

## EFFECT OF DIFFERENT TEMPERATURES ON GROWTH AND SEX RATIO OF NILE TILAPIA (*Oreochromis niloticus*) FRY

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### ABSTRACT

The present study was carried out at Fish Research Center (FRC), Suez Canal University, Ismailia, Egypt, in two consecutive experiments. First experiment, was conducted to determine the effect of different water temperatures on growth performance of Nile tilapia (*Oreochromis niloticus*) fry. Second experiment, was carried out to study the effect of different water temperatures on sex ratio of progenies of Nile tilapia (*Oreochromis niloticus*), during their early life stages. In experimental 1, twenty-day-old Nile tilapia fry, weighing  $0.30 \pm 0.03$ g, were distributed at a density of 30 fry per 45-L fiberglass aquarium for 60 days on four thermal regimes (20, 25, 30, and 35°C) three replicate for each. The physico-chemical parameters of water in all aquaria were recorded. Fish were fed three times daily using a commercial diet containing 46% protein at a rate of 12% of body weight/day. Growth performance was estimated throughout the experiment. Results showed that the final mean weight was significantly higher at 25 and 30°C than at 20 and 35°C. Daily weight gain (DWG) and feed conversion ration (FCR) were better at 25 and 30°C. At all temperatures, survival rates were not affected.

In experiment 2, Nile tilapia fry (first 20 days after yolk sac resorption) were distributed at a density of 100 fry per 45-L fiberglass aquarium for 28 days in four different temperature regimes (22, 27, 32, and 37°C), three replicate for each. Results showed that the high temperature (37°C) treatment produced a significantly higher proportion of males (89%) with low survival rates (79%). Whereas the sex ratio of progenies reared at temperature below 37°C never deviated significantly from the balanced sex ratio. The study suggested that the best growth and feed utilization of *O. niloticus* juveniles may be higher at 25 and 30°C and it is possible for producing higher males (sex ratio) population of Nile tilapia by thermal sex reversal.

**Keywords:** Nile tilapia (*O. niloticus*), growth, temperature, sex ratio, survival rate.

### INTRODUCTION

Tilapias are members of the Cichlidae family which are a group of warm water fish and have become the most important warmwater aquaculture fish group in the world (FAO, 2003).

Saber *et al.* (2004) showed that young *O. niloticus* prefer temperature between 30-36°C and suggested to avoid higher temperatures (about 41°C) being lethal.

High temperature of rearing (from 34 to 37 °C) applied after hatching (around 10 days post fertilization) and lasting from 10 to 28 days significantly skewed sex ratios towards males (Baras *et al.*, 2001 and Tessema *et al.*, 2006).

Growth of fish is a complex process affected by many behavioral, physiological, nutritional, and environmental factors; however, temperature is

recognized as one of the most important single abiotic factor affecting growth, feed intake, and feed conversion of fish (Rougeot *et al.*, 2008).

Several problems face fish production in Egypt. One of these problems is that most tropical species die when temperature drops below 10°C (El-Sherif and El-Feky, 2009).

Therefore, this study aimed to ( i ) test the effect of different water temperatures on growth, feed utilization efficiency, and survival rate of Nile tilapia fry reared in fiberglass aquaria (45-L) for 60 days; and ( ii ) to investigate the effect of different temperatures on sex ratio of progenies of Nile tilapia reared in fiberglass aquaria (45- L) during the first 28 days of exogenous feeding.

## **MATERIAL AND METHODS**

This study was carried out from 15<sup>th</sup> March to 13<sup>th</sup> May, 2009, at Fish Research Center (FRC), Suez Canal University, Ismailia, Egypt.

### **Experimental design:**

The present study was carried out in two experiments:

#### **Experiment 1:**

Twenty-day-old Nile tilapia fry (from FRC), weighing  $0.30 \pm 0.03g$ , were obtained from one female and were dispatched into 12 fiberglass aquaria with capacity of 45-L de-chlorinated water at initial stocking density of 30 fry per aquarium (Azaza *et al.*, 2008). Photoperiod was kept at a constant 12:12 (light:dark) ratio throughout the study. Each treatment was tested in three replicates. Temperature treatment was carried out in four recirculating systems, each unit consisted of an independent temperature regulation (reservoir tank), biofilter, UV-sterilization lamp and three fiberglass aquaria. A 1kW immersion thermostatic heater was installed in each reservoir tank to maintain the preselected water temperature (Rougeot *et al.*, 2008). Four different temperature regimes were maintained at 20, 25, 30, and 35°C. Supplemental aeration was provided to maintain dissolved oxygen levels near saturation. In all aquaria, water was constantly replaced by continuous flow at the rate of 1L/ min to provide oxygen and remove excess nitrogenous wastes.

