İSTANBUL UNIVERSITY PRESS

EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2019; 34(1): 22-28 • DOI: https://doi.org/10.26650/ASE2019499991

Research Article

Effects of Water Temperature on Sex Differentiation and Growth Parameters of the Mozambique Tilapia (*Oreochromis mossambicus*, Peters, 1852)

Karl Christofer Kingueleoua Koyakomanda, Muamer Kürşat Fırat, Cüneyt Süzer, Serhat Engin, Müge Hekimoğlu, Hülya Sayğı, Osman Özden, Fatih Güleç, Şahin Saka

Cite this article as: Kingueleoua Koyakomanda, K. C., Fırat, M. K., Süzer, C., Engin, S., Hekimoğlu, M., Sayğı, H. … Saka, Ş. (2019). Effects of water temperature on sex differentiation and growth parameters of the Mozambique tilapia (Oreochromis mossambicus, Peters, 1852). *Aquatic Sciences and Engineering, 34*(1), 22–28.

ABSTRACT

In this study, the effects of water temperature on growth, survival rate, gonad development and sex ratios of Mozambique Tilapia (*Oreochromis mossambicus*) were studied by applying different temperature applications (28, 30, 32, 34 and 36°C) for a single period of 40 days. At the end of the study, in the control group (28°C), the males proportion was 47.62%. In fishes reared at 30°C, average male ratio was found as 73.68%. The highest male proportion was obtained in the 36°C temperature group (86.67%). Growth performances, feed utilization and gonad development were found to increase with elevating temperatures up to 34°C unlike the survival rate which has been found to fall with higher temperatures. In this study, sex differentiation rates differed significantly between all groups ($p<0.05$). However, the highest male rate obtained in group A and D. As hypothesized, results suggest that fish performed better at 30-32°C than 28°C or 34-36°C water temperature and the optimum temperature for a better expression of growth parameters in Mozambique Tilapia could be 30°C.

Keywords: Mozambique Tilapia (*Oreochromis mossambicus*), growth, survival, water temperature

ORCID IDs of the authors:
K.C.K.K. 0000-0002-4614-0127;
M.K.F. 0000-0003-1470-209;
C.S. 0000-0003-3535-2236;
S.E. 0000-0002-1663-1769;
M.H. 0000-0003-4685-7475;
H.S. 0000-0002-2327-566X;
O.Ö. 0000-0003-3638-6657;
F.G. 0000-0002-7077-7363;
Ş.S. 0000-0001-7300-4860

Ege University, Faculty of Fisheries, Aquaculture Dept, Izmir, Turkey

Submitted:
20.12.2018

Accepted:
26.02.2019

Online published:
18.03.2019

Correspondence:
Müge A. Hekimoğlu
E-mail:
mhekimoglu@gmail.com

INTRODUCTION

In the last 30 years, the amount of global aquaculture has increased by 12 times, resulting in the highest growth rate in the food production sector close to 8% (Osvald and Mikolasek, 2016). Aquacultural production, which was estimated at 7.36 million tons in the 1980s, reached 16.48 million tons in the 90's and 54.8 million tons in 2005. The actual annual production turns around 73.8 million tons (FAO, 2016). A portion of this increase in global aquaculture production (around 12.31%) is provided by Tilapia culture (FAO, 2016). Tilapia is one of the earliest fish species cultivated in the world and as understood from the paintings in ancient Egyptian graves its cultivation dates back to 3000 years BC (Popma and Masser 1999). Several studies on the reproduction of a variety of fish species have shown that sex ratio is often influenced by the ambient temperature of the water (Patino et al., 1996; Blazquez et al., 1998; Yamamoto, 1999; Goto et al., 2000; Baroiller and D'Cotta, 2001; Azuma et al., 2004). This has been amply demonstrated in several species, including the Nile Tilapia. Unfortunately the Tilapia of Mozambique, which is very close to its cousin of the Nile, has not enjoyed the same ardor in scientific researches. The aim of this study is to investigate the effect of altered water temperature on Mozambique tilapias's (*Oreochromis mossambicus*) sex differentiation and growth parameters. In addition, it will also help to enrich the scientific literature on this

subject so as to provide up-to-date sources to future researchers who will want to deepen research in this field.

