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The Effects of 17 β-Estradiol on Growth, Survival and Feminization of Green Tiger Shrimp, *P. semisulcatus* (Decapoda: Penaeidae)

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Abstract: The effects of 17 β-estradiol were investigated on survival, feminization and growth of green tiger shrimp, *Penaeus semisulcatus* in different life stages. Egg, nauplius, protozoea, mysis and postlarvae were immersed in water containing 50 μg L^{-1} 17 β-estradiol from the beginning of the each stage to the end of the stage. The grow out were carried out about 4 months to emerging the secondary sexual characters in ventral side of shrimp in this ponds. The results of the experiment showed that the method of immersion 50 μg L^{-1} 17 β-estradiol in eggs, nauplius, protozoea stages decreases the survival rate. The best feminization rate (71.88%) were observed in naupli group and this result was important statistically than control and other groups ($p<0.001$). Respect of the growth rate, the highest mean weight was 14.15±2.41 g in nauplii group and this followed by control (13.66±2.48 g) and egg groups (12.04±0.68 g) and were significantly different from the other groups ($p<0.05$).

Key words: 17 β-estradiol, shrimp, *Penaeus semisulcatus*, survival, feminization, Turkey

INTRODUCTION

In some crustaceans, growth rate is different between sexes (Garcia, 1985; Hartnoll, 1985; Dall *et al*., 1990; Hansford and Hewitt, 1994; Diaz *et al*., 2001; Perez-Rostro and Ibarra, 2003). The penaeid shrimp also exhibit a sex-dependent dimorphic growth pattern with females growing faster and reaching a larger ultimate size than males (Choe, 1971; Hartnoll, 1985; Bray and Lawrence, 1992; Primavera *et al*., 1998).

It has been reported that females have a higher food efficiency utilization and apparent digestibility of energy (Moss and Moss, 2006; Gopal *et al*., 2010). So, it is considered that mono-sex populations consisted of all females are preferred in shrimp culture. Among several monosex production techniques, hormonal sex reversal directly or indirectly, through breeding of sex-reversed fish has commonly been used to produce monosex populations in aquaculture (Hunter and Donaldson, 1983; Piferrer and Lim, 1997).

While 17 β-estradiol, the natural estrogen has been shown to be an effective feminization hormone in some fish (Pandian and Sheela, 1995), the administration of the steroids in crustaceans focused on reproduction has been attempted but the results are varied and sometimes inconsistent (Wilder *et al*., 2002; Okumura, 2004). Hence, hormonal induction of feminization may be an important and interesting subject for penaeid shrimp species and to improve economic gains in shrimp farming. *P. semisulcatus* which is an Indo-Pacific species distributed along the coast of the Eastern Mediterranean and is one of the most important commercial species in this region. A few commercial farms and research center in Turkey practice its culture on small scale. This study was therefore aimed to investigate the effect of 17 β-estradiol on survival, feminization and growth of *P. semisulcatus*.

MATERIALS AND METHODS

Experimental shrimp stock, their maintains, experimental design: This study was carried out at the Marine Research Station (Faculty of Fisheries, Mustafa Kemal University, Kale Village (36°17'29.98"N-35°47'4.40"E), Iskenderun, Hatay, Turkey. The eggs obtained from females which caught in the fourth gonadal stage in Iskenderun Bay, North East Mediterranean sea (36°22'35.39"N-35°44'15.10"E and 36°38'23.08"N-36°3'0.57"E) by gill net operation in early morning July 2007. Females (65.55±6.1 g weight and 182.50±7.10 mm total length) transferred in 250 L plastic tank (in oxygenated sea water) to the station approximately in 2 h by boat (Mustafa Kemal 1 RV, 23 m, 300 Hp) and spawned in 100 L plastic tank at 38 g L^{-1}

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salinity and 28°C in the same day night. Following spawning, fertilization rate was determined under the inverted-microscope (CKX31 Olympus) in 10 min. The eggs taken from 2 females were pooled and concentrated onto a 100 μm sieve. The eggs were then counted and stocked into 2 L round bottom glass flasks containing UV filtered sea water in four replicates at a density of 100 eggs L^{-1}.

