Sustainable Aquatic Research, 2(1): 1-23
DOI: 10.5281/zenodo.7881895
e-ISSN: 2822-4140

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RESEARCH PAPER

Temperature influences masculinity, sex reversal after mono-sex, and the hormonal residue in the flesh of Nile tilapia (*Oreochromis niloticus*)

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Citation

Sarker, B., Hossain, M. A., Alam, M. M. M., Mian, S. & Iqbal, M.M. (2023). Temperature influences masculinity, sex reversal after mono-sex, and the hormonal residue in the flesh of Nile tilapia (*Oreochromis niloticus*). *Sustainable Aquatic Research,* 2(1), 1-23.

Article History

Received: 3 January 2023
Received in revised from:18 February 2023
Accepted: 20 February 2023
Available online: 30 April 2023

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Keywords

Sex reversal
Mono-sex tilapia
Masculinization
Hormone residue
Fish flesh
Tilapia aquaculture

Abstract

This study was conducted to investigate the impact of temperature on masculinity and growth in hatchery and production phases, and to assess the hormonal residue in fish flesh of Nile tilapia (*Oreochromis niloticus*). In cemented tanks with hapa setting hatchlings were released and fed with 60 g/kg hormone and maintained at 30 ºC, 33 ºC, 36 ºC and 39 ºC for 28 days and 60 days. At 36 ºC no female fish was observed during the 28 days of hormone treatment and 60 days of trial production. The average body weight gain, daily weight gain, growth trends and survival rate were higher in 36 ºC. The 36 °C treatment was repeated in the production pond for 178 days. Several females (5-6 individuals) were discovered. The survival rate, other growth parameters, and water quality parameters were found to be comparatively optimum during the experiment. Another setup was used to assess the hormonal residue and found at the end of the experiment 42.1 pg/mL 17α-methyltestosterone rest in fish muscle was observed in 178 days. The water quality parameters used in this investigation were also consistent and did not change noticeably during the course of the experiment. This study determined the ideal temperature producing mono-sex tilapia in hatchery and production conditions. The results of this study might enable Nile tilapia mono-sex hatcheries to operate more cheaply and produce pure male tilapia, however, it may also help the government establish a limit for the permissible level of hormone residue in commercial tilapia products.

Introduction

Oreochromis niloticus has a reputation as a highly prolific and regularly traded fish species, with its origins perhaps in Africa and the Middle East (Eknath & Hulata, 2009; Suresh & Bhujel, 2012; Yongo et al., 2021). The Nile tilapia, for instance, is said to have been produced by Egypt more than 4,000 years ago, making this nation the origin of the species (Mir et al., 2018). Worldwide aquaculture has expanded by 12 times in the past 30 years, resulting in the food production sector's greatest growth rate of about 8% (Subasinghe et al., 2009; Naylor et al., 2021). In 2020, global aquaculture production reached a record 122.6 million tonnes, while only tilapia farming contributed 6.03 million tonnes covering 5.27 % of total production (Miao & Wang, 2020; FAO, 2022). The water temperature has a considerable impact on regulating the sex ratio of tilapia, according to several studies on the reproductive biology of this fish species (Tessema et al., 2006; Pankhurst & Munday, 2011; Nivelle et al., 2019). In a pond with only male tilapia, the mono-sex culture strategy may be used to prevent tilapia from reproducing in an unregulated manner (El-Greisy & El-Gamal, 2012; Fuentes-Silva et al., 2013; Gómez-Márquez et al., 2015). The hormonal sex reversal strategy is the one that is most frequently used and the most economical technique for producing male tilapia (Barbosa et al., 2013; Megbowon & Mojekwu, 2014; Hoga et al., 2018; Karaket et al., 2022). In addition to these methods, there are also genetic engineering, hormone sex reversal, hand sexing, and hybridization (Phelps & Popma, 2000; Fuentes-Silva et al., 2013; Budd et al., 2015; Rahma et al., 2015). When Nile tilapia were administered 60 mg MT per kg of feed for 28 days, various researchers discovered that approximately 100% of the fish were male (Desprez et al., 2003; El-Greisy & El-Gamal, 2012; Al-Hakim et al., 2013; Jensi et al., 2016; Palupi et al., 2020). It was discovered that the hormonally treated tilapia's production rate was not appropriately recorded. This research was crucial in determining the real levels of hormone residue in fish bodies, allowing for the production of 100% mono-sex tilapia fries. Hormones are employed to improve fish output in aquaculture when one sex of a species can develop larger and more rapidly than the other sex. In order to maximize fish yield, scientists have turned to the usage of estrogens and androgens, which play a key role in the phenomenon of sexual dimorphism.

There are several instances of temperature-dependent sex determination in the animal kingdom (Ospina-Álvarez & Piferrer, 2008; Baroiller et al., 2009; Rhen et al., 2011; Brown et al., 2014; Blackburn, 2018). The temperature was discovered to be a factor in determining sex in fish for the first time in Atlantic Silverside fry (Conover & Kynard, 1981). At lower temperatures, *Menidia menidia* evolved as females, but at higher temperatures, they developed as males (Conover & Fleisher, 1986; Lagomarsino & Conover, 1993; Penman & Piferrer, 2008). It was discovered that high-temperature treatments during the early embryonic stage (labile period) in *Oreochromis niloticus* result in a considerable divergence of the sex ratio in favor of males (Tessema et al., 2006; Azaza et al., 2008; Rougeot et al., 2008; Nivelle et al., 2019; Valdivieso et al., 2022). The effects of temperature on tilapia sex reversal should be more thoroughly investigated in sub-tropical or tropical regions.

