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Sex Ratio of Genomar Supreme Tilapia Strain Exposed to Elevated Temperature

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The study evaluated the sex ratio of Nile tilapia exposed to elevated temperature. It also investigated the best family from Genomar Supreme Tilapia (GST) strain with the best response on temperature induced sex differentiation. The study used 2x5 factorial design with Factor A, the two temperatures (28℃ and 36℃) and Factor B, the 5 families of GST strain. The study consisted of ten treatments with three replicates. Yolk sac fry undergo a 10-day exposure to temperatures 28°C (T28) and 36°C (T36), after exposure to these temperatures, the fish were nursed in indoor tanks for 30 days before gonad squashing. Mean survival rate of different families of tilapia exposed to the two temperatures was significantly affected by the interaction between family and temperature (P<0.05) and were also significantly different between families and between temperatures. Mean survival rate of Family 3 (F3; 71.47%) was significantly higher than those of the other families. Moreover, Family 5 (F5, 69.33%), Family 4 (F4, 69.00%) and Family 1 (F1; 62.83%) were comparable to each other but significantly higher than Family 2 (F2, 45.22%). Mean of survival rate after 30-day nursing of previously exposed GST strain in T28 and T36 was not affected by the interaction between families and temperatures and was also not significantly different between families and between temperatures. Generally the survival rate was high ranging from 85.46±17.32 – 96.66±1.25% and this might be attributed to the ideal temperature of 28℃ during the nursing period. Comparing results of the two temperature treatments showed there was apparent increase of male at T38 as compared to T28. Chi-square test for fixed ratio analysis, however, showed that all families including those exposed to T28, deviated from the expected sex ratio of 1:1. Percentage male was significantly affected by the interaction between family and temperature (P<0.05) and was also significantly different between families and between temperatures. On the effect of family, mean male percentage of F3 (78.00%) was highest but was comparable to that of F5 (77.83%) and F2 (76.91%), then, followed by that of F4 (73.67%), that was not significantly different to F1 (72.42%). Moreover, significantly lowest mean male percentage was recorded in F1.
The study concluded the following: 1) survival rate of GST was affected by the temperature of the water with significantly lower survival rate at elevated temperature; 2) elevated temperature

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of 36 ℃ was effective in sex reversal of GST; and 3) F3 had the best response on temperature induced sex differentiation based on sex ratio and survival rate.

Keywords: Genomar Supreme Tilapia Strain, Yolk sac fry, Gonad Squashing, Sex reversal, Temperature induced

Introduction

Nile tilapia (*Oreochromis niloticus*) is one of the most important species in aquaculture. It provides one of the major sources of animal protein and income throughout the world (Barriga-Sosa *et al.,* 2004). Its farming continues to expand due to easiness for handling and its tolerance to a wide range of environmental conditions including factors such as pH, temperature, nitrogen wastes, and dissolved oxygen (Noor El Deen and Zaki, 2010). In view of the increasing commercialization and continuing growth of tilapia industry, the commodity is not only the second most important farmed fish globally, next to carps but is also described as the most important aquaculture species of the 21st century (Shelton, 2002). According to Mair *et al.,* (1997) the desirability of monosex populations for tilapia culture is well established. Grow-out of monosex male populations prevents or minimizes recruitment and thereby competition between recruits and stocked fish which, in mixed sex populations, can significantly reduce harvested yields (Mair *et al.,* 1997). Male is the desired sex of cultured tilapia as it grows faster and diverts less energy into reproduction (Phelps and Popma, 2000). Sex reversal, if properly applied, can be effective in producing sex ratios greater than 98% male. However, inconsistencies in application often result in lower sex ratios and environmental and human health concerns have been raised about the direct use of synthetic hormones in aquaculture (Mair *et al.,* 1997).

Currently male monosex populations are produced mainly by androgen treatments. Due to various environmental issues related to hormone use i.e. possible effects of treatment residues on water quality and biodiversity with the growing concerns for food security, finding a sex control alternative based on nonhazardous, consumer and environment-friendly methods represents a major challenge for aquaculture. Heat treatment as a means of producing monosex tilapia has been tried in several laboratories (Abucay *et al.,* 1999; Baroiller and D'Cotta, 2001; Angienda *et al.,* 2010). Sexual differentiation of gonads in Nile tilapia is triggered by temperature during the critical developmental period. Exposure to elevated temperature for 10 or more days between post fertilization days 9–13 increases the proportion of male individuals (Baroiller *et al.,* 1995a, Hendry *et al.,* 2002; Angienda *et al.,* 2010). Temperature lability may provide evolutionary advantages to tilapia species by providing higher numbers of

males with increased capacity for dispersal (Baras *et al.*, 2000; Devlin and Nagahama, 2002).