MATERIALS AND METHODS

The experiment was carried out in the Fish Breeding Aquarium Unit of Ege University in Izmir (Turkey). The tilapia offspring used in the experiment were obtained naturally from the tilapia rootstocks in the laboratory of Ege University Aquaculture Faculty Aquaculture Department. The eggs were incubated at 28°C for 8 days at the root of the rootstock individual. During the incubation period of the eggs, water parameters (temperature, oxygen, and pH) were followed. In aquariums, cartridge water (10 microns) was used and the rested tank water was used. In order to keep the aquarium water clean, air-assisted mechanical filters have been used, and as a result of daily siphoning, fresh water has been introduced as water.

The desired amount of oxygen in aquariums is provided by the ventilation lines in the unit. The system uses a dual fluorescent system (950 lux) for a 14 hour dark period. In order to adjust the water temperatures in the aquarium, Eheim 100-300 W two different thermostatic heaters are used. In addition, Kern brand precision balance (0.01 g) for measuring fish weights, HI 9142 brand oxygen meter for oxygen measurements, Merck Pharo 100 Visible Water / Wastewater Analysis Spectrophotometer for measuring ph-nitrogen compounds and 2 mercury thermometers for temperature measurements were used.

The experiments were carried out in 15 glass aquariums, 30 * 30 * 30 cm in size and 18 liter in water volume. 255 new hatched *O. mossambicus* larvae were placed under 5 different experimental temperatures (28 (group K), 30 (group A), 32 (group B), 34 (group C) and 36°C (group D) with 3 replicates and then reared during 40 days. In the study, 1-1.5 mm sized extruder commercial trout start feed was used.

Estimation of Growth Parameters

Specific Growth Rate (SGR)
Specific growth rate SGR. was calculated according to the following equation (De Silva and Anderson, 1995):

SGR (% body wt.gain/day) =

$$\left[\frac{\text{Log}_n \text{ Final fish wt.} - \text{Log}_n \text{ initial fish wt.})}{\text{Time interval}}\right] \times 100$$

Feed conversion rate
FCR = Amount of feed consumed (g) / Live weight gain (g) (Santinha et al., 1999).

Condition Factor (K)
The weight-length relation of Fulton, which was used to determine the health of fish, was calculated using the following formula (Ricker, 1975). $K = W.100 / L^3$

Gonadosomatic index (GSI)
Calculation of the gonadosomatic index of the groups was made by the following formula (Halver, 1989; Hepher, 1990). GSI = (Gonad weight (g) x 100 / Fish weight (g))

Survival Rate (SR)
The survival rate of fish is calculated by the formula reported by Pechsiri and Yakupitiyage (2005). SR % = (Ns / Ni) x 100.
Ns: Number of fish at the end of the trial; Ni: Number of fish at the beginning of the trial.

Histological Analyzes of Gonad and Sex rate (Masculinity rate)

For histological analysis, gonad samples taken from fish were fixed with 10% formaldehyde and stored. Tissue samples were fixed in 10% buffer formol solution for sectioning and kept until histological examination was performed. Blocked specimens were fixed to formol and 5 µm thick sections were taken from Leica 2125 Rotary microtome. Hematoxylin-Eosin staining was performed to examine the general structure (Luna, L.G. 1968, Bancroft et al., 1996; Presnell and Schreibman, 1997). The preparations were examined under different types of Olympus JX31 type phase contrast microscope and photographed with the Olympus E330 type microscope. In this way, sex rates of fish were determined.

Statistical Analyzes

The growth parameters (Live Weight (LW), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Condition Factor (K) and Survival Rate (SR)) analyzed in this trial were obtained by One-way ANOVA test. The difference between groups was tested at a significance level of 5% ($p \leq 0.05$) with the Student-Newman-Keuhls multiple comparison test. For sex ratios, X2 test and Likelihood ratio test were applied. The results are given as Mean±Standard Deviation. All data were analyzed in SPSS 15.0 (SPSS, Chicago, IL) statistical package program.

RESULTS AND DISCUSSION

Water Aquality

The average and standard deviations values of water temperatures measured daily for each treatment throughout the study are given in Table 1. The lowest value was measured in the control group (28.06±0.23°C) and the highest water temperature was measured in D group (35.66±1.89°C). Even if differences between groups in terms of the amount of dissolved oxygen during the experiment were observed ($P \leq 0.05$), all values were found to be comprised in an acceptable range all along the study. The K (control) and A (30°C) groups showed the highest oxygen concentrations (5.05±0.74 ppm and 5.05±0.72 ppm respectively). The group with the lowest amount of oxygen was D group (4.40±0.75 ppm).