The hormone 17α-methyltestosterone (MT) is an artificial androgenic steroid that promotes the development of male sexual characteristics as well as the growth of muscle tissue (Khalil et al., 2011; El-Greisy & El-Gamal, 2012; Liñán-Cabello et al., 2013). The degree of efficiency attained through fish farming can be significantly impacted by differences between males and females in terms of their development, size, behavioral habits, and breeding season (Desjardins et al., 2006; Budd et al., 2015). Exogenous steroid injections may be useful for controlling tilapia sexual maturation (Phelps & Popma, 2000; Budd et al., 2015; Yu et al., 2022). The majority of the hormone stays inside the fish's body, where it may continue to function, despite some of it being ejected back into the water. If not properly regulated, these substances might pollute water, food, and food products, endangering the health of both people and animals (Mlalila et al., 2015; Hoga et al., 2018; Vinarukwong et al., 2018; Azizi-lalabadi & Pirsaheb, 2021). The European Economic Community (EEC) enacted restrictions on the dosage of growth-promoting anabolic steroids in cattle reared for human consumption as a direct result of this (Kicman, 2008; Hirpessa et al., 2020; McVeigh et al., 2021; Skoupá et al., 2022). Residues, which are chemical compounds that contain the parent substances and/or their metabolites, may be present in any edible tissue from an animal, fowl, fish, or other animals (O'Keeffe & Farrell, 2000; Kim et al., 2013; Okocha et al., 2018; Rana et al., 2019). However, the Food and Drug Administration of the United States of America has approved several food items derived from hormone-treated animals as being both safe and effective (Mlalila et al., 2015). The lowest allowable amount of 17α-methyltestosterone in muscle is 1 ppb (Garcey, 1986; Marzouk et al., 2016). Meat, poultry, fish, and shellfish are just a few of the items that may contain residues. Additionally, the waste contains contaminants linked to veterinary drugs (Treiber & Beranek-Knauer, 2021; Wang et al., 2021). People who consume fish flesh with 17α-methyltestosterone residue run the risk of developing several harmful health conditions, such as mutagenicity, carcinogenicity, hepatotoxicity, hepatic adenocarcinoma, fetotoxicity, and environmental toxicity (Megbowon & Mojekwu, 2014; Hoga et al., 2018; Suseno et al., 2020). It was discovered that residues in tilapia fish had not been thoroughly studied after they reached harvesting size. Considering the research gap, this study was conducted to quantify the sex-reversal (male to female) propensity of Nile tilapia during six months, to evaluate the impact of androgen duration on the development rate and viability of all-male Nile tilapia by the use of stable thermal treatment technology for growing fingerlings in tanks, and to estimate hormonal residues of testosterone in Tilapia (*Oreochromis niloticus*) flesh, after using of 17α-methyltestosterone (60 mg/kg of food) during the stage of gonadal differentiation to produce mono sex production which is compatible with an ELISA technique. Therefore, the current study was aimed at identifying temperature influence on masculinity, sex reversal after mono-sex, and the hormonal residue in the flesh of *Oreochromis niloticus*.

Materials and Methods

Experiment site and periods

This study was conducted in the Bangladesh Rural Advancement Committee (BRAC) tilapia hatchery at Sreemongal upazila, Moulvibazar district, Bangladesh (Figure 1). The laboratory analysis of hormone residue was carried out at the Faculty of Veterinary, Animal, and Biomedical Sciences and Faculty of Fisheries, Sylhet Agricultural University, Sylhet. The experiment was conducted in four cemented tanks (2.5 m × 1.2 m × 1.1 m) and the water area of the pond is 404.6 m^2.

Hapa setting

After being filled with water using a blue filter net, a nursery hapa was constructed. Each breeding hapa needed four bamboo poles (Fregene et al., 2020). Three nursery hapas (2.5 m × 2 m × 1 m), each measuring 5 m^3, were built and put in nursery ponds where they were attached to bamboo poles and nylon ropes. The upper surface of the hapa was at least 0.30 cm above the waterline. Each treatment in the experiment consisted of three replications (R_1, R_2, and R_3) containing 1500 tilapia hatchlings.

Preparation of tank and pond

The cemented tanks (2.5 m × 1.2 m × 1.1 m) were cleaned and rinsed with Potassium permanganate. The ponds were dried after the water was removed with an electric pump (DM-30, Pedrollo Company, Dhaka, Bangladesh). The muck was physically removed after seven days of letting the pond dry. After drying, lime was applied at a rate of one kilogram per 40.46 m^2. Water was stocked after three days of liming.

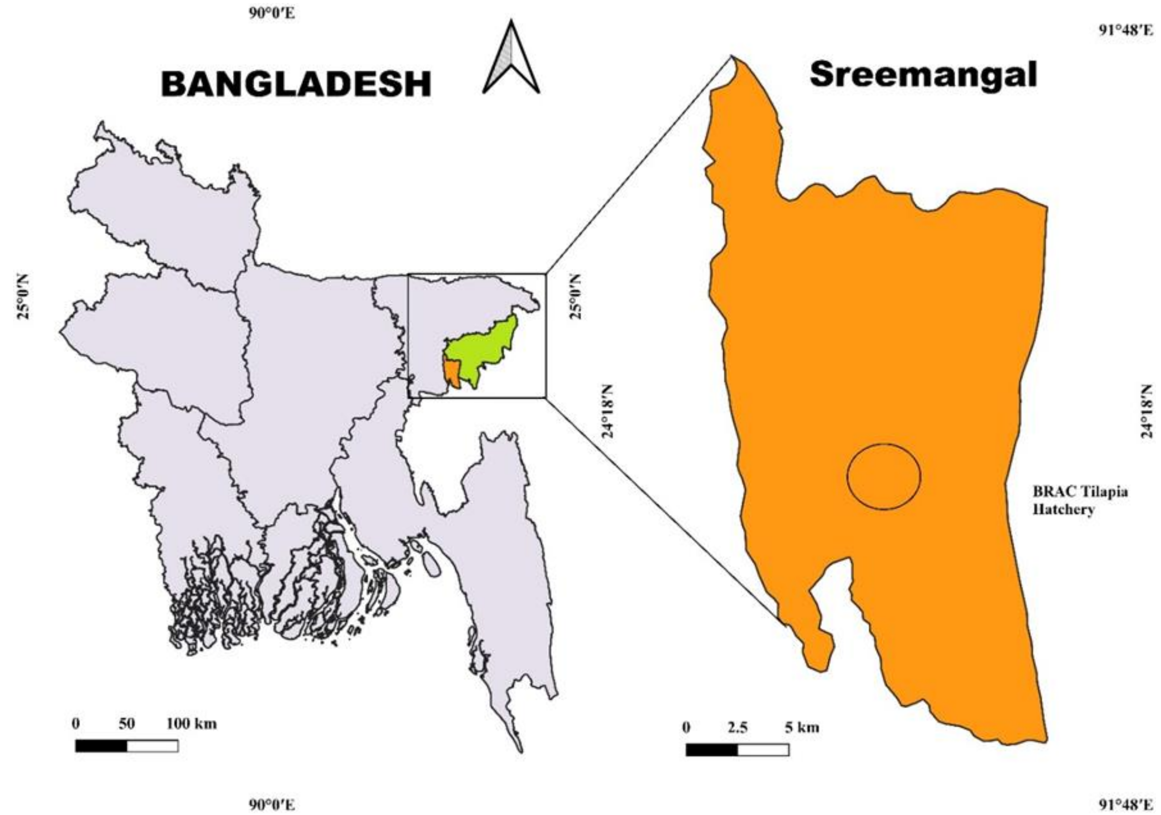

Figure 1. Location of BRAC tilapia hatchery at Moulvibazar district.

Stocking of fries

The fry (weight 0.01 g and length 6 mm) utilized in this experiment came from the BRAC Fish Hatchery at Sreemongal. A total of 1500 larvae were released in three cement tanks with 1000-liter water capacity during the 28-day hormone treatment period.