Since it is well known that temperature can dramatically influence the structure and function of proteins and other macromolecules, temperature fluctuations as are encountered by fish in different habitats can alter sex-determination pathways and influence the probability that development would be male or female (Devlin and Nagahama, 2002).

Objectives: Generally, this study was conducted to evaluate the sex differentiation of Nile tilapia using elevated temperature. Specifically, this study was intended to:

1. Determine the effect of elevated temperature on the survival rate and sex ratio of Nile tilapia fingerlings, and
2. Evaluate the family under the Genomar Supreme Tilapia strain that has the best response on temperature-induced sex differentiation.

Materials and methods

Experimental Fish

Genomar Supreme Tilapia strain is a Genetically Improved Farmed Tilapia (GIFT) derived strain of the 9th generation of genetically improved tilapia. The biggest breeding and genetic improvement was the GIFT project that originally produced 8 strains of the Nile Tilapia collected from Africa in the 1980's, initiated and implemented by the International Center for Living Aquatic Resources Management (ICLARM, now World Fish); a new strain was produced and distributed globally (Panorama Acuicola, 2012).

Commercial rights of the portion of the 9th generation of GIFT genetic line was sold to Genomar (a Norweigian venture capital genetic improvement company) few years ago and is now marketed globally as Genomar Supreme Tilapia (GST), thus it is called a GIFT-derived strain (Panorama Acuicola, 2012).

Yolk sac fry from five different families, Generation 26, Batch 4 of GST strain, the offsprings of Generation 25 from Generation 24 selected breeders, were used in the study (Figure 2). The yolk sac fry were placed in hapa (0.46 m x 0.30 m) installed inside 300-400 liters capacity indoor glass aquaria. Aquaria were supplied with aeration system, 6-in-1 aquarium top filter and heater thermostat with the capacity of 2000 wattage. The fry were fed ad libitum in 10 days using booster 1 (Tateh Aquafeeds).

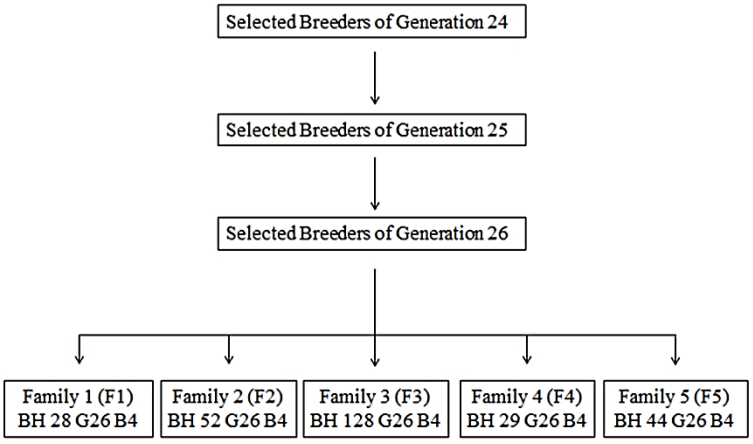


Figure 2. Selection of experimental fish

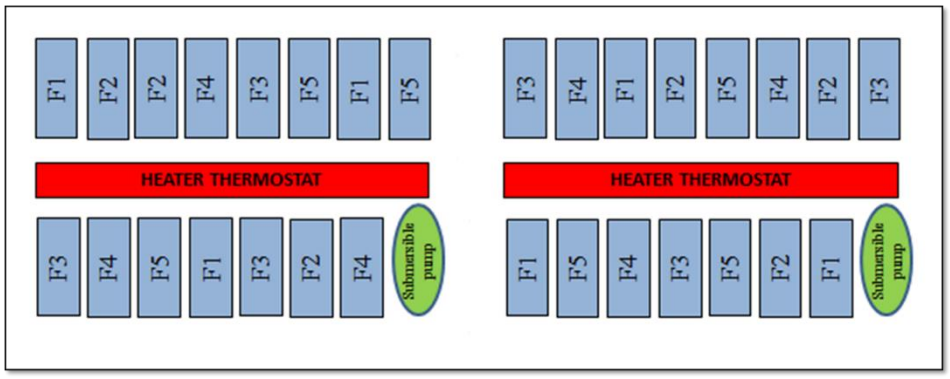
Experimental Units and Design

There were two units of rectangular aquaria with the capacity of 300-400 liters were used throughout the experiment. The study utilized a 2x5 factorial design with Factor A being the two temperatures and Factor B consisted of 5 families of GST strain (Table 1). The study consisted of ten treatments with three replicates (Figure 3). Fifteen hapas measuring 0.46 x 0.30 m were installed in each aquarium. Each aquarium was installed with aeration system to supply dissolved oxygen, 6-in-1 aquarium top filter system to filter dirt and circulate water inside the aquarium.

