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Effects of Freshwater and Seawater on Growth, Total Testosterone Levels and Testis Development of Tilapias

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Abstract: Effects of freshwater and seawater on growth, total testosterone levels, testis development of *Oreochromis niloticus*, *Oreochromis aureus* and *Tilapia zilli* were determined in this study. Although seawater affected the growth of fishes positively ($p<0.05$) and all testis development stages (immature, maturing and maturated) were observed in all species in seawater, total testosterone concentrations of the fishes grown in this environment did not reach the levels seen in the fishes grown in freshwater ($p<0.05$). In addition, in seawater, gonad development was observed later and the last 2 gonadal stages were seen together in all species.

Key words: Tilapia, growth, total testosterone, gonad, freshwater, seawater

INTRODUCTION

Tilapias have been preferred by culturists because of many positive characteristics (Sarıhan and Toral, 1982; Ali, 1983; Stickney, 1986; Ballarin and Haller, 1987). One of the most important properties of tilapias is its being an group of eurohaline fish that can be cultured in both brackish and seawater (Watanabe *et al*., 1985; Watanabe and Kuo, 1985; Al-Amoudi, 1987a, b; Ridha and Lone, 1990). This is extremely important for regions where have limited freshwater supply.

In aquaculture, gonadal development and production of gametes have importance in not only females but also males. The growth of many fish is inhibited during late maturation stage. The growth of male fish shows the poorest growth during testis development. Reproduction of tilapias is limited or inhibited by salt water (Ballarin and Haller, 1987; Shepherd and Bromage, 1988; Fineman, 1989) and depends directly or indirectly on the activity of hormonal glands that have stimulatory and inhibitory effects on the maturation process. There is not enough research about testosterone levels and gonadal development through growth of tilapia held in seawater.

For this reason, this study was conducted to determine growth, total testosterone levels and testis development stages of three commercially important tilapia species in freshwater and seawater and to contribute especially to following culture studies and activities of tilapia in seawater.

MATERIALS AND METHODS

In the present study *Oreochromis aureus* (Staindachener, 1864), *O. niloticus* (Linnaeus, 1758) and *T. zilli* (Gervais, 1848) were used. Mean initial weights of the three species were 0.70±0.09 g, 0.45±0.04 g, 0.60±0.03 g in freshwater and 0.23±0.08 g, 0.24±0.01 g, 0.22±0.09 g in seawater, respectively (All values in the study were given with standard error of the means (SEM)).

Experiments were carried out at the Fresh and Marine Water Fisheries Research Stations of the Fisheries Faculty of Cukurova University, Turkey. The freshwater (FW) study lasted 28 weeks and the seawater (SW)18-20 weeks. The study was carried out in triplicate. Six fiberglass tanks (diameter 4 m) divided in two by net frames were used. Each side of the tanks was stocked with 500 fish for each repetition of each species.

Water was pumped into the tanks at 4 L min^{-1}. FW was pumped from an irrigation channel of the Seyhan Dam Lake and SW (35% 0) was pumped directly from the sea. Water temperature, dissolved oxygen and salinity were measured twice a day; pH was measured daily. Means of these parameters were calculated separately for each sampling period (once every 15 day). During the experiment, in FW tanks, water temperatures were maintained between mean 20.8±0.2°C (mean minimum temperature) and 27.2±0.1°C (mean maximum temperature). Dissolved oxygen ranged between means of 7.09±0.32 and 8.02±0.32 mg L^{-1}. pH ranged between means of 7.00±0.44 and 7.88±0.09. The temperature of SW tanks changed

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between means of 22.1±0.1 and 27.6±0.1°C, dissolved oxygen between 7.16±0.12 and 7.84±0.01 mg L^{-1}, pH between 7.10±0.15 and 7.76±0.11 and salinity between 34.73±0.05 and 35.98±0.02 ppt (all figures are mean minimum and maximum).

Fish were fed 0.25, 1 and 2 mm trout granules with crude protein level of 55% until week 11, when they weighed approximately 10 g and than 2 mm pellets with a crude protein level of 45%, depending on the sizes of the fish and their mouth opening. The food ration was 5 % of body weight, distributed four times daily.

Samples were taken randomly once every fifteen days. Weight and length measurements were carried out using a 0.01 g sensitive scales and milimetric ruler. Twenty-five specimens of every species were sampled for analysis of hormone at first two measurements. Until 12th measurement period 20 specimens and then 15 specimens were sampled. Whole fish bodies were homogenized in 3% trichloroacetic asid (Rothbard *et al*., 1987). Homogenates were stored for further study at -25°C. The samples thawed at room temperature and homogenates were extracted with diethyhlether 3-4 times of sample. The diethyhlether was evaporated and the steroid dissolved in RIA (radioimmunoassay) buffer.

