SINET: Ethiop. J. Sci., 45(3):347-358, 2022 ISSN: 0379–2897 (PRINT)
 eISSN: 2520–7997

Date received: January 31, 2022; Date revised: August 03, 2022; Date accepted: August 05, 2022
DOI: *https://dx.doi.org/10.4314/sinet.v45i3.9*

Effect of elevated fry rearing temperature on survival rate, growth performance, and sex ratio of three *Oreochromis niloticus* populations of Ethiopian Rift Valley Lakes

Megerssa Endebu[1*], Abebe Getahun[2] and Misikire Tessema[3]

[1.] Oromia Agricultural Research Institute, Batu Fishery and Other Aquatic Life Research Center, East Shoa, Ethiopia. E-mail: iamendebu@yahoo.com
[2.] Addis Ababa University, College of Natural and Computational Sciences, Department of Zoological Sciences. Addis Ababa, Ethiopia. E-mail: abebe.getahun@aau.edu.et
[3.] Ethiopian Biodiversity Institute. Addis Ababa, Ethiopia. E-mail: misikiret2010@gmail.com

ABSTRACT: Sex-reversal in *Oreochromis niloticus* is used to produce mono-sex males which are desired in aquaculture for better growth performances and population control. The present study was aimed at studying the effect of elevated fry rearing temperature on survival, sex ratios, and growth performances of *O. niloticus* populations of Lakes Chamo, Koka, and Ziway. Fries from five separate brood pairs of each population were either treated (T) in elevated fry rearing temperature of 36 ± 1°C or kept at room temperature of 24 ± 1°C as control (C) groups for 10 days (phase-I) and then grown in outdoor ponds for six months (phase-II). Survival rates in T groups (74.0 to 91.7%) were lower than that of C groups (83.3 to 97.0%) in all the three populations in phase-I but not in phase-II. Chamo population was better in survival rate (88.4% and 95.4% in Phase-I, and 95.4% and 95.3% in phase-II) than Koka population (85.8% and 91.3% in Phase-I, and 93.9% and 93.5% in phase-II) and Ziway population (80.2% and 88.6% in Phase-I and 90.2% and 91.9% in phase-II) both in T and C groups respectively. The Chamo population also attained significantly ($p<0.05$) higher mean final weight of 31.58 ± 6.78 g and 24.26 ± 6.67 g in T and C groups respectively, followed by that of Koka population with 21.70 ± 5.10 g and 18.83 ± 4.16 g while Ziway population with mean final weight of 17.49 ± 4.60 g and 16.81 ± 4.15 g was the least both in T and C groups. The overall sex ratios in T groups were skewed towards male but balanced in all C groups of each population. Better growth was achieved only in T groups with higher male ratio than their corresponding C groups. The number of sensitive brooding pairs and the overall male ratio in T groups were higher in the Koka population (40%, 61.68%) than in Ziway (20%, 56.78%) and Chamo (20%, 56.39%) populations respectively. However, the sensitivity of sex reversal to elevated fry rearing temperature was wide between individuals within each population than across the three populations. Hence, sensitive individuals can be selected from the populations and their degree of sensitivity can be improved through continuous selection of progenies from temperature-sensitive parents.

Keywords: Heat treatment, Koka, Lakes Chamo, Nile tilapia, Sex reversal, Ziway

INTRODUCTION

Nile Tilapia (*Oreochromis niloticus*) is a freshwater fish species that is cultured worldwide because of its adaptation to a wide range of environmental conditions, rapid growth rate, easy and rapid propagation, and tolerance to stress in handling (El-Sayed, 2006; Getinet GebreTsadik and Bart, 2007). The growth performance of the tilapias in aquaculture is affected by genetics and environment, both of which can be modified in fish culture to improve production and productivity. Accordingly, genetically improved strains such as genetically improved farmed tilapia (GIFT) and genetically improved red tilapia have been used in aquaculture production (Ansah *et al.*, 2014; Lago *et al.*, 2016). Aquaculture business, in general, is not yet developed in Ethiopia where the *Oreochromis niloticus* species, the dominant catch in the country's fishery production (Yared Tigabu, 2010; Gashaw Tesfaye and Wolff, 2014) are the targeted and promising species for the sector's development. The *O. niloticus* are indigenous to the country and are found in water bodies of different drainage basins situated in different agro-ecologies (Tenalem Ayenew and Dagnachew Legesse, 2007; Abebe Getahun, 2017). Chamo, Koka, and Ziway are among the major Rift Valley lakes known for

*Author to whom correspondence should be addressed.

their fishery production (Abebe Getahun, 2017). The *O. niloticus* from these lakes have been evaluated for their growth performances in pond culture (Kassaye Balkew and Gjoen, 2012; Daba Tugie *et al.* 2017) and indicated as potential populations for further genetic improvement. The establishment of improved core strains of *O. niloticus* populations is, therefore, required for aquaculture development in the country.

The sex of the fish has also effects on growth performance in *O. niloticus* culture. Mixed-sex culture of the species in the ponds often produces undesired offspring, which increases fish numbers beyond the recommended density and creates stressful conditions by competing for the limited resources such as feed, oxygen, and space in the pond (Diana and Lin, 2004). The uncontrolled multiplication of fish in mixed-sex culture results in a stunted fish population of poor market value (Hepher and Pruginin, 1981; Coleman, 2001). Moreover, males grow faster than their female counterparts (Macintosh and Little, 1995; Megerssa Endebu *et al.,* 2016) in tilapia culture that mono-sex male populations of the species are desired in aquaculture to improve production and productivity.