To ensure uniform temperature of water inside the aquaria, heater with thermostat (2000 watts capacity) was installed in each aquarium. The heater thermostat was connected to a controller to regulate the heat from the heater thermostat. Thermometer was also installed to further check temperature in the aquaria.

Table 1. Factors assessed in the study.

FACTOR A (Temperature)	FACTOR B (Families of GST strain)
28 °C 36 °C	Family 1 Family 2 Family 3 Family 4 Family 5



10 days at 28 ℃ Temperature Experiment (T28)

10 days at 36 ℃ Temperature Experiment (T36)

Figure 3. Experimental units

Experimental Methods

Broodstock management

Male and female tilapia breeders with ratio of 1:1 were placed in a 1.5 x 1m fine mesh hapa net prior to breeding. The breeders were fed at 1.2% body weight for 7-10 days. After 7-10 days, checking and collection of eggs inside the mouth of female breeders were done. During this period, monitoring of water quality parameters like dissolved oxygen and temperature during morning (8-9am) and afternoon (3-4 pm) were monitored using D.O meter.

Egg collection

Eggs were collected after 7-10 days of breeding. The eggs were cleaned in running water after collection in the pond to remove excess dirt/mud. Eggs were subjected to 10 minutes aerated water bath using Iodine (5ml/liter) for egg surface disinfection and hydrogen peroxide (0.7 ml/ liter) for the treatment of newly collected eyed eggs. After the disinfection process, the eggs were flushed with water, and samples were weighed before transferring to artificial incubator (AI). In AI, egg, eyed egg and yolk sac fry were flushed with 1ppt salinity of water with filtration system of 1-6 psi. During artificial incubation, dead eggs were removed using a scoop, plastic pipette and brush. Eggs remained in the artificial incubator until it reached the 50-60% yolk sac stage.

Elevated temperature treatment

After the eggs reached the 50-60% yolk sac stage, three hundred pieces of fry were placed in plastic bag with 50 ml of water and oxygenized prior to stocking. The plastic containers with fry were placed in the aquarium for 30 minutes before releasing the fry into the water. After the fry were released, they were exposed to elevated temperature for 24 hours (Figure 4) beginning with 29℃ with 0.5℃ increase in every 2 hours and 30 minutes until it reached 36℃ . The fry were exposed to elevated temperature for 10 days. During this period the fry were fed ad libitum using fry booster 1 (Day and Night). Cleaning of aquaria was done to remove dead fry and excess feeds. After 10 days of exposing the fry into elevated temperature, the temperature was reduced every 2 hours and 30 minutes until it reached normal temperature. After the temperature was reduced to normal the total weight and total number of fry were determined and survival rate in each treatment was computed.

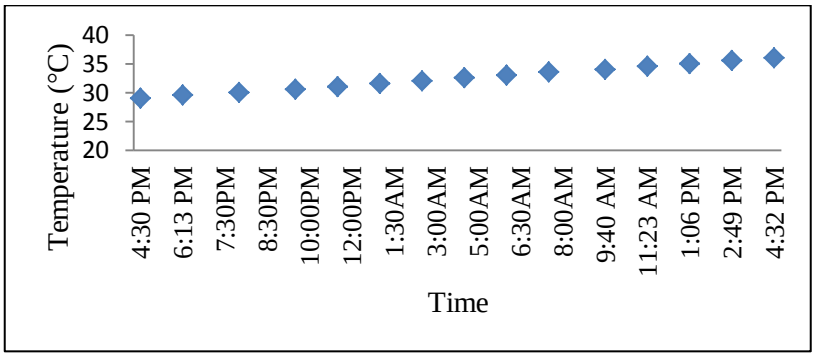


Figure 4. 24-hour acclimatization to elevated temperature

Nursing of Nile tilapia to a desirable size for sexing

After 10-day of exposing yolk sac fry to elevated temperature, experimental fish were raised separately in indoor tanks for 30 days until the microscopic inspection of gonad squashes. Fish were fed ad libitum every three hours (day and night). After 30 days, the experimental fish were collected and total weight and total number of the experimental fish was determined prior to gonad squashing. One hundred pieces of Nile tilapia were randomly sampled in each replicate of the two treatments and then subjected to gonad squashing.

