High temperature effect on the growth performance of hybrid tilapia *Oreochromisniloticus*X *Oreochromisaureus*juveniles reared in a recycling system

Comment [FE1]: The effect of high temperature on the growth performance of hybrid tilapia *Oreochromisniloticus* X *Oreochromisaureus* juveniles reared in a recycling system

Running title: High temperature effect on hybrid tilapia juveniles.

ABSTRACT

Tilapia juveniles live in littoral regions of freshwater ecosystems which have temperatures that may reach critical values for growth, especially in face of the onslaught of climate change when continental aquatic environments may experience increases in temperature that could exceed the tolerance degree of many species especially because temperature influences the growth of different tilapia strains. This study analyzes the effect of different temperatures (25, 30, 35, 40°C) on the growth and survival of hybrid tilapia juveniles in a re-circulating system. Duplicate groups of 30 juvenileswere stocked in 200 L tanks in a closed, re-circulating system. Automatic heaters with thermostats were used to establish the four water temperatures to which the fish were subjected, commercial food was supplied at 5% of their weight as daily ration. Fish were weighed and measured at 15-day intervals for a period of 3 months. Water temperature and dissolved oxygen were monitored daily. Ammonia, nitrates and dissolved reactive phosphoruswere monitored every third day. Fish responses to different temperatures were estimated through variations in length, weight and inter-circuli space of fish scales. Average initial and final weight and length, Specific growth rate (SGR), Fulton Index, Length-weight relationship and survivalwere used to assess tilapia growth performance.Results indicated that the weight, length, SGR and Fulton Index were significantly affected ($p < 0.05$) by water temperature. The growth of the fish reared at 30°C was almost one third greater than that at 25°C and one sixth greater than

Comment [FE2]: Why?

that at 35°C. Except for the fish reared at 40°C, the other treatments showed a positive allometric growth, with the better results recorded for the 30 and 35°C treatments.The fish reared at 40°C survived only two weeks while the survival rate of 98, 90 and 88.3% for 25, 30 and 35 ^{o}C, respectively.

Key words: tilapia, growth, climate change, temperature, cichlids, tilapia hybrids.

1. INTRODUCTION

Cichlids tend to live in lentic aquatic system and are good at hiding on swamp banks. These littoral areas, regularly inhabited by juveniles, have temperatures that may reach critical values for growth, especially in face of the onslaught of climate change when continental aquatic environments may experience increases in temperature that could exceed the high tolerance degree of many species. Both experimental and field studies have reported an optimum temperature range (for reproduction, growth and survival) for the tilapia *Oreochromisniloticus*(Linnaeus, 1758) of 25 to 32°C, with the greater survival and the optimal metabolic rate recorded at 28°C [1,2]. Tilapia tolerate temperatures up to 40°C [3], though this temperature induces stress, disease and mortality. The best growth of *O. niloticus* juveniles was recorded at 26 and 30°C [4]. Young *O. niloticus* preferred temperatures of 30 to 36°C, while a temperature of around 41°C was lethal [5]. Likewise, [6] reported 100% mortality at 39°C, high survival rates at 33°C, the best growth at 27 to 35°C, and an optimum temperature of 27°C.

Organisms belonging to the same species that possess strains, such as many fish, present differential physiological responses [7]. Cold tolerance of tilapia species and hybrids have showed that the genetic variation have a large dominance component [8] and that even different stocks of tilapia show very different temperature tolerances [9]. However, for tilapia hybrids there are actually few studies on temperature tolerance [10, 8, 11, 9] and it is also necessary to study the effect cause under various conditions, such as extreme temperature in case of future climate change that induced temperature rise.

This study was therefore designed to analyze the effect of different temperatures (25, 30, 35, 40°C) on the growth performance and survival rate of hybrid tilapia juveniles (*Oreochormisniloticus* x *O. aureus*) in a re-circulating system, considering the increase in water temperature along the littoral zone fringe of freshwater dams.

2. METHODOLOGY

This study was carried out at the indoor Fish Biology wet laboratory of the Autonomous Metropolitan University, Campus Iztapalapa, in Mexico City. Nile tilapia juveniles were obtained from the "Rubio" fish farm in Morelos State. The specimens were homogenous in size and body weight, and visibly healthy. They were fed the same diet for one week prior to introducing them to the experimental conditions. Duplicate groups of 30 juveniles (1.9 ± 0.45 g and 4.8 ± 0.32 cm average initial weight and length respectively) were stocked in 200 L plastic tanks in a closed, re-circulating system where each tank was independent and received a separate treatment system. Automatic heaters with thermostats were used to establish the four water temperatures (25, 30, 35, 40°C) to which the fish were subjected. Each tank was provided with aeration through an air compressor (Fluval mod MS306). A commercial diet with 32% crude protein was provided (Nutripec brand) at a daily rate of 5% of the body weight in two rations. The fish were weighed and measured at 15-day intervals, and their daily ration was re-adjusted accordingly to size.