Fish fry were acclimated to experimental system for 1 week prior to the start of experiment. Experimental water temperatures were attained gradually by heating or cooling water (using ice bags) at 1°C/ 2 hours (Wessels and Hörstgen-Schwark, 2007).

The fish were fed on a commercial diet of 46% crude protein according to (NRC, 1983), produced by the Sinai Shrimps 21 Compony, Port Said. The ingredients composition and proximate analysis of feed are provided in Table (1). The feed was offered by hand three times daily (Wessels and Hörstgen-Schwark, 2007) at a rate of 12% BW/day (Parker, 1994). Initially, fish were fed powder diets to accommodate the fry. From day 20 until day 60, fish were fed on pellets size of 3 mm. The diet was stored in refrigerator (4°C) during the experimental duration to avoid the nutrients deterioration. All fish in each aquarium were weighed and measured at the beginning and at the end of the experiment. Mortality was recorded daily.

**Table (1): Ingredients composition and proximate analysis of the diet offered to Nile tilapia in this experiment, on dry matter basis**

Ingredients	% dry weight
Fish meal <sup>a</sup>	59.9
Soluble fish protein concentrate <sup>b</sup>	01.0
Cod liver oil	05.8
Gelatinized starch <sup>c</sup>	29.8
Vitamin premix <sup>d</sup>	01.0
Mineral premix <sup>e</sup>	01.0
Choline chloride (50%)	00.5
Lignin sulphate	01.0
<b>proximate analysis (%DMB)</b>	
Dry matter	95.5
Crude protein	46.0
Crude fat	11.7
Crude Ash	08.4
Crude fiber	04.3
Nitrogen free extract (NFE)	29.6

a- Triple Nine, Denmark (CP: 78.6% DM; GL: 9.8% DM).

b- Sopropêche G, France (CP: 72.7% DM; GL: 18.0% DM).

c- C-Gel Instant-12016, Cerestar, Mechelen, Belgium.

d- Vitamins (mg kg<sup>-1</sup> diet): retinol, 18,000 (IU kg<sup>-1</sup> diet); calciferol, 2000 (IU kg<sup>-1</sup> diet); alpha tocopherol, 35; menadion sodium bis., 10; thiamin, 15; riboflavin, 25; Ca pantothenate, 50; nicotinic acid, 200; pyridoxine, 5; folic acid, 10; cyanocobalamin, 0.02; biotin, 1.5; ascorbyl monophosphate, 50 and inositol, 400 (Pfizer).

e- Minerals (mg kg<sup>-1</sup> diet): cobalt sulphate, 1.91; copper sulphate, 19.6; iron sulphate, 200; sodium fluoride, 2.21; potassium iodide, 0.78; magnesium oxide, 830; manganese oxide, 26; sodium selenite, 0.66; zinc oxide, 37.5; potassium chloride, 1.15 (g kg<sup>-1</sup> diet); sodium chloride, 0.40 (g kg<sup>-1</sup> diet) and dibasic calcium phosphate, 5.9 (g kg<sup>-1</sup> diet) (Pfizer).

## Experiment 2:

Second experiment was carried out in the same recirculating system mentioned in experiment 1. This experiment was conducted using Nile tilapia fry (first 20 days after yolk sac resorption) at a density of 100 fry per fiberglass aquarium (45-L de-chlorinated water) for 28 days. Four different temperature regimes (22, 27, 32, and 37°C) were applied to sibling fry. Each temperature treatment was tested in three replicates. Experimental water for each aquarium was cooled or warmed to adjust level of temperature at a rate of 1°C per 2 h in order to avoid thermal shock (as used in experiment 1). Experimental temperature was maintained throughout 28 days (Baras *et al.*, 2001 and Rougeot *et al.*, 2008). At the end of the experiment, water temperature was adjusted at 28°C (1°C / 2 h). From each aquarium, fish were transferred into larger tanks (150 L) and held at 28°C to promote growth. When juveniles reached at age of 3 months, they were removed for sexing (genital papilla) (Rougeot *et al.*, 2007 and 2008).