Gonad squashing

Experimental fish was first anaesthetized using MS 222 (tricaine methanesulfonate). After this, using the dissecting kit, the fish was dissected to remove the gut and exposed the gonads. Using forceps, the gonads were

removed and put into glass slide with acetocarmine and viewed under the microscope. Acetocarmine was used to stain the gonads for easy identification. Results were recorded in a tally sheet.

Statistical Analyses

Data on the sex ratio of male to female were analyzed using the Chi square test (for fixed ratio) to determine deviation from the expected sex ratio of 1:1. Percentage data were Arcsine transformed prior to analysis. Means were compared using two-way ANOVA and Duncans Multiple Range Test. Statistical analyses were done using Sirichai Statistics 0.7 software.

Results

General Condition of the Experimental Fish

On the event of 10-day of exposing the fish to elevated temperature (36 ℃), it was observed there was no mortality among samples during the first five (5) days. Meanwhile, during the 6th and 7th days of exposing them to elevated temperature, several mortalities among samples had risen and were recorded. High mortality was observed during the 8th day of experiment.

Fish exposed at elevated water temperature (38 ℃) had comparable growth than those exposed at 28 ℃. However during the nursing period, fish previously exposed to elevated water temperature (38 ℃) had higher weight gain than those exposed to 28 ℃ (Table 2).

According to Drummond *et al.* (2009). , increase of water temperature in fry of Nile tilapia caused better weight gain, size and survival. Water temperature for the best performance of growth and survival rate of tilapia in the process of sex reversal is 30 ℃. However, they observed that at temperatures above 35 ℃, there was significant decrease in survival and growth of fish and in almost all treatments that used temperature of 39 ℃ (considered by some researchers as ideal for masculinization). Contrary results in the present study may possibly be due to the number of fish that survived in each treatment that affected the gain in weight of tilapia after exposure to temperatures 28 and 36 ℃ and after nursing of tilapia. It is well known that fish cultured at higher density will have slower growth than those cultured at lower density.

Table 2. Weight (g) of the experimental fish throughout the duration of the study

	Egg Collection		After A.I	After Exposure In Temperature		After Nursing	
Family	Color	Weight		28 ℃	36 ℃	28 ℃	36 ℃
1	Orange egg	0.006	0.007	0.023	0.027	0.371	0.901
2	Orange egg	0.006	0.007	0.027	0.027	0.687	0.861
3	Orange egg	0.006	0.007	0.028	0.028	0.777	0.797
4	Yellow egg	0.006	0.007	0.030	0.020	0.557	0.725
5	Orange egg	0.006	0.007	0.028	0.040	0.307	0.564

Survival Rate

Mean survival rates of Genomar Supreme Tilapia (GST) strain exposed to 28 ℃ (T28) and 36 ℃ (T36) are shown in Table 3. Highest survival rate at T28 was recorded in Family 1 (F1) with 90.55±2.88% survivability compared to other families while the lowest survivability was recorded in Family 2 (F2) with 53.89±9.88%. On the other hand, families treated at T36 were observed to have decreased rate of survivability compared to families treated at T28. The highest survival rate was observed in Family 3 (F3) with 63.60±3.46% survival, while the lowest rate of survival was observed in F1 with 35.11±5.59% survival.

Mean survival rate of different families of tilapia exposed to the two temperatures was significantly affected by the interaction between family and temperature and was also significantly different between families and between temperatures (P<0.05). Mean of survival rate of F3 (71.47%) was significantly higher than those of the other families.

Moreover, Family 5 (F5, 69.33%), Family 4 (F4, 69.00%) and F1 (62.83%) were comparable to each other but significantly higher than F2 (45.22%).

Table 3. Mean of the survival rate of Genomar Supreme Tilapia strain exposed to normal (28 ℃) and elevated (36 ℃) temperatures

	Survival Rate (%)					
Temp	Family					Mean
	1	2	3	4	5	
36 ℃	35.11±5.59	36.56±4.22	63.60±3.46	38.78±3.06	39.22±2.99	42.65^{a}
28 ℃	90.55±2.88	53.89±9.88	79.33±9.64	99.22±0.42	99.44±0.79	84.49^{b}
Mean	62.83^{b}	45.22^{c}	71.47^{a}	69.00^{b}	69.33^{b}	63.57

Note: Means with different superscript letters in a row or column are significantly different with each other (P<0.05).