Feeding trial

One kilogram of androgen hormone-treated feed (60 mg/kg) was prepared using 250 mL of the resultant combination after being mixed with ethanol at a concentration of 99.99% and allowed to stand for an entire night to guarantee a uniform mixture. This meal had been air-dried for 24 hours at room temperature before being put in an airtight container and cooled to 4 °C. The hormone-treated feed was stored in airtight containers (Pant et al., 2020). The hormone-feeding phase was 28 days in the tank providing feeding five times a day (8:00 AM, followed by 10:00 AM, 12:00 PM, 2:00 PM, and 4:00 PM). For the next six months in hapas, the fish were given a hormone-free commercially available diet twice a day (Haq et al., 2017). The feed was administrated to the tilapia according to their age and percent (%) body weight (Table 1).

Table 1. Feed administration schedule to tilapia fish during the study.

Days	Feed applied (% body weight)
1-7 days	40%
8-14 days	30%
15-21 days	25%
22-28 days	20%
29-60 days	15%
61-90 days	12%
91-120 days	10%
121-150 days	8%
151-178 days	7%

Temperature and hormone-based masculinity

The experiment was carried out in cement tanks with temperature control. A 300-watt water radiator (Model-CY 84, Guangdong, China) thermostatically controlled the water temperature in each tank. For the control group, cement tank water was heated to a temperature of 30 °C, and for the second, third, and fourth groups, to 33 °C, 36 °C, and 39 °C, respectively. Every three days, a quarter of the water in each tank was changed to ensure water quality and reduce the gathering of organic waste. The maximum tank capacity was 3000 liters. Air movement kept the oxygen concentration in the water at or above 5 ppm (YSI digital DO meter, Model 58). The tanks were cleaned each morning before feeding by siphoning out the leftover food and waste. In temperature-controlled cement tanks, tilapia larvae were reared for 28 days with hormone-mixed feed and then next 60 days rearing fries in the cemented tank with feed that was hormone free.

Identification of sex and reversal

A total of 100 fish were randomly selected from each replication at one-month intervals during the rearing period to determine their sexes following the method described by Guerrero & Shelton, 1974; Islam & Yasmin, 2016. This experiment was conducted for 178 days (28 days hormone treatment period in the tank with 36 °C and 150 days of observation in hapa) to determine the sex reversal ratio of the experimental fish. A six months experiment was conducted in the tank and the water area of the pond is 404.6 m^2.

Aceto carmine squash process for gonad

A standard Aceto carmine squash test was done according to Guerrero & Shelton (1974). The stain (Aceto carmine) is prepared by adding 0.5 g of carmine to 100 ml of 45% acetic acid and then boiling it for 2 to 4 minutes. When cool the solution is filtered with filter paper to remove coarse materials. For microscopic examination, a sample of gonad was placed on a glass slide. Then the aceto-carmine solution was added to the sample. Gonad was then examined with a coverslip under the Optical microscope; model B-383PL with Optika Digital Camera; model B9, Italy using 10X magnification.

Determination of hormonal residue

The hormone 17α-Methyltestosterone residue was determined using an ELISA assay (SunLong Biotech Co. Ltd., China) that was bought from a nearby commercial provider. The amount of MT residues in tilapia flesh was measured; previously validated by Risto et al. (2013) and Passini et al. (2018). The ELISA assay was validated following the manufacturer's protocol. This experiment was conducted for 178 days (28 days of hormone treatment in the tank at 36 °C and 150 days of observation in hapa) to determine the hormonal residue in the experimental fish from March 1, 2021, to August 26, 2021.

Monitoring of water quality parameters

Weekly readings of parameters such as pH (PH5011, GOnDO Electronic, Taiwan), dissolved oxygen (DO), temperature (HI98193, Hanna Instruments, USA), and transparency (Secchi disk) were taken and documented properly. The ammonia-nitrate of the water was assessed only in the hormone residual experiment using an NH_3-N kit (Sera, Heinsberg, Germany).

Sampling

Samples for assessed growth were obtained at the fry's stomach's peak emptying time in the early morning. A random selection of 100 fry from each hapa and tank was weighed (mean weight 10.89±0.03 mg). The weights of fishes were calculated using an electric balance (FGH, AND, Korea). Every fifteen days following the end of the experiment, seven fishes were randomly chosen, and their flesh was examined for the presence of hormones. To measure the fish growth parameters such as weight, survival rate, and water quality parameters such as temperature, pH, and DO samples were obtained on the first day of the 28-day hormone therapy phase, afterward, and every fifteen days during the experimental period.

Growth parameters

The growth parameters of tilapia were measured using different formulas prescribed by previous studies (Haq et al., 2017; Zafar et al., 2017; Shajib et al., 2018; Billah et al., 2020; Mondal et al., 2020; Islam et al., 2021). The equations were,

Weight gain = Mean final weight - Mean initial weight,

% Weight gain = [(Mean final weight – Mean initial weight) / Mean initial weight] x 100,

Average daily weight gain = (Mean final weight – Mean initial weight) / (T2 – T1),

where T2-T1 expresses the duration of the experimental period.

Specific growth rate (SGR, %/day) = [(loge W2 – loge W1) / (T2 – T1)] x 100,

where, W1 = the initial live body weight (g) at time T1 (day) and W2 = the final live

body weight (g) at time T2 (day).

The survival rate of fish was calculated by counting the number of fish harvested at the end of the experiment from each treatment and control.

Survival rate (%) = (Total number of harvest / Total number of stock) x 100.

Statistical Analysis

For the hormone feeding experiment, one-way analysis of variance (ANOVA) was used to compare the effects of the treatments on the fish's total weight gain, daily weight gain, and specific growth rate using SAS 9.4 (SAS Institute, 2014).

Results

Sex differences at different temperatures

At the 30ºC and 33 ºC temperatures 3% of female fish were found during the 88 days of investigation, while at 36 ºC and 39 ºC temperatures no female was observed (Figure 2).