Sex reversal is one of the mechanisms used to produce male mono-sex in *O. niloticus* production. Sex reversal of the *O. niloticus* in aquaculture can be achieved in a variety of ways such as through hormonal treatment or elevated fry rearing temperature at the early swimming stage of the fish. *O. niloticus* displays a genetic sex-determination system (XX-female, XY-male) where exposure to high temperatures (above 32˚C to 36.5˚C) during a thermo-sensitive period (10 to 30 days post-fertilization), induces masculinization (Baroiller *et al.,* 1995; Hendry *et al.,* 2002; Misikire Tessema *et al*., 2006; Angienda *et al.,* 2010; Nivelle *et al*., 2019). Proportion of males in the *O. niloticus* population increases with increasing fry rearing temperature up to 36°C, a temperature beyond which the number of males does not increase (Abucay *et al.* 1999; Baroiller and D'Cotta, 2001; Angienda *et al.* 2010; Misikire Tessema *et al*., 2006; Khater *et al*., 2017).

Sensitivity of the *O. niloticus* to temperature treatment depends on genetics. In, the evaluation of the effects of rearing temperatures on *O. niloticus* populations, Misikire Tessema *et al*. (2006) observed significant differences in degree and range of responses to a high temperature between populations and breeding pairs within the population. Angienda *et al.,* (2010) indicated that the sex reversal efficiency of heat treatment in tilapia is affected by autosomal genes; whereby tilapia with a genetic marker called Abur36 achieved higher sex reversal efficiency up to 95% male.

The aquaculture activity in Ethiopia mainly involves pond culture of *O. niloticus* which has been challenged by poor fish growth performance because of a number of problems among which over population due to unwanted recruitment is one of the factors (Megerssa Endebu *et al*., 2016) In order to alleviate the overpopulation problem of *O. niloticus* in pond culture, predatory control of the unwanted recruitment by introducing predatory fish such as *Clarias gariepinus* has been practiced in Ethiopia (Megerssa Endebu *et al*., 2016). Efficiency of the predatory control method, the availability of predator fish are some of the problems related to predatory control of unwanted population in mixed sex culture.

Growth rate of female *O. niloticus* was also reported to be slower than their male counterpart whereby the mean weight of female *O. niloticus* at harvest was half of its male batches in mixed-sex population grown in pond culture practiced in Ethiopia (Megerssa Endebu *et al.,* 2016).

Use of male mono-sex is practiced in commercial production of *O. niloticus* worldwide but not practiced in Ethiopia. Tilapia sex-reversal using androgen hormone (Methyl testosterone) has been used by commercial producers of tilapia in many Asian countries, but hormone treatment in fish rises human health and environment concerns that it is not recommended in fish production (Mlalila *et al*., 2015). Sex reversal of *O. niloticus* by using elevated fry rearing temperature is acceptable but the responses of different *O. niloticus* populations to the heat treatment were not evaluated.

Therefore, the aim of the current experiment was to evaluate survival rate, growth rate, and sex ratio of *Oreochromis niloticus* populations of Chamo, Koka, and Ziway Lakes after treating them in elevated fry rearing temperature.

MATERIALS AND METHODS

Sources of the Oreochromis niloticus populations

The parental stocks of three different *Oreochromis niloticus* populations were collected from lakes of Chamo, Koka, and Ziway and brought to Batu Fish and Other Aquatic Life Research Center, quarantined and kept as separate populations in outdoor concrete ponds for two to four months earlier to the commence of the experiment. The source lakes are located in separate drainage basins and had different water qualities (Table 1).

Table 1. Water quality parameter values (mean ±SE) at Lakes Chamo, Koka, Ziway and Ground water used in experimental ponds at Batu.

Water quality parameters	Chamo	Koka	Ziway	Experimental ponds
Temperature (°C)	28.09 ± 1.41[a]	22.77 ± 1.20[c]	24.24 ± 2.23[b]	24.8 ± 3.1
Secchi depth (cm)	22.73 ± 5.85[a]	13.05 ± 5.49[c]	14.19 ± 2.15[b]	27.44 ± 4.80
pH	9.21 ± 0.43[a]	8.26 ± 0.36[c]	8.54 ± 0.46[b]	8.9 ± 0.5
Conductivity (µS/cm)	1,622.26 ± 193.93[a]	395.07 ± 208.93[c]	516.50 ± 51.26[b]	2,262.5 ± 431.7
Salinity (ppt)	0.826 ± 0.06[a]	0.19 ± 0.11[c]	0.25 ± 0.03[b]	1.4 ± 0.2
TDS (mg/L)	1,179.52 ± 118.41[a]	268.45 ± 149.38[c]	350 ± 37.19[b]	1,993.2 ± 327.4
SRP-P(µg/L)	79.05 ± 18.72[a]	72.48 ± 40.19[a]	38.67 ± 8.62[b]	-
NO-3 - N (µg /L)	11.11 ± 7.09[b]	613.43 ± 910.73[a]	51.91 ± 53.18[b]	-

[a,b,c] values with different superscript are statistically different at 0.05 level of significance. SRP = soluble reactive phosphorus; TDS = total dissolved solids. (Seasonal data of two years; May 2018 to April 2020 for the three lakes and monthly data of 6 months for experimental months)

Progenies of the three *O. niloticus* populations were evaluated for their survival, sex ratio and growth rate responses to elevated fry rearing temperature at 36°C for 10 days starting from the first swimming. This temperature was reported to result in higher sex reversal efficiency with minimal mortality rate (Angienda *et al.* 2010). The responses were also evaluated in the growth period of the fish after the treatment. The current experiment was conducted at Batu Fish and Other Aquatic Life Research Center, located in Central Ethiopian Rift Valley, for about three years, from Nov 2017 to Oct 2020. The Batu Research Center is found near Lake Ziway at 7°56' N latitude, 38° 43' E longitude, and an altitude of about 1640 m.a.s.l.

Fry production

Generations of similar age groups were produced in the concrete ponds at the research center from each of the Chamo, Koka and Ziway *O. niloticus* populations under similar pond condition. They were grown to sexual maturity and similar sizes of sexually mature male and female broods of these generations after age of one year were taken from each of the three populations and kept separately in ponds for conditioning before commencing the experiment. The broods were fed on pellets supplemented with 25-30% crude protein during this conditioning period.