Table 4 shows the mean of survival rate after 30-day nursing of previously exposed GST strain in T28 and T36. Mean survival rate of different families of tilapia previously exposed to the two temperatures was not affected by the interaction between families and temperatures and was also not significantly different between families and between temperatures. Generally, the survival rate was high and this may be attributed to the ideal temperature during the nursing period.

Table 4. Mean of the survival rate of Genomar Supreme Tilapia strain previously exposed to normal (28 ℃) and elevated (36 ℃) temperatures after nursing

Survival Rate (%)						
Temp	**Family**					**Mean**
	1	2	3	4	5	
36 ℃	92.72±0.51	96.18±4.65	92.82±5.04	90.47±6.73	93.06±9.24	93.05[a]
28 ℃	96.66±1.25	94.17±2.50	85.46±17.32	91.19±10.84	95.10±5.27	92.52[a]
Mean	94.69[b]	95.17[b]	89.14[b]	90.83[b]	94.08[b]	92.78

Note: Means with the same superscript letters in a row or column are not significantly different with each other (P>0.05)

Sex Ratio of Nile Tilapia (Oreochromis niloticus) Exposed at 28 ℃ (Control) and 36 ℃ (Elevated) Temperature

In the present study, the comparison of the two temperature treatments showed there was apparent increase of male at T38 compared to T28 as shown in Table 5. Chi-square test for fixed ratio analysis, however, showed that all families including those exposed to T28, deviated from the expected sex ratio of 1:1. The deviation of the sex ratio towards male even in the population exposed to T28 indicates that male tilapia in the different families may have higher hatching rate and survival rate than females during the early life stages.

Table 5. Percent male and female after 30 days nursing (previously exposed to 28 ℃ (control) and 36 ℃ (elevated) temperatures, (Chi-square; for fixed ratio)).

FAMILY	TEMPERATURE			
	28 ℃		36 ℃	
	% Male	%Female	% Male	%Female
1	60.67±2.05**	39.33±2.05**	81.67±7.93**	9.67±1.25**
2	69.00±4.90**	34.33±2.49**	87.33±8.06**	6.00±1.63**
3	65.33±4.11**	34.67±4.11**	90.67±3.68**	9.33±3.68**
4	62.33±2.49**	37.67±2.49**	85.00±1.41**	15.00±1.41**
5	67.00±1.63**	33.00±1.63**	88.67±7.32**	6.00±7.79**

Note: Means with ** indicates that they deviated from the expected sex ratio of 1:1 (P<0.01).

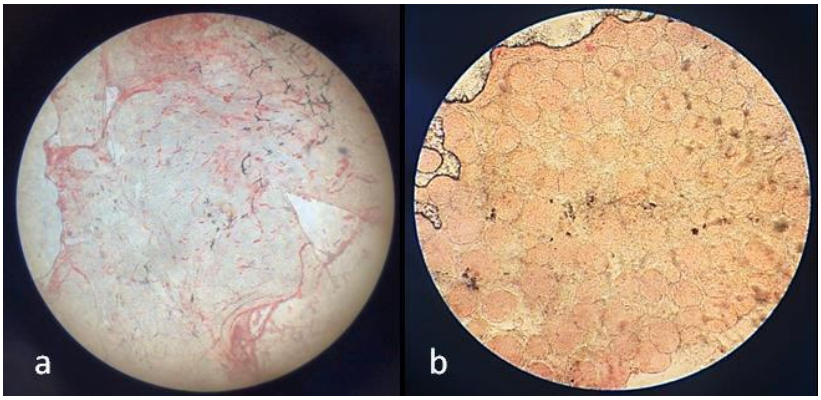


Figure 5. Male (a) and female (b) gonads under the microscope

Table 6 represents the mean male percentage of GST strain previously exposed to T28 and T36. Percentage male was significantly affected by the interaction between family and temperature ($P<0.05$) and was also significantly different between families and between temperatures. Results show that F3 has the highest male percentage among families reared under T36 with 90.67±3.68% while the lowest male percentage was observed in F1 with 84.17±7.93%. On the other hand, for families reared under T28, highest male percentage was recorded in F2 (69.00±4.90 %) while the lowest male percentage was observed in F1 (60.67±2.05 %). However, comparison between means revealed that families exposed to T36 had significantly higher mean male percentage of 86.67% than families exposed to T28 with 64.87% males.

