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EFFECTS OF FRESH WATER AND SEA WATER ON GROWTH, ESTRADIOL-17 β CONCENTRATION AND OVARIAN DEVELOPMENT IN JUVENILE TILAPIA

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Abstract

The effects of fresh water and sea water on growth, estradiol-17 β levels, ovarian and oocyte development of *Oreochromis niloticus*, *O. aureus* and *Tilapia zilli* were determined. Sea water affected growth positively ($p < 0.05$) but estradiol-17 β concentrations in fish reared in sea water did not reach the levels in fish reared in fresh water. Ovarian stages developed differently in sea and fresh water. Ovulation occurred only when the fish were grown in fresh water whereas mature and atretic oocytes were seen simultaneously in all species when grown in sea water. There were no statistically significant differences in oocyte diameter between the groups grown in fresh and sea water ($p > 0.05$).

Introduction

Tilapia are preferred by culturists because of many positive characteristics (Sarihan and Toral, 1982; Ali, 1983; Stickney, 1986; Balarin and Haller, 1987). One of the most important is its being a euryhaline fish that can be cultured in both brackish and sea water (Watanabe et al., 1985; Watanabe and Kuo, 1985; Ridha and Lone, 1990).

The growth of many fish is inhibited during late maturation stages. The growth of female tilapia is poorest during sexual maturation (Bromage, 1989). Reproduction is inhibited by salt water (Balarin and Haller, 1987) and depends directly or indirectly on the activity of hormonal glands that have stimulatory and inhibitory effects on the maturation process.

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There is not enough research about steroid hormone levels and gonadal development of tilapia held in sea water. The present study was conducted to compare somatic growth, estradiol-17 β levels and ovarian development stages in three commercially-important tilapia species grown in fresh and sea water.

Materials and Methods

For this study, *Oreochromis aureus* (Steindachner, 1864), *O. niloticus* (Linnaeus, 1757) and *Tilapia zilli* (Gervais, 1848) were used. Mean initial weights of the three species were 0.70 $\pm$ 0.09 g, 0.45 $\pm$ 0.04 g, 0.60 $\pm$ 0.03 g in fresh water and 0.235 $\pm$ 0.08 g, 0.245 $\pm$ 0.01 g, 0.22 $\pm$ 0.09 g in sea water, respectively.

Experiments were carried out at the Fresh and Marine Water Fisheries Research Stations of the Fisheries Faculty of Çukurova University, Turkey. The fresh water study lasted 28 weeks and the sea water 18-20 weeks. Fish were stocked into six fiberglass tanks (diameter 4 m), one for each treatment. The tanks were divided in two by net frames and each side was stocked with 500 fish. Water was pumped into the tanks at 4 l/min. Fresh water (35 ppt) was pumped from an irrigation channel of the Seyhan Dam Lake and sea water was pumped directly from the sea. Water temperature, dissolved oxygen concentrations and salinity were measured twice a day; pH was measured daily. In freshwater tanks, water temperatures were maintained between 20.8 $\pm$ 0.2°C (mean minimum temperature) and 27.2 $\pm$ 0.1°C (mean maximum temperature). Dissolved oxygen ranged between means of 7.09 $\pm$ 0.32 and 8.02 $\pm$ 0.32 mg/l. pH ranged between means of 7.00 $\pm$ 0.44 and 7.88 $\pm$ 0.09. The temperature of seawater tanks ranged between means 22.1 $\pm$ 0.1°C and 27.6 $\pm$ 0.1°C, dissolved oxygen between 7.16 $\pm$ 0.12 mg/l and 7.84 $\pm$ 0.01 mg/l, pH between 7.10 $\pm$ 0.15 and 7.76 $\pm$ 0.11 and salinity between 34.73 $\pm$ 0.05 and 35.98 $\pm$ 0.02 ppt (all figures are mean minimum and maximum).

Fish were fed 0.25, 1 and 2 mm trout granules with a crude protein level of 55% until week 11, when they weighed approximately

10 g, and then 2 mm pellets with a crude protein level of 45%, depending on the sizes of the fish and their mouth openings. The food ration was 5% of the body weight, distributed four times daily.