Hapa nets of 1 mm mesh size and volume of 1 m^3 were suspended in outdoor concrete ponds of each 5 m^2 sizes and used for fry production. The hapa nets in ponds were changed when clogged by algae and the dirt cleaned by washing for reuse. Five hapa nets were allocated for each of the three *O. niloticus* populations' spawning pairs. Five males and five females were selected from the conditioned brood fishes of each population and pairs of one male and one female of the selected individuals were put into each of the hapa nets to spawn. The males were mouth clipped by cutting the upper lip using sharp scissors while anesthetised in 0.1 ml/L clove oil solution and treating the wound with iodine before pairing, to reduce their aggressiveness toward their female partners. Each pair of the broods was checked every three days for the release of fry in the hapa nets. The breeding pairs were replaced with other pairs in case of mortality or inability to spawn in a month after pairing.

The water used in these experimental ponds was pumped from a shallow well with known water

quality; water temperature of 24.8 ± 3.1°C, pH 8.9 ± 0.5; conductivity 2,262.5 ± 431.7 µs/cm; TDS 1,993.2 ± 327.4 mg/L and salinity 1.4 ± 0.2 ppt (Table 1). The produced tilapia fry in hapa nets from the breeding pairs were then transferred to experiment unit in Lab at age of first swimming date.

Temperature treatment and some management aspect of the fry (phase-I)

The *O. niloticus* progenies from each breeding pair of the three populations (Chamo, Koka, and Ziway) produced in the outdoor hapa nets were collected at age of first swimming date, transferred to plastic water tankers of 500L capacity in lab. The water tankers were prepared as treatment unit having thermostat heater fixed and control units without heater. Additionally, a tanker of temperature acclimatizing unit with thermostat heaters fixed were used to introduce and exit the fish to and from treatment unit.

The elevated fry rearing temperature adjusted to a fixed temperature of 36 ± 1°C was used as a treatment unit for the treatment groups in order to induce masculinisation (Angienda *et al.*, 2010; Abou El-Fotoh *et al*., 2014). In the treatment, constant elevated water temperature was maintained by the use of thermostat water heaters (Themowatt type R-T-M 15A/T105/250V adjustable functional temperature with maximum temperature of 80°C), aerator pumps (110-130V/60HZ 6.0W with 3.0 L/minute pumping capacity), and Aquarium thermometer (analogue thermometer with temperature range 0 – 40 °C and accuracy ± 1 °C, Mowtom brand). The elevated temperature was allowed to stabilize for three to five days before introducing the fry into the treatment.

Two groups of sixty to one hundred fry each from each of the breeding pairs were randomly taken at age of their first day of swimming and assigned into treatment and control groups. The treatment groups were first put into temperature acclimatizing unit in lab for one day where the water temperature raises gradually from room temperature of about 24°C to 36°C before transferring to treatment unit. The fry were then transferred into treatment unit for masculinisation experiment under elevated fry rearing temperature for 10 treatment days. The control groups were directly put into the control unit having water at room temperature for a stay of similar period in lab. Fry of each pair were kept separate in perforated but screen-covered plastic bottles of five-litre capacity for 10 experimental days. The plastic bottles were arranged in the thermostat bath treatment unit randomly.

Temperature was monitored three times a day (morning at 8:00 – 8:30, Afternoon 2:30-3:00 pm and evening at 7:30–8:00 pm) using digital thermometer of SX723 pH/mV/Cond meter, Xi'an China. Feeding the juveniles of both the treatment and control groups was commenced with filtered zooplanktons consisting of copepods and rotifers collected from fertilized outdoor ponds. During the elevated fry rearing temperature treatments, the fries were observed daily and deaths were recorded when occurred. After 10 days of the elevated fry rearing temperature treatment, the fries were transferred to the temperature acclimatizing unit in lab where the thermostats of the heating unit was switched off and the unit was allowed to cool down slowly to room temperature of 24°C, normally at about one day. The final numbers of the fries in each container were recorded before transfer to outdoor ponds.

In the control groups, the corresponding fry from each breeding pairs were kept in tanks with similar management with the treatment group. The water temperature in the control groups was 24 ± 1°C. Aeration, feeding, treatment duration and other managements were similar with that of the treatment groups.

Growth of the fry in outdoor ponds (phase-II)

After the end of the treatment in lab, the temperature acclimatized fries of both treatment and control groups of each population and siblings were counted and separately taken to the outdoor concrete ponds where they were grown to size of sexual differentiation. The fries were acclimatized to the pond water for five to ten minutes during the transfer by gradually mixing the pond water into the containers carrying the fries before releasing the fries into the suspended hapa within the ponds. In these outdoor concrete ponds, the siblings in treatment groups and their corresponding control groups were arranged in two separate blocks, treatment and control blocks. In each block, fry from different parents were placed in hapa nets with random arrangement.

The concrete ponds were fertilized with poultry manure for its higher nutrient content that the water harbored phyto- and zooplankton (copepods, rotifers and few daphnia) which were used as live feeds for the fingerlings. The fingerlings were also supplemented with a feed prepared from mixture of wheat bran (50%) and noug cake (50%) at an estimated 40% of their body weight daily in powder form and gradually decreased to 5% body weight daily from the 2nd month onwards (Daba Tugie *et al*., 2017). Finally, the fish were sexed at 180 days when it was easy to identify their sexes by visual observation to their genital papilla.

DATA COLLECTION AND ANALYSIS

Survival rates

Survival rates of the progenies were calculated based on the numbers of fish that survived up to the end of the experiment period and expressed as percentages of the initial numbers stocked (Putri *et al*., 2020). The survival rates in this experiment were calculated at two stages, phase I and phase II. Phase-I was the survival rate during the first 10 days in the laboratory experiments both for treatment and control groups while phase-II was the survival rate in outdoor ponds during the 180 days of growth period. Similar method and time of data collection were employed to the treatment groups and their corresponding control groups.

$$\mathbf{SR} = \frac{\mathbf{No -- Nt}}{\mathbf{No}} \mathbf{X\ 100\%}$$

Where: SR = S*urvival (%)*
No = *Number of fish at the start of the experiment*
Nt = Number of fish dead during the experiment

The survival rates were presented in table 2 for each of the progenies in treatment group (36°C/10 day) and the control group for both phase-I (10 days) and phase-II (180 days) for each of the populations. The differences between mean survival rates for treatment groups and control groups as well as the mean for the three populations were analyzed using independent-samples T test and one-way ANOVA, respectively at a 0.05 significance level in IBM SPSS statistical package, version 20.0.