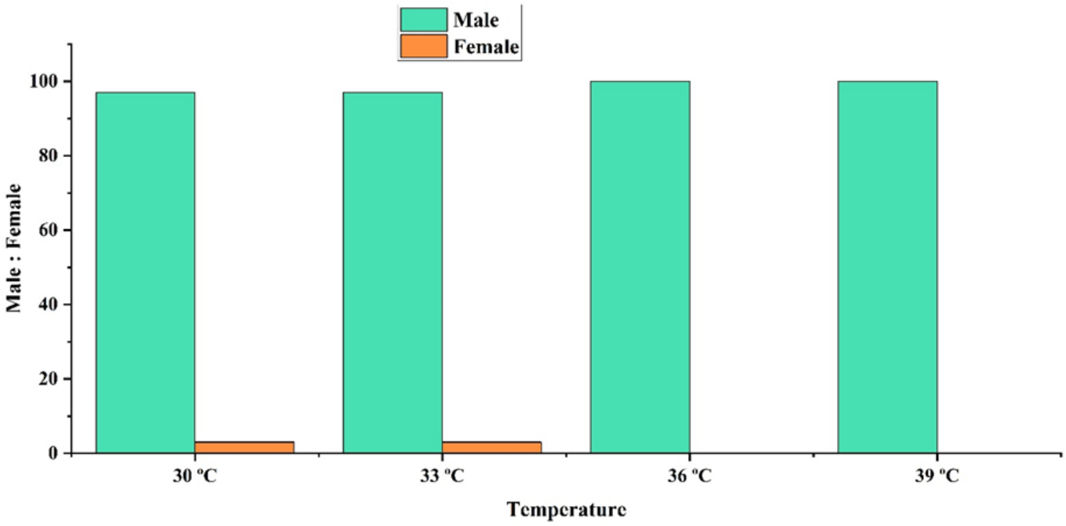

Figure 2. The number of male and female fish after 88 days by aceto-carmine test.

Growth performance parameters at different temperatures

At 30 ºC, the highest daily average body weight gain was observed on day-88 (2.13 g), followed by day-70 (1.55 g), day-56 (0.9 g), day-42 (0.69 g), day-28 (0.29 g), day-14 (0.021 g), and day-1 (0.009 g). At 33 ºC, the highest daily average body weight gain was observed on day-84 (2.38 g), followed by day-70 (1.84 g), day-56 (1.10 g), day-42 (0.77 g), day-28 (0.30 g), day-14 (0.03 g), and day-1 (0.009). At 36 ºC, the highest daily average body weight gain was observed on day-84 (3.15 g), followed by day-70 (2.43 g), day-56 (1.57 g), day-42 (1.09 g), day-28 (0.40 g), day-14 (0.03 g), and day-1 (0.009). At 39 ºC, the highest daily average body weight gain was observed on day-84 (3.02 g), followed by day-70 (2.35 g), day-56 (1.24 g), day-42 (0.98 g), day-28 (0.39 g), day-14 (0.03 g), day-1 (0.009) (Figure 3).

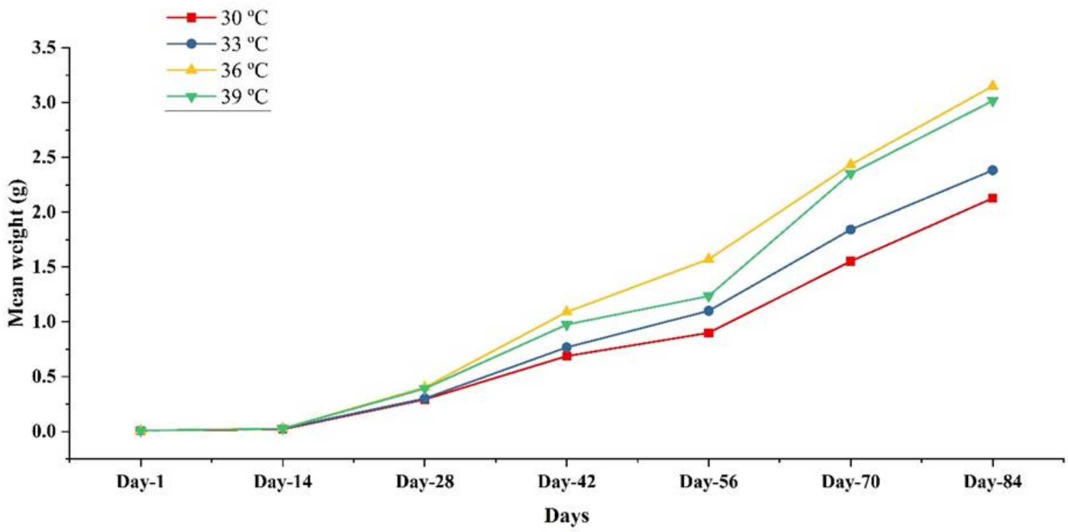

Figure 3. Growth (daily body weight gain) of *O. niloticus* fries of different replications at different temperatures.

Growth parameters during temperature experiment

The growth parameters such as initial weight, final weight, weight gain, percent weight gain, and average daily weight gain did not differ at various temperatures in 28 days of hormone treatment (Table 2).

At 36 ºC, the growth parameters such as final weight (94.67±0.18 g), weight gain (94.41±0.17 g), and average daily weight gain (1.57±0.00 g) were found significantly (P=0.0014; 0.0003) higher than other water temperatures (Table 3).

Table 2. The growth parameters of hormone administrated (28 days) *O. niloticus* in different water temperatures.

	30 ºC	**33 ºC**	**36 ºC**	**39 ºC**	***P* value**
Initial weight (g)	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	1.0
Final weight (g)	0.24±0.01^{a}	0.24±0.01^{a}	0.26±0.01^{a}	0.26±0.01^{a}	0.3665
Weight gain (g)	0.23±0.01^{a}	0.23±0.01^{a}	0.25±0.01^{a}	0.25±0.01^{a}	0.3665
% weight gain	2293.33±63.60^{a}	2260±65.06^{a}	2480.00±117.90^{a}	2466.67±145.30^{a}	0.3665
Average daily weight gain (g)	0.008±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.3559

Table 3. The growth parameters of cultured (60 days) *O. niloticus* in different water temperatures.

	30 ºC	**33 ºC**	**36 ºC**	**39 ºC**	***P* value**
Initial weight (g)	0.24±0.01^{a}	0.24±0.01^{a}	0.26±0.01^{a}	0.26±0.01^{a}	0.3665
Final weight (g)	92.90±0.32^{c}	93.33±0.07^{bc}	94.67±0.18^{a}	94.03±0.18^{ab}	0.0014
Weight gain (g)	92.66±0.32^{c}	93.10±0.07^{bc}	94.41±0.17^{a}	93.78±0.17^{ab}	0.0014
Average daily weight gain (g)	1.54±0.00^{b}	1.55±0.00^{b}	1.57±0.00^{a}	1.56±0.00^{a}	0.0003

Effect of temperature on survival ratio

The highest survival rate of Nile tilapia was observed at 36 ºC (94.9%), followed by 39 ºC (93.57%), 33 ºC (91.61%) and 30 ºC (87.95%) (Figure 4).