Measurements of total testosterone were made with a Iso-Data Gamma Counter at Medical Faculty of Cukurova University. For measurement, 50 μL sample was pipetted into tube-coated serum. One mililiter tracer ^{125}I testosterone was added, then vortexed and incubated 3 h at 37°C in water bath (Coat-A-Count Total Testosterone Kit; DPC, Los Angeles). After incubation, tubes were decanted and steroid hormone levels were determined using Coat-A- Count Kits (DPC, Los Angeles) according to the protocol of the manufacturer. Statistical analyses were carried out with Duncan's Multiple Range (among species in the same environment) and t tests (between the same species in FW and SW) at SPSS 8.0 package programme for windows (SPSS, 1998).

Histological analysis: After autopsy, gonads were fixed in Bouin's solution then embedded in paraffin blocks. Paraffin sections were sliced into 5 μm thick and stained with hematoxylin and eosin (Rothbard *et al*., 1987). Testis development stages were determined in dissecting microscope.

RESULTS AND DISCUSSION

Growth: There were no significant differences in body weight at week 28 between *O. niloticus* and *O. aureus* reared in freshwater (Table 1, Fig. 1 and 2) but both were significantly larger than *T. zilli* ($p<0.05$). Due to powder break-down in the seawater supply that caused the death of the fish, the *O. aureus* and *T. zilli* experiments ended in week 18 and the *O. aureus* 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 ($p>0.05$), but they were somewhat lower than that of *O. aureus* ($p<0.05$). Mean daily weights gain in fresh and seawater were 0.124±0.22 and 0.144±0.01 g day^{-1} for *O. niloticus*, 0.128±0.01 and 0.151±0.30 g day^{-1} for *O. aureus* and 0.115±0.20 and 0.131±0.01 g day^{-1} for *T. zilli*. In both environments, *O. aureus* grew faster than *O. niloticus*, which grew faster than *T. zilli*. The differences in growth rates became apparent in week 8. In each species, growth in seawater was significantly better ($p<0.05$) than growth in freshwater.

For both fresh and seawater, the descending order of growth performance was *O. aureus*, *O. niloticus* and *T. zilli*. This order concurs with Liao and Chang (1983),

Table 1: Mean body weight of three tilapia species grown in FW and SW

	FW			SW		
Sample (week)	*O. niloticus*	*O. aureus*	*T. zilli*	*O. niloticus*	*O. aureus*	*T. zilli*
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±0.05	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