Ten fish were randomly sampled every two weeks for histological analysis. For hormonal analysis, 25 fish were sampled in the first two samplings, 20 during weeks 4-12, and 15 thereafter. Whole fish bodies were homogenized in 3% trichloroacetic acid (Rothbard et al., 1987). Homogenates were stored for further study at -25°C. The samples were then thawed at room temperature and homogenates were extracted with diethyl ether 3-4 times. The diethyl ether was evaporated, and the steroid dissolved in RIA (radioimmunoassay) buffer. Samples of 100 μ l were pipetted into serum-coated tubes, 1 ml tracer ¹²⁵I estradiol was added, then vortexed and incubated for three hours at room temperature (Coat-A-Count Estradiol Kit; DPC, Los Angeles). After incubation, tubes were decanted and steroid hormones determined using an Iso-Data Gamma Counter. For histological analysis, after autopsy, gonads were fixed in Bouin's solution then embedded in paraffin blocks. Paraffin sections were sliced into 5 μ m sections and stained with hematoxylin and eosin. The lengths and widths of oocytes were measured with a micrometric ocular in a dissecting microscope.

Statistical analyses were carried out with Duncan's Multiple Range (among species in the same environment) and *t* tests (between the same species in fresh and sea water) using the SPSS package program.

Results

Growth. There were no significant differences in body weight at week 28 between *O. niloticus* and *O. aureus* reared in fresh water (Table 1) but both were significantly larger than *T. zilli* ($p < 0.05$). Due to a power break-down in the sea water supply that caused the death of the fish, the *O. aureus* and *T. zilli* experiments ended in week 18 and the *O. niloticus* experiment ended in week 20. At that time, the mean body weights of *O. niloticus* and *T. zilli* did not statistically differ from one another ($p > 0.05$),

Table 1. Mean body weights of three tilapia species grown in fresh or sea water.

Sample (week)	Fresh water			Sea water		
	<i>Oreochromis niloticus</i>	<i>Oreochromis aureus</i>	<i>Tilapia zilli</i>	<i>Oreochromis niloticus</i>	<i>Oreochromis aureus</i>	<i>Tilapia zilli</i>
0	0.45±0.04a	0.70±0.09b	0.60±0.03ab	0.24±0.01a	0.23±0.08a	0.22±0.09a
2	1.81±0.04a	2.16±0.01b	1.79±0.04a	0.86±0.02ab	0.91±0.01a	0.77±0.01a
4	3.72±0.04a	4.18±0.03b	3.36±0.05c	1.77±0.01a	1.97±0.10a	1.42±0.09a
6	4.87±0.12a	5.92±0.02b	4.47±0.03c	4.16±0.04a	4.38±0.75a	3.39±0.14b
8	6.81±0.15ab	7.52±0.10a	5.81±0.30b	7.04±0.12a	8.28±0.09b	7.48±0.12a
10	9.65±0.13a	9.59±0.02a	8.50±0.29b	9.93±0.46a	11.70±0.13a	10.19±0.75a
12	12.08±0.49a	12.81±0.08a	10.30±0.27b	12.19±0.19a	14.28±0.29b	12.96±0.43b
14	14.52±0.04ab	15.25±0.05a	13.53±0.45b	15.69±0.52a	17.12±0.63a	15.06±0.06a
16	17.26±0.02ab	18.40±0.28a	15.18±0.46b	17.72±0.15a	20.14±0.02b	16.24±0.09c
18	18.72±0.04ab	19.29±0.33a	17.31±0.07b	21.70±0.09a	22.73±0.41b	20.03±0.79a
20	20.35±0.04ab	21.25±0.03a	19.42±0.01b	23.73±0.35	*	*
22	21.87±0.02a	22.60±0.04a	19.88±0.02b	*		
24	23.60±0.09a	23.10±0.07b	20.50±0.02c			
26	24.10±0.04a	24.56±0.09b	21.52±0.02c			
28	25.07±0.04a	25.91±0.15a	22.07±0.04b			

Different superscripts indicate significant differences between species according to Duncan's Multiple Range Test.

*Experiment ended due to fish mortality.

but they were somewhat lower than that of *O. aureus* ($p<0.05$). Mean daily weight gains in fresh and sea water were 0.124 ± 0.02 and 0.144 ± 0.01 g/day for *O. niloticus*, 0.128 ± 0.01

and 0.151 ± 0.30 g/day for *O. aureus*, and 0.115 ± 0.20 and 0.131 ± 0.01 g/day for *T. zilli* (Fig. 1). In both environments, *O. aureus* grew faster than *O. niloticus*, which grew faster than

Fig. 1. Growth of three tilapia species grown in (a) fresh water and (b) sea water.

T. zilli. The difference in growth rates became apparent in week 8. In each species, growth in sea water was significantly better ($p < 0.05$) than growth in fresh water.