## Physico-chemical analysis of water:

Water quality parameters from every aquarium were monitored daily throughout the experimental period. Water temperature, dissolved oxygen and levels of pH were measured twice daily by using oxygen-temperature meter (YSI model L 57) (Marques *et al.*, 2000) and pH meter (model 56, NR 8

BB 203) (Tessema *et al.*, 2006). Water temperature in each treatment remained nearly constant throughout the experimental period (ranges: 20-35°C), dissolved oxygen ranged between 4.9 and 7 mg/l and pH ranged between 6.6 and 8.

**Parameters used:**

Growth performance was evaluated through specific growth rate (SGR), daily weight gain (DWG), feed intake (FI), feed conversion ratio (FCR), coefficient of condition (KC), and survival rate (SR). The following formulae were used:

$$\text{SGR (\%/day)} = 100 (\ln W1 - \ln W0) / t$$

(According to De- Silva and Anderson, 1995)

$$\text{DWG (g/day)} = (W1 - W0) / t$$

(According to De- Silva and Anderson, 1995)

$$\text{FI (g/day)} = \text{g dry matter/day}$$

$$\text{FCR} = (\text{dry matter FI}) / (\text{live DWG})$$

(According to De- Silva and Anderson, 1995)

$$\text{KC} = \text{mean weight/length}^3 \times 100$$

$$\text{SR (\%)} = \text{Ni} \times 100 / \text{N0}$$
 (According to Harrell *et al.*, 1990)

**Where:**

ln = natural log

W1 = Final wet weight (g)

W0 = Initial wet weight (g)

T = Time interval in days

Ni = Number of fish at the end

N0 = Number of fish initial stocked

**Statistical analysis:**

The data obtained in this study were analyzed by one-way ANOVA procedure of statistical analysis system (SAS, 1988). Means were compared by Duncan's new multiple range test (Zar, 1996).

## **RESULTS AND DISCUSSION**

### **Experiment 1:**

Table 2 summarizes the final body weight, total length, survival rates, weight gain, SGR, daily weight gain (DWG), FCR and condition factor for all treatments of Nile tilapia fry reared in aquaria for 60 days. At the end of the experiment, the final body weights (ranged 5.54–18.24 g) were significantly ( $P < 0.05$ ) greater at 25 and 30°C than at 20 and 35°C. The FBW at 20°C and 35°C decreased by 5.54 and 9.71g, respectively, which was attributed to a decrease in feed consumption (Larsson and Berglund, 2005). Temperature of 30°C showed the highest body weight (18.24g) followed by temperature 25°C (13.16g). From these results it could be concluded that the average body weight of tilapia observed in the experimental groups was found to be related to temperature. This is in agreement with the findings of Devline and Nagahama (2002) and Saber *et al.* (2004), who reported that the best growth rate was at 30°C than at 25°C. The differences among treatments were significant ( $P < 0.05$ ). Final mean lengths ranged 6.73-9.34 cm among

treatments and showed the same trends as observed for weights (Saber *et al.*, 2004). The growth rates over the 60 experimental days, expressed as mean DWG and SGR, significantly ( $P < 0.05$ ) increased, from a minimum (0.087 g/day and 4.795% BW/day, respectively) at 20°C to a maximum (0.299g/day and 6.781% BW/day) at 30°C.

Generally, growth rate increased with increasing water temperature and reached its optimal at 30°C, then declined significantly ( $P < 0.05$ ) at 35°C. The effect of temperature on growth depends on the interaction between feed consumption and metabolism (Baroiller and D'Cotta, 2001). This is mainly due to the feed consumption and metabolic scope, where a substantial amount of feeding energy is used to cover maintenance metabolism at the expense of somatic growth. Similar results were obtained by Saber *et al.*, (2004) and El-Sherif and El-Feky (2009). There were significant differences ( $P \leq 0.05$ ) in FBW and SGR at different temperatures, but the differences in SGR was not significant ( $P \geq 0.05$ ) between 25 and 30°C.

Survival rates (SR) throughout the experiment ranged 86.6–90.0% among treatments El-Sherif and El-Feky (2009) noted that optimal water temperature for optimum growth and survival of tilapia are 25-30°C. No significant differences ( $P \geq 0.05$ ) in survival were recorded among the treatments (temperatures 20, 25, 30 and 35°C).

