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Sex Differences in the Responses of Serum Calcium Concentrations to Temperature and Estrogen in Tilapia, *Oreochromis mossambicus*

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Ching-Lin Tsai and Li-Hsueh Wang (2000) Sex differences in the responses of serum calcium concentrations to temperature and estrogen in tilapia, *Oreochromis mossambicus*. *Zoological Studies* **39**(1): 55-60. Sex differences in the responses of serum calcium concentrations to temperature and estrogen in tilapia, *Oreochromis mossambicus*, were investigated. Prespawning males and females were kept at 26, 29, and 32 °C aquatic temperatures for 21 d. The serum calcium levels increased by elevated temperature in females but were not altered in males. Females showed higher serum calcium levels than males at the 26, 29, and 32 °C exposure groups, respectively. On the other hand, males and females were gonadectomized and treated with different dosages of 17β-estradiol for 21 d. Serum calcium levels significantly decreased after gonadectomy in females but were not altered in males. There was no difference in serum calcium levels between gonadectomized males and females. Treatment with 17β-estradiol at 50 or 100 mg/kg diet significantly increased serum calcium levels dose-dependently in both gonadectomized males and females. The hypercalcemic effect of 17β-estradiol was greater in castrated males. These results suggest that the estrogen induced calcium regulatory system is temperature dependent in females and not in males. On the other hand, estrogen induced elevation of blood calcium level is more pronounced in males than in females.

Key words: Estradiol, Serum calcium, Sex difference, Temperature, Tilapia.

The natural reproductive cycle of teleosts is synchronized with seasonal and other environmental cues (Kojima 1981, Crim 1982, Asahina and Hanyu 1983, Shimizu and Hanyu 1983, Foscarini 1988, Okuzawa et al. 1989, Scott and Pankhurst 1992, Francis 1994, Shimizu et al. 1994, Cornish and Smit 1995). Low temperature induces gonadal regression and terminates the natural spawning period; whereas, high temperature induces spawning in tilapia (Cornish and Smit 1995). In female teleosts, serum calcium displays a seasonal variation and increases together with the ovarian maturation (Fleming et al. 1964, Woodhead 1969a, Balbontin et al. 1978, Srivastava et al. 1985, Whitehead et al. 1985, Srivastava and Srivastava 1994), whereas, in male teleosts, serum calcium levels show no change at different stages of gonadal maturation during the annual reproductive cycle (Fleming et al. 1964, Balbontin et al. 1978). However, the effect of temperature on serum calcium of male and female teleosts is little known.

Serum concentrations of estrogen increase during the spawning phase and vary consistently with the natural reproductive cycle (Crim and Idler 1978, Norberg et al. 1989, Mol et al. 1994, Srivastava and Srivastava 1994). The increase of estrogen secretion results in a rise of serum calcium during ovarian maturation. Exogenous estrogen increases serum calcium concentrations in female goldfish (Bailey 1957), *Fundulus kansae* (Fleming et al. 1964), and salmonids (Bjornsson et al. 1989). Neither castration nor testosterone treatment affects serum calcium levels in male killifish, *Fundulus heteroclitus* (Pang and Balbontin 1978). In contrast, injections of estrogen in male goldfish (Beiley 1957), *Fundulus kansae* (Fleming and Meier 1961), *Gadus morhua* (Woodhead 1969b), and killifish, *Fundulus heteroclitus* (Pang and Balbontin 1978) increase the level of serum calcium. Estrogen has a hypercalcemic effect in both male and female teleosts as has been reported by other workers (Bailey 1957, Fleming and

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Meier 1961, Fleming et al. 1964, Woodhead 1969b, Pang and Balbontin 1978, Bjornsson and Haux 1985, Bjornsson et al. 1989). However, sex differences in the responses of serum calcium to estrogen are little known.

In the present study, sex differences in the responses of serum calcium concentration to temperature and estrogen treatment in tilapia are investigated.

