Korean J. Ichthyol. 9(2), 195~201, 1997

韓魚誌, 9(2), 1997

# Effects of Temperature and Estradiol-17β Treatment on Phenotypic Sex Determination in Different Genotypes of Nile Tilapia (*Oreochromis niloticus*)

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Environmental sex determination by temperature was revealed in different genotypes (putative XX : XY=1 : 1, 1 : 0, 0 : 1) of nile tilapia (*Oreochromis niloticus*). There was no significant deviation from expected sex ratio in control group treated with 27℃, while the temperature regimes of 33℃ and 36℃ during labile period induced the differentiation of phenotypic sex into female with a clear trend forward percent female with increasing temperature. The administration of 240 and/or 480 mg estradiol - 17β ($E_2$)/kg diet also showed the additional effect to derive the phenotypic sex to female in putative XX : XY=1 : 1 and 0 : 1 progeny groups.

## Introduction

The sex determination mechanism of fish involving sex chromosomes, with either female or male homogamety in different species, are based mainly on genetic factors. In many species, however, sex determination may be entirely polygenic and also be affected by various environmental factors such as temperature, pH, light and salinity etc. (Conover and Kynard, 1981 ; Conover and Heins, 1987). Since the first evidence of temperature controlled sex determination has been reported in a gonochoristic species *Menidia menidia* (Conover and Kynard, 1981), a few studies have made on other species (Sullivan and Schultz, 1986 ; Korpelainen, 1990 ; Strüman *et al.*, 1996).

In nile tilapia (*Oreochromis niloticus*), the sex determination mechanism has been evidenced by a lot of models including XX femlae - XY male based genetic factor, temperature, and genotype/temperature interaction (Baroiller *et al.*, 1995 ; Abucay *et al.*, 1997). However, studies on the temperature controlled sex determination of nile tilapia revealed the quite variable results depending on the strains and genotypes. Baroiler *et al.* (1995) reported that high water temperature (36℃) treatment significantly increased the percent male in both genetically normal and all XX progenies. Abucai *et al.* (1997) also reported the same results in putative all XX progenies treated at high water temperature (37℃) but the same 37℃

treatment increased the percent female in putative all XY and YY progenies.

The objective of this study was to re-examine the effect of temperature on phenotypic sex determination of different genotypes in nile tilapia and to evaluate the effectiveness of temperature on estradiol-17$\beta$ ($E_2$) treatment for sex reversal.

## Materials and methods

### Fish and genotypes

The fish used for the experiment was originated from a Japanese strain of nile tilapia (*Oreochromis niloticus*). Three experimental progeny groups with different genotypes were used for examining the effect of temperature on phenotypic sex determination. The groups representing different genotypes were produced by chromosome-set manipulation and sex reversal technique as described by Kim *et al.* (1996).

The notification for 3 experimental groups with different genotypes are as follow :

GNT (genetically normal tilapia) : the progeny group expected to have normal sex ratio (XX : XY=1♀ : 1♂) that produced by crossing normal female (XX) with normal male (XY).

GFT (genetically female tilapia) : the progeny group expected to have all-female sex ratio (XX : XY=1♀ : 0♂) that produced by crossing normal female (XX) with sex reversed male (♂ XX).

GMT (genetically male tilapia) : the progeny group expected to have all-male sex ratio (XX : XY=0♀ : 1♂) that produced by crossing superfemale (♂YY) with sex reversed male (♂ XX).

### Temperature treatment

All experimental progenies were incubated initially at 27±0.5℃ of nursery cage under closed-recirculated system until the temperature treatment. The desired temperatures (33 and 36℃) were obtained by gradually increasing the temperature with 4℃/day. Control treatment (27℃) was also made for each genotype group. Treatment duration was 28 days that has been thought to be labile period for sex differentiation in this species (Kim *et al.*, 1993). There were 2 replicate tanks per each treatment combination (genotype/temperature). At the end of treatments, the survival rate of each treatment was evaluated as a percent survived fish of initially stocked fish, and then the temperatures of all experimental groups except control groups were decreased to 27℃ with a rate of 4℃/day.