Salinity and temperature were measured with a digital salinometer (YSI 30 model USA). Moderate aeration (approximately 4 bubble sec^{-1}) was maintained through a silicon rubber tube with a glass rod at the tip. After completion of the stocking, the glass flasks containing eggs, separated 6 different groups (Control, eggs, nauplii, protozoea, mysis and postlarvae) for hormone treatment. To determine the proper hormone dosage, the effects of 5 different levels 17 β-estradiol (1000, 500, 250, 100 and 50 μg L^{-1}) were tested take into account of eggs and larval survival for a week. Because of survival rate were found high only 50 μg L^{-1} Eastrodiol 17 β levels, hormone immersion dosage were chosen as 50 μg L^{-1}.

Hormone immersions: Stock solution of hormone were prepared by dissolving 0.1 g 17 β-estradiol (Sigma, St. Louis, MO, USA) in 1 mL 95% ethanol (Merck). Hormone immersions were given to eggs, nauplius, protozoea and mysis from the beginning of the each stage to the end of the stage. Hormone immersion of PL were started at PL1 stage and ended at PL5. After completion of the hormone immersion (following at the end of the each stages), the flasks were emptied, washed and they were filled with filtered sea water. The experiment were maintained to the PL stage in this flasks. The larvae were fed a mixture of *Tetraselmis chuii* (20 cells μL^{-1}), *Isochrysis galbana* (25 cells μL^{-1}) and *Chaetoceros calcitrans* (50 cells μL^{-1}) produced in a batch culture system. Newly hatched *Artemia nauplii* (5 mL^{-1}) were introduced into the culture flask when entered mysis1 stage. After completion of the hormone immersion, some samples were transferred into 10 mL test tubes and stored at -70°C in an ultra cold freezer before the analysis of hormone were conducted.

When the larval stages was to be completed and attaining PL1 stage, the replications of the groups were incorporated transferred to the fiberglass tanks (500 L capacity). PL's reared to the stage of PL15 in this tanks.

Following this culture period (in PL15 stage) all PL's were harvested and stocked to the earthen ponds as 5 PL m^{-2}. The grow out were carried out about 4 month to emerging the secondary sexual characters in ventral side of shrimp in this ponds. Following the harvest, female-male rate, survival rate (Number of larvae at the end of each treatment/number of larvae at the beginning of the treatment×100), mean growth rate were determined for each experiment groups, respectively.

Statistical analysis: Differences in growth, survival rate were assessed by one-way ANOVA. Differences were considered significant at the level of $p<0.05$. Sex ratio obtained in the experiments was subjected to the χ^2-test. Differences from the expected 1:1 ratio (female: male) were considered significant at the level of $p<0.001$.

RESULTS AND DISCUSSION

Survival and growth rate: The survival rates of control and experimental groups (Eggs, nauplii, protozoea, mysis and PL) taken from different culture periods were shown in Table 1. All larvae died within a short period of time at the level of 1000, 500, 250 and 100 μg L^{-1} 17 β-estradiol, except 50 μg L^{-1}. The survival rates of control and experimental groups (Eggs, nauplii, protozoea, mysis and PL) 50 μg L^{-1} hormone treated *P. semisulcatus* varied between 20.37±4.66 and 77.25±7.81% to the PL1 stage. The survival rates of PL, mysis and control groups were found statistically different from the eggs, nauplii and protozoea groups ($p<0.05$). The survival rate of *P. semisulcatus* from PL1 to the PL15 stage varied between 56.93% (PL group) and 75.09% (Nauplii group). The survival rates of control and experimental groups were found for 78.06% control, 67.32% eggs, 61.94% nauplii, 65.82% protozoea, 80.00% mysis and 73.55% PL groups, respectively at the end of the harvest. The average weight of the shrimp taken from control and experimental groups at the end of the study ranged between 14.15±2.41 and 11.39±0.54 g (Table 1). The highest mean weight was 14.15±2.41 g in nauplii group and this followed by control (13.66±2.48 g) and egg groups (12.04±0.68 g) and were significantly different from the other groups ($p<0.05$).