MATERIALS AND METHODS

Tilapia, *Oreochromis mossambicus*, were obtained from the Tung Kang Marine Laboratory of the Taiwan Fisheries Research Institute. Sexually mature males weighing 12-15 g and 8-10 cm long, and females weighing 10-13 g and 7-9 cm long were used. Fish were kept in a glass aquarium of 60×30×45 cm^3. Water was constantly circulated in a closed system. The water temperature was regulated by an automatic controller and maintained at 24 ± 2 °C. The light/dark cycle was 14/10 h. Fish were acclimated for 2 wk before the experiments. Fish in the prespawning phase with a gonadosomatic index of 0.38 ± 0.07 in males and 4.60 ± 0.02 in females were used. The prespawning phase of testes shows spermatocytes and spermatids while that of the ovary shows yolk vesicles and yolk globules, according to the reports of Dadzie (1969 1974) and Htun-Han (1978a, b). Figure 1 shows the transverse sections of testes and ovary in the prespawning phase, respectively. Three experiments were performed in the present study.

Experiment 1

Males and females in the prespawning phase were each divided into 3 groups which were exposed to aquatic temperatures of 26 (n = 6), 29 (n = 6), and 32 °C (n = 6) for 3 wk at which time serum calcium concentrations were measured.

Experiment 2

Prespawning males were divided into 4 groups which were orchidectomized (ORX) (n = 6), orchidectomized and treated with 50 mg 17β-estradiol (E_2, Sigma, St. Louis, MO, USA)/kg diet (ORX+50E_2) (n = 6), orchidectomized and treated with 100 mg E_2/kg diet (ORX+100E_2) (n = 6), or left intact (a control group) (n = 6), respectively. On the other hand, prespawning females were divided into 4 groups which were ovariectomized (OVX) (n = 6), ovariectomized and treated with 50 mg E_2/kg diet (OVX+50E_2) (n = 6), ovariectomized and treated with 100 mg E_2/kg diet (OVX+100E_2) (n = 6), or left intact (a control group) (n = 6), respectively. Estradiol-17β was dissolved in ethanol and mixed with the commercial diet. Each fish was fed 1 pellet once per day. Each pellet contained 0.3 g of food. After 3 wk of the treatments, serum calcium concentrations of males and females were quantified.

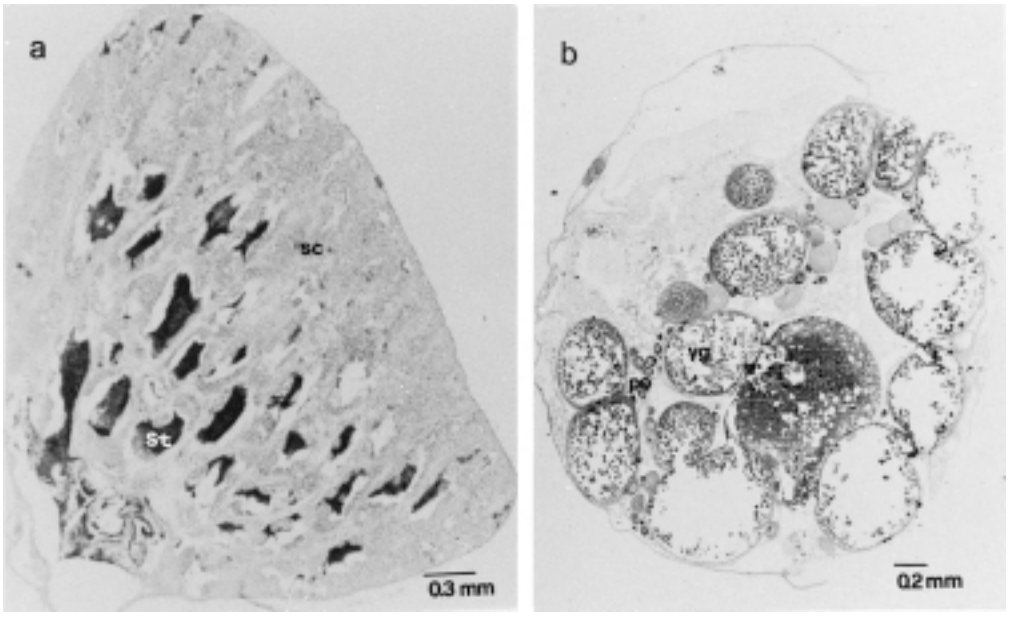

Fig. 1. Testis (a) and ovary (b) of tilapia in the prespawning phase. po, primary oocyte; sc, spermatocyte; st, spermatids; yg, yolk globule.