### Hormonal sex reversal with temperature treatment

To examine the combined effectiveness of temperature and estradiol-17$\beta$ ($E_2$) treatment for feminization of nile tilapia, the progenies of different genotypes were subjected to E2 treatment under different temperature (27, 33, and 36℃). Effectiveness of the dose levels, 240 ($E_2$ 240) and/or 480 mg ($E_2$ 480) E2/kg diet were examined in each temperature by oral administration of hormone diet on an ad libitium basis. Treatment duration was also 28 days. Detailed procedures for $E_2$ treatments involving making hormone diet and management of fish was according to the procedure described by Kim *et al.* (1993).

### Phenotypic sexing

At the age of 120 days, the phenotypic sex of experimental fish was determined. The gonads were surgically removed, and the sex were examined by squash method under light microscope.

### Statistics

Difference in growth and sex ratio were

assessed by ANOVA and/or chi-square tests. Difference was considered to be significant at the level of $P<0.05$.

## Results

### Survival of artificially incubated eggs

No significant difference in mean survival rates in 4 detection points was observed among the progeny groups of different genotypes (GNT, GFT and GMT). The overall survival rates at swim up stage was ranged from 82.7 to 86.2% (Table 1).

### Effects of temperature and $E_2$ treatment on the sex ratio of GNT

Mean survival rates of the groups treated with different temperature did not differ with the range from 78.1 to 85.5% ($P>0.05$). There was no significant difference in survival rates between the groups treated with temperature alone and those treated with temperature and $E_2$. The control (27℃) group showed the slightly higher percent of male, but the deviation was not significant from the normal 1♀ : 1♂ ratio. Percent female was gradually increased with only increasing the temperature ; 63.9% females were produced in 33℃ - treated group ($P<0.05$) and 74.9% females in 36℃ - treated group ($P<0.001$). The percent females under each temperature were significantly increased by $E_2$ treatment with dose level of 480 mg/kg diet ($E_2$ 480). The 94% of female obtained in $E_2$ treated group under 27℃. The portion of females in $E_2$ treated groups were slightly increased, but not significantly, with increasing the temperature, 97.3% in 33℃ and 98.4% in 36℃ (Table 2).

### Effects of temperature and $E_2$ treatment on the sex ratio of GFT

There was no significant difference in the percent survival among treated groups ($P>0.05$) regardless of temperature and hormone treatment. The sex ratio of genetically all female groups were not affected by water temperature. The experimental groups revealed all - femaleness consistently in all temperature treatments (Table 3). The 480 mg/kg diet of $E_2$

**Table 1. Survival rates of artificially incubated nile tilapia eggs[1]**

| Parent genotype | Developmental stages | | | |
|---|---|---|---|---|
| | Two cell | Blastula-gastrula | Hatching | Swim up |
| GNT (XX♀ × XY♂) | 97.0±0.4 | 91.4±0.7 | 89.8±3.4 | 86.2±3.9 |
| GFT (XX♀ × ♂XX♂) | 94.5±2.9 | 91.4±3.1 | 87.5±2.9 | 85.5±3.4 |
| GMT (♂YY♀ × ♂XX♂) | 95.2±4.9 | 89.6±4.3 | 84.1±2.0 | 82.7±1.9 |

[1]No significant differences among values (mean±s.d. of triplicates) were observed.

**Table 2. Effects of rearing water temperature with estradiol-17β administration on the sex ratio of GNT nile tilapia progenies produced from cross of normal female (XX) with normal male (XY)[1]**

| Putative genotype Water temperature | | | | | | |
|---|---|---|---|---|---|---|
| XX : XY=1 : 1 | Control (27℃) | | 33℃ | | 36℃ | |
| (GNT) | $E_2$0[2] | $E_2$480[3] | $E_2$0 | $E_2$480 | $E_2$0 | $E_2$480 |
| % Survival | 78.1±6.7[a] | 86.0±3.7[a] | 85.5±6.5[a] | 86.7±5.8[a] | 80.5±3.9[a] | 77.5±4.7[a] |
| % Female | 43.1±3.9[a] | 94.0±3.3[d] | 63.9±2.6[b] | 97.3±3.1[d] | 74.9±1.7[c] | 98.4±1.4[d] |
| $X^2$ against ♀1 : ♂1 | ns* | P<0.001 | P<0.001 | P<0.001 | P<0.001 | P<0.001 |

[1] Values (mean±s.d. of triplicates) within a row having the different superscripts are significantly different ($P<0.05$).
[2] Non treated group, [3] 480 mg/kg diet of $E_2$ treated group.
* Not significant, $X^2$ test.