Growth Performances

The statistical significance levels of the growth parameters such as body weight (BW), total length (TL), condition factor (K), gonadosomatic index (GSI), specific growth rate (SGR), feed conversion ratio (FCR) are given in Table 1. Except for the condition factor, the differences between these parameters were found to be significant ($p \leq 0.05$). The groups with the highest body weight according to the last measurements were respectively A (30.497±1.98 g). B (27.762±1.81 g) and Control (23.780±1.60 g) groups. C and D groups reached 16.829±1.53g and 11.212±2.34g respectively and were found to be statistically lower than the previous groups ($p \leq 0.05$).

Table 1. Environmental parameters, growth performances, survival rate and masculinity rate of the experiment

Parameters	A (30°C)	B (32°C)	C (34°C)	D (36°C)	K (28°C)	F Value	P Value
Average water temperature (°C)	29.7±0.7 ^{d}	32.16±0.59 ^{c}	33.84±1.1 ^{b}	35.66±1.89 ^{a}	28.06±0.23 ^{e}	809.26	0.00
Average DOC (ppm)	5.05±0.7 ^{a}	4.64±0.66 ^{b}	4.48±0.74 ^{bc}	4.40±0.75 ^{c}	5.05±0.74 ^{a}	25.06	0.00
Weight (g)	29.9±11.8 ^{a}	31.23±10.3 ^{a}	17.76±5.03 ^{b}	9.42±4.12 ^{c}	25.74±7.82 ^{a}	20.84	0.00
Total Length (cm)	12±1.96 ^{a}	11.84±1.43 ^{a}	10.21±0.84 ^{b}	8.13±1.25 ^{c}	11.17±1.42 ^{a}	20.06	0.00
Gonodosomatic Index (GSI)	0.2±0.18 ^{a}	0.27±0.27 ^{a}	0.26±0.22 ^{a}	0.004±0.004 ^{b}	0.23±0.24 ^{a}	36.78	0.00
Specific Growth Rate (SGR)	6.3±0.66 ^{a}	6.37±0.50 ^{a}	5.85±0.37 ^{a}	5.00±0.85 ^{b}	6.05±0.50 ^{a}	15.06	0.00
Condition Factor (CF)	1.9±0.30 ^{a}	1.95±0.23 ^{a}	2.00±0.00 ^{a}	2.07±0.60 ^{a}	2.00±0.00 ^{a}	0.54	0.71
Food Conversation Rate (FCR)	0.45±0.3 ^{b}	0.44±0.37 ^{b}	0.66±0.30 ^{b}	1.90±1.26 ^{a}	0.56±0.27 ^{b}	6.33	0.00
Survival rate (%)	41.18 ^{b}	49.02 ^{b}	68.63 ^{a}	29.41 ^{c}	62.75 ^{b}	-	0.034
Masculinity rate (%)	73.68 ^{b}	50.00 ^{c}	42.86 ^{c}	86.67 ^{a}	47.62 ^{c}	-	0.034

For length measurement, average values of 11.678±0.36 cm and 11.218±0.33 cm were obtained respectively from A and B groups. However, the fish in group D showed the lowest length (7.99±0.43 cm). A strong relationship has been noticed between the two parameters as shown in figure 3.

The highest SGR was determined in group B (6.37±0.50) followed by group A (6.33±0.66) and the lowest in the group D (5.00±0.85). K did not differ statistically between groups ($p \leq 0.05$). The highest K-value was found in group D (2.07±0.60) and the lowest in group A (1.90±0.30).

The GSI differences were found statistically significant among the treatment groups and D group ($p \leq 0.05$). Accordingly, the highest GSI was obtained from group B (0.27±0.27) and the weakest from group D (0.004±0.004).

Temperature was found to influence significantly feed consumption in *O. mossambicus* ($p \leq 0.05$). Feed consumption was statistically similar in the groups C (16.50±2.50 g). D (17.93±5.18 g) and Control (18.3±3.48 g). But fishes kept at 30°C (20.33±2.11 g) and 32°C (25.42±6.14 g) showed a higher feed intake.

