



# Effect of Estradiol-17 $\beta$ on Embryonic Tolerance, Growth, and Muscular compactness of Giant Freshwater Prawn, *Macrobrachium rosenbergii*

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## ABSTRACT

Effects of estradiol-17 $\beta$  on the giant freshwater prawn were observed in case of the embryonic tolerance, growth, development, survival rate, yolk adsorption, eye appearance, and muscular compactness. The methods were designed in two steps; those were the tolerance and the growth. In the two hours after spawning, embryos were immersed in 10, 50, 100, or 150  $\mu$ g/ml estradiol-17 $\beta$  solution for two days, and the controls were treated with 15% artificial seawater. The tolerance was monitored in a separate experiment; they were immersed in 10, 50, 100, or 150  $\mu$ g/ml estradiol-17 $\beta$  solutions showed that the mortality rate at 150  $\mu$ g/ml estradiol-17 $\beta$  was 31.66%. The growth represented by yolk adsorption, hatching rate, and eye appearance. The results showed that the eyes appeared earlier than those of the controls. The survival rates were investigated in other experiments. The results showed that the highest survival rate was 48.16%, observed in the 50  $\mu$ g/ml estradiol-17 $\beta$  treatment. Therefore, estradiol might accelerate the growth as indicated by the number of days for eye appearance being shorter period than in the control treatment. The eyes of the embryos treated with 50, 100, and 150  $\mu$ g/ml estradiol-17 $\beta$  appeared on day 10, whereas those in the control were observed on day 16. Hatching rate was tending to high in 150  $\mu$ g/ml estradiol-17 $\beta$  but those were not significance with the control. Yolk adsorption was found in treated embryo rather than those of the controls. The pattern of yolk cluster distribution was differing from the control. The muscle tissue was observed on day 20 after the histological process. The results showed that the bundles muscle cells were more compactness and were larger, denser, and stronger with increasing concentrations of estradiol-17 $\beta$  than that the controls. Therefore, estradiol-17 $\beta$  should be applied to stimulate growth and might be introduced with the feed to the prawn industry and manufacturing.

## 1. INTRODUCTION

Prawn is an economical animal in many countries in Asia such as Bangladesh, China, Vietnam, and Taiwan including Thailand. A low production yield, small size, diseases and a different size in one batch are problems. Therefore, the management of reproduction of prawn is interesting to increase the production yields. Several projects have improved the management of heterogeneous prawn populations in grow-out ponds and recognition of special requirements during harvesting and processing to ensure that only high-quality products reach the market. The giant freshwater prawn, *Macrobrachium*

*rosenbergii*, is one of the most economically important aquatic animals. It belongs to the Phylum Arthropoda, Class Crustacean, Order Decapod, Infra-order Caridea, Superfamily Palaemonoidea, Genus *Macrobrachium*, Species *rosenbergii*. It is the largest of all known *Macrobrachium* [1]. *M. rosenbergii* lives in tropical freshwater environments with turbid conditions caused by adjacent brackish water areas. Due to the fact that its larval development must take place in brackish water, gravid females migrate downstream into estuaries where the eggs hatch as free-swimming larvae. From egg hatching to postlarvae, the planktonic larvae pass through several zoeal stages. After metamorphosis, postlarvae assume a more benthic lifestyle and begin to migrate upstream towards freshwater to become adults [2]. A low production yield, small size, diseases and size differences are some of the problems of production. Therefore, management of the reproduction of prawn is important to increase production yields.

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Estrogens are steroid hormones which play a significant role in the reproductive system. Although estrogens are regarded as female steroid hormones, they have known to have profound effects on both female and male reproductive systems. The occurrence of estradiol-17 $\beta$  (E2) has been reported in decapods such as red mud crab *Scylla serrata* [3] and copepods *Acartia tonsa* [4]. In flies, E2 induces vitellogenin synthesis [5]. In corals, *Montipora verrucosa*, E2 was

found in the homogenates of tissues and spicules. It plays a role in the regulation of gametogenesis and spawning of coral; therefore, E2 acts as a bioregulator of coral reproduction [6]. In Asteroidea, *Sclerasterias mollis*, the sizes of oocytes and protein levels in the ovaries of E2-treated groups were higher than the control [7]. In copepod, *Acartia tonsa*, E2 stimulates sexual maturation and egg production [4].