Significant effects of temperature ( $P < 0.001$ ) on feed intake (FI) were observed. Feed intake increased with increasing temperature until 30°C (0.552g/day/fish) as given by Azaza *et al.* (2008). Feed conversion ratio (FCR) differed between groups ( $P < 0.05$ ) and decreased from 2.890 at the lowest temperature (20°C) to 1.851 at 30°C (Table 2). In this study, FCR of tilapia was the best at 30°C then followed by 25°C. These results are in full agreement with the findings of Tessema (2001) and Saber *et al.* (2004). The difference between 25 and 30°C was not significant ( $P \geq 0.05$ ).

The obtained results indicated that, fish reared at 30 and 25°C had the highest and the lowest condition factor (KC), respectively. The other treatments had intermediate and similar ( $P > 0.05$ ) condition factors (Altena and Hörstgen-Schwark, 2002).

#### **Experiment 2:**

Survival rates after temperature treatments and percentage of males and females after sexing for all treatments are given in Table 3. The percentage of males reared over the first 28 days of exogenous feeding at temperatures among 22, 27 and 32°C (46.55, 57.34 and 61.67%, respectively) never deviated significantly from equilibrated sex ratio. In the other side, treatment at 37°C for 28 days led to a significantly ( $P < 0.01$ ) higher percentage of males (89%). In this regard, Baras *et al.* (2001) reported that ambient water temperature during the first weeks of exogenous feeding strongly influences the phenotypic sex of Nile tilapia, and that a significant masculinising effect is obtained at temperatures of circa 37°C. They also found that low temperature rearing did not affect the sex ratio of progenies. Tessema *et al.* (2006) and Rougeat *et al.* (2008) showed that high temperature treatments could be increase the percentage of males in the offspring of Nile tilapia. Compared to the 36°C treatment, a further increase in

males was not obtained with a treatment of 38 °C in the majority of the tested progenies in either population. On the other hand, Azaza (2004) found that low rearing temperatures (19°C) did not affect the sex ratio of progenies, confirming results obtained for other strains and species of tilapia.

The mean survival rates in treatments reared at 22 and 37°C (65.1 and 79.3%) were significantly ( $P<0.05$ ) lower than those observed at 32 and 27°C (87.66 and 95). This was in agreement with (Baras *et al.*, 2001 ;Tsai *et al.*, 2003 and Matsuoka *et al.*, 2006). They found that tilapia reared over the 28 first days of exogenous feeding at temperatures from 24°C to 33°C showed high and similar survival rates (mean of 96.7%) over 70 days, in contrast to siblings reared at lower (21°C) or higher (35°C and 37°C) temperatures (means of 79.0%, 85.0% and 64.7%, respectively). The rearing temperature producing the highest survival rate during the first 28 days of exogenous feeding was modeled as 27.5°C. Tessema *et al.* (2006) noticed that the overall rate of survival of progeny groups subjected to the temperature treatments of 18 °C or 38 °C was significantly lower ( $P<0.001$ ) than the ones in the corresponding controls (27°C).

**Table (2): Growth performance of Nile tilapia (*O. niloticus*) fry exposed to different water temperatures (Mean±SE)**

Parameters	Temperature (°C)			
	20	25	30	35
IBW (g)	0.30±0.03	0.31±0.01	0.31±0.02	0.30±0.01
FBW (g)	5.540±0.260 <sup>d</sup>	13.16±0.341 <sup>b</sup>	18.24±0.315 <sup>a</sup>	9.710±0.152 <sup>c</sup>
LI (cm)	2.030±0.012	2.010±0.003	2.020±0.013	2.020±0.007
LF (cm)	6.730±0.211 <sup>c</sup>	8.990±0.164 <sup>a</sup>	9.340±0.132 <sup>a</sup>	7.980±0.056 <sup>b</sup>
DWG (g/day/fish)	0.087±0.007 <sup>d</sup>	0.214±0.004 <sup>b</sup>	0.299±0.002 <sup>a</sup>	0.157±0.004 <sup>c</sup>
SGR (%/day)	4.795±0.057 <sup>b</sup>	6.231±0.061 <sup>a</sup>	6.781±0.029 <sup>a</sup>	5.735±0.041 <sup>a,b</sup>
FI (g/day/fish)	0.251±0.008 <sup>c</sup>	0.411±0.012 <sup>b</sup>	0.552±0.004 <sup>a</sup>	0.402±0.011 <sup>b</sup>
FCR (g/g)	2.890±0.043 <sup>a</sup>	1.921±0.035 <sup>b</sup>	1.851±0.046 <sup>b</sup>	2.561±0.052 <sup>a</sup>
SR (%)	86.63±2.012	90.00±2.027	88.90±1.353	86.60±2.012
KC	1.817±0.012	1.811±0.015	2.239±0.052	1.912±0.044

Means within a row marked with different letters are significantly different ( $P<0.05$ ).