Experiment 3

Prespawning males were orchidectomized, and then divided into 3 groups which were fed a diet containing 0, 50, or 100 mg methyltestosterone (MT, Sigma, St. Louis, MO, USA)/kg diet, respectively. Methyltestosterone was dissolved in ethanol and mixed with the commercial diet. Each group contained 6 animals. Serum calcium concentrations were quantified after 3 wk of treatment.

Serum calcium concentrations

Blood at 0.2 ml was collected from the caudal vessel using a heparinized syringe and stored overnight at 4 °C. The serum was separated by centrifugation at 3000 g for 30 min. Then, 25 µl of serum was reacted with 2.5 ml of working reagent for in vitro quantitative determination of calcium in serum (Diagnostic Chemicals Limited, Oxford, CT, USA) for 5 min. Total serum calcium was measured by the absorption at 570 nm with a spectrophotometer (U-3210, Hitachi, Tokyo, Japan). Serum calcium concentration was expressed as micromoles per milliliter of serum.

Statistical evaluations

The statistical significance was determined by Student's *t*-test, linear-regression analysis, and one-way ANOVA with Duncan's multiple-range test ($p < 0.05$).

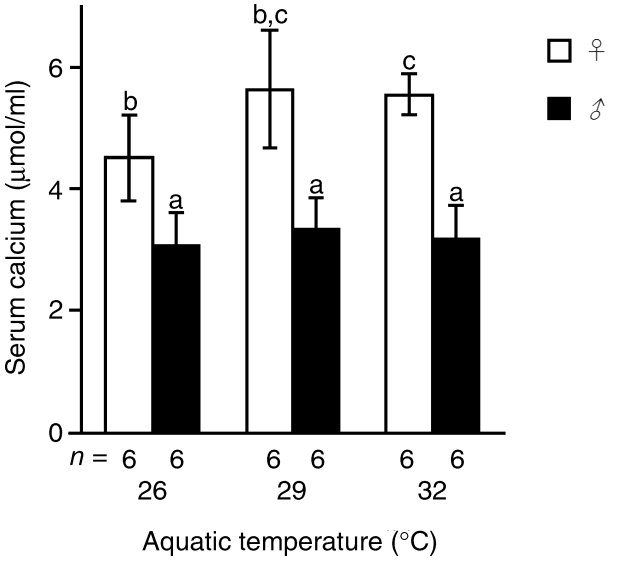

Fig. 2. Effects of temperature on serum calcium concentrations in male (♂) and female (♀) tilapia, *Oreochromis mossambicus*. Data are represented as means ± S.D. Different letters indicate statistically significant differences ($p < 0.05$) between groups, analyzed by one-way ANOVA with Duncan's multiple-range test.

RESULTS

Effects of temperature on serum calcium of males and females

Effects of environmental temperature on serum calcium levels in intact prespawning males and females are shown in figure 2. In intact prespawning females, the concentrations of serum calcium in the 29 and 32 °C exposure groups were significantly increased compared to the 26 °C exposure group ($F = 4.44$, $p < 0.05$), whereas, in intact prespawning males, no significant differences were found among the 26, 29, and 32 °C exposure groups ($F = 0.68$, $p > 0.05$). Females showed higher serum calcium levels than males in the 26 °C ($t = 3.69$, $p < 0.005$), 29 °C ($t = 5.09$, $p < 0.005$), and 32 °C ($t = 8.12$, $p < 0.0001$) exposure groups.

Effects of castration and estrogen on serum calcium of males and females

Table 1 shows that serum calcium concentrations in intact prespawning females were higher than those of intact prespawning males ($t = 2.97$, $p < 0.05$). After ovariectomy, serum calcium concentrations were significantly decreased in females ($t = 3.30$, $p < 0.01$), but not altered in males after castration ($t = 1.56$, $p > 0.05$). However, there was no significant difference between gonadectomized males and females ($t = 0.79$, $p > 0.05$). Effects of E_2 on serum calcium levels of castrated males and females are also shown in table 1. In castrated males, serum calcium concentrations of the ORX+50E_2 and