Growth rate

Progenies with similar stocking densities (50±3 fish/pond) in each population were selectively considered for growth performance evaluation among the progenies in the experimental ponds (phase-II). At monthly intervals, up to 6 months post-heat treatment, statistical samples of fingerlings were drawn from the ponds for weight measurements used for determination of growth rate of the populations. The progenies' monthly growth rates of the three *O. niloticus* populations in terms of mean weight (g) were presented in graph under treatment and control groups (Fig 1). The mean final weights (g) of the progenies in each population were analyzed and compared using independent-samples T test for the treatment and control groups. The mean final weights of the progenies across the populations under treatment and control groups were analyzed using one-way-ANOVA. The mean differences were separated at 0.05 level of significance, LSD Post Hoc Multiple Comparison in SPSS statistical package. The fingerlings drawn at 180 days post heat treatment were also used for sexing.

Sex ratios

Sexing was done by observing into their genital papilla. The numbers of males and females of each group were recorded for each population both for the treatment and the control groups. Sex ratios were calculated as proportions of the numbers of male individuals per group. Deviation from 1:1 sex ratio in all the treated and control groups were analyzed by using chi-square test for each of the population.

RESULTS

Survival rates

The survival rates of the *O. niloticus* progenies during the first 10 days of lab experiment (phase-I) and during the growth period in outdoor experimental ponds for 180 days (phase-II) were presented in Table 2. In phase-I, the survival rate of the progenies of the treatment group ranged from 81.6%, 78.3% and 74.0% to a maximum of 91.7%, 90.0% and 86.7% in Chamo, Koka and Ziway populations, respectively. Similarly, the survival rate of the progenies in the control group

ranged from a minimum of 93.3%, 85.0% and 83.3% to a maximum of 97.0%, 98.3% and 91.7% in Chamo, Koka and Ziway populations, respectively (Table 4).

Mean survival rates of the *O. niloticus* populations in the laboratory (phase-I) during the treatment phase were given in Table 2. The mean survival rates of the progenies in treatment groups were significantly lower than the mean survival rates in control groups for all the three populations. Comparing the survival rates among the three *O. niloticus* populations progenies under treatment groups, the mean survival rates of the Chamo population (88.38 ± 4.12%) was significantly ($p < 0.05$) higher than that of the Ziway population (80.20 ± 4.95%), while the survival rate for Koka population (85.78 ± 4.91%) was not statistically different ($p > 0.05$) from the values of the two populations. Similarly, in the control groups, the mean survival rate of Chamo population (95.40 ± 1.50%) was significantly higher than that of the Ziway populations (88.6 ± 3.16%), while the survival rate for Koka population (91.32 ± 5.32%) was not statistically different ($p > 0.05$) from the values of the two populations. Mean survival rates of the *O. niloticus* populations in outdoor experimental ponds (phase-II) during the 180 days growth period were also presented in Table 2. It was found that the mean survival rates of the progenies of treatment groups were not statistically different from the mean survival rates of their corresponding control groups for all the three *O. niloticus* populations. When the progenies mean survival rates were compared, the values were not statistically different among the populations both for the control groups and the treated groups, except that the mean survival rate of Ziway population (90.18 ± 6.60%) was significantly lower than that of Chamo population (95.38 ± 1.17%) in the treatment group.

Table 2. Survival rates (Mean ± SD) of the progenies of the three *O. niloticus* populations in phase-I and phase-II under treatment and control groups

Treatment	**Mean survival rate (%) in phase-I**		
	Chamo	**Koka**	**Ziway**
Treated	$88.38 \pm 4.12^{b,c}$	85.78 ± 4.91^{c}	80.20 ± 4.95^{d}
Control	95.40 ± 1.50^{a}	$91.32 \pm 5.32^{a,b}$	$88.60 \pm 3.16^{b,c}$
Treatment	**Mean survival rate (%) in phase-II**		
	Chamo	**Koka**	**Ziway**
Treated	95.38 ± 1.17^{a}	$93.94 \pm 3.91^{a,b}$	90.18 ± 6.60^{b}
Control	95.32 ± 2.63^{a}	$93.52 \pm 4.05^{a,b}$	$91.86 \pm 2.65^{a,b}$

^{a,b,c} *significantly different mean survival rates (%) at 0.05 level of significance*

Growth performance

The growth performance of progenies of *O. niloticus* populations treated in elevated fry rearing temperature and their corresponding control groups was presented in Fig.1. Of the totally fifteen progenies in the current sex reversal experiment, progenies which were produced at the same batch and having nearly similar densities were considered in the growth performance evaluation (Ch5, K2 and Z2 in Table 4). The average fry weight at their first month in ponds was 1.17 g with insignificant differences among treatment and control groups and across the populations. But, average weight of the fry/fingerling started to segregate gradually between the populations, with the Chamo population attaining better weight than Koka and Ziway both in treatment (Fig.1a) and control (Fig.1b) groups.

