17β-estradiol has no effect on reproduction success of calanoid copepod, *Acartia omorii*

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ABSTRACT

The natural estrogen 17β-estradiol (E2) is responsible for the endocrine disruption and also has potential effect on the reproduction of vertebrate. However, its effect on invertebrate reproduction has scarcely been studied. The present study was conducted to determine the effect of E2 on survival, egg production and egg hatch of calanoid copepod, *Acartia omorii* under bath administration. Survival was observed every 24h for 10 days. Each 10 individuals of adult female were incubated at different concentration of E2 (0.001, 0.01, 0.1, 1, 10 and 100μg/L) with food (*Isochrysis* sp.+*Tetraselmis* sp., ratio 1:1). Egg production was observed through the incubation of single gravid female with above same conditions. Egg hatching success was examined for each 100 eggs at different concentration of E2, which were obtained from pre-exposed female at the same concentration. More than 80% of survival was obtained in 100μg/L level after 96h exposure periods. Egg production was slightly increased in 100μg/L but not significant. No significant (P<0.05) effect of E2 was observed in hatching rate up to 100μg/L level. Result suggests that there is no effect of E2 on the reproduction success of the calanoid copepod, *A. omorii* in the coastal and marine environment.

Keywords: Copepod, 17β-estradiol, survival, egg production, egg hatch

INTRODUCTION

Due to growing populations and rapid increasing farming system, higher amount of natural estrogens from human and animal origin (including livestock and wildlife) have been constantly released into surface water through municipal and industrial waste way, which result the elevation of hormonal level in the aquatic environment (Ying *et al*., 2002). For example, women excrete 2-12μg/person/day and men 1.6μg/person/day with their urine (Gower, 1975). Hormonal growth promoter is widely used in the world in livestock farms. Evidence was found, in the year 2000, around 33tons of estrogens hormone excreted from various farm animals in European Union and 49 tons in USA and faecal estrogen consists mainly of 17α-estradiol, 17β-estradiol (E2) and estrone (Lange *et al*., 2002). The enrichment of E2 in surface water varies in different water environment at different levels, such as, in Germany up to 3.6ng/L (Kuch and Ballschmiter, 2001); Italy (Tiber river), 0.11ng/L (Baronti *et al*., 2000); Japan (109 major rivers), up to 24ng/L (Tabata *et al*., 2001); Netherlands (coastal/estuarine water and rivers), 5.5ng/L (Belfroid *et al*., 1999).

E2 might endanger for aquatic animals. Many researches have been conducted to determine the impact of E2 to aquatic animals. Among them most of research works were on the reproduction related. In vertebrates mainly in fish: intersex induction in Medaka (Koger *et al*., 2000); alter reproductive behavior in threespine stickleback (Wibe *et al*., 2002); adverse reproductive effect in fathead minnows (Panter *et al*., 1998). Attention has also been paid on the reproduction and development of invertebrates: disruption of the timing of larval development in barnacle (Billinghurst *et al*., 2001); inhibition on the oocyte growth of echinoids (Wasson *et al*., 2000). A little study was found on the affect to zooplankton: development and reproduction of harpacticoid copepod (Htchinson *et al*., 1999a; Breitholtz and Bengtsson, 2001). Moreover, Billinghurst *et al*. (1998) observed the toxic effect of E2 on the barnacle settlement at environmentally realistic concentrations. Generally natural estrogen, E2 is considered as an aquatic pollutant (Shore *et al*., 1993) and also is partially responsible for the disruption of reproduction and development in some wildlife populations (Tyler *et al*., 1998). Report was found that estradiol concentration in water as low as 10ng/L may affect the aquatic animals (Shore *et al*., 1995).

Zooplankton is important in aquatic ecosystems and if E2 gives adverse effect to them, aquatic ecosystem might collapse. In zooplankton community, copepod comprise as much as 70% of them (Steele, 1970). In this paper, the most common calanoid copepod in coastal water, *Acartia omorii* were used to determine the reproductive effect of E2 by testing: (i) acute toxicity for adult female survival, (ii) egg production capabilities of exposed animals subjected to lethal and sub lethal concentrations and (iii) egg hatching success in different concentration of E2 exposed eggs (i.e. eggs were collected from the same concentration of exposed animal in the same condition).