Table 1. Effects of 17β-estradiol (E_2) administration on serum calcium levels in gonadectomized male and female tilapia, *Oreochromis mossambicus*

Treatment	*n*	Serum calcium concentration (µmol/ml)	
		Male	Female
Intact (prespawning phase)	6	3.06 ± 0.31	4.16 ± 1.23*
Castrated	6	2.87 ± 0.47	2.90 ± 0.28
Castrated+E_2, 50 mg/kg diet	6	5.88 ± 1.19	3.35 ± 0.04*
Castrated+E_2, 100 mg/kg diet	6	7.43 ± 1.17	5.20 ± 0.65*

Levels of serum calcium concentration are expressed as means ± S.D. Asterisks indicate significant differences ($p < 0.05$) between males and females, as analyzed by Student's *t*-test.

ORX+100E_2 groups were significantly increased compared to the ORX group (F = 25.49, p < 0.0001). On the other hand, in ovariectomized females, serum calcium concentrations in the OVX+100E_2 group were significantly increased but were not altered in the OVX+50E_2 group as compared to the OVX group (F = 27.35, p < 0.001). The hypercalcemic effect of E_2 in both males and females was dose dependent. However, linear-regression analysis showed that the hypercalcemic effect of E_2 was significantly different between castrated males and females, as shown in figure 3. The hypercalcemic effect of E_2 was more potent in males than in females (F = 28.09, p < 0.0001).

Effects of androgen on serum calcium of males and females

The effects of MT on serum calcium concentrations in castrated males are shown in figure 4. Serum calcium concentrations of castrated males were significantly increased by 50 mg MT/kg diet but were not influenced by 100 mg MT/kg diet (F = 21.48, p < 0.001).

DISCUSSION

As observed in the present study, serum calcium concentrations increased consistently with elevated temperatures in female tilapia but not in males in the prespawning stage (Fig. 2). This result indicates that the calcium regulatory system in female tilapia in reproduction active stage is temperature dependent. Temperature-dependent hypercalcemia might be geared to the needs of natural reproductive cycles in female tilapia. Seasonal environmental changes in water temperatures control gonadal maturation and the natural spawning period in some female teleosts (Asahina and Hanyu 1983, Shimizu and Hanyu 1983, Lam 1984, Okuzawa et al. 1989, Johnston et al. 1992, Shimizu et al. 1994, Cornish and Smit 1995). Serum calcium levels are consistent with the natural reproductive cycle and ovarian maturation (Fleming et al. 1964, Woodhead 1969, Balbontin et al. 1978, Srivastava et al. 1985, Whitehead et al. 1985, Srivastava and Srivastava 1994). On the other hand, vitellogenesis is synchronized with the natural reproductive cycle and is dependent on the stage of growth of ovarian eggs in females (de Vlaming et al. 1980, Van Boheman et al. 1981, Yu et al. 1981, Copeland et al. 1986, Norberg et al. 1989, Specker and Sullivan 1994). Vitellogenin is a female-specific serum protein which is synthesized by the liver as the egg-yolk precursor in all oviparous vertebrates (Yu et al. 1980 1981, Wallace 1985). Calcium is necessary for the formation of vitellogenin molecules (de Vlaming et al. 1980, Yeo and Mugiya 1997). Vitellogenesis is evoked by direct estrogenic action upon the liver (Yu et al. 1981, Wallace 1985). Vitellogenic gene expression increases with temperature in rainbow trout (Mackay and Lazier 1993). It is thought that temperature may exert an effect on fish reproduction by a direct action

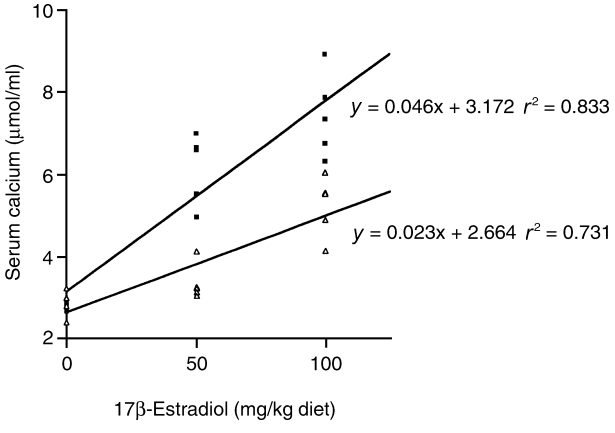

Fig. 3. Linear-regression analysis showing that 17β-estradiol increased serum calcium concentrations in castrated males (■) and females (Δ) in a dose-dependent manner.