**Table (3): Effects of ambient water temperatures on survival rate (SR %) and sex ratio of Nile tilapia (*Oreochromis niloticus*) during the first 28 days of exogenous feeding (Mean±SE).**

Temperature (°C)	Parameter			Test $\chi^2$ 1:1
	Survival (%)	Males (%)	Females (%)	
22	65.10±6.10 <sup>d</sup>	46.55	53.45	NS
27	95.33±2.24 <sup>a</sup>	57.34	42.66	NS
32	87.66±2.35 <sup>b</sup>	61.67	38.33	NS
37	79.30±5.50 <sup>c</sup>	89.00	11.00	**

Means within SR column with different letters are significantly different ( $P<0.05$ ).

\*\* $P<0.01$ .

P is the probability of accepting the hypothesis that sex ratio is balanced.

NS: no significant difference ( $P>0.05$ ).

$\chi^2$ : represents the deviation of sex ratio of the treated progeny from that of balanced (1:1) sex ratio.

## CONCLUSION

This study showed that water temperature (30°C) can be optimum for growth of *O. niloticus* fry. Also, the possibility of producing higher males sex ratio population of Nile tilapia may be obtained by thermal sex reversal.

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## تأثير درجات الحرارة المختلفة على كفاءة النمو والنسبة الجنسية لزريعة البلطي النيلي

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أجريت هذه الدراسة بمركز بحوث الأسماك، جامعة قناة السويس، الإسماعيلية، مصر في تجربتين بهدف دراسة تأثير درجات الحرارة المختلفة على كل من كفاءة النمو وكفاءة الاستفادة من الغذاء ومعدل البقاء (التجربة الأولى) وكذا تأثيرها على النسبة الجنسية (التجربة الثانية) ليرقات البلطي النيلي في مراحل نموها الأولى.

التجربة الأولى: تم رعاية زريعة البلطي النيلي بعمر ٢٠ يوماً وبمتوسط وزن ٠.٣٠ ± جم في أحواض فيبرجلاس (سعة ٤٥ لتر ماء) بكثافة تخزينية ٣٠ زريعة لكل حوض لمدة ٦٠ يوماً في أربع معاملات حرارية مختلفة (٢٠، ٢٥، ٣٠، و ٣٥ م°). سجلت التحليلات الفيزيوكيماوية للماء. تم تقدير قياسات كفاءة النمو المختلفة خلال التجربة. غذيت الأسماك ثلاث مرات يومياً على عليقه تجاريه تحتوى على ٤٦% بروتين وبمعدل ١٢% من وزن الجسم يومياً. أوضحت النتائج وجود اختلافات معنوية عالية في الوزن النهائي عند درجات الحرارة ٢٥ و ٣٠ م° عنه عند ٢٠ و ٣٥ م°. لوحظ أن كل من الوزن المكتسب اليومي ومعدل تحويل الغذاء كانا أفضل عند درجات الحرارة ٢٥ و ٣٠ م° - أما معدل البقاء فلم يتأثر. ولهذا يمكن الحصول على أفضل نمو ومعدل استفادة من الغذاء ليرقات البلطي النيلي المرعاة على درجات حرارة من ٢٥ الى ٣٠ م°.

التجربة الثانية: تم رعاية زريعة البلطي النيلي بعد امتصاص كيس المح وبمتوسط وزن ٠.٣٠ ± ٠.٣ جم في أحواض فيبرجلاس (سعة ٤٥ لتر ماء) بكثافة تخزينية ١٠٠ زريعة لكل حوض لمدة ٢٨ يوماً في أربع معاملات حرارية مختلفة (٢٢، ٢٧، ٣٢، و ٣٧ م°) وبنفس ظروف الرعاية في التجربة الأولى. أوضحت النتائج أن رعاية زريعة البلطي النيلي على درجة الحرارة العالية (٣٧ م°) أدى للحصول على أعلى نسبة من الذكور (٨٩%) مع معدل بقاء منخفض (٧٩%). ومع ذلك فإن النسبة الجنسية للنسل المربي في درجات حرارة أقل من ٣٧ م° لم تتأثر معنوياً. ولذلك اقترحت هذه التجربة إمكانية إنتاج نسبة عالية من الذكور لزريعة البلطي النيلي باستخدام الحرارة في تحويل الجنس.

### قام بتحكيم البحث

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