Water temperature (measured with a mercury thermometer) and dissolved oxygen (measured with HI947 HANNA Oxygen meter) were monitored daily. Ammonia, nitrates anddissolved reactive phosphorus (measured withHI83203 HANNA Photometer) were monitored every third day. Fish responses to different temperatures were estimated through variations in length, weight and inter-circuli spaces in order to test if inter-circuli distances are a good indicator of growth. An inverted microscope Invertoscop-D, Karl Zeiss, was used to make the inter-circuli distances using a micrometric eyepiece Kpl W8x/20, calibrated with a micrometric push bar of 1 mm with 100 subdivisions (0.01 mm each division). The last four external inter-circuli spaces were measured (from the focus of the scale at an angle of 45^{o}), considered these as indicators of the last environmental conditions.Four scales were sampled at the initial and final stages. The subsequent parameters were used to assess tilapia growth performance such as: Average initial and final weight and length, Specific growth rate SGR = 100 x (Ln Wf – Ln Wi)/t, Fulton Index K = 100 x (W/Lt^3) and Length-weight relationship $W_t = a\ Lt^b$, where: W_f = Final wet weight, W_i = Initial wet weight, Lt_f = Final total length, Lt_i = Initial total length, t = Time interval in days, *a*and *b* = parameters of the allometric ratio between Total weight (W_t) and Total length (L_t), and Ln = the Napierian logarithm.Also, a *t*-test ($\alpha = 0.05$) was carried out to test the slope between temperature treatments, where $t_b$was the *t*-value for the null hypothesis $b = 3$. Survival rate was also estimated. Due to a filter failure, specimens in the 30°C tank suffered high mortality two weeks before the end of the experiment, for which reason a maximum likelihood EM method was used [12]and six data were recovered; SPSS ver. 15 was used for this analysis.

In order to justify a parametric analysis, a Mauchly's Test of Sphericity was carried out. However, as the data did not reach the condition of sphericity[13, 14], a mixed model ANOVA was calculated with weight and length data. In order to calculate differences between averages, a Bonferroni post hoc test was applied. All tests were performed with SPSS Ver. 15.

Mauchly'ssphericity test rejected the H_o (H_o = the sphericity assumption was fulfilled). Due to this, an ANOVA for repeated measures was applied. No differences were obtained for the two replicates ($P > 0.05$). At the beginning,no significant differences were recorded for the length and weight values. Finally, a regression analysis for temperature versus mean size was run.

3.RESULTS

The results indicated that weight, length, SGR and Fulton Index were significantly affected ($p < 0.05$) by water temperature. The growth of the fish reared at 30°C was almost one third greater than that at 25°C and one sixth greater than that at 35°C (Table 1). Except for the fish reared at 40°C, the other treatments showed a positive allometric growth, with the better results recorded for the 30 and 35°C treatments (Table 1). Fish length obtained at 30°C was greater throughout the experiment, followed by that obtained at 35°C (Fig. 1).

Table 1. Effects of water temperature on initial and final weight (Mean ± SE), Species growth rate (SGR); Fulton Index, *b* parameter of length-weight relationship and survival (%) of tilapia hybrid juveniles.

Temperature °C	W_i (g)	W_f (g)	SGR (%/day)	Fulton Index	*b*	Survival (%)
25	1.91 ± 0.52	31.61 ± 8.53	3.34	1.69	3.07 (+)	95.0
30	1.91 ± 0.51	53.24 ± 13.88	3.96	1.87	3.14 (+)	43 (90*)
35	1.97 ± 0.34	44.71 ± 10.78	3.71	1.80	3.14 (+)	88.3
40	1.95 ± 0.40	2.22 ± 0.32	0.14	1.67	2.72 (–)	0

W_i =Average initial weight; W_f=Average final weight; SGR, species growth rate; Fulton Index.

*In parenthesis is the survival estimation for specimens in 30°C treatment if mortality wasn't operated by a filter failure.