**Table 3. Effects of rearing water temperature with estradiol-17$\beta$ administration on the sex ratio of GFT nile tilapia progenies produced from cross of normal female (XX) with sex reversed male ($\Delta$XX)[1]**

| Putative genotype | Water temperature | | | | | |
|---|---|---|---|---|---|---|
| XX : XY=1 : 0 | Control (27℃) | | 33℃ | | 36℃ | |
| (GFT) | $E_2$ 0[2] | $E_2$ 480[3] | $E_2$ 0 | $E_2$ 480 | $E_2$ 0 | $E_2$ 480 |
| % Survival | 83.2±13.2a | 94.2±0.4a | 85.7±5.7a | 88.9±3.9a | 85.4±12.9a | 91.6±2.4a |
| % Female | 100.0±0.0a | 99.0±1.4a | 100.0±0.0a | 100.0±0.0a | 100.0±0.0a | 100.0±0.0a |
| X2 against ♀1 : ♂0 | ns* | ns | ns | ns | ns | ns |

[1] Values (mean±s.d. of triplicates) within a row having the different superscripts are significantly different ($P<0.05$).
[2] Non treated group, [3] 480 mg/kg diet of $E_2$ treated group.
* Not significant, X2 test.

treatment group ($E_2$ 480) also showed the 100% females in all 3 different temperatures.

### Effects of temperature and $E_2$ treatment on the sex ratio of GMT

Mean survival rate of 36℃ - treated group (74.0%) was slightly lower than both that of control group (82.2%) and that of 33℃ - treated group (76.2%). However the differences were not significant. There was a clear trend toward percent females with increasing temperature (Table 4). The genetically all male groups showed nearly 100% males in control groups. It was coincident with the expected sex ratio (♀ : ♂=0 : 1) ($P>0.9$). But the percent female was dramatically increased in 33 and 36℃ treated groups. About 54% of genetically male progenies differentiated into phenotypic females in 33℃ -treated group ($P<0.001$), and much more percent females (77%) was obtained in 36℃ ($P<0.001$) (Table 4).

The effect of 240 and 480 mg/kg diet of $E_2$ for sex reversal into female was also demonstrated in all temperature treatments without any significant adverse effect on survival ($P>0.05$). The extent of feminization was dependent on the concentrations of $E_2$. The significant increase in female percent was observed in GMT progeny groups under 27℃ ; 3.7% of females ($E_2$ 0), 84.4 ($E_2$ 240) and 96.5% ($E_2$ 480). Percent females of 93.7 ($E_2$ 240) and 97.3 ($E_2$ 480) were observed in 33℃ treatments, while 81.4 ($E_2$ 240) and 98.7 ($E_2$ 480) of females were shown in 36℃ treated groups.

## Discussion

The sex determination mechanism of nile tilapia has been thought to be based on the XX female - XY male system (female homogame-

**Table 4. Effects of rearing water temperature with estradiol - 17$\beta$ treatment on the sex ratio of GMT nile tilapia progenies produced from cross superfemale ($\Delta$YY) with sex reversed male ($\Delta$XX)[1]**