Estradiol-17 β concentrations. Estradiol-17 β concentrations are shown in Table 2. In fresh water, the concentrations peaked (Fig. 2) two weeks later in *O. niloticus* than in *O. aureus* and *T. zilli*. In sea water, peaks occurred two weeks later in *O. aureus* and *O. niloticus* than in *T. zilli*. The value and week of the hormone peak in each species differed between fresh and sea water, with levels that were significantly different ($p < 0.05$).

Histological results. Six stages in ovarian development were determined: chromatin nucleolus, perinucleolar, cortical alveolar, vitellogenesis, maturation and ovulation during oogenesis (Fig. 3, Table 3). Oocyte diameters in the different development stages are given in Table 4. There were insignificant differences in the lengths and widths among species and between fresh and sea water ($p > 0.05$). Ovulation did not occur in sea water. Moreover, atretic oocytes were seen in ovaries of all species reared in sea water. The oocytes developed later in sea water specimens than in fresh water.

Discussion

For both fresh and sea water, the descending order of growth performance was *O. aureus*, *O. niloticus*, and *T. zilli*. This order concurs with Liao and Chang (1983), Pullin (1983), Al-Ahmad et al. (1988a) and Suresh and Lin (1992). Stickney (1986) showed that the growth performances of *O. niloticus* in sea water and fresh water are similar. Payne (1983) found that the growth performance of *T. zilli* in different salinities (5-31‰) had small differences. While others reported that tilapias in general have higher growth performance in sea water (Liao and Chang, 1983; Al-Ahmad et al., 1988ab; Cruz et al., 1990; Suresh and Lin, 1992), the body weight of fish reared in sea water in the present study began to surpass that of fish in fresh water after week 8. *O. aureus* reached 381.9 g from 70 g in 158 days in sea water (Balarin and Haller, 1987). According to Al-Ahmad et al. (1988a),

Chervinski and Yashouv (1971) determined that *O. aureus* gained 1.97 g/day in sea water (54 ppt). In the present study, daily weight gains were lower probably because of differences in the environment as well as culture and nursery conditions.

Estradiol-17 β levels of all species in both environments increased concurrently with fish growth until peak levels. The week during which estradiol-17 β peaked in all species in fresh water differed from the peak week in sea water. The peak levels in sea water specimens were lower than in fresh water specimens. Apparently, estradiol-17 β secretion was influenced by the sea water, as reported by Al-Amoudi (1987a,b), Bromage (1989) and Suresh and Lin (1992).

Increases and decreases in estradiol-17 β concentration differ among species. The level in rainbow trout and other salmonids (Kagawa et al., 1983a) and *Pagrus major* (Matsuyama et al., 1988) increases until ovulation, then decreases sharply. On the other hand, in *Carasius auratus* (Kagawa et al., 1983b) it increases until ovulation and remains high. In the present study, the estradiol-17 β concentration in all species increased gradually during the initial stages of the ovarian cycle and more rapidly in later stages. It decreased during ovulation but did not drop to the initial values.

Tilapias have an asynchronous type of ovary (Bogomolnaya et al., 1984; Yaron, 1995), i.e., they have ovulating oocytes simultaneously with early vitellogenic oocytes. Therefore, estradiol-17 β levels in tilapia are not low after ovulation, increasing during later periods. Atretic oocytes were seen in ovaries of all three species reared in sea water together with mature oocytes. At this time, the estradiol-17 β levels of the fish reared in sea water were lower than those of fish reared in fresh water, again emphasizing the inhibition of estradiol-17 β on oocyte development.

Ovarian development stages were harmonious with those in other species, as reported by Micale (1986), Kucuktas (1987), Sarmasik (1992), Cihangir (1996) and Unal et al. (1996).

O. niloticus ovulated in the sixth week

Table 2. Estradiol-17 β levels (pg/ml) of three tilapia species grown in fresh or sea water.