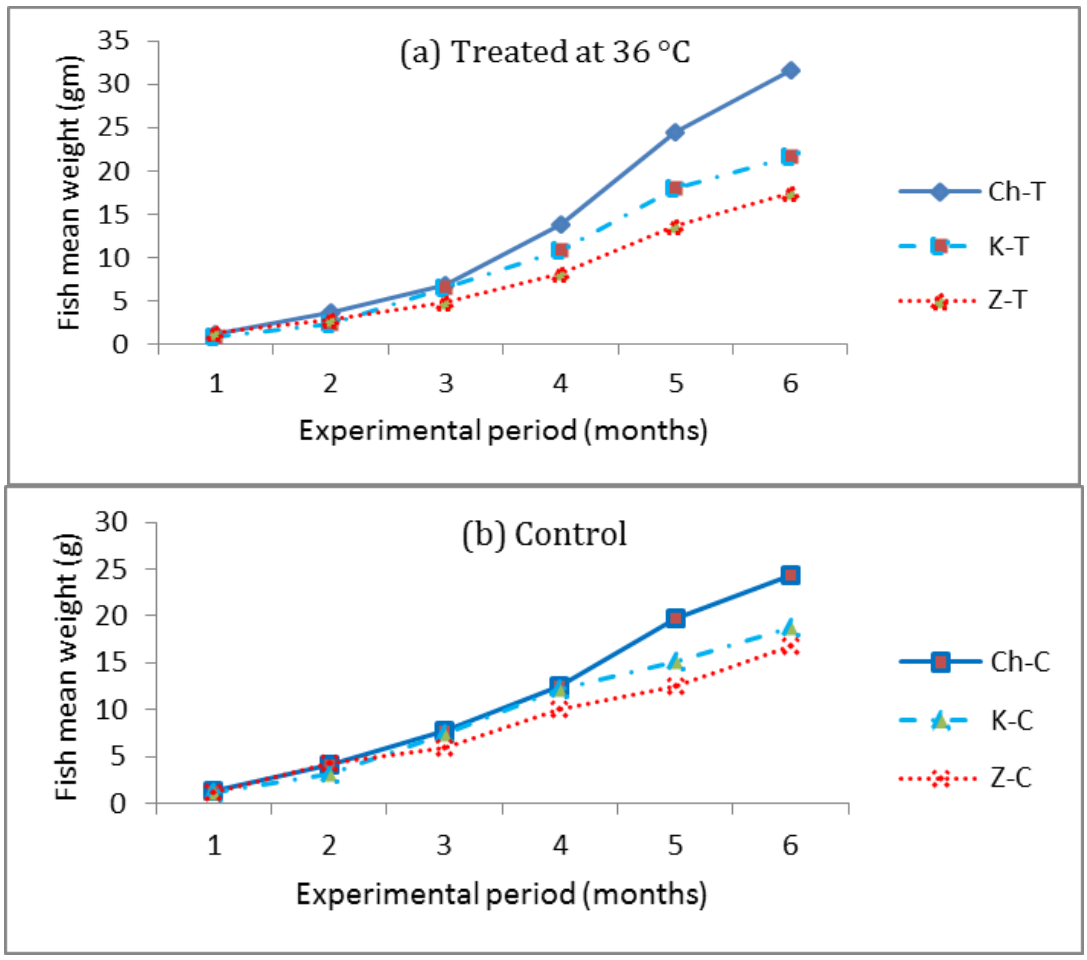

Figure 1. Growth performance of *O. niloticus* populations in phase-II, treated (a) and control (b) (n = 52, 47, 50, 55, 52, 50 for Ch-T, K-T, Z-T, Ch-C, K-C, Z-C respectively at month 6).

The mean final weight of the *O. niloticus* progenies after six months of growth was significantly different between treatment and control groups in Chamo and Koka populations, but not significant in Ziway population (Table 3). However, the treatment groups had higher male ratios in Chamo (63.46%) and Koka (65.96%) populations. The final mean weight of male *O. niloticus* was higher than their female counterparts in all the progenies. As a result, the mean weight of the treated groups was higher than that of their corresponding control groups only when the sex ratio was skewed towards males as observed in Chamo and Koka populations.

Table 3. Final weight (mean ± SD g) of treatment and control groups of *O. niloticus* populations of Chamo, Koka and Ziway.

Treatment	Mean weight (g) of *O. niloticus* populations		
	Chamo	Koka	Ziway
Treated	31.58 ± 6.78^{a}	21.70 ± 5.10^{c}	$17.49 \pm 4.60^{d,e}$
n	52	47	50
Control	24.26 ± 6.67^{b}	18.83 ± 4.16^{d}	16.81 ± 4.15^{e}
n	55	52	50

^{a,b,c,d,e} *significantly different mean weights (g) at 0.05 level of significance*

Sex ratios

The proportions of male *O. niloticus* individuals varied among treatment and control groups (Table 4). The sex ratio presented as "% male" ranged from 49.06% to 64.29% in the treatment groups, while it ranged from 47.27% to 53.70% in the control groups of Chamo population. Among five independent siblings obtained from different breeding pairs of Chamo population, only one sibling, the Ch1, resulted in a significantly higher ($p<0.05$) proportion of males than females (Table 4). The overall sex ratio of the treated siblings group of Chamo (Ch1-Ch5) was significantly skewed towards males ($p<0.05$). However, the sex ratio of their corresponding siblings in the control group and the overall total sex ratio in the control group did not significantly ($p> 0.05$) deviate from 1:1 (Table 4).

In the case of *O. niloticus* population from Koka, the sex ratio presented as "% male" ranged from 53.09% to 76.92% in the treatment group with the overall 61.68% male, while it ranged from 46.17% to 57.45% in the control group with the overall total sex ratio of 50.52% (Table 4). Among five independent siblings obtained from different breeding pairs of Koka population, two treated groups of siblings, the K2 and K4, resulted in a significantly higher ($p<0.05$) proportion of male individuals (Table 4). The cumulative sex ratios of the treated group of Koka population (K1-K5) were significantly ($p<0.001$) skewed towards males, while the sex ratios of their corresponding siblings under the control group and the overall total sex ratio of the control group did not significantly ($p>0.05$) deviate from 1:1 (Table 4).

The sex ratio of *O. niloticus* population of Ziway in five different sibling groups ranged from 44.00% to 78.69% in the treatment groups with an overall ratio of 56.78% males, while it ranged from 46.81% to 54.00% in the control groups with overall sex ratios of 49.35% (Table 4). Among five independent siblings obtained from different brood pairs of Ziway population, only one siblings group, the Z1, resulted in a significantly higher ($p<0.05$) proportion of male individuals (Table 4). The cumulative sex ratio of the treatment group of Ziway population (Z1-Z5, 56.78%M) was also significantly ($p<0.05$) skewed towards the male, while the sex ratios of their corresponding siblings under control group and overall total sex ratio of the control group did not significantly ($p>0.05$) deviate from 1:1 (Table 4). The interaction effect of population and heat treatment on sex-ratio was not significant.