MATERIALS AND METHODS

The detailed description on the culture of *A*. *omorii* and the testing method for a harmful substance was mentioned in the 'materials and methods' of Hossain *et al*. (2004). The test substance, 17β-estradiol was purchased from Wako Pure Chemical Industries, Ltd. Japan (molecular weight: 273.38; melting point: 174-180°C) was dissolved in 99.5% acetone. All exposure media were prepared before 12h of application with filtered seawater (25‰), stored in Pyrex media bottles and preserved in an incubator (20°C) at dark condition during the study.

In this experiment, the exposure concentrations were applied for all observations as 0.001, 0.01, 0.1, 1, 10 and 100μg/L levels. Exposure periods for all observations were 10 days at temperature 20±1°C. For survival observation, 10 females were used for one exposure level incubating single individual in each test tube. In egg production observation, one gravid female was incubated in a 100ml (working volume) exposure medium. In egg hatching observation, each 10 eggs were randomly selected from the laid of 10 gravid female at 72-96h exposure periods and incubated in a micro plate chamber containing 10ml exposure medium with the same concentration of E2. 10 replications were conducted in latter two observations. In all observation, we first carried out control, solvent control and E2 exposure treatments, which were considered as different treatment groups. Because the data from the control and the solvent control did not differ significantly in any of the measured variables, we treated these two groups into a single group for analysis, which will be referred to as control (C) group.

RESULTS AND DISCUSSION

Adult survival

No mortality was observed after 48h exposure period in all treatment. After 96h, more than 80% were survived at highest concentration 100µg/L and after 240h exposure periods only 30% were died in this treatment (Fig.1). No individual was died in control treatment up to144h and were 80% survived after 240h. It should be considered as normal because all individual were picked up from mass culture system, where several age groups were present. In this study, adult individuals (♀) were selected through the superficial viewing with the help of microscope; so all individuals might not be in the same age group.

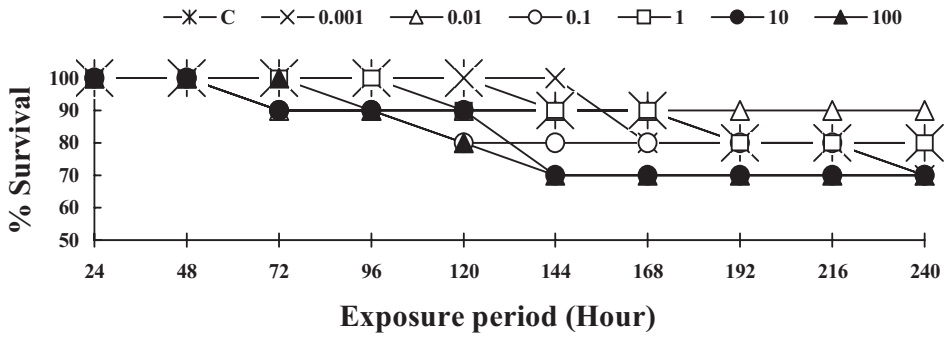

Figure 1. Total survival (%) of adult copepod (♀) at different exposure time.

At the end of 10 days observation, survival was ascertained as 70, 90, 70, 80, 70 and 70% at concentrations of 0.001, 0.01, 0.1, 1, 10 and 100µg/L, respectively. So, in this exposure treatment, increasing level of E2 has not any progressive effect on adult survival. A few reports were found on the effect of E2 on adult survival of copepod. Hutchinson *et al*. (1999a) observed above 90% survival after 240h exposure for adult copepod *Tisbe battagliai* at 100µg/L concentration. Breitholtz and Bengtsson (2001) obtained only 10% mortality in harpacticoid copepod *Nitocra spinipes* after 18 days exposure at 500µg/L. Andersen *et al*. (2001) found, up to 1mg/L of E2 does not have any effect on adult survival of calanoid copepod *Acartia tonsa* after 48-hour of exposure period. These findings, together with our result clearly show very low toxicity of E2 for adult copepod survival. In comparison with androgenic hormone testosterone, which has adverse effect observed; 216h- LC_{50} at 101µg/L bath exposure in *Acartia omorii*, not as male hormone but as an ecdysteroid (Hossain *et al*., 2004), E2 has no mentionable effect on this species. We tried to determine the survival rate of copepod in present persistent level in natural water. The reported level of E2 in natural water are less than 1µg/L (See introduction), it might give no effect on the survival of adult copepods in natural waters.