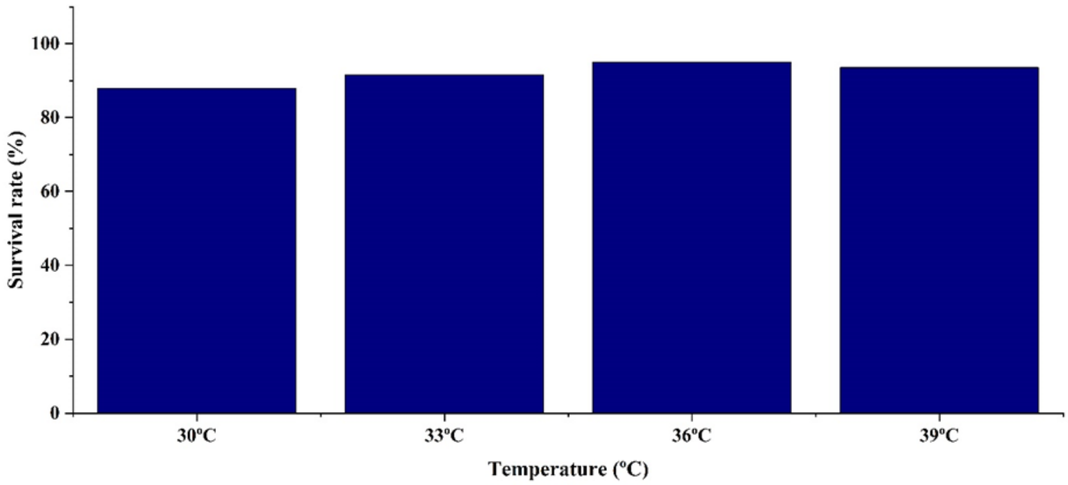

Figure 4. The survival rate of Nile tilapia in different temperatures.

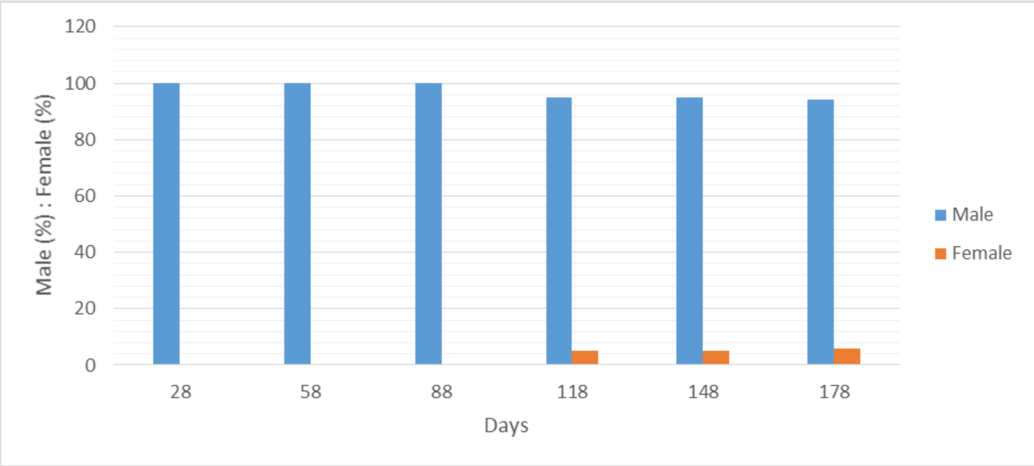

Figure 5. Nile tilapia sex ratios during the study periods.

Masculinization of Nile tilapia

It was found that, from the beginning of the experiment (0 days) until 88 days, there was no female tilapia observed, while at 118 days five female tilapia were observed, followed by 148 days (5 females), 178 days (6 females) (Figure 5).

Growth and survival rate during 178 days

In this study, after 28 days of hormone administration, using 36 °C constant temperature by water heater then releasing in pond. We found the average weight of Nile tilapia was 0.23 g, while at 58 days, the weight was found 62 g, followed by 88 days (112.80 g), 148 days (157.00 g) and 178 days (191.11 g) (Figure 6).

It was also found that at the 28 days of hormone administration use 36 °C constant temperature by the water heater, the survival rate was 96.2%, followed by 58 days (94.8%), 88 days (93.4%), 148 days (92.06%) and 178 days (90.01%) (Figure 7).

The growth parameters such as initial weight, final weight, weight gain, percent weight gain, and average daily weight gain in 28 days and 178 days were found to be significantly different (Table 4).

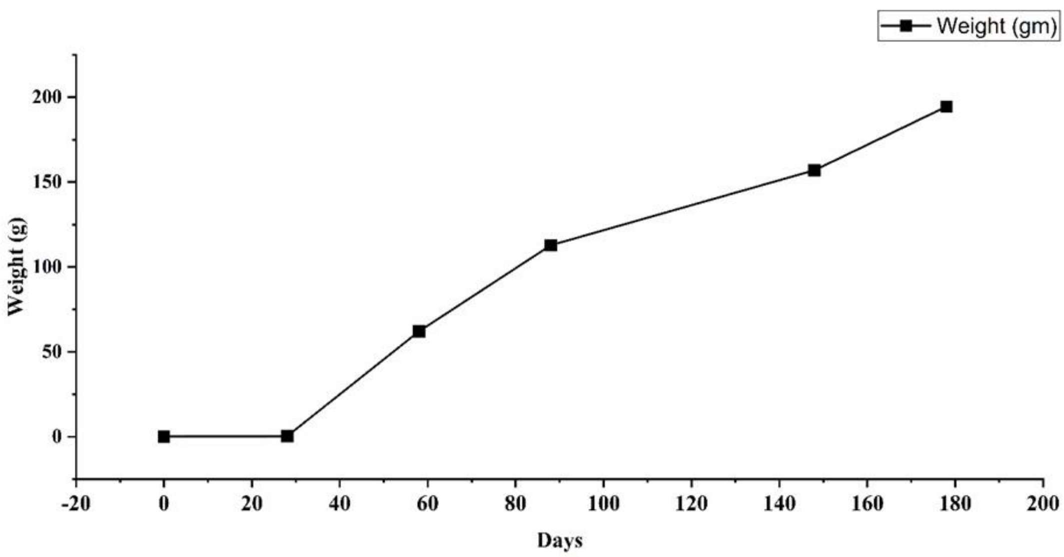

Figure 6. The average weight of the Nile tilapia observed during the experimental period.