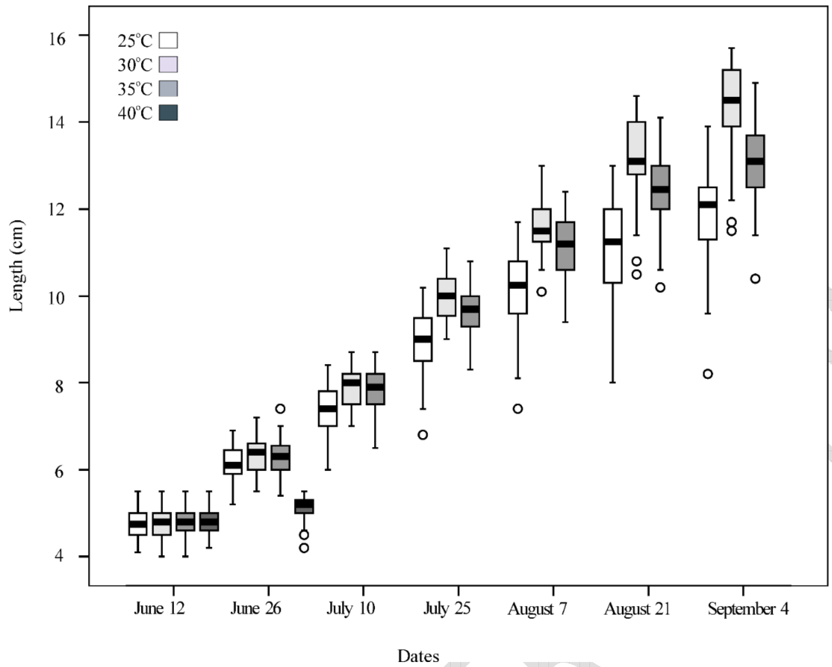

Fig. 1. Boxplot depicting size (cm) of *Oreochromisniloticus*X *O. aureus*for the treatments 25 °C, 30°C, 35°C and 40°C along the seven dates measured.

The best growth was obtained at 30°C and was significantly different from that obtained at 25 and 35°C ($p < 0.05$), whereas the least growth was obtained at 40°C ($P< 0.05$).Fish survival ranged between 0% and 95% and was significantly different ($P< 0.05$). The fish reared at 40°C survived only two weeks, while the low survival recorded at 30°C was caused by a failure in the filter system two weeks before finishing the experiment.

A quadratic effect ($P< 0.01$) was recorded for temperature versusmean size (Fig. 2), where 31°C was the optimum temperature for the species, probably because they were not stressed at 25 and 30°C, while the fish in the 35°C treatment showed red eyes and had a softer skin. Fish at 40°C lived only two weeks, hardly ate, were very stressed, and presented red eyes, very smooth skin and blood spills (Fig. 3a). Distension of the belly was also observed (Fig. 3b).

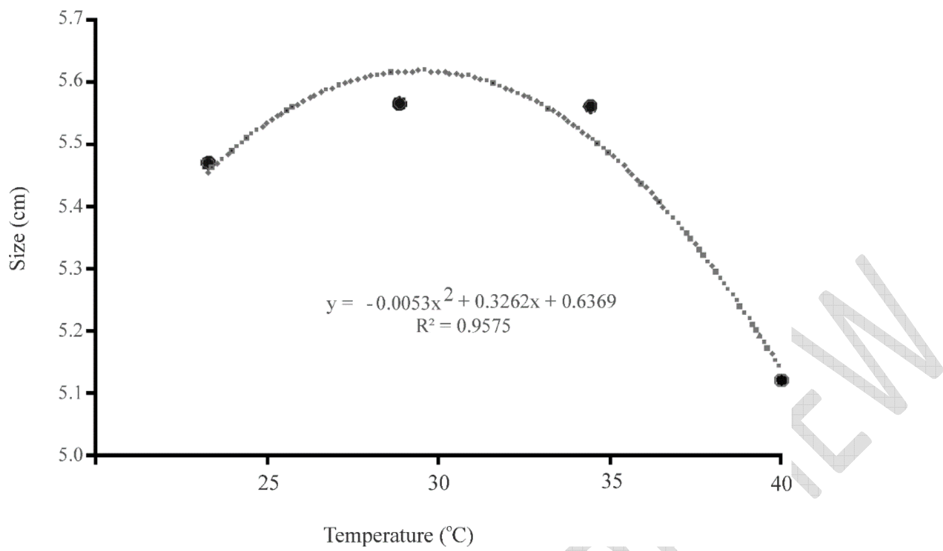

Fig. 2. Quadratic relationship ($P < 0.01$) between temperature and mean size.

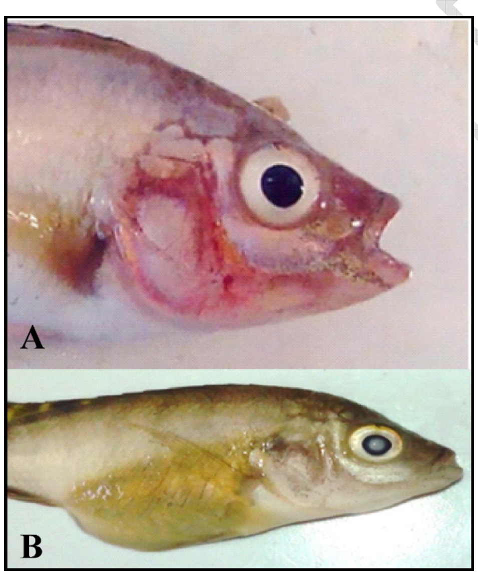

Fig. 3. A. *OreochromisniloticusX* X *O. aureus* juveniles of treatment 40°C after fifteen days. Red eyes very smooth skin and spills blood; B. Belly distension and spleen and pancreas sickness.