Table 6. Mean of the male percentage of Genomar Supreme Tilapia strain exposed to normal (28 ℃) and elevated (36 ℃)

Male Percentage (%)						
Temp	**Family**					**Mean**
	1	2	3	4	5	
36 ℃	84.17±7.93	84.82±8.06	90.67±3.68	85.00±1.41	88.67±7.32	86.67a
28 ℃	60.67±2.05	69.00±4.90	65.33±4.11	62.33±2.49	67.00±1.63	64.87b
Mean	72.42b	76.91a	78.00a	73.67b	77.83a	75.70

Note: Means with different superscript letters in a row or column are significantly different with each other ($P<0.05$).

On the effect of family, mean male percentage of F3 (78.00%) was highest but was comparable to that of F5 (77.83%) and F2 (76.91%). Then followed by that of F4 (73.67%), that was not significantly different from F1 (72.42%). Moreover, significantly lowest mean male percentage was recorded

in F1 (Table 6). Furthermore, results of the present study also showed that male percentage exposed in T36 increased about 20% compared to the percentage of male exposed in T28. However, low increase in male percentage may support the earlier interpretation that increased male percentage after exposure to elevated temperature maybe possibly due the high mortality rate of the female fish in the experiment.

Results presented in Table 7 are means of female percentage of GST strain previously exposed to T28 and T36. Percentage female was significantly affected by the interaction between family and temperature ($P<0.05$) and was also significantly different between families and between temperatures. Results show that F4 had the highest female percentage among families reared under T36 with 15.00±1.41% while the lowest male percentage was observed in F2 with 5.74±1.63%. On the other hand, for families exposed under T28, highest female percentage was recorded in F1 (39.33±2.05%), while the lowest male percentage was observed in F5 (33.00±1.63%). However, comparison between temperature means revealed that families exposed under T28 had significantly higher mean female percentage of 35.80% than families exposed under T36 with mean of 9.25%.

Table 7. Mean of the female percentage of Genomar Supreme Tilapia strain previously exposed to normal (28 ℃) and elevated (36 ℃) temperatures

Female Percentage (%)						
Temp	**Family**					**Mean**
	1	2	3	4	5	
36 ℃	10.17±1.25	5.74±1.63	9.33±3.68	15.00±1.41	6.00±7.79	9.25[a]
28 ℃	39.33±2.05	34.33±2.49	34.67±4.11	37.67±2.49	33.00±1.63	35.80[b]
Mean	24.75[ab]	20.04[c]	22.00[bc]	26.33[a]	19.50[c]	22.52

Note: Means with different superscript letters in a row or column are significantly different with each other ($P<0.05$).

Statistical results also show there were significant differences among families and mean female percentage in F4 (26.33%) was significantly higher than those from the rest of the families, except that of F1 (24.75%). Moreover, mean female percentage in F1 was not significantly different than that from F3 (22.00%). Meanwhile, that F3 was comparable to those of F2 (20.04%) and F5 (19.50%, Table 7).

Water Quality

Water quality parameters [temperature and dissolved oxygen (DO)] during broodstock maintenance and breeding were measured. Daily readings of temperature and DO are presented in Appendix Table 7.

Daily readings of temperature were optimum. The lowest was 31.3 ℃ in the morning and the highest was 36.6 ℃ in the afternoon. Lowest temperature readings were recorded in Day 15 of the experiment. Majority of DO readings were optimum, with the lowest reading of 3.44 mg/L.

Discussion

This research study evaluated the sex ratio of Nile tilapia exposed in elevated temperature to elucidate its effect on the sex ratio of fingerlings. Furthermore, this research has investigated the best family from Genomar Supreme Tilapia (GST) strain with the best response on temperature induced sex differentiation. The study utilized a 2x5 factorial design with Factor A being the two temperatures (28 ℃ and 36 ℃) and Factor B consisted of 5 families of GST strain. The study consisted of ten treatments with three replicates. Fifteen hapas measuring 0.46 x 0.30 m were installed in each aquarium. To ensure uniform temperature of water inside the aquaria, heater with thermostat (2000 watts capacity) was installed in each aquarium. The heater thermostat was connected in a controller to regulate temperature. Thermometer was also installed to further check the temperature in the aquaria.