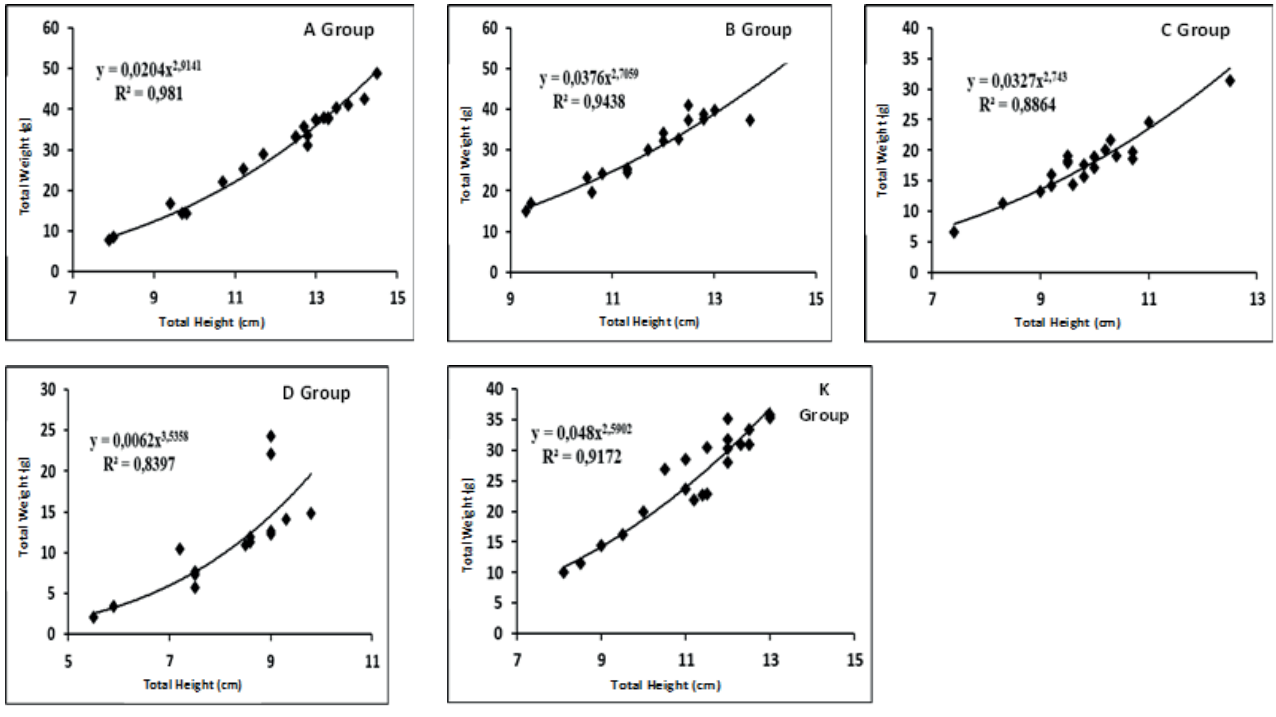

Figure 3. Length (Height)-weight relationship of the experimental groups (n = 51)

Table 2. Average egg diameter between experimental groups

Groups	Average egg diameter (µm)	F value	p value
A (30°C)	170.390±107.58'b (104.300-236.481)	14.017	0.00
B (32°C)	261.154±113.93'b (195.063-327.245)		
C (34°C)	457.517±137.15 a (391.427-523.608)		
K (28°C)	240.309±113.24'b (174.218-306.399)		

In terms of FCR, fish belonging to group B had the lowest average value of 0.44±0.37. Then followed A Group (0.45±0.28). Control group fishes were observed to have better FCR (0.56±0.27) than those grown at 34°C (0.66±0.30).

Histological Analized for Gonad

Ovarium

Egg sizes calculated by using Gonadal-Squash method are presented in Table 2.

The difference between groups was statistically significant ($p \leq 0.05$). Individuals reared under 34°C were found to have larger egg diameters than the others (457.52±137.15 µm). After that, group B follows (261.15±113.93 µm). Control and group A had egg diameters of 240.31±113.24 µm and 170.39±107.58 µm respectively.

At the end of the experiment (90 days after eclosion), there were no mature eggs in all treatment groups. The totality of the eggs recorded were at an intermediate stage of development (previtellogenic and vitellogenic stages) in general. Unfortunately, no egg cells were seen in D group because of its excessive immaturity (Figure 1).

Testes

In Figure 2 representative testis images by treatment are given below.