**Table 1:** Mortality rates of embryos treated with different estradiol-17 $\beta$  concentrations.

Tolerance Dose	Number of Embryos Death			Total Mortality	Mean $\pm$ SD	Mortality rate (%)
	rep 1	rep 2	rep 3			
0 $\mu$ g/ml	30	22	21	73	24.33 $\pm$ 3.84	24.33
10 $\mu$ g/ml	32	20	21	73	24.33 $\pm$ 3.84	24.33
50 $\mu$ g/ml	30	31	19	80	26.66 $\pm$ 3.84	26.66
100 $\mu$ g/ml	22	26	31	80	26.66 $\pm$ 2.90	26.66
150 $\mu$ g/ml	38	20	37	95	31.66 $\pm$ 5.84	31.66

**Table 2:** Survival rates of embryos after subjects with different E2 concentrations for two days and reared in fresh 15% ASW until day 19.

Estradiol-17 $\beta$ concentrations	Survival rates (%)
15%ASW	46.83 $\pm$ 1.64
10 $\mu$ g/ml	45.00 $\pm$ 5.07
50 $\mu$ g/ml	48.16 $\pm$ 2.62
100 $\mu$ g/ml	45.83 $\pm$ 0.88
150 $\mu$ g/ml	43.66 $\pm$ 1.20

Invertebrate, E2 induces ovary-like gonad (primordial follicle) formation of Tamar Wallaby [8] and act as a Chemotactic-like agent, to guide PGCs engagement into the chick gonads [9]. During sexual differentiation in carp, *Cyprinus carpio*, E2 increased vitellogenin in blood and then was taken up by the developing oocytes [10] resulting in the full-grown gonad and increase number of spermatozoa in male fish, eelpout, *Zoarces viviparus* [11]. Moreover, E2 was also responsible for the biosynthesis of egg yolk protein, vitellogenin, in the liver (the major target organ for estrogen) of fishes [12]. Also, it was found in hemolymph, ovaries, and hepatopancreas in female shrimp, *Penaeus monodon* [13]. In tadpoles, E2 stimulated aromatase activity to cause vitellogenesis in developing oocytes in the gonads [14]. It also stimulated lipogenic activity in the ovary and play a role in stimulation of female sexual maturation and egg production of the giant freshwater prawn [15].

This present study showed the determination of the effect of estradiol-17 $\beta$  on embryonic tolerance, survival rate, and growth, as indicated by eye appearance and differences in muscle bundles of giant freshwater prawn embryos. The lethal dose was monitoring for *M. rosenbergii* and the survival rates were investigated.

## 2. MATERIALS AND METHODS

The mature male giant freshwater prawns weighed 60-100 g and females weighing 25-30 g were purchased from prawn farm in Supanburi Province, Thailand. They were acclimatized in large fiberglass tanks for 1-2 days and were separated in an individual compartment and were laboratory-maintained as previously described [16]. After premating molt of the female, mating was allowed by placing a male with the female. The mating was ascertained by the presence of the spermatophore at the sternum of the female. After spawning, a cluster of embryos was removed within 2 h and were subjected to treatments.

E2 was prepared as described previously [17]. The 1-cell stage embryos on day 1 after oviposition were placed in a 100 ml-evaporating dish containing about 30 ml of 15% (v/v) artificial seawater (ASW) which comprised 0, 10, 50, 100, 150  $\mu$ g/ml E2.

Tolerance dose was tested for 48 hours before subject to treatments 0, 10, 50, 100, 150  $\mu$ g/ml E2.

Embryos were treated for 2 days before transferring to fresh 15% ASW and were cultured to hatching. Eyes appearances were monitored every 2 days of developmental progress. The survival rates were determined on day 19 after spawning. The embryos were sampled on day 19 to process for histological study.

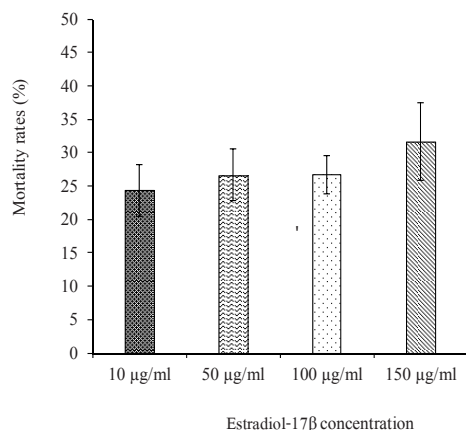
Sections of 5  $\mu$ m thickness were prepared and stained with hematoxylin-eosin. The muscle development was observed under a bright-field microscope (Olympus BX 51) and photographs were taken (Olympus DP 50 digital camera).