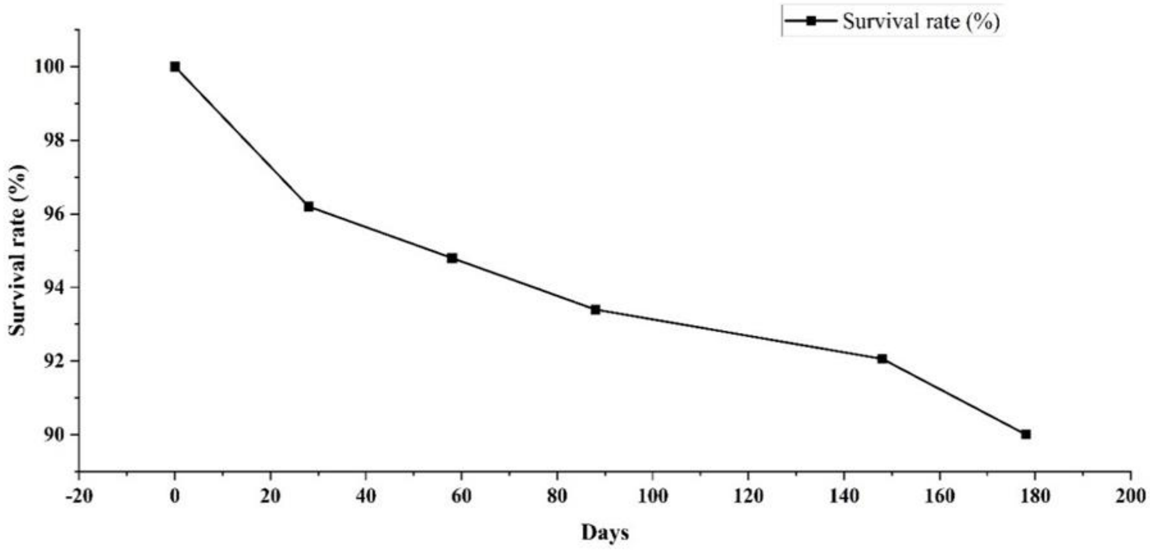

Figure 7. The survival rate of the Nile tilapia observed during the experimental period.

Table 4. Growth parameters of Nile tilapia at 28 days and 178 days.

Parameters	**28 Days**	**178 Days**
Initial weight (g)	0.01±0.00	0.235±0.004
Final weight (g)	0.235±0.004	191.11±1.75
Weight gain (g)	0.225±0.004	190.87±1.74
% Weight gain	2250±40.41	81246.83±1387.92
Average daily weight gain (g)	0.008±0.00	1.07±0.01

Water quality parameters during 178 days sex reversal experiment

The mean value of pH was 7.69±0.03. The statistical analysis showed a minimum pH of 7.6±0.02 (weeks 3, 10, and 13) and maximum pH of 8.5±0.01 (weeks 17 and 18). The mean value of dissolved oxygen (DO) was found 6±0.05, while the minimum DO has observed 4.2±0.10 ppm (week 18), and the maximum DO was found 7.4±0.08 ppm (week 10) (Figure 8).

The mean transparency of water was 37.65±0.23 cm, while the minimum transparency of water was 32±0.45 cm (weeks 4, 12, 18, and 19) and the maximum transparency was 47±0.59 cm (week 16). The mean temperature of the water was 31.18±0.27 ºC, while the minimum temperature of the water was observed at 24.08±0.35 ºC (weeks 26), and the maximum temperature was found to be 34.37±0.47 ºC (week 15) (Figure 9).

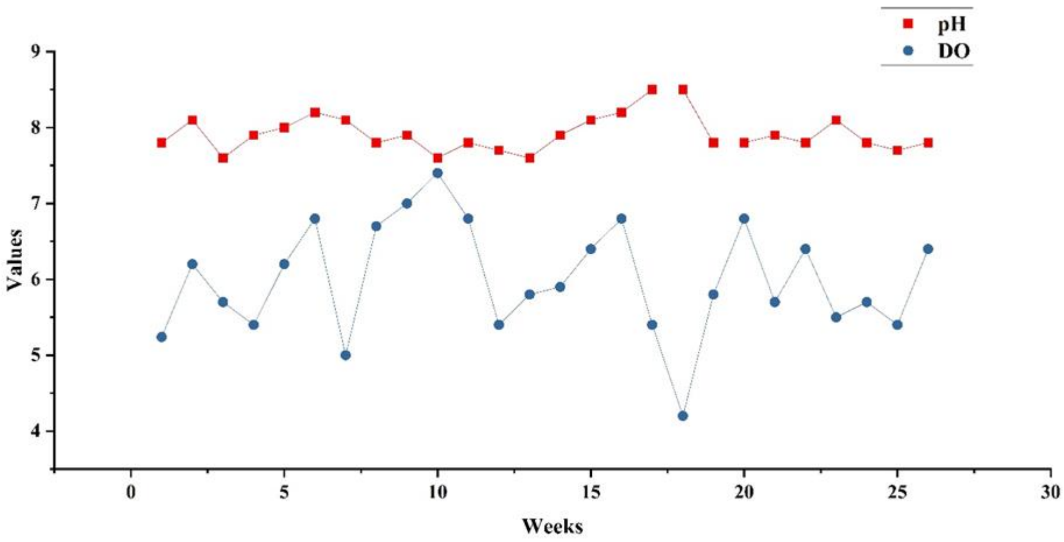

Figure 8. Water quality parameters (pH and dissolved oxygen) during experimental period.

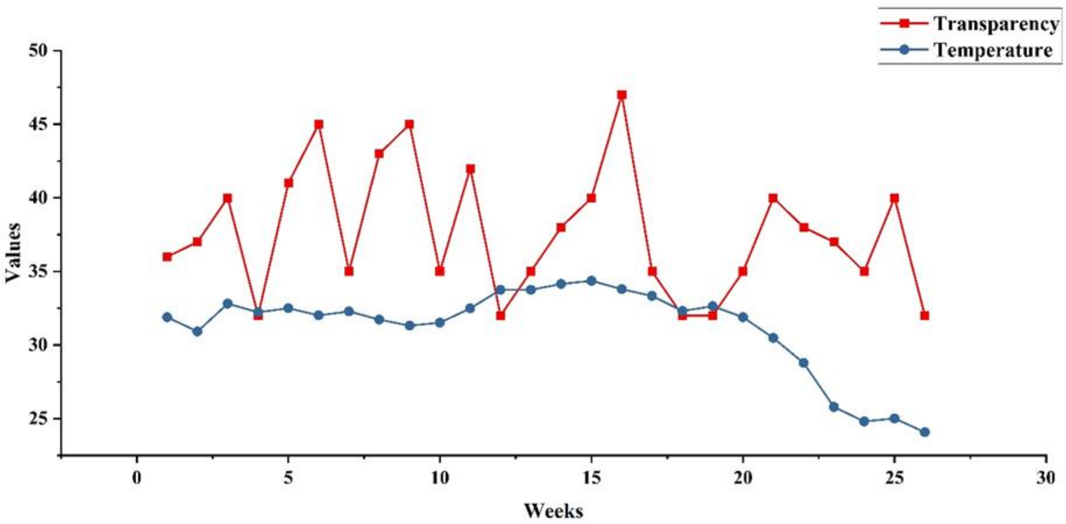

Figure 9. Water quality parameters (transparency and temperature) during experimental periods.