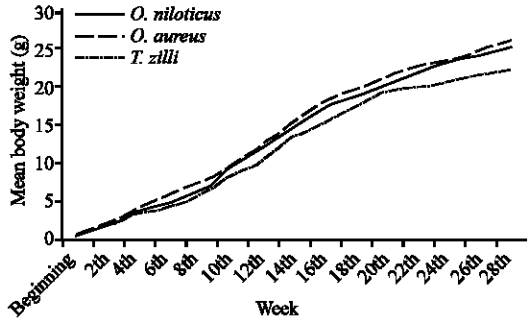

Fig. 1: Mean body weight of three tilapia species grown in FW

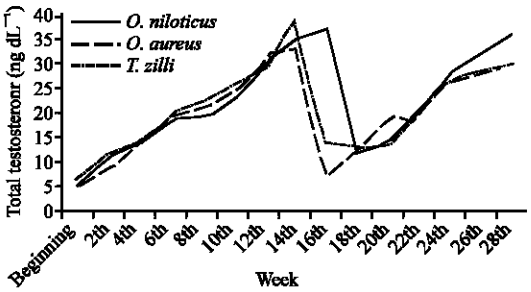

Fig. 3: Total testosterone levels of three tilapia species grown in FW

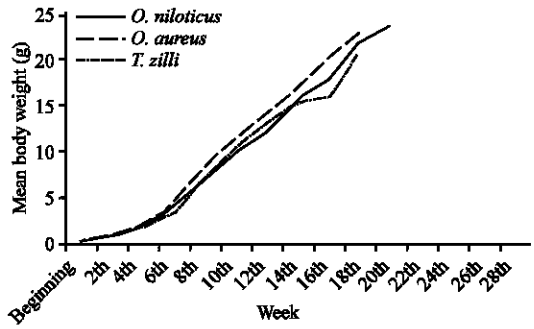

Fig. 2: Mean body weight of three tilapia species grown in SW

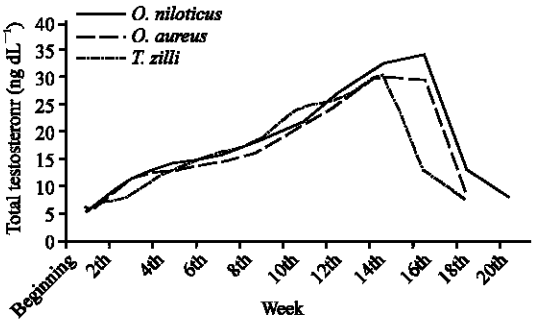

Fig. 4: Total testosterone levels of three tilapia species grown in SW

Pullin (1983), Al-Ahmad *et al.* (1988b) and Suresh and Lin (1992). Stickney (1986) showed that he growth performance of *O. niloticus* in seawater and freshwater 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 seawater (Liao and Chang, 1983; Al- Ahmad *et al.*, 1988a, b; Suresh and Lin, 1992; Cruz *et al.*, 1990), the body weight of fish reared in seawater in the present study began to surpass that of fish in freshwater after week 8. *O. aureus* reached 381.9 g from 70 g in 158 days in seawater (Ballarin and Haller, 1987). According to Al-Ahmad *et al.* (1988a), determined that *O. aureus* gained 1.97g day $^{-1}$ in seawater (54 ppt). In the study, daily weight gains were lower probably because of differences in the environment as well as culture and nursery conditions.

Total testosterone concentration and gonadal histology: Total testosterone concentrations of species rared in both environments are shown in Table 2. Although, a week of the hormone peak in each species did not differ between fresh and seawater, the value of the peak (Fig. 3 and 4) in each species differed with levels that were significantly different ($p<0.05$).

Three stages in testis development of the fish were determined in the study: immature, maturing and maturated during spermatogenesis (Table 3 and Fig. 5). Especially stage III was seen in different week in each species in both fresh and seawater. It was determined 3 stages in testis development as immature, maturating and maturated.

Total testosterone levels of all species in both environments increased concurrently with fish growth and gonadal development until its peak levels. The week during which total testosterone peaked in all species in freshwater did not differ from the peak level in seawater. The peak levels in seawater specimens were lower than in freshwater specimens. This study showed that the seawater influenced total testosterone secretion of tilapias.

Plasma testosterone level of female in *Carasius auratus* increased in the month before ovulation peaked after ovulation (Kagawa *et al.*, 1983). Although testosterone level in males of *O. mossambicus* increased parallel with gonadosomatic index values, when water temperature increased, it suddenly peaked and caused earlier maturation of sperm (Cornish and Smith, 1991).

According to Paulidis *et al.* (1994), Scott *et al.* (1980) and Lou *et al.* (1986) reported that testosterone was in

Table 2: Total testosterone levels of three tilapia species grown in FW and SW (ng dL^{-1})

	FW			SW		
Sample (week)	*O. niloticus*	*O. aureus*	*T. zilli*	*O. niloticus*	*O. aureus*	*T. zilli*
0	5.01±0.05a	5.01±0.07a	6.08±0.05b	5.02±0.07a	5.01±0.04a	6.09±0.09b
2	11.00±10.4ab	8.17±10.4a	12.91±0.04b	11.22±0.09a	11.27±0.02a	8.19±0.24a
4	13.87±0.02a	13.42±0.01a	14.32±.0.1a	14.36±0.12a	13.23±0.01a	13.99±0.01a
6	18.33±0.08a	18.34±0.01a	19.00±0.02a	15.91±0.02a	14.25±0.02b	16.32±0.04a
8	19.31±0.08a	20.75±0.07a	22.53±0.04b	18.30±0.05a	16.28±0.04b	18.85±0.04a
10	22.36±0.01a	24.47±0.07b	25.56±0.00b	21.34±0.01a	20.33±0.07b	24.43±0.02c
12	29.43±0.02a	30.52±0.01b	29.10±0.02c	27.00±0.02a	25.35±0.07b	26.11±0.02b
14	34.71±0.09a	32.65±0.04b	38.97±0.07c	32.67±0.01a	30.59±0.09b	30.60±0.02c
16	36.87±0.19a	7.16±0.02b	14.37±0.02ca	34.77±0.02a	29.48±0.02b	13.50±0.07c
18	12.85±0.9ab	12.22±0.01a	13.32±0.02b	13.57±0.19a	9.17±0.04b	7.23±0.04c
20	14.22±0.12a	18.31±0.14a	13.81±0.9ac	8.32±0.04a	*	*
22	20.44±0.01a	19.65±0.04a	19.33±0.03a	*		
24	27.65±0.11a	26.41±0.04a	26.10±0.03a			
26	31.64±0.1a	28.41±0.05b	28.80±0.02b			
28	35.82±0.11a	29.55±0.04b	29.51±9.04b			