The statistical test was used One-way ANOVA on SPSS version 11.5 for the window to compare mean values of various treatment groups, followed by Duncan multiple range test (multiple comparisons between the means of each treatment) and were considered significant at  $P < 0.05$ .

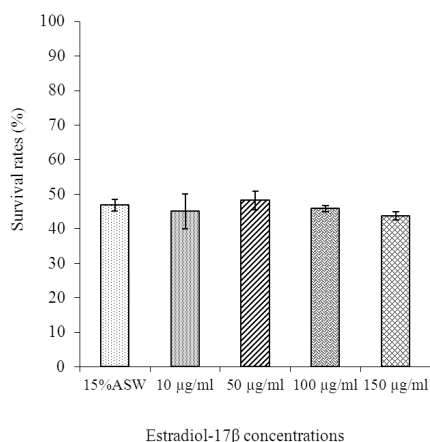
## 3. RESULTS

### 3.1. Tolerance, Survival Rates, and Hatching Rates

The mortality rates among the embryos treated with different estradiol-17 $\beta$  concentrations were not significant to that of the control ( $p > 0.05$ ). The highest concentration in this study was 150  $\mu$ g/ml; and therefore, the results showed that therefore the highest mortality rate was 31.66% observed in the 150  $\mu$ g/ml estradiol-17 $\beta$  treatment. The tolerance dose may be more than 150  $\mu$ g/ml estradiol-17 $\beta$  (Figure 1). The lowest mortality rate was 24.33% observed in the 10  $\mu$ g/ml estradiol-17 $\beta$  treatment. In addition, the survival rate decreased with increase in the estradiol-17 $\beta$  concentrations. The lowest survival rate was 43.66% observed in the 150  $\mu$ g/ml estradiol-17 $\beta$  treatment. The highest survival rate was 48.16% observed in the 50  $\mu$ g/ml estradiol-17 $\beta$  treatment (Figure 2). However, there were no differences from the control ( $p > 0.05$ ). Therefore, the tolerance of the prawn embryos in this study was found to be over the highest concentration of the estradiol-17 $\beta$  (150  $\mu$ g/ml) tested. The hatching rates tend to higher with increasing E2 concentrations. However, it is lesser than that of the control, 10  $\mu$ g/ml being no effect with E2.



**Figure 1:** Mortality rates of embryos treated with different estradiol-17β concentrations.



**Figure 2:** Survival rates of embryos treated with different estradiol-17β concentrations.

### 3.2. Eye Appearance

Eyes were observed at days 8, 10, 12, 14, and 16 after spawning. The results showed that the eyes of the embryos treated with 50, 100, and 150 μg/ml estradiol-17β almost all appeared on day 10, whereas those of the control appeared on day 16. The present study investigated the day of eye appearance, survival rate, muscle tissue, and yolk mass to indicate the growth of the prawn embryos. The result showed that the eyes of almost all embryos treated with 50, 100, and 150 μg/ml estradiol-17β were observed clearly on day 10, whereas this was not found in the controls. Thus, this study indicated that estradiol-17β either reduced the number of days for the eye appearance or increased the yolk used for development.

### 3.3. Yolk Adsorption

On day 19, differences in the eye sizes were observed among the treatments with degenerated yolk masses. The yolk mass of the embryos from the higher treatment concentrations was more degraded (Figure 3). The yolk is adsorbed gradually during development. The yolk mass was investigated to indicate the growth of the prawn embryos. The results showed that the yolk masses of the 100 μg/ml and 150 μg/ml estradiol-17β treated embryos were reduced. Moreover, degradation of the yolk was observed while the activity of the prawns was increased by estradiol-17β.

### 3.4. Muscular compactness

The embryos after hatching were collected for determining muscle bundles. The results showed that the muscle cells and bundles became more compact with increasing concentrations of estradiol-17β (Figure 4). The embryos treated with 50 μg/ml estradiol-17β had larger and stronger bundles of muscles than the control.

Moreover, the embryos that were treated with 150 μg/ml estradiol-17β had larger bundles that were denser and stronger than under the other treatments (Figure 4E). The growth of the prawn embryos in this study was promoted by estradiol-17β that resulted in the size of the treated embryos being larger than the controls because of muscle compactness. Estrogen has been proposed as a potential therapeutic agent for female humans, as these agents may potentially limit muscle damage and inflammation by stimulating the repair of muscles.