| Putative genotype | Water temperature | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| XX : XY=0 : 1 | Control (27℃) | | | 33℃ | | | 36℃ | | |
| (GMT) | $E_2$ 0[2] | $E_2$ 240[3] | $E_2$ 480[4] | $E_2$ 0 | $E_2$ 240 | $E_2$ 480 | $E_2$ 0 | $E_2$ 240 | $E_2$ 480 |
| % Survival | 82.2±2.0[a] | 82.2±5.7[a] | 77.0±2.7[a] | 76.2±5.8[a] | 79.9±5.4[a] | 75.3±4.6[a] | 74.0±7.4[a] | 70.5±8.8[a] | 71.7±9.7[a] |
| % Female | 3.7±2.1[a] | 84.4±1.4[c] | 96.5±0.5[d] | 54.2±5.5[b] | 93.7±3.7[d] | 97.3±1.2[d] | 76.8±6.5[c] | 81.4±2.4[c] | 98.7±1.2[d] |
| $X^2$ against ♀0 : ♂1 | ns* | $p<0.001$ | $p<0.001$ | $p<0.001$ | $p<0.001$ | $p<0.001$ | $p<0.001$ | $p<0.001$ | $p<0.001$ |

[1] Values (mean±s.d. of triplicates) within a row having the different superscripts are significantly different ($P<0.05$).
[2] Non treated group, [3] 240 mg/kg diet of $E_2$ treated group, [4] 480 mg/kg diet of $E_2$ treated group.
* Not significant, $X^2$ test.

ty), however, many studies have been reported to claim that other factors such as autosomal sex modifying factors and environmental conditions could influence the sex determination in this species (Mair *et al.*, 1991).

Since the first time evidence of temperature dependent sex determination has been reported in *Menidia menidia* (Conover and Kynard, 1981), it has been believed that one of the most important environmental factors for phenotypic sex determination is temperature in several species including olive flounder (Kim, unpublished data), cyprinid loach (Arai *et al.*, 1997), pejerrey (Strüsmann *et al.*, 1996) and tilapia (Baroiller *et al.*, 1995).

The results from the present study indicated that the sex determination of our strain of nile tilapia has been significantly affected by water temperature with the trend forward percent female with increasing temperature, regardless of progeny groups with different genotypes. Previous studies considering the temperature controlled sex determination in nile tilapia have reported quite variable results contradictory to each other. Baroiller *et al.* (1995) have reported that the high temperature (36℃) would induce the phenotypic male in nile tilapia ("Bouake strain") and Florida red tilapia. However, Abucay *et al.* (1997) reported that high temperature treatment (37℃ for 21 days) induced the differentiation into phenotypic male in XX genotype, but the same 37℃ treatment increased the percent female in XY and YY genotypes of nile tilapia (presumed as "Lake Manzala strain"). This variability may be caused by strain-specific genetic background or possible differences in experimental conditions. The strain and/or sire dependent variabilities on percent male in progenies produced by YY ♂ × XX ♀ has already been reported (Tuan *et al.*, 1997).

In the present study, additional effect on the feminization of GNT and GMT nile tilapia could be observed when 240 and/or 480 mg/kg diet of $E_2$ were administered under different water temperature. The effectiveness of $E_2$ for direct feminization has been reported in many species including nile tilapia (see the review, Pandian and Sheela, 1995). It may be valuable to perform the further research to examine if successful feminization can be achieved by combining the very lower amount of hormone and high temperature treatment. Further research also will be needed to determine the more defined optimal condition for improving yield of the sex reversal based on temperature regime.

This study proved that phenotypic sex of nile tilapia was thermolabile, and the sex differentiation of this species could be directed by temperature regime, which may be a valuable mean for genetic improvement program of nile tilapia. In future study, elucidation of the mechanism of temperature driven sex determination should be need.

## References

Abucay, J. S., G. C. Mair, D. O. F. Skibinski and J. A. Beardmore, 1997. Environmental sex determination : The effects of temperature and salinity on sex ratio in *Oreochromis niloticus* L.. In : The Sixth International Symposium on Genetics in Aquaculture, Stirling, Scotland. 24~28 June 1997. University of Stirling, Scotland.

Arai, K., T. Nomura, T. Hayashi and R. Suzuki, 1997. Sex~reversal by high water temperature in the loach, *Misgurnus anguillicaudatus*. In : The Sixth International Symposium on Genetics in Aquaculture, Stirling, Scotland, 24~28 June 1997. University of Stirling, Scotland.