The analysis of these different images allows us to make the following explanations:

- The gonad from the control group is in the process of maturation. Although there are some black dots that prove the existence of spermatozoa, it can be said that the gonad is generally full of primitive reproductive cells;
- Sperm cloud is common in the gonads of fish grown under 30 and 32°C; these clouds are a sign that they are full of spermatozoa. They also accumulate so much as to form a real warehouse;
- In those kept below 34°C, sperm clouds are less noticeable than in the previous group. But they tend to accumulate. Here, too, it can be said that the gonad is on the maturation stage;
- The gonads of fish grown at 36°C are almost completely immature. There is no sperm cloud. It is full of spermatogonies confirming that the gonad remains immature.

Survival Rate (SR)

The difference between the groups for SR was statistically significant (Table 1), and the effect of temperature on this topic was significant ($p \leq 0.05$). The highest survival rate was obtained at 34°C (68.63%). The lowest one was seen in the 36°C group (29.41%). The differences between the groups A, B and control groups were not significant ($P > 0.05$).

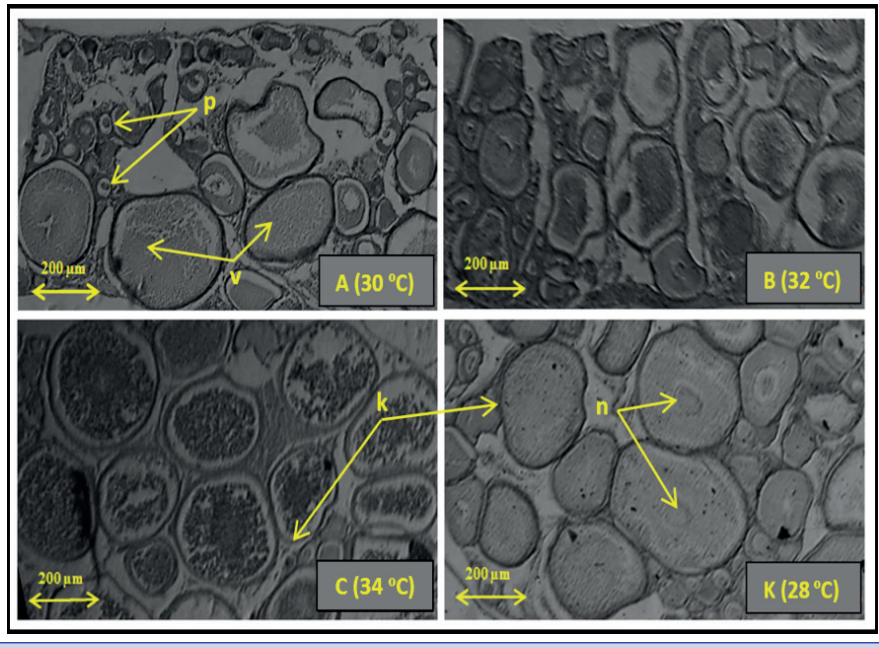

Figure 1. Female gonad sections by temperature (X 10, HE) (n: nucleus, p: previtelogenic oocytes, v: vitellin globules, k: korion)