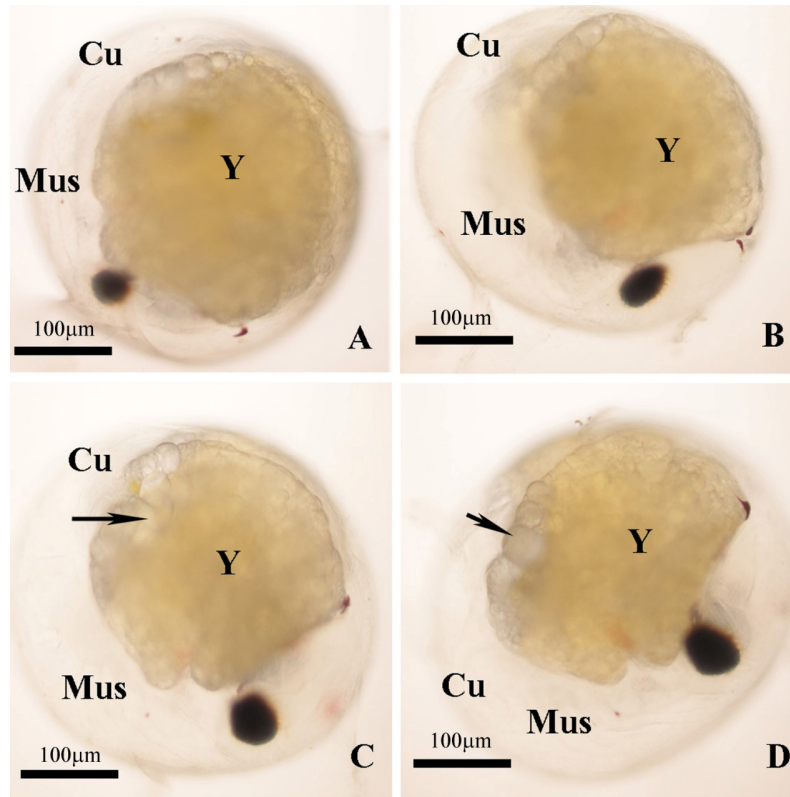
## 4. DISCUSSION

The lethal dose ( $LD_{50}$ ) was more than 150 μg/ml estradiol-17β. Therefore, estradiol-17β did not affect the survival rates (Pakdeenarong and Damrongphol, 2006). The tolerance dose was more than 150 μg/ml estradiol-17β. When estradiol-17β was injection into adult giant freshwater prawns it affected the enzyme activities [18]. Which indicated that estradiol-17β can affect the embryonic metabolism. Whereas, our previous study on the growth of estradiol-17β treated embryos showed that it promoted the survival rate [17].

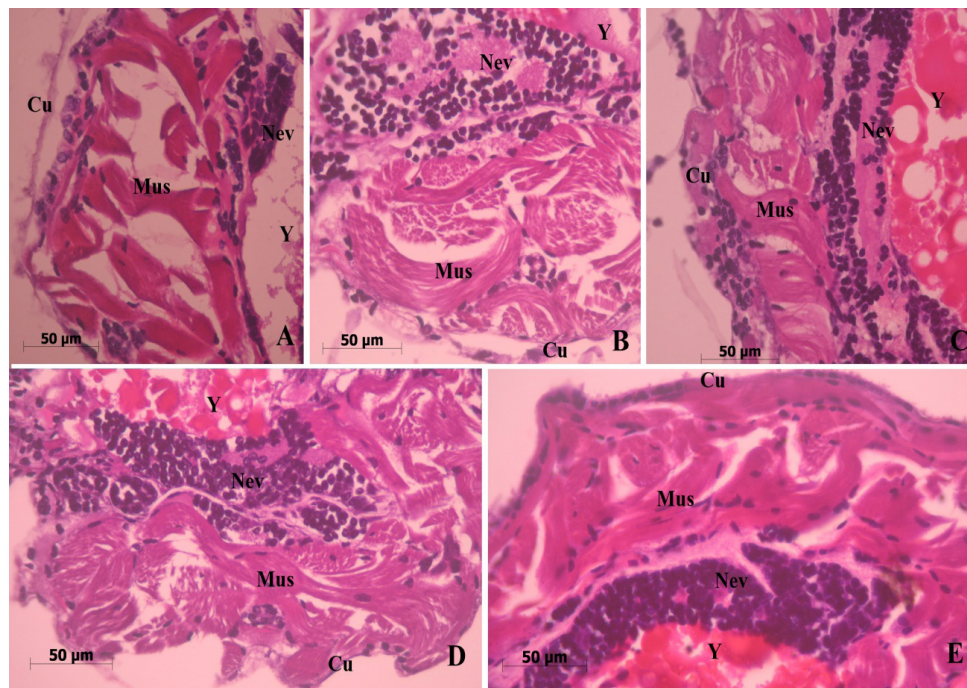
The recent study was to investigate the day of eye appearance, survival rate, muscle pathology, and yolk mass to indicate the growth of prawn embryos. The result shows that eyes were observed at day 8, 10, 12, 14, and 16 after spawning. The result shows that the eyes of almost treated embryos in 50, 100 and 150 μg/ml estradiol-17β were observed on day 10 distinctly whereas those not found in the controls. The results showed that the lowest of survival rate was 43.66% observed in 150 μg/ml estradiol-17β. The highest survival rate was 48.16% observed in 50 μg/ml estradiol-17β. The survival rate was 46.83%. The muscular tissue was studied on day 20 after spawning. The result showed that the muscle cells and bundles were gradually more compactness with increasing of estradiol-17β concentration. Since the embryos treated with 50 μg/ml estradiol-17β had larger and stronger of bundles of muscles than the control. Moreover, embryos were treated with 150 μg/ml estradiol-17β had larger bundles, denser and stronger than the others. E2 plays a role in body mass index in human [19], although the growth of prawn embryo of this study to promote by E2 resulting in the size of treated embryos was larger than the controls. Estrogen has been proposed as potential therapeutic agents for women, as these agents may potentially limit muscle damage and inflammation with stimulating and repair in muscles. The effects of estrogen on skeletal muscle to play a significant role in stimulating muscle repair and regenerative processes, including the activation and proliferation of satellite cells [20], although E2 plays an important role in the muscles both in vertebrate and invertebrate according to this study.