Table 4. Effects of higher fry rearing temperature on survival and progeny sex ratios of *O. niloticus* populations of Lakes Chamo, Koka and Ziway.

Spawn	Treatment group (36°C)				Control group				
	Survival (%)		Sexed	male	Survival (%)		Sexed	male (%)	$\chi 2$
	10 days	180 days	(No.)	(%)	10 days	180 days	(No.)		
Ch1	88.0	95.5	84	64.29[b]	97.0	93.8	91	51.65	0.099
Ch2	89.0	96.6	86	51.16	95.0	91.6	87	48.28	0.103
Ch3	91.7	96.4	53	49.06	96.7	98.3	57	50.88	0.018
Ch4	81.6	93.9	46	52.17	93.3	96.4	54	53.7	0.296
Ch5	91.6	94.5	52	63.46	95.0	96.5	55	47.27	0.164
Overall	88.4	95.4	321	56.39[a]	95.4	95.3	344	50.29	0.012
K1	89.0	91.0	81	53.09	85.0	96.5	82	48.78	0.049
K2	88.3	88.7	47	65.96[a]	90.0	96.3	52	46.15	0.308
K3	78.3	95.7	45	62.22	88.3	88.7	47	57.45	1.043
K4	90.0	96.3	52	76.92[c]	98.3	96.6	57	52.63	0.158
K5	83.3	98.0	49	55.1	95.0	89.5	51	49.02	0.020
Overall	85.8	93.9	274	61.68[c]	91.3	93.5	289	50.52	0.031
Z1	74.0	82.4	61	78.69[c]	89.0	93.3	83	48.19	0.108
Z2	76.7	96.2	50	48.84	90.0	92.6	50	54.00	0.320
Z3	82.0	95.1	78	53.85	89.0	92.1	82	48.78	0.049
Z4	76.7	93.5	43	44.00	83.3	94.0	47	46.81	0.191
Z5	81.6	83.7	41	53.66	91.7	87.3	48	50.00	0.000
Overall	80.2	90.2	273	56.78[a]	88.6	91.9	310	49.35	0.052
Grand	84.8	93.2			91.8	93.6			

Note: Ch1 - Ch5 = progenies of Chamo population, K1 - K5 = progenies of Koka population, Z1 - Z5 = progenies of Ziway population, n = number of individuals included in statistical analysis and χ^2 = Chi-square test value for deviation of sex ratios of control from 1:1; [a,b,c] sex ratio of treatment group significantly different from 1:1 (P<0.05, 0.01, 0.001).

DISCUSSION

Based on the results obtained in the present study, rearing fry produced from three *O. niloticus* populations at 36 ± 1°C for the duration of 10 days decreased the survival rates of all the treatment groups but not affected the survival rate during the growth phase after the treatment in all the three populations. Though the difference between the treatment temperature (36 ± 1°C) and the temperature in the populations' natural environment was relatively higher for Koka (14°C) and Ziway (12°C) as compared to that of Chamo (8°C) to affect the survival rate, the experimental fish from the three populations in the current study were grown in similar environments and exposed to the experiment similarly. Optimum rearing temperature range for *O. niloticus* is 27-32°C (Drummond *et al.*, 2009; Samuel Bekele *et al.*, 2019). Increasing water temperature beyond the optimum range is a stressful condition which may induce mortality. The elevated fry rearing temperature of 36°C decreased the survival rate of the fish in treated group in the present study. Similar temperature effect on survival rate was also reported by Khater *et al.* (2017). Though different between treated and control groups, the survival rates in the current study were within ranges reported in earlier studies (Khater *et al.*, 2017; Nivelle *et al.*, 2019). Effect of population on survival rate was observed both in treatment groups and control groups, whereby the Chamo population performed better in survival rate, followed by Koka population while Ziway population was the least. The Chamo tilapia were vigor in size at egg and fry stages as compared to Koka tilapia, while the fry of Ziway tilapia were very small in size and prone to death easily in handling during this experiment; which perhaps resulted in lower survival rate. Moreover, the three *O. niloticus* populations have shown different phenotypic and reproductive characters in their respective natural environments (Megerssa Endebu *et al.*, 2021a; 2021b), suggesting potential genetic differences among the populations. Both genetics and environment affect early fry survival rate in Nile tilapia (Yonas Fessehaye *et al.*, 2007) which influenced the survival rates of the populations differently in the current study.

Growth rate and mean final weight of the *O. niloticus* differed among the populations in the present study, both in control and treated groups with Chamo population performing better than Koka and Ziway. Growth rate of an organism is affected both by environmental and genetic factors (De Verdal *et al.*, 2018). The environmental factors were kept similar for all the *O. niloticus* progenies under the present study, except the deliberately adjusted temperature differences between the treated and control groups in their early age. Hence, the differences in growth rates between the progenies of the *O. niloticus* populations were more likely due to genetic effect. Feed conversion rate and growth rate in juvenile tilapia is determined by genetics (De Verdal *et al.*, 2018). Similar growth differences were also observed for the *O. niloticus* populations of Ethiopian Rift Valley in pond experiments (Kassaye Balkew and Gjoen, 2012; Daba Tugie *et al.* 2017). However, the differences in growth rate and final mean weight between treated and control groups within a population were observed only when the sex ratios were different between the groups. The weight differences were attributed to differences in sex ratios induced by the elevated fry rearing temperature treatment, for which progenies with similar sex ratio had similar growth rate in a population, regardless of temperature treatments. The progenies with higher proportions of male individuals achieved faster growth rate and attained higher mean final weight than those with balanced sex ratios, in spite of the fact that the males grow faster than females in *O. niloticus* populations (Macintosh and Little, 1995; Chakraborty and Banerjee, 2010; Megerssa Endebu *et al.*, 2016).