Egg production

In lower exposure level (up to 10µg/L), almost equal egg production was observed. A little increased (but not significant) egg production was found at highest concentration 100µg/L. Average egg production for all treatments in different exposure days that's ranged was 11-38

eggs (female^{-1}day^{-1}) (Fig.2A-J). In control treatment, mean egg production per a single individual was 11-33 eggs (female^{-1}day^{-1}). In another study, Hossain *et al*. (2003) also reported the 15-26 eggs in *Acartia omorii* under the same condition. So, normal egg production result was obtained in this observation. A large difference was found within the treatment in different exposed day but in average production was not related to the level of E2 (Fig.2K). These results indicate that E2 has no significant effect on the egg production of calanoid copepod up to 100μg/L level.

Andersen *et al*. (1999) observed that E2 stimulated the maturation of ovaries in calanoid copepod *Acartia tonsa* and also increased the egg production rate in different conjunctive day even at low concentration (23μg/L). Sarojini *et al*. (1990) reported that estrogens have stimulating effect on maturation of ovaries of the marine crab, *Scylla serrata*. On the contrary, Breitholtz and Bengtsson (2001) reported that E2 has no hormonal effect on reproduction of harpacticoid copepod *Nitocra spinipes* up to 160μg/L concentration. No effect also found in nauplii production up to 100μg/L concentration in harpacticoid copepod *Tisbe battagliai* after 21 days exposure period (Hutchinson *et al*., 1999a). In this study, enhancing tendency in egg production was observed in calanoid copepod at higher concentration of E2 in long exposure duration but not significant. So, it is indicate the possibility of insensitivity and higher concentration test is need. In the effect of male hormone testosterone, which significantly decreased the egg production at above 9μg/L concentration in *Acartia omorii* under bath exposure (Hossain *et al*., 2004), estrogens E2 have no adverse effect on the reproduction of copepod.

Egg hatch

Only successfully hatched number was counted as hatched nauplii (live or dead). In control, hatching rate was around 81% for eggs laid during 10days incubation period. The same hatch percentages was reported by Hossain *et al*. (2003) at 20°C and Uye (1980) reported that 70 to 98% of eggs hatched at 20°C and maximum 86% egg hatch at 22.2°C in 25‰. Compare to previous investigations, the egg hatch observation of *A*. *omorii* is considered to be performed properly in this study. Hatching rates were nearly the same in all exposure treatment, where average range was 74-83% (Fig.3). Egg hatching rate was fluctuated within the treatment but average values was obtained above 70% in all treatments suggesting that most of laid eggs were viable. Statistically no difference was observed in egg hatching success in any treatment. Therefore, there are no effects of E2 on hatch of copepod eggs up to 100μg/L concentration.

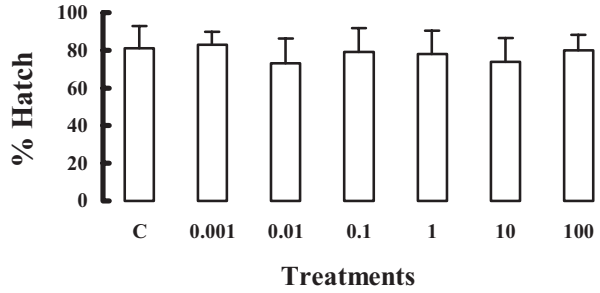

Figure 3. Average egg hatch (%) in each treatment after 10 days of exposure observation.

Hypothesis was being considered that, exposure to breeders also affects their newly produced eggs. So far, no report was found on the egg hatching activities related to E2 in any animal. Therefore, the observed result in hatching success under exposure treatment probably resulted from the absence of hormonal effect and the condition of female when the eggs were produced. So, bath exposure of E2 does not give any effect on the egg hatching success of copepod eggs. In comparison with androgenic hormone testosterone, significantly decreased the egg hatch at above 9µg/L concentration in *Acartia omorii* under bath exposure (Hossain *et al*., 2004), E2 has no mentionable effect on hatch of copepods eggs.

CONCLUSION

A little enhancement was obtained in egg production at 100µg/L concentration of E2 but not significant. There were no hormonal effects ascertained in adult survival and also in egg hatch. It's indicating that E2 has not any effect on adult survival and egg hatch of calanoid copepod up to 100µg/L concentration. Thus, environmental level of hormone E2 is not harmful for the reproduction success of calanoid copepod *A*. *omorii* at the persistent level in coastal and marine environment.

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