Temperature at 28 ℃ (T28) was used as a control and 36 ℃ (T36) for the application of elevated temperature to yolk sac fry (sexless stage) of tilapia from five different families of Generation 26, Batch four (4) of GST strain. Yolk sac fry undergo 10-day exposure in temperatures T28 and T36. Even a short temperature treatment (10 days) (Baroiller *et al.,* 1995a; Baroiller *et al.,* 2009) can be just as efficient as a longer (21 days) hormonal treatment (Baroiller and Toguyeni, 2004; Baroiller *et al.,*, 2009), both of them cover the same initial critical period comprising the 14-24 days after fertilization (Baroiller *et al.,* 2009) , after exposure, the fish were nursed in indoor tanks for 30 days before gonad squashing.

Fish exposed at elevated water temperature (38 ℃) had comparable growth than those exposed at 28 ℃. However during the nursing period, fish previously exposed to elevated water temperature (38 ℃) had higher weight gain than those exposed to 28 ℃ (Table 2). Results of the present study are contrary to results of the study by Drummond *et al.* (2009).

According to them, increase of water temperature in fry of Nile tilapia caused better weight gain, size and survival. Water temperature for the best performance of growth and survival rate of tilapia in the process of sex reversal is 30 ℃. However, they observed that at temperatures above 35 ℃, there was significant decrease in survival and growth of fish and in almost all treatments that used temperature of 39 ℃ (considered by some researchers as ideal for

masculinization). Contrary results in the present study may possibly be due to the number of fish that survived in each treatment that affected the gain in weight of tilapia after exposure to temperatures 28 and 36 ℃ and after nursing of tilapia. It is well known that fish cultured at higher density will have slower growth than those cultured at lower density.

The mean survival rate of different families of tilapia exposed to the two temperatures was significantly affected by the interaction between family and temperature ($P<0.05$) and were also significantly different between families and between temperatures. Mean of survival rate of Family 3 (F3, 71.47 %) was significantly higher than those of the other families. Moreover, Family 5 (F5, 69.33 %), Family 4 (F4, 69.00%) and Family 1 (62.83%) were comparable to each other but significantly higher than Family 2 (F2, 45.22 %). Previous studies by Azaza (2008) and Rahma *et al.* (2015) disclosed that survival was reduced if masculinizing temperature increases. Similarly, Rougeot *et al.* (2008) reported that high temperature treatment affects survival more than temperaturw duration. Meanwhile, Drummond *et al.* (2009) observed that temperatures above 35 ℃ produced significant decrease in survival and growth of the fish, as well as for studies that used temperature of 39 ℃ (considered by some researchers as ideal for masculinization) which produced 100% mortality in 3 weeks.

However, 39 ℃ is considered in tilapia to be stressful and, the temperature in which mortality begins to juveniles (Martinez *et al*., 2014) which is in congruence to the results of this current study wherein increase in temperature from 28 ℃ and 36 ℃ dramatically decreased the rate or survivavility of tilapia. The effectiveness of temperature treatments in the masculinization of fish has also been traced to increased stress level leading to higher blood cortisol levels although the mechanism is unclear (Martinez *et al*., 2014). Tilapia is a thermo-sensitive species and its male to female ratio increases with high temperatures and/or ovarian differentiation in induced by low temperatures (Fuentes-Silva *et al.*, 2013).

Mean of survival rate after 30-day nursing of previously exposed GST strain in T28 and T36 was not affected by the interaction between families and temperatures and was also not significantly different between families and between temperatures. Generally, survival rate was high (85.46±17.32 – 96.66±1.25%) and this may be attributed to the ideal temperature during the nursing period.

Comparing the results of the two temperature treatments, show that there was apparent increase in the number of male at T36 as compared to T28. Chi-square test for fixed ratio analysis, however, showed that all families including those exposed to T28, deviated from the expected sex ratio of 1:1. The

deviation of the sex ratio towards male even in the population exposed to T28 indicates that male tilapia in the different families may have higher hatching rate and survival rate than females during the early life stages. This interpretation of the results should be studied further as the increase in percentage male during exposure to elevated temperature may mostly be due to differences in survival rate of male and female tilapia when exposed to stressful condition like elevated temperature rather than the effect of elevated temperature during sexual differentiation like disruption of the normal development processes causing the switch to males for the genetic females and the high temperature may influence the structure or action of a hormone or hormones acting during sex differentiation (Hunter and Donaldson,1983).