Minimum and maximum values of dissolved oxygen were 3.2 and 7.4 mg/L. Average values of ammonia ranged from 0.4 to 0.7 mg/L. Average values of nitrates and dissolved reactive phosphorus ranged from 0.19 to 7.1, and 1.2 to 6.3 mg/L respectively. No significant differences in the parameters were observed among treatments ($P > 0.05$).

The initial value for the inter-circuli distance was 35.05 ± 0.0008 µm, with no significant differences between treatments. The final value did show significant differences ($P < 0.05$) between the 30°C treatment (80.18 ± 0.007 µm) and the 25 and 35°C treatments (51.80 ± 0.00095 µm and 62.70 ± 0.000029 µm respectively).

4. DISCUSSION

The present study verified that rearing Nile tilapia juveniles at the optimum water temperature (30°C) results in a significant improvement in growth performance. However, fish growth rates and survival were significantly reduced at 25 and 35°C. The length-weight relationship was not a good parameter to separate well-being since only the fish reared at 40°C showed differences in growth performance. In most species of fish, the exponent *b* is close to 3, but this index may change due to strong variations in temperature or through starvation [15, 16]. Fish reared at 40°C hardly ate and no growth was observed during the 15 days they survived. As was observed in this study, [17] mentioned that *O. niloticus* reared at 37°C had a reduced survival and a low food intake. [18]reported 25°C as the optimum temperature for nutrient digestibility in the tilapia *O. niloticus*. Nevertheless, it has been mentioned that a greater temperature increases the rate of passing digesta through the intestinal tract, reducing the digestibility and assimilation of nutrients [19]. Therefore, the reduction in growth performance at 35°C could be attributed to a high rate of gastric evacuation. As in this study, [4] recorded a maximum *O. niloticus* fry growth at 30°C in relation to a maximum feed consumption, and also reported that high-temperature (almost 37°C) treatments yielded a significantly higher proportion of males with lower survival rates. This masculinization effect at high temperatures should be considered in the aquaculture industry, although most aquaculture takes place in waters that are not thermo-regulated and undergo more or less pronounced daily variations [20]. It is also important to mention that the temperature used in cultures influences the growth of different tilapia strains [21]. According to [9], the *Oreochromismossambicus* x Wami Tilapia *O. urolepis* hybrid from aquaculture was most tolerant to higher temperatures (33-38 °C), while the Blue Tilapia *O. aureus* was the most tolerant to colder temperatures (11-16 °C). Regarding the importance of water temperature observed in tilapia growth [22,4, 20, 1], behavioral factors produce a slower growth of small specimens and a resulting greater size dispersal.

The inter-circuli space was useful to compare growth rates, as the inter-circuli space of the scales and growth are related[23].The range of dissolved oxygen values was suitable for Nile tilapia feeding and growth, this in agreement with [24]. Ammonia tolerance ranges for tilapia of 0.001 to 0.2 mg/L were reported by [25] and of 0.35 to 0.5 mg/L by [26]. [27]reported nitrate tolerance values at concentrations of up to 100 mg/L, and dissolved reactive phosphorus tolerance values of 0.6 to 1.5 mg/L [28]. Water quality was not within the recommended values or the tolerance range of the species, particularly in the case of ammonia and dissolved reactive phosphorus. However, since high values were recorded for all the treatments, the quality of the water was not considered to have an effect on the results or to be an additional stressor.

According to [29], several tropical dams located at 17 and 18º North latitude have surface temperatures of 30 to 35°C (Caracol Dam: up to 30°C during March-July, Nezahualcoyotl Dam: 28-30°C,Aguamilpas Dam: 24-35°C), and some dams located at 27º North latitude may have temperatures above 30°C during the summer days. In agreement with the [30], global temperature will increase 2 to 6°C this century. However, the temperature of aquatic systems in many regions could reach greater temperatures sooner than expected during the summers, and global warming scenarios could affect the survival and growth of shallow-water species and lead to the extinction of the affected species. The strain *Oreochromisniloticus*X *Oreochromisaureus*tolerates temperatures above 30°C as was recorded in this study, but it does not survive at 40°C.

CONCLUSIONS

We can conclude that the better growth for *Oreochromisniloticus*X *O. aureus*was for 30 °Cfollowed by 35°C treatments while the juveniles reared at 40°C survived only two weeks and almost no growth was recorded during that time.

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