Table 1: Survival rate of *P. semisulcatus* treated 17 β-estradiol in early life stages

Groups	No. (100 L^{-1})	17 β-estradiol (μg L^{-1})	Survival rate egg-PL1 (%)	Survival rate PL1-PL15 (%)	Stocking density in pond (No m^{-2})	Survival rate PL15-harvest (%)
Control	800	0	67.50±5.83^a	62.96	5	78.06
Eggs	800	50	34.25±4.15^b	73.33	5	67.32
Nauplii	800	50	20.37±4.66^b	75.09	5	61.94
PZ	800	50	37.00±6.41^b	70.95	5	65.81
Mysis	800	50	76.88±3.05^a	65.04	5	80.00
PL5	800	50	77.25±7.81^a	56.93	5	73.55

*Mean values in rows with different superscripts are significantly different ($p<0.05$)

Table 2: Female/male rate of *P. semisulcatus* treated 17 β-eastrodiol in early life stages

		Percentage		Number		
Dosage (μg L^{-1})	Application stages	Female♀	Male ♂	Female ♀	Male ♂	χ^2
0	Control	49.59	50.41	60	61	-
50	Egg	56.31	43.69	58	45	1.640
50	Nauplius	71.88	28.13	69	27	18.380*
50	Protozoea	46.08	53.92	47	55	0.627
50	Mysis	48.39	51.61	60	64	0.129
50	PL1	49.12	50.88	56	58	0.035

Significance levels (*p<0.001) are for χ^2values in comparison with an expected sex ratio of 1 Female:1 Male

Sex ratio: The sex rations of experimental groups hormone treated *P. semisulcatus* were shown in Table 2. Sex rations in nauplii groups did differ from a 1:1 ratio (p<0.001). Sex rations of control, egg, protozoea mysis and post larvae groups didn't differ from 1:1 ratio.

The survival rates of hormone treated eggs, nauplii and protozoea of *P. semisulcatus* were very low comparing with control, mysis and PL stages. Low survival of early stages of *P. semisulcatus* treated with hormones was not surprising because 17 β-estradiol treatments result in higher mortality of most aquaculture species (Kim *et al*., 1997; Kirankumar *et al*., 2003; Sower *et al*., 1984; Herman and Kincaid, 1988; Karayucel *et al*., 2006). It is most likely as the result of exhaustion subsequent to enhanced liver metabolism. It was also reported that 17 β-estradiol at 62.5 μg L^{-1} and higher levels may cause growth suppression and low survival for mysid shrimp *Americamysis bahia* (Hirano *et al*., 2004).

CONCLUSION

This result may show that 17 β-estradiol treatment to the eggs and early larval stages of *P. semisulcatus* may cause high mortality. Survival rates from PL1 to the harvest in experimental groups were not significantly differ control and other groups. As seen from the present study, the average weight of the shrimp of control and experimental groups ranged between 14.15±2.41 and 11.39±0.54 g at the end of the study. Although, there were significant differences point of average weight gained between hormone treated and control groups, it is not confirmed clearly present study results. So, it is not meaningful to say whether the estradiol had a positive or negative effects on growth for *P. semisulcatus*. It is needed more detailed study in the future to determine the effects of estradiol on growth in penaeid shrimp. A treatment of 17 β-estradiol at the concentration of 50 μg L^{-1} produced 71.88% females in early larval stages of *P. semisulcatus* and the level and proportion of feminization in hormone treated groups appeared to be administration time and duration. Because feminization rate were found higher in early stages (eggs and nauplii) than the latter stages. To the knowledge, the present study is the first report related to the effect of estradiol on feminization, survival and growth of *P. semisulcatus*. 17 β-estradiol didn't have a clear influence on growth of *P. semisulcatus* not at the dose range tested in the study. However, feminization rates of the nauplii and eggs were observed to be better than those of the control and other hormone treated groups. Further research is needed to fully understand the effects of 17 β-estradiol on feminization, survival and growth of penaeid shrimps.

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