Different superscripts indicate significant differences between species according to Duncan's Multiple Range Test. *Experiment ended due to fish mortality

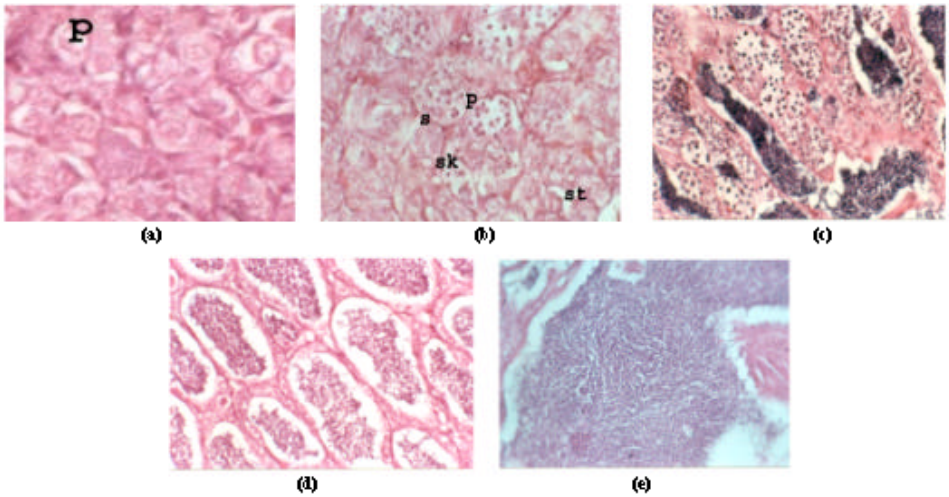

Fig. 5: Testis development stages (stained with hematoxylin and eosin): (a) immature testis stage ×40, (b and c) maturing stage ×40 and ×100, (d and e) matured sage × 40; p: primer spermatogonium, sk: seconder spermatogonium; s: seminifer tubule; st: setoli cells

high level during last stage of spermatogenesis. In this study testosterone levels were obtained from whole body extract of both male and female. However the results of the hormone level do not agree with those informed by Scott and Boynes (1982), Boynes and Scott (1985), Scott and Sumpte (1989) according to Paulidis *et al*. (1994). In the present study testosterone levels of each fish species, especially as seen in fish in freshwater, increased concurrently with male gonad development.

Testis development, as informed for many fishes by Unal *et al*. (1996), was seen in three different stages in the present study. Each three stage was recognized clearly in freshwater specimens, but stage III was seen not alone but together especially stage II in seawater specimens of

Table 3: Testis development stages of three tilapia species grown in FW and SW

	FW			SW		
Sample (week)	*O. niloticus*	*O. aureus*	*T. zilli*	*O. niloticus*	*O. aureus*	*T. zilli*
0	-	-	-	-	-	-
2	-	-	-	-	-	-
4	I	I	I	I	I	I
6	I	I	II	I	I	I
8	II	II	II	I	I	I
10	II	II	III	I-II	I	II
12	II	III	III	I-II	II	II
14	III	III	III	I-II	II	III-II
16	III	I	I	II	II-III	I
18	I	I	I	II-III	I	I
20	I	I	I	I	*	*
22	I-II	I	I	*		
24	II	II	I			
26	II	II	II-III			
28	III	II	III			

I, II and III indicate the stages of immature, maturing and maturated, respectively

each species. Gonadal degeneration is observed in tilapia maintained for a long time in sea or salt water. Female maturation in seawater specimens delayed (Altun *et al*., 2004). In this study, degeneration was not seen in gonadal tissues of males in seawater. To be seen together of stages III with II can be due to low testosterone levels of the fish in seawater.

CONCLUSION

According to these results, seawater affected fish growth, testosterone levels and gonadal development. However, results belonging to hormone levels and gonadal stages show that spermatogenesis in seawater is possible. Moreover further studies on additional applications in order to obtained gametes at last stage of spermatogenesis of fish or to stimulate the reproduction of tilapia grown in seawater will able to be necessary and useful.

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