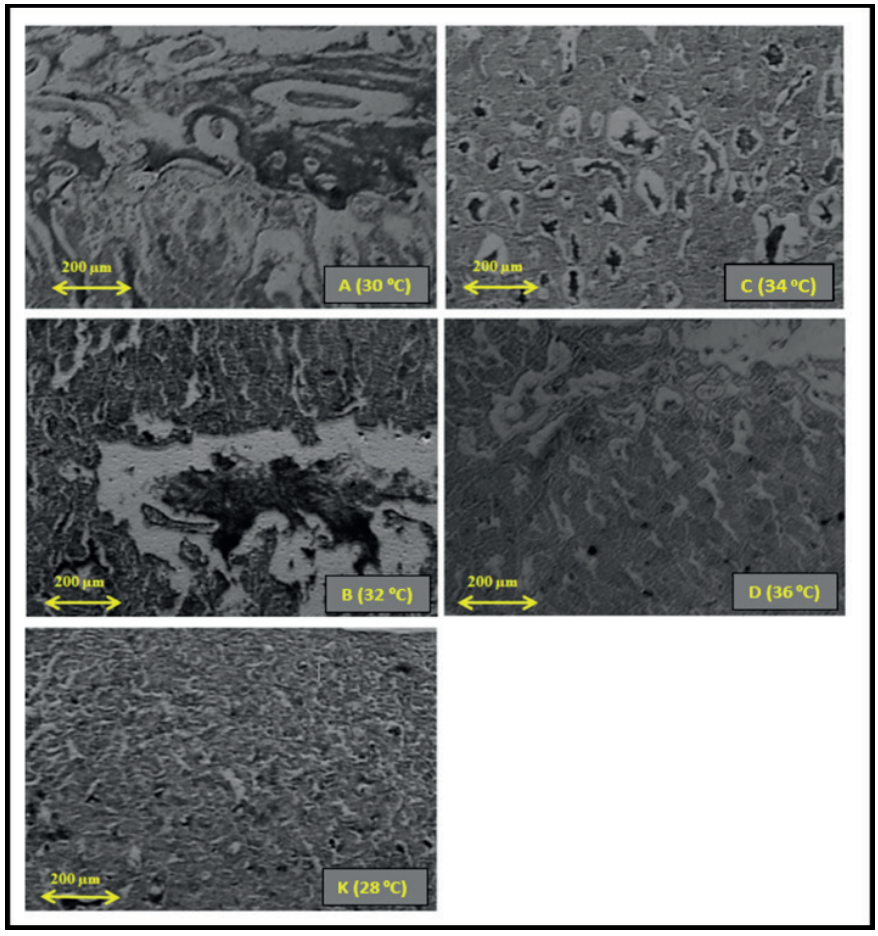

Figure 2. *O. mossambicus gonad* (testes) sections showing sperm cloud and spermatogenic cells in different development stages

Sex Ratios

The sex ratios obtained from the groups at the end of the trial were given in Table 1. The differences between groups were statistically significant ($p \leq 0.05$). The highest masculinity rate was obtained from the groups reared under 36 and 30°C (86.67% and 73.68%, respectively). No intersex individuals were observed after the examination of the gonad. The lowest male ratio was recorded as 42.86% in the C group (34°C). A balanced sex-ration was obtained in control (28°C) and B group (32°C).

The results of the present study show that survival rate and sex ratio, gonad development, mean weights, lengths and growth rates of juvenile *O. mossambicus* were significantly influenced by temperature. The highest masculinity rate was obtained from fishes reared under 30 and 36°C respectively (73.68% and 86.67%). No intersex gonad has been found. Our results are similar those published by Baroiller et al. (1995), Tessema et al. (2006); Azaza et al., (2008) and Soltan et al. (2013) who investigated the effects of temperature on Nile Tilapia (*Oreochromis niloticus*) of which juvenile fries were exposed to different temperatures ranged from 22 to 36°C. At the end of their studies, the highest male ratio was obtained with individuals reared at 36°C and ranged between 80 and 84 %. The conversion rate to the male character in tilapias may not always result in the success of the transformation activity. In some cases, the resistance of the two characters to the difficult conditions may affect this. That is, if a gender is more resilient than the other in a difficult condition, the gender ratio cannot be evaluated only with classical information. For example, in some scientific studies, males are more resistant to temperature than females (Altun et al., 2006: El Said, 2013). In addition, differences in gender ratio are caused by parental influence (Baroiller, 1996). In addition, these differences may result from the heterogeneity of the population, that is, the mixture of two or more strains. First of all, we did not practice an adaptation phase before exposing fishes to different temperatures. In the work conducted by Çetinkol (2012), fish were kept at 27°C for 1 week before being exposed to three different temperatures (24, 28, 32°C). Many scientific researches stated that sudden temperature changes can be damaging for the offspring (Sultan et al., 2009; Altun et al., 2006; Wang and Tsai, 2000). According to these researchers, exposing fish to masculinizing temperatures put their survival chances at risk. Secondly, Wang and Tsai, 2000; Borges et al., 2005 demonstarted that Tilapine species begin to struggle considerably at temperatures of 34°C. They emphasized that in *Oreochromis mossambicus*, deformity and cannibalism ratios rose to signifi-

cant levels with increasing temperature from 32 to 35°C. Concerning the gonad development, exposure of new-hatched Mozambique Tilapia fries to 36°C resulted in insignificant ovary size, reduced testicular size (immaturation) and in weaker growth performances as well (Table 1 and 2). Our findings are in line with those obtained by Byerly (2003) who examined the effects of elevated sub-lethal temperatures on the development of gonadal germ cells as well as somatic growth of channel catfish fries Ictalurus punctatus. For that 2 days-old channel catfish fries were exposed to different temperatures ranging from 27 (control) to 36°C for a period of four weeks. The results indicated that exposure to 34°C reduced gonad size with a slight decrease in overall body weight while the one to 36°C resulted in a significant reduction in oocyte number and ovarian/testicular area.