Sample (week)	Fresh water			Sea water		
	<i>Oreochromis niloticus</i>	<i>Oreochromis aureus</i>	<i>Tilapia zilli</i>	<i>Oreochromis niloticus</i>	<i>Oreochromis aureus</i>	<i>Tilapia zilli</i>
0	0.22 $\pm$ 0.0a	0.3 $\pm$ 0.7b	1.5 $\pm$ 0.03ac	0.4 $\pm$ 0.0a	0.4 $\pm$ 0.1a	0.6 $\pm$ 0.02a
2	8.3 $\pm$ 10.8a	12.0 $\pm$ 0.0b	17.9 $\pm$ 0.4c	5.0 $\pm$ 0.0a	9.0 $\pm$ 1.0b	6.8 $\pm$ 0.6ab
4	24.7 $\pm$ 0.0a	55.3 $\pm$ 2.5b	39.7 $\pm$ 1.0c	17.5 $\pm$ 0.2a	12.3 $\pm$ 1.3b	16.4 $\pm$ 0.0a
6	278.6 $\pm$ 0.5a	295.0 $\pm$ 6.1b	288.9 $\pm$ 1.2ab	182.2 $\pm$ 5.4a	152.1 $\pm$ 6.7b	124.5 $\pm$ 0.6c
8	330.4 $\pm$ 0.2a	352.0 $\pm$ 3.8b	336.5 $\pm$ 3.7a	294.9 $\pm$ 2.5a	630.0 $\pm$ 0.2b	226.7 $\pm$ 3.2c
10	721.8 $\pm$ 2.6a	793.7 $\pm$ 0.2b	743.1 $\pm$ 2.5c	372.9 $\pm$ 9.2a	269.4 $\pm$ 6.3b	689.0 $\pm$ 4.5c
12	1014.2 $\pm$ 4.0a	1428.7 $\pm$ 8.2b	1290.4 $\pm$ 7.9c	463.7 $\pm$ 8.6a	507.4 $\pm$ 1.7b	976.7 $\pm$ 6.4c
14	1224.8 $\pm$ 0.7a	1531.0 $\pm$ 7.7b	1340.5 $\pm$ 3.3a	991.1 $\pm$ 5.49a	652.930 $\pm$ 5.235b	1273.0 $\pm$ 4.7c
16	1591.7 $\pm$ 0.2a	202.1 $\pm$ 5.5b	1230.5 $\pm$ 5.9a	1326.1 $\pm$ 7.7a	872.815 $\pm$ 6.724b	207.195 $\pm$ 3.5c
18	277.7 $\pm$ 1.6a	319.8 $\pm$ 12.0a	1212.5 $\pm$ 2.1b	1570.6 $\pm$ 6.4a	1119.9 $\pm$ 6.694b	63.2 $\pm$ 0.5c
20	363.9 $\pm$ 2.3a	457.9 $\pm$ 0.4b	6.99.9 $\pm$ 1.1c	291.41 $\pm$ 11.343	*	*
22	418.9 $\pm$ 3.5a	751.6 $\pm$ 2.5b	570.6 $\pm$ 2.5c	*		
24	661.8 $\pm$ 6.2a	991.4 $\pm$ 0.9b	444.8 $\pm$ 2.0c			
26	992.6 $\pm$ 3.8a	1105.7 $\pm$ 0.3b	1465.0 $\pm$ 5.6b			
28	1182.3 $\pm$ 6.6a	1019.2 $\pm$ 6.3b	943.8 $\pm$ 9.2c			

Different superscripts indicate significant differences between species according to Duncan's Multiple Range Test.

*Experiment ended due to fish mortality.

Fig. 2. 17 β -estradiol levels of three tilapia species grown in (a) fresh water and (b) sea water.

Fig. 3. Ovarian development stages (stained with hematoxylin and eosin): (a) chromatin nucleolus stage x 40, (b) perinucleolar stage x 40, (c) cortical alveolar stage x 100, (d) vitellogenesis stage x 40, (e) maturation stage x 100.

while *O. aureus* and *T. zilli* in the fourteenth. Fish were measured twice during the ovulating periods. The *O. niloticus* grown in sea water were smaller at the beginning of the experiment than the other species. Perhaps they were too small to enter ovulation as early as four weeks, as did the other species. Fineman (1989) reported that *O. niloticus* reared in sea water had mature oocytes but did not release them, as we also found. Oocyte development in all the species was slower in sea water than in fresh, perhaps resulting from the lower estradiol-17 β levels.

There was a close relationship between hormone level and gonadal development in all species. The hormone levels peaked while the fish had mature gonads. However the ovarian tissues of fish grown in sea water did not mature as early as in the fresh water specimens. The fish grown in sea water did not ovulate at the same time as those grown in fresh water. Oocyte diameters during the development stages were similar among species. Apparently, environmental differences did not affect oocyte size.

According to our results, the three tilapia species can be raised in marine fish cultures since the average body weights and daily growth rates in sea water were higher than in fresh water. Reproduction, however, still requires fresh water.