Yolk mass to indicate the growth of prawn embryos were investigated. Yolk composes of vitellogenin and protein which produce from hepatopancreas of brooder to egg [21]. Freshwater prawn treated with estradiol-17β increase mitochondrial  $Na^+-K^+-ATPase$ , cytosolic malate dehydrogenase, and cytosolic glucose-6-phosphate dehydrogenase activities [22]. The result showed that the yolk mass of 100 μg/ml and 150 μg/ml estradiol-17β treated embryos were reduced. Moreover, degradation of yolk was observed by mean the activities of prawn was increased by E2.





**Figure 3:** Embryos on day 19 after immersed in different E2 concentration for 2 days after spawning. A) Embryos immersed in 15% ASW without E2 served as control; B) Embryos immersed in 10  $\mu\text{g/ml}$  E2 for 2 days after spawning; C) Embryos immersed in 50  $\mu\text{g/ml}$  E2 for 2 days after spawning shown yolk degeneration indicated by an arrow; D) Embryos immersed in 150  $\mu\text{g/ml}$  E2 for 2 days after spawning shown yolk degeneration indicated by the arrow. Abbreviation: Cu = cuticle; Mus = muscle; Y = yolk; = yolk degeneration; scale bar = 100  $\mu\text{m}$ .



**Figure 4:** Muscular tissues of embryos treated with different estradiol-17 $\beta$  concentrations. A) Muscles tissue of embryo without treated with E2 had no fiber characteristics. Muscle fiber arranged in disorder and tissue mass was loose and less. B) Muscles tissue of embryo treated with 10  $\mu\text{g/ml}$  E2 showed an arrangement of fiber, however, the mass of fibers was the loose arrangement. C-E) Muscles tissue of embryo treated with 50, 100, 150  $\mu\text{g/ml}$  E2, respectively. The gradual concentration of E2 induced the greater of the mass of muscular tissue. Striated muscle characteristics were observed as multiple nuclei, and fibers were arranged in order. The budding muscle was observed in high concentration indicate a more strength of muscular function. Abbreviation: Cu = cuticle; Mus = muscle; Y = yolk; Nev = ventral nerve cord.

## 5. CONCLUSION

That E2 concentration range was no affects to the mortality rate in this study indicated by LD50 was higher than 150 µg/ml of E2. Likewise, the tolerance and survival rates had no significance with the control that implies that E2 was no affects to tolerance and survival rates. Yolk adsorption, compactness muscular bundles, and early eye appearance were observed. Therefore, E2 effects on growth of prawn embryos. The optimum E2 concentration was 50 µg/ml. Nevertheless, all E2 treated embryos had more compactness muscle cells and bundles than those of controls. This study indicated that E2 may accelerate growth activities of embryo periods and farmers can use E2 for prawn hatcheries and nurseries.

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