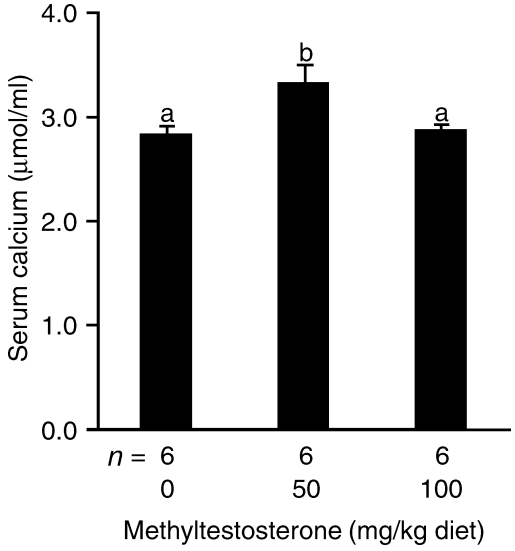

Fig. 4. Effects of methyltestosterone treatment on serum calcium concentrations of orchidectomized male tilapia, *Oreochromis mossambicus*. Data are represented as means ± S.D. Different letters indicate significant differences (p < 0.05) between groups, analyzed by one-way ANOVA with Duncan's multiple-range test.

on the responsiveness of liver to E_2 in vitellogenesis (Yaron et al. 1980, Mackay and Lazier 1993). However, the role of temperature-dependent hypercalcemia in the reproduction of females needs further investigation.

In female tilapia, serum calcium levels decreased after ovariectomy and increased after treatment with E_2 (Table 1). These results indicate that the gonads play an important role in the calcium regulatory system of females. In males, neither orchidectomy nor treatment with 100 mg MT/kg diet had an effect on serum calcium (Table 1; Fig. 4). But, 50 mg MT/kg diet had a hypercalcemic effect on males (Fig. 4). The hypercalcemic effect of MT might be mediated by the effect of E_2, which is converted from testosterone by aromatase (Naftolin et al. 1975). Methyltesosterone used in the present study is a synthetic and aromatizable androgen (de Leeuw et al. 1986). 17β-Estradiol had a hypercalcemic effect in both castrated males and females (Table 1), the effect being greater in castrated males (Fig. 3). The results indicate that the calcium regulatory system in castrated males has a higher sensitivity to E_2. To understand the different sensitivities to E_2 in the calcium regulatory systems of male and female teleosts, more detailed studies are required. In summary, this study has provided the evidence that sexual differences exist in the effect of temperature on the calcium regulatory system. The calcium regulatory system is temperature dependent in female tilapia and temperature independent in males at prespawning stage. On the other hand, E_2 has a hypercalcemic effect on both males and females, with this effect being greater in males.

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吳郭魚血鈣濃度對溫度及雌性素反應之性別差異

蔡錦玲[1]　王立雪[1]

本研究以吳郭魚探討溫度及雌性素對魚體血鈣濃度之影響並比較雌、雄之差異。結果發現吳郭魚分別飼養在 26 ℃、 29 ℃及 32 ℃， 經 21 天後，雌魚之血鈣濃度隨溫度而顯著增加；雄魚則不受溫度影響。而且，飼養於同一水溫下，雌魚之血鈣濃度皆顯著高於雄魚。另一方面，排卵前期雌魚之血鈣濃度顯著高於排精前期之雄魚。兩者去除性腺後則無顯著差異。去除性腺並投予雌性素，經 21 天後，雌雄吳郭魚之血鈣濃度皆顯著增加，其增加量與雌性素投予量成正相關，但是，雌性素引發之高血鈣效應在雄魚顯著高於雌魚。此結果說明溫度對吳郭魚血鈣調節系統之影響具性別差異，雌魚具溫度相關性而雄魚則無。

關鍵詞： 雌性素，血鈣，性別差異，溫度，吳郭魚。

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