Hormonal residue in tilapia fish

The current study found that, at the start of the hormone treatment, the concentration of residue was the highest. On day 2, it was measured 73.50 pg/mL residue, followed by day-28 (62.3 pg/mL), day-43 (59.07 pg/mL), day-58 (55.22 pg/mL), day-73 (51.08 pg/mL), day-88 (47.8 pg/mL), day-103 (45.09 pg/mL), day-118 (44.59 pg/mL), day-133 (44.62 pg/mL), day-148 (43.02 pg/mL), day-163 (43.01 pg/mL) and day-178 (42.1 pg/mL) (Figure 10).

Average weight and survivability

The final average weight of tilapia was 223.70±1.99 g, while the final average survivability was determined 92.00±0.00% (Table 5).

Water quality parameters during residue experiments

The water temperature of experimental ponds fluctuated between 27.60 °C (day-88) to 31.30 °C (day-178). The highest pH values of water were measured at 8.15 (day-193), and the lowest pH was observed at 7.3

(day-0). The dissolved oxygen fluctuated between 5.8 (day-28) to 7.1 (day-193) mg/L, while the NH3-N was found to vary from 0.01 to 0.02 mg/L during the study periods (Figure 11).

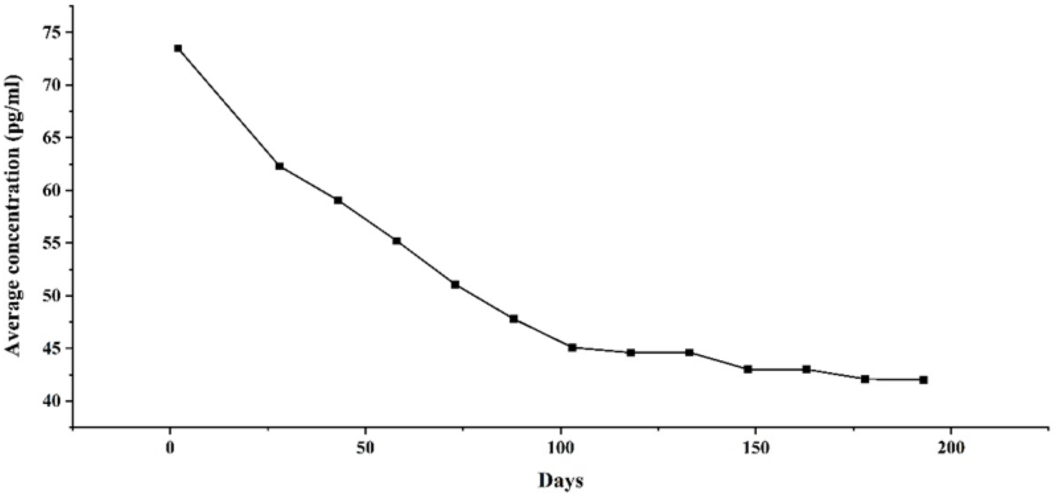

Figure 10. Average hormone concentration on different days.

Table 5. The average growth and survivability during the hormone residue experiment.

Days	Average weight	Average survivability
1	0.01±0.00	100±0.00
28	1.11±0.06	96±0.33
58	45.27±1.47	96±0.33
88	93.80±1.70	96±0.58
118	129.83±2.28	94±0.33
148	194.17±1.76	92±0.33
178	223.70±1.99	92±0.00

Discussion

When a person or animal changes their sex, the appropriate female or male sexual characteristics are replaced with those of the other sex (Todd et al., 2016; Weber & Capel, 2018). Animals, including fish, typically experience one of two sex changes: An organism that is original of the male sex but later changes to that of the female sex is said to be practicing protandry (Cook & Munguia, 2015; Casas et al., 2016; Roberts et al., 2021). When an organism changes its sex after birth from female to male, it is said to have protogyny (Kuwamura et al., 2002; Benvenuto et al., 2017).

The current study found, with a trial of 84 days at 36 ºC and 39 ºC, that no female was

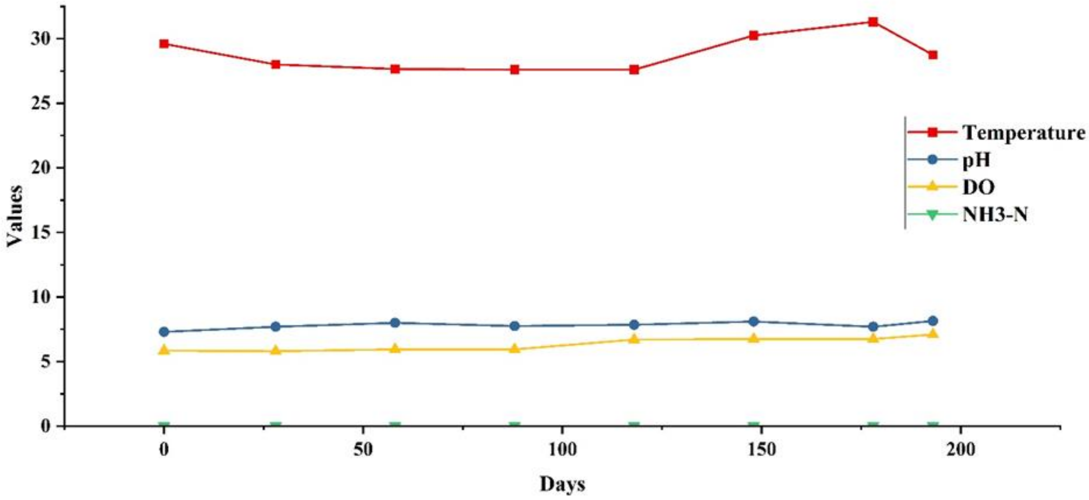

Figure 11. Water quality parameters during the hormonal residue experimentation.

observed, while 3% of female was observed at 30 ºC and 33 ºC, suggesting the higher temperature produces 100% male compared to the lower temperatures. The study of Baroiller et al. (1996) showed that at 36ºC higher male tilapia was observed with high survivability rates, which supported the findings of Ali (2009) who revealed the higher temperature (37 ºC) might inhibit the sex reversal of tilapia fish. In *O. niloticus*, the temperature can have an impact on sex ratios; raising temperatures beyond 34 °C during sexual differentiation have a masculinizing effect (Baroiller & D'Cotta, 2001). The lethal temperature for *O. niloticus* has been reported as 42 °C (Nivelle et al., 2019). *O. mossambiccus*, a species of tilapia, was found to be affected by the water temperature in terms of growth and survival rates, gonad development, and fish sex ratios (Koyakomanda et al., 2019).