Baroiller, J. F., F. Clota and E. Geraz, 1995. Temperature sex determination in two tilapia, *Oreochromis niloticus* and the red tilapia (red Flori-

da strain) : Effect of high or low temperature, p.158~160. In : Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish (eds. Goetz, F. and P. Tomas), 2~8 July 1995. The University of Texas, Austin.

Conover, D. O. and B. E. Kynard, 1981. Environmental sex determination : interaction of temperature and genotype in a fish. Science 213 : 577~579.

Conover, D. O. and S. W. Heins, 1987. The environmental and genetic components of sex ratio in *Menidia menidia* (Pisces : Atherinidae). Copeia 1987 : 732~743.

Kim, D. S., J.~Y. Jo and I. C. Bang, 1993. Effects of 17$\beta$-estradiol on the sex reversal of nile tilapia, *Oreochromis niloticus*. J. Aquacul. 6 : 125~132.

Kim, D. S., C. H. Noh, Y.-H. Choi and Y. K. Nam, 1996. Production of supermale (YY) and superfemale ($\Delta$YY) nile tilapia (*Oreochromis niloticus*) by sex reversal and chromosome manipulation. II. Progeny tests with supermale and superfemale nile tilapia. J. Aquaculture 9 : 101~106.

Kim, K. K., I. C. Bang and Y. Kim, 1996. Studies on the production of all-female populations of olive flounder, *Paralichthys olivaceus*. IV. Sex determination by water temperature during sex differentiation period. J. Aquacul. 9 : 429~435.

Korpelainen, H., 1990. Sex ratios and conditions requires for environmental sex determination in animals. Biol. Rev. 65 : 147~184.

Mair, G. C., A. G. Scott, D. J. Penman, J. A. Beardmore and D. O. F. Skibinski, 1991. Sex determination in the genus *Oreochromis*. 1. Sex reversal, gynogenesis and triploidy in *O. niloticus* (L.). Theor. Appl. Genet. 82 : 144~152.

Strüsmann, C. A., S. Moriyama, E. F. Hanke, J. C. Calsina Cota and F. Takashima, 1996. Evidence of thermolabile sex determination in pejerrey. J. Fish Biol. 48 : 643~651.

Sullivan, J. A. and R. J. Schultz, 1986. Genetic and environmental basis of variable sex ratio in laboratory strains of *Poeciliopsis lucida*. Evolution 40 : 152~158.

Tuan, P. A., D. C. Little, G. C. Mair and J. A. Beardmore, 1997. Sex determination and feasibility of genetically male tilapia production in the Thai-Chitralda strain of *Oreochromis niloticus* (L.). In : The Sixth International Symposium on Genetics in Aquaculture, Stirling, Scotland. 24~28 June 1997. University of Stirling, Scotland.

Varadaraj, K., S. S. Kumari and T. J. Pandian, 1994. Comparison of conditions for hormonal sex reversal of mozambique tilapias. Prog. Fish Cult. 56 : 81~90.

Effects of Temperature and Estradiol-17$\beta$ Treatment on Phenotypic Sex Determination of Nile Tilapia

# 수온 및 estradiol－17$\beta$ 처리가 나일틸라피아(*Oreochromis niloticus*)의 성결정에 미치는 효과

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수온 및 estradiol－17 ($E_2$) 처리가 나일틸라피아의 성결정에 미치는 효과를 조사하기 위하여 세가지의 유전자형 집단(XX : XY=1 : 1, 1 : 0, 0 : 1)을 대상으로 실험을 실시하였다. 실험군들간 수온 및 $E_2$ 처리에 따른 생존율의 유의한 차이는 없었다. 사육 수온에 따른 성비는 대조군(27℃)에서는 각 유전자형에 따라 예상되는 암수 비율이 관찰되었다. 그러나 성분화 시기에 사육 수온을 33 및 36℃로 증가시킨 실험군에서는 암컷의 비율이 유의적으로 높았으며, 33℃에 비해 36℃ 실험군의 암컷률이 높았다. 서로 다른 처리 수온 조건에서 $E_2$ 처리를 병행한 결과 모든 처리 수온에서 호르몬 처리를 하지 않은 실험군들보다 유의적으로 높은 암컷률을 보였다.