In mammals, heat-dependent germ cell loss has been observed only in males (Ito et al, 2003). But for fishes germ cell loss occur also in females indicating that they are also heat sensitive (Strussmann et al., 1998, Fat-Hallah. 2000. Ito et al, 2003). Exposure to elevated temperature has been reported by many researchers (Scott et al, 1979; Pinart et al, 1999; Fat-Hallah, 2000) to cause an inhibition of testicular development. Temperature influence the physiological processes (Brett and Groves, 1979) and the growth in many fish species (Jobling 1981; Elliott, 1982; Jonassen et al., 1999). The specific growth rate, feed rate, average live weight gain, feed conversion rate and condition factor obtained at the end of our study are shown in Table 2. The analyzes of results help to notice that fish groups reared at temperature comprised within 28 and 32°C showed better performances than the other groups. The highest performances was seen in B group (32°C), while the fish kept at 36°C showed the weakest ones. This is in accordance with findings from Dikel (2009) who stressed that every fish species is known to have a specific temperature optimum to survive and grow. Within this thermal tolerance range, maximal growth is obtained at optimal temperature. With increasing temperature, the uptake of nutrients also increases to maximum and then decreases when approaching the upper tolerance level (Warren and Davis, 1967; Dikel, 2009). At elevated temperatures, feed intake becomes more vigorously and the digestive processes of the fishes are then accelerated (Cossins and Bowler, 1987). According to Heap and Thorpe (1987), the 0-group malpigmented and normally pigmented turbot (*Scophthalmus maximus.* L) And turbot-brill hybrids, *S. maximus x S. rhombns* (L) developed faster at elevated temperatures not only because of much improved appetite but also because of an increase in food conversion efficiency (FCR). Similar results have been reported by Cai and Curtis (1990) in which the growth rate and food consumption, expected the assimilation efficiency in triploid grass carp (*Ctenopharyngodon idella*) increased with environmental temperature. The results of the present study are in line with these findings. But they differ slightly from those obtained by Al-Asgah and Ali (1997), who reported that the total weight gain and specific growth rate of Nile Tilapia differed significantly ($p<0.05$) at different water temperatures and increased with higher temperature up to 29°C, after which no significant increase was observed. They then suggest that the optimum temperature for *Oreochromis niloticus* lies somewhere near 29°C. We think that the variability in the reported results may be by the species differences.

In our study, the values for the feed conversion ratio (FCR) decreased with the increase in water temperature up to 32°C indicating a better utilization of feed per unit live weight gain. It corresponds to the results of Al-Asgah and Ali (1997). According to Dikel (2006)'s study, Tilapia's health has been reported to be seriously threatened by lowering the water temperature below 12-13°C. Under normal conditions, Tilapia, which has optimum growth around 27-28°C, loses its growth potential as it goes under this temperature. However, as the temperature is increased above 30-32°C, a significant decrease in growth rate is observed. Similar results were obtained from Fattah et al. (2008).

CONCLUSION

Our studies demonstrated that growth performance of juvenile Mozambique Tilapia (Oreochromis mossambicus) was affected by water temperatures. Exposure of fries to 36°C had a marked inhibitory effect on both gonadal and somatic development. Accordingly, the 36°C exposure caused several physical and physiological abnormalities in the exposed fish. It is then clear that an exposure temperature of 36°C is deleterious to Mozambique Tilapia. These observations help us to suggest shortening of the exposure time to 1-2 weeks maximum in order to minimize the effects on health and growth of this fish for in case of its large scale production.

Since the studies conducted on this species are limited, the data obtained are considered to be important. Same experiments should be performed in another rearing environment such as concrete ponds, with different feeds in order to better understand the environmental influences on the reproduction of this fish because it is inevitable that continuing studies on Mozambique Tilapia under the above assessments will lead to more information on this species, which is important for aqua culturists.

Conflict of Interests: The authors declare that for this article they have no actual, potential or perceived conflict of interests.

Ethics Committee Approval: This study was carried out in accordance with animal welfare and the ethics of trial. All procedures were performed in accordance Law on Veterinary and Medical Activities and National Animal Welfare Act. Therefore ethical approval was not required.

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