The current study found that the average body weight gain at 36 ºC was highly stable within 84 days of experiments, while the day at the end of the study was found as 3.15 g, which was significantly higher than the other treatments (30, 33, and 39 ºC). On the other hand, the growth curve of average daily weight gain at 39 ºC temperature was similar, but this temperature was close to the lethal temperature of experimental fish species, suggesting not to culture at that temperature (Nivelle et al., 2019). The daily average body weight gain at higher temperatures was reported by different studies (El-Sherif & El-Feky, 2009; Tine et al., 2022), suggesting the temperature might influence the daily body weight gain, which was found to confirm the present study.

The growth performance parameters such as final weight, weight gain, percent weight gain, and average daily weight gain in 28 days of hormonal treatment found no significant differences among temperatures. This was due to short treatment periods, hormonal effects on the fish body, and all the temperatures maintained with similar amenities (Drummond et al., 2009; Ferdous & Ali, 2012; Singh et al., 2017). On the other hand, at 36 ºC, the growth parameters for instance, final weight, weight gain, and average daily weight gain were found to be significantly higher than at other water temperatures. It was because of excellent management that particular temperature was maintained for 84 days of culture and proper dietary nutrition ratio. The ideal growth of tilapia following hormone treatment, according to several other studies, was found to be sufficient for achieving higher production when acceptable environmental and feeding practices were maintained (El-Greisy & El-Gamal, 2012; Hossain et al., 2021). The survival rate of Nile tilapia was observed to be higher at 36 ºC in the current study, compared to the other temperature treatments. The survival rate of the juvenile tilapia might be influenced by temperature, while 42 ºC can be lethal for the fish species (Baroiller et al., 1995). But sometimes higher temperatures might help to get the best survival rate (Pandit & Nakamura, 2010).

At 118 days five female tilapia were observed, followed by 5 females at 148 days, and 6 females at 178 days in the current experiment. This occurrence was brought on by the tilapia fish's body having less activity of testosterone levels (Schreiber et al., 1998; El-Greisy & El-Gamal, 2012). The weight gain after hormone treatment showed an increase with days in Nile tilapia, suggesting that, they received proper dietary supplements and environmental condition was also optimum. Other studies also suggested that the growth curve after hormone treatment in Nile tilapia was ascending in nature (Khalil & Mousa, 2013; Dergal et al., 2016). The survival rate during culture periods decreased over time, and at the end of the experiment, it was 90.01%. The study by Hossain et al. (2021) found an 87.6% survival rate during the experiment period of one year, however, with a high temperature, the current survival rate in Nile tilapia was optimum in aquaculture production. The growth parameters such as initial weight, final weight, weight gain, percent weight gain, and average daily weight gain in 28 days and 178 days were found significantly different. The final weight of the experimental fish would be higher than that of the initial weight. This condition can be implemented in other parameters such as weight gain, percent weight gain, and average daily weight gain as well.

The mean value of pH was found as 7.6. The statistical analysis showed the minimum pH as 7.6 and the maximum pH as 8.5. The mean value of dissolved oxygen (DO) was found as 6.00, while the minimum DO was observed as 4.2 ppm, and the maximum DO was found as 7.4 ppm. The mean transparency of water was 37.65 cm and the mean temperature of the water was 31.18 ºC. The ideal water quality parameters for Nile tilapia aquaculture have been examined in some studies over the past ten years, but their findings on pH, dissolved oxygen, transparency, and temperature were very similar to those of the present study; and no outlier or significant different parameters were found (Caldini et al., 2011; Makori et al., 2017; Choudhary & Sharma, 2018; Martins et al., 2019).

The ELISA method was used in the study to identify 17α-methyltestosterone in fish flesh. To avoid both false negative and false positive results, the method being used should be sensitive, specific, accurate, and have a high level of precision. The results of the current investigation showed that the hormone residue decreased with time, reaching its lowest level of 42.1 pg/mL at day 178 of the experiment and showing a downward growth trend from the start to the end of the study. The study by Vinarukwong et al. (2018) suggested that 3.224 ng/g of hormonal residue could be found after 23 days of withdrawal periods, which was optimal and acceptable in export quality products. Another study by Dergal et al. (2016) found 0.09 µg/kg hormone residue in Nile tilapia flesh after 60 days of withdrawal. The current study found far less residue in the flesh of Nile tilapia.

The current study found that the final average weight of tilapia was 223.70±1.99 g, while the final average survivability was determined 46.00±0.00% after 178 days of treatment. The survivability of fish was very low due to the sampling of fish from the experimental tanks every week to get the residual samples. Otherwise, this current result was coincident with previous studies (Drummond et al., 2009; Dergal et al., 2016; Yusuf et al., 2019; Costa e Silva et al., 2022; Karaket et al., 2022).

The current study discovered that the experimental ponds' water temperature varied from 27.60 °C to 31.30 °C. The pH of the water ranged from 7.3 to 8.15. During the study period, the dissolved oxygen varied between 5.8 and 7.1 mg/L, while the NH_3-N was observed to vary between 0.01 and 0.02 mg/L. The ideal water quality parameters for Nile tilapia aquaculture have been studied over the past ten years, but none of them found any outliers or significantly different parameters for pH, dissolved oxygen, transparency, or temperature (Caldini et al., 2011; Makori et al., 2017; Choudhary & Sharma, 2018; Martins et al., 2019).

Conclusions

It is clear that at a constant 36 °C and using androgen hormone, tilapia fish grow only in males. It will also add to the corpus of knowledge on the subject, guaranteeing that scholars in the future will have access to current, pertinent information. The results of the current analysis concluded that methyl testosterone residues are present in tilapia fish at trace levels. Even though they were present in very small amounts, many of these traces were in the samples. Our findings add to the mounting proof that MT is safe to provide during sexual inversion in juvenile tilapia. More thorough research is needed, both in terms of the sample network and sampling frequencies, due to the growing concern around the world over persistent pollutants and their detrimental impact on human health.

Ethical approval

This research had been approved by the animals' ethics committee of Sylhet Agricultural University Research System (SAURES), Bangladesh.

Data availability statement

The authors declare that data are available from authors upon a reasonable request.

Funding

The study was supported by the "Optimization of Hormone Dose and Management Measures for Quality Mono sex Tilapia Fry Production" project funded by the University Grants Commission (UGC) Research Grants through Sylhet Agricultural University Research System (SAURES), Sylhet, Bangladesh.

persistent pollutants and their detrimental impact on human health.

Author Contribution

BS: Conceptualization, Data curation, Formal analysis, Methodology, Writing-original draft
MAH: Data curation, Investigation, Methodology, Review and editing
MMMa: Conceptualization, Data curation, Formal analysis, Review and editing
SM: Conceptualization, Data curation, Formal analysis, Methodology, Review and editing
MMI: Conceptualization, Formal analysis, Methodology, Review and editing.

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