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Table 3. Ovarian development stages of species grown in freshwater and seawater

Sample (week)	<i>Oreochromis niloticus</i>		<i>Oreochromis aureus</i>		<i>Tilapia zilli</i>	
	Fresh water	Sea water	Fresh water	Sea water	Fresh water	Sea water
0						
2						
4						
6	Cromatin nucleolus & perinucleolar	Cromatin nucleolus	Cromatin nucleolus	Cromatin nucleolus	Cromatin nucleolus	Cromatin nucleolus
8	Perinucleolar	Perinucleolar	Perinucleolar Cortical alveola	Perinucleolar	Perinucleolar & cortical alveolar & atretic oocytes	Perinucleolar
10	Perinucleolar & cortical alveolar	Perinucleolar	Vitellogenesis	Perinucleolar	Vitellogenesis	Cortical alveolar & atretic oocytes
12	Vitellogenesis	Cortical alveolar	Maturation	Cortical alveolar	Maturation	Vitellogenesis
14	Maturation	Vitellogenesis	Maturation	Vitellogenesis	Maturation	Maturation
16	Maturation	Maturation	Ovulation	Vitellogenesis	Ovulation	Maturation & atretic oocytes
18	Ovulation	Maturation & atretic oocytes	Perinucleolar	Maturation & atretic oocytes	Perinucleolar	Maturation & atretic oocytes
20	Perinucleolar	Perinucleolar	Perinucleolar & cortical alveolar		Perinucleolar & cortical alveolar	
22	Perinucleolar & cortical alveolar		Cortical alveolar		Perinucleolar & cortical alveolar	
24	Perinucleolar & cortical alveolar		Cortical alveolar		Perinucleolar & cortical alveolar	
26	Cortical alveolar & vitellogenesis		Vitellogenesis		Cortical alveolar	
28	Cortical alveolar & vitellogenesis		Vitellogenesis		Cortical alveolar	

Table 4. Diameters (lengths x widths) of oocytes of tilapia grown in fresh or sea water (μm).

Stage	Oreochromis niloticus		Oreochromis aureus		Tilapia zilli	
	Fresh water	Sea water	Fresh water	Sea water	Fresh water	Sea water
Cromatin nucleolus	5 $\pm$ 0.1 x 5.1 $\pm$ 0.1	5.1 $\pm$ 0.1 x 5.3 $\pm$ 0.1	4.8 $\pm$ 0.2 x 4.9 $\pm$ 0.1	5.0 $\pm$ 0.2 x 5.0 $\pm$ 0.3	5.0 $\pm$ 0.2 x 5.2 $\pm$ 0.2	4.8 $\pm$ 0.1 x 5.0 $\pm$ 0.2
Perinucleolar	8.43 $\pm$ 0.2 x 10.62 $\pm$ 0.1	8.5 $\pm$ 0.2 x 10.5 $\pm$ 0.2	9.0 $\pm$ 0.3 x 11.0 $\pm$ 0.4	8.8 $\pm$ 0.4 x 10.8 $\pm$ 0.8	9.6 $\pm$ 0.4 x 11.3 $\pm$ 0.3	9.5 $\pm$ 0.3 x 11.0 $\pm$ 0.3
Cortical alveolar	10.2 $\pm$ 0.4 x 13.0 $\pm$ 0.4	10.5 $\pm$ 0.4 x 13.4 $\pm$ 0.2	10.8 $\pm$ 0.5 x 13.8 $\pm$ 0.1	10.3 $\pm$ 0.1 x 13.0 $\pm$ 0.2	10.5 $\pm$ 0.4 x 13.4 $\pm$ 0.2	10.7 $\pm$ 0.2 x 13.5 $\pm$ 0.6
Vitellogenesis	34.0 $\pm$ 0.4 x 35.0 $\pm$ 0.5	32.7 $\pm$ 0.4 x 34.0 $\pm$ 0.6	33.0 $\pm$ 0.6 x 34.0 $\pm$ 0.5	33.0 $\pm$ 0.7 x 43.0 $\pm$ 0.7	32.0 $\pm$ 0.7 x 35 $\pm$ 0.5	31.0 $\pm$ 0.4 x 33.0 $\pm$ 0.4
Maturation	61.0 $\pm$ 0.1 x 64.5 $\pm$ 0.3	63.0 $\pm$ 0.4 x 65.0 $\pm$ 0.4	62.0 $\pm$ 0.5 x 68.0 $\pm$ 0.5	63.0 $\pm$ 0.5 x 64.0 $\pm$ 0.6	62.0 $\pm$ 0.4 x 68 $\pm$ 0.6	64.0 $\pm$ 0.3 x 63.0 $\pm$ 0.3
Ovulation	125.0 $\pm$ 0.9 x 130.0 $\pm$ 0.8		122.0 $\pm$ 0.8 x 126.0 $\pm$ 0.7		129.0 $\pm$ 0.5 x 131.0 $\pm$ 0.8	

Differences in lengths and widths are insignificant ($p>0.05$), according to Duncan's Multiple Range Test.

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