : 2.5 and 5 μM) not much higher than adult lethal concentrations and no other effect than simple toxic effect was observed (ANDERSON *et al.*, 2001). MU and LE BLANC (2002) reported that testosterone elicits embryo toxicity to daphnids by interfering the activity of ecdysteroid which regulate the critical process of embryo development. They also note testosterone elicits direct toxicity to the daphnid embryos and the maternal organism can serve as the vector for exposure. In copepod, maternal exposure seems to have no effect on egg hatch process.

In this study, the acute toxicity of testosterone to adult female of *A. omorii* was ascertained and the effect was time dependent as ordinary toxic substances. Testosterone adversely affects on adult female survivability at above 1 μM, giving 96 h-LC_{50} of 8 μM. The

effects of testosterone on egg production and egg hatch success occur in concentrations higher than 0.1 and 2 μM, respectively, and the effects were not exposure-time dependent. Maternal exposure seems to have no effect on egg hutching success.

In crustaceans, the roles of steroid hormones have not been fully elucidated. SUMMAVIELLE *et al.*, (2003) suggested through *in vitro* incubation of ovary and hepatopancreas of penaeid shrimps, that steroid hormones have physiological role in the maturation cycle. On the contrary, testosterone is thought to elicit adversely effects not as androgen but as an antagonist of ecdysteroids (MU and LEBLANC, 2002) , and synthetic estrogenic agent such as endsulfan and diethyl bestrol also act as antagonists of ecdysteroids (ZOU and FINGERMAN, 1997). Inhibition of egg production by *A. omorii* was occurred at much lower concentration of testosterone than the lethal concentration. Therefore, testosterone might act as endocrine disruptor not as a simple toxic agent. Egg production test using *A. omorii* could be a good biological model for assessing the possible endocrine disruptor in coastal environment.

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