Male gonad differentiation can be achieved by applying high temperature treatment during the critical period of sex differentiation (Rahma *et al.,* 2015)**.** Temperature of the culture environment has been shown to affect sex ratios of some fish, tilapia inclusive (Rahma *et al.,* 2015). The change in sex of tilapia from female to male increased with high temperature, while ovarian differentiation is induced by low temperatures (Baroiller and D'Cotta, 2001). Tessema *et al.* (2006) reported that masculinization rate did not increase with increasing temperature from 36℃ to 38℃ but strain response to high temperature treatment differed. Drummond *et al.,* (2009) demonstrated that the temperatures between 26 to 32℃ were not sufficient to induce sex reversal because there was no change in the proportion of males in groups.

Similar results were reported by Baras *et al.,* (2001) that range of temperature between 20℃ and 33 ℃ produced the same proportion of males (42 to 60%) than in temperature of 27 ℃. Abucay *et al.* (1999) disclosed that sensitivity of the fish to high temperature is likely to be related to effects during sexual differentiation. There are two possible developmental pathways whereby temperature can affect this process. First, an environmental shock like high temperature may disrupt the normal development processes during sex differentiation causing the switch to males for the genetic females and switch to females for the genetically male progeny. Second, high temperature might influence the structure or action of hormone or hormones acting during sex differentiation (Hunter and Donaldson, 1983). Tessema *et al.* (2006) revealed that temperature of 36℃ and 38℃ increased the proportion of males by 78%. However, temperature of 38℃ increased mortality rate.

The percentage male was significantly affected by the interaction between family and temperature ($P<0.05$) and was also significantly different between families and between temperatures. Family 3 had the highest male percentage among families reared under 36℃ with 90.67±3.68% while the lowest male percentage was observed in F1 with 84.17±7.93%. On the other hand, for

families reared under 28 ℃, highest male percentage was recorded in F2 (69.00±4.90%), while the lowest male percentage was observed in F1 (60.67±2.05%). However, comparison between means revealed that families exposed to T36 had significantly higher mean male percentage of 86.67% than families exposed to T28 with 64.87% males.

On the effect of family, the mean male percentage of F3 (78.00%) was highest but was comparable to that of F5 (77.83 %) and F2 (76.91%). Then followed by F4 (73.67 %), that was not significantly different from F1 (72.42%). Moreover, significantly lowest mean male percentage was recorded in F1. Significant effects of interaction between family and temperature nd temperature alone are in congruence with the reports of Baroiller and D'Cotta, (2001) that changes in sex of tilapia from female to male increases with high temperature, thus inducing gonadal masculinization during its restricted developmental period

Temperature-induced sex masculinization has been demonstrated in sensitive tilapia progenies (79% males at 39 ℃ and 46% males at 27 ℃): after reaching sexual maturity, high-temperature-treated (HTT) and control males were individually progeny-tested (Baroiller *et al.*, 1999). A strong effect of temperature on sex differentiation has been demonstrated in various tilapia species and in a hybrid (Baroiller *et al.*, 1995a, b; 1996; Baroiller and Clota, 1998; Desprez and Melard, 1998; Wang and Tsai, 2000; Baroiller *et al*., 2009). The percentage female was significantly affected by the interaction between family and temperature (P<0.05) and was also significantly different between families and between temperatures. Family 4 had the highest female percentage among families exposed to T36 with 15.00±1.41%, while the lowest male percentage was observed in F2 with 5.74±1.63%. On the other hand, the families in T28, with highest female percentage was recorded in F1 (39.33±2.05%), while the lowest male percentage was observed in F5 (33.00±1.63%). However, comparison between temperature means revealed that families exposed to T28 had significantly higher mean female percentage of 35.80% than families in T36 with mean of 9.25%.

Statistical results also show there were significant differences among families and the mean female percentage in F4 (26.33%) was significantly higher than those from the rest of the families, except that of F1 (24.75%). Moreover, mean female percentage in F1 was not significantly different than that from F3 (22.00%). Meanwhile, F3 was comparable to those of F2 (20.04%) and F5 (19.50%).

Based from the initial results, the following conclusions were made: 1. Survival rate of GST strain was affected by the two temperatures, 28 ℃ and 36 ℃, of the water and with significantly lower survival rate at elevated

temperature; 2. Elevated temperature of 36 ℃ was effective in sex reversal of Nile tilapia based upon the results of sex ratio of Nile tilapia; and 3. Family 3 had the best response on temperature induced sex differentiation based on sex ratio and survival rate.

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