The overall sex ratios of the *O. niloticus* in the present study were skewed toward males for the heat-treated groups in all the three populations (Chamo, Koka and Ziway) while the ratio was balanced in all the control groups of each population. However, the response in sex reversal after exposure to elevated fry rearing temperature varied among individuals, breeding pairs within each population and also across the populations. The proportion of males in the responded groups also varied suggesting differences in the degree of sensitivity among individuals within each population. The number of breeding pairs whose progenies were sex-reversed and the overall male ratio in the treated groups was higher in Koka *O. niloticus* population than in Ziway and Chamo populations; the higher response in the population with the higher temperature difference between

treatment and their natural environment. Different *O. niloticus* populations can show significant differences in the degree and range of responses to elevated temperature treatments (Misikire Tessema *et al*., 2006). The differences in sensitivity of the *O. niloticus* progenies within the populations suggest the responsiveness variability among individuals (Angienda *et al*., 2010).

CONCLUSIONS

Progenies of three *O. niloticus* populations of Chamo, Koka and Ziway responded to elevated fry rearing temperatures differently in terms of survival rate and growth performance. Survival rate was affected both by population and the elevated fry rearing temperature treatment. The elevated fry rearing temperature decreased the survival rate in all the three *O. niloticus* populations. Chamo population attained a higher fry survival rate than Koka and Ziway both in treated and control groups. The growth performance of the *O. niloticus* progenies was affected by population whereby the Chamo population followed by Koka attained better size at final harvest. Elevated fry rearing temperature influences growth rate only when the sex ratio is altered in a population. Elevated fry rearing temperature induces a shift in sex ratios toward the male in *O. niloticus* populations of Lakes Chamo, Koka and Ziway. However, variation in sex reversal sensitivity was wide between individuals within each population than across the three populations. This indicates that elevated fry rearing temperature reduces survival rate in all populations and alter sex ratio in sensitive individuals regardless of their population origin while the fish growth rate is affected by its population origin.

Therefore, sensitive individuals to heat treatment in sex-reversal should be selected from the Chamo population as this population has higher survival and growth rate in pond culture. The degree of sensitivity of the selected individuals should also be improved through consequent breeding of sensitive parents, followed by selection in order to achieve higher male percentage required in aquaculture development of Ethiopia.

ACKNOWLEDGMENTS

This activity was jointly supported and monitored by Addis Ababa University and Agricultural Growth Program II (AGP-II) via Batu Fish and Other Aquatic Life Research Center of Oromia Agricultural Research Institute. The authors would like to thank Addis Ababa University and Ethiopian Biodiversity Institute for supervision and follow-up of the research; thematic research fund and AGP-II for the financial support; the Batu Fish and Other Aquatic Life Research Center for the logistic and technical support.

REFERENCES

1. Abebe Getahun (2017). The fresh water fishes of Ethiopia: diversity and utilization. pp349. View Graphics and Printing, Addis Ababa, Ethiopia.
2. Abou El-Fotoh, E.M., Ayyat, M.S., Abd, G.A. and Farag, M.E. (2014). Mono sex male production in Nile tilapia (*Oreochromis niloticus*) using different water temperatures. Zagazig *J. Agric. Res.*, **41(1**).
3. Abucay, J.S., Mair.G.C., Skibinski, D.O.F. and Beardmor, J.A. (1999). "Environmental sex determination: the effect of temperature and salinity on sex ratio in *Oreochromis niloticus* L." Aquaculture, 173: 219–234. *Aquaculture*, ***173***: 219–234
4. Angienda, P.O., Aketch, B.O. and Waindi, E.N. (2010). Development of All-male Fingerlings by Heat Treatment and the Genetic Mechanism of Heat Induced Sex Determination in Nile Tilapia (*Oreochromis niloticus* L.). *Int. J. Bio. and Life Sci.* ***6(1):*** *38-43*
5. Ansah, Y.B., Frimpong, E.A. and Hallerman, E.M. (2014). Genetically improved tilapia strains in Africa: Potential benefits and negative impacts. *Sustainability*, **6**: 3697-3721. DOI: 10.3390/su6063697
6. Baroiller, J.F., Chourrout, D., Fostier, A. and Jalabert, B. (1995). Temperature and sex chromosomes govern sex ratios of the mouth brooding cichlid fish *Oreochromis niloticus*. *J. Exp Zool.*, **273**, 216–223.
7. Baroiller, J.F. and D'Cotta, H. (2001). Environment and sex determination in farmed fish. *Comp Biochem Physiol,* ***130***,399–409.
8. Chakraborty, S. B. and Banerjee, S. (2010). Comparative Growth Performance of Mixed-Sex and Monosex Nile Tilapia Population in Freshwater Cage Culture System under Indian Perspective. *Int. J. biology,* ***2****:44-50. DOI:10.5539/ijb.v2n1p44.*
9. Coleman, R. (2001). Cichlids and Science: Bad Cichlids. *Cichlid News Magazine*,32-34.

10. Daba Tugie, Megerssa Endebu, Darbew Hailu and Alemu Lema (2017). Screening of *Oreochromis niloticus* strains for their important production traits in high stocking density and low CP feed quality without aerating devices in concrete Tanks, Batu, Oromia, Ethiopia. *Int. J. Fisheries and Aquatic Res*, **2(6):** 07-10.
11. De Verdal, H., Vandeputte, M., Mekkawy, W., Chatain, B. and Benzie, J.A.H. (2018). Quantifying the genetic parameters of feed efficiency in juvenile Nile tilapia *Oreochromis niloticus*. *BMC Gene,* 19, 105 (2018). DOI: 10.1186/s12863-018-0691-y
12. Diana, J.S., Yi, Y. and Lin C.K. (2004). Stocking densities and fertilization regimes for Nile tilapia (*Oreochromis niloticus*) production in ponds with supplemental feeding, in Proceedings of the Sixth International Symposium on Tilapia in Aquaculture, R. Bolivar, G. Mair and K. Fitzsimmons, Eds. Manila, Philippines, BFAR, Philippines. pp 487-499
13. Drummond, C.D., Murgas, L.D.S. and Vicentini, B. (2009). Growth and survival of tilapia *Oreochromis niloticus* (Linnaeus, 1758) submitted to different temperatures during the process of sex reversal. *Ciênc. agrotec., Lavras*, **33(3**): 895-902.
14. El-Sayed, A.F.M. (2006). Tilapia culture. CABI publishing, oxfordshire fatty acid metabolism. *J Nutrition*, **132**: 222-230.
15. Gashaw Tesfaye and Wolff, M. (2014). The state of inland fisheries in Ethiopia: a synopsis with 4 updated estimates of potential yield. *Ecohydrology and Hydrobiology*, 14: 200-219. DOI: 10.1016/j.ecohyd.2014.05.001
16. Getinet GebreTsadik and Bart, A. N. (2007). Effects of feeding, stocking density and water-flow rate on fecundity, spawning frequency and egg quality of Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture*, **272**:380-388.
17. Hendry, C.I.,Martin-Robichaud, D.J and Benfey, T.J. (2002). Gonadal sex differentiation in Atlantic halibut. *J. Fish Biol*. ***60*:**,1431-1442.
18. Hepher and Pruginin. (1981). *Commercial Fish Farming*. John Wiley and Sons, New York. pp. 291.
19. Kassaye Balkew and Gjoen, H.M. (2012). Comparative studies on the growth performance of four juvenile *Oreochromis niloticus* L., (1758) strains in pond culture, Ethiopia. *Int. J. Aquaculture*, **2(7**): 40-47.
20. Khater, E.G., Ali, S.A. and Mohamed W.E. (2017). Effect of Water Temperature on Masculinization and Growth of Nile Tilapia Fish. *J Aquac Res Development* 8:9 DOI: 10.4172/2155-9546.1000507
21. Lago, A.A., Rezende, T.T., Dias, M.A.D., de Freitas, R.T.F. and Hilsdorf, A.W.S. (2016). The development of genetically improved red tilapia lines through the backcross breeding of two *Oreochromis niloticus* strains. *Aquaculture*, **472**: 17-22. DOI: 10.1016/j.aquaculture.2016.06.042
22. Macintosh, D.J. and Little, D.C. (1995). Nile tilapia (*Oreochromis niloticus*). In: Bromage, N.R., Roberts, R. J. (Eds.). *Broodstock management and egg and larval quality*. Cambridge: Blackwell,. chap. 12, 277-320.
23. Megerssa Endebu, Abebe Getahun and Misikire Tessema (2021a). Differences in phenotypic characters of Nile tilapia (*Oreochromis niloticus* L) in three Ethiopian Rift Valley lakes; Screening strains for aquaculture. *Fish Aquac J.* 12(s1):001. DOI: 10.35248/2150-3508.21.s1.001
24. Megerssa Endebu, Abebe Getahun and Misikire Tessema (2021b). Variations in reproductive characteristics among *Oreochromis niloticus* populations of three Ethiopian Rift Valley lakes. *J. Biol. Sci.,* **21**: 19-28. DOI: 10.3923/jbs.2021.19.28
25. Megerssa Endebu, Daba Tugie and Tokuma Negisho (2016). Fish growth performance in ponds integrated with poultry farm and fertilized with goat manure: a case in Ethiopian Rift Valley. *Int. J. Fishery Sci. Aquac*. 3(2): 40-45.
26. Misikire Tessema, Müller-Belecke,A. and Hörstgen-Schwark, G. (2006). Effect of rearing temperatures on the sex ratios of *Oreochromis niloticus* populations. *Aquaculture*, 258, 270–277 doi:10.1016/j.aquaculture.2006.04.041
27. Mlalila, N., Mahika, C., Kalombo, L., Swai, H. and Hilonga A. (2015). Human food safety and environmental hazards associated with the use of methyltestosterone and other steroids in production of all-male tilapia. *Envir. Sc. and Poll. Res.*, **22**, 4922-4931. DOI: 10.1007/s11356-015-4133-3
28. Nivelle, R., Gennotte, V., Kalala E.J.K., Ngoc, N.B., Muller, M., Melard, C. and Rougeot, C. (2019). Temperature preference of Nile tilapia (*Oreochromis niloticus*) induces spontaneous sex reversal. *PLoS ONE,* 14(2): e0212504. DOI: 10.1371/journal.pone.0212504
29. Putri, T.A. Maya, S. and Santanumurti, M.B. (2020). Nile tilapia (*Oreochromis niloticus*) fish hatchery technique: the survival rate evaluation in IBAT Pandaan, Pasuruan, East Java. *IOP Conf. Ser.: Earth Environ. Sci.* 441 012052. DOI:10.1088/1755-1315/441/1/012052

30. Samuel Bekele, Mulder, H.A., Benzie, J.A.H. and Komen H. (2019). A systemic literature review of the major factors causing yield gap by affecting growth, feed conversion ratio and survival in Nile tilapia (*Oreochromis niloticus*). *Reviews in Aquaculture*, **12(2)** : 524 - 541. DOI: 10.1111/raq.12331

31. Tenalem Ayenew and Dagnachew Legesse (2007). The changing face of the Ethiopian rift lakes and their environs: Call of the time. *Lakes and reservoirs research and management*, **12(3)**: 149-165. DOI: 10.1111/j.1440-1770.2007.00332.x

32. Yared Tigabu (2010). Stocking based fishery enhancement programmes in Ethiopia. *Ecohydrology and Hydrobiology*, **10(2-4)** : 241-246. DOI : 10.2478/v10104-011-0012-9

33. Yonas Fessehaye, Komen, H., Rezk, M.A. and Arendonk, J.A.M. (2007). Effect of inbreeding on survival, body weight and fluctuating asymmetry (FA) in Nile tilapia, *Oreochromis niloticus. Aquaculture* **264(1-4)**: 27-35. DOI: 10.1016/j.*aquaculture*.2006.12.038