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# Investigating the Effects of Extended Acclimatization Period on the Growth and Survival of Sex-Reversed Nile Tilapia (*Oreochromis niloticus*) Fingerlings through Communal Rearing


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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*


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## ABSTRACT


The right acclimatization procedure can be the first step toward successful tilapia production. However, the lack of standardized acclimatization periods poses challenges, highlighting the need for focused research to develop effective protocols that improve growth performance and survival


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rates in tilapia and potentially other aquatic species. Therefore, this study was conducted to investigate the effects of extended acclimatization periods on the growth and survival of sex-reversed Nile tilapia (*Oreochromis niloticus*) fingerlings through communal rearing. Conducted at the Freshwater Aquaculture Center of Central Luzon State University, the study utilized nine experimental units (hapa nets) within a 1000 m² pond. Three acclimatization durations— (T1) 20, (T2) 30, and (T3) 40 minutes—were tested with corresponding replicates. Growth performances, water quality parameters, length-weight relationships, and survival rates were analyzed over a 15-day period. No significant differences were observed in the growth performances of the fingerlings across the three treatments (MWG: T1- 0.58±0.01 g, T2- 0.60±0.07 g, T3- 0.55±0.02 g; MLG: T1- 0.72±0.05 cm, T2- 0.74±0.13 cm, T3- 0.66±0.01 cm; SGR: T1- 11.48±0.12 %/day, T2- 11.15±0.45 %/day, T3- 11.32±0.23 %/day; ADG: T1- 0.030±0.001 g/day, T2- 0.032±0.005 g/day, T3- 0.029±0.001 g/day). Survival rates were 86±2.75% (T1), 83±4.86% (T2), and 84±3.23% (T3). These results indicate that sex-reversed Nile tilapia fingerlings could be acclimatized for 20 to 40 minutes without significant differences in growth performances and survival rates. Length-weight relationship analysis revealed that the fingerlings had negative allometric growth, probably caused by decreasing space in the experimental units as the fish grew. However, the obtained K values (K>1) show that the fingerlings were still in good condition. The water quality parameters in the study were within acceptable levels, though some were within the upper limit. The findings underscore the importance of standardized acclimatization protocols to enhance the resilience and growth performance of Nile tilapia in aquaculture settings.

*Keywords: Nile tilapia; acclimatization; standardized; growth performance; survival rate; length-weight relationship; condition factor.*

## 1. INTRODUCTION

Global fisheries and aquaculture production reached a record 214 million tonnes in 2020, consisting of 178 million tonnes of aquatic animals and 36 million tonnes of algae. This significant increase was mainly due to the growth of aquaculture, particularly in Asia [1]. Tilapia became the second most important species group of cultured aquatic animals in 2014 in terms of quantity when its production first exceeded 5 million tonnes. In 2018, farmed tilapia accounted for 5.27% of global aquaculture production [2]. In the Philippines, the tilapia industry produced 263,871.29 metric tons, accounting for 11.4% of the country's total aquaculture production of 2,324,000 metric tons in 2020 [3].

Propagating and farming tilapia involves minimal effort, and the technology used can be straightforward and easily adapted for small-scale fish farmers [4]. The successful culture of this fish can begin with the proper execution of the acclimatization process. Acclimatization is the process by which an organism becomes physically adjusted to the temperature of its environment [5]. It plays an important role in how well an organism can tolerate heat and cold [6]. This process usually involves floating the sealed bag containing the fish in the culture environment to equalize the temperature, and gradually adding a small amount of water to the bag before finally releasing the fish [7].

However, a current problem with this practice is the lack of a standardized acclimatization period due to varying recommendations that usually range from 15 to 60 minutes. On the internet, accounts of these varying practices stem from different websites of aquaculturists and ornamental fish hobbyists. For example, on the website Hoffman's Water X Scapes [8], the suggested duration is about 15 to 60 minutes, while on another, Advanced Aquarium Concepts [9], it is about 20 to 45 minutes. Moreover, the recommended duration by Aquaforest [10], which is 15 to 30 minutes, is longer than that of The Biota Group [11], which is only about 10 to 15 minutes.

Additionally, the literature appears to lack studies focusing on the assessment of various acclimatization methods for tilapia in warm environments. Specifically, there are no related studies on the impacts of prolonged acclimatization periods for tilapia and other aquaculture species. Existing studies mostly revolve around acclimatization in saline environments and colder temperatures, such as the study conducted by King and Sardella [12], which investigated upper and lower thermal tolerances and the effect of environmental salinity in Mozambique tilapia.

With the presented knowledge gap, it can be said that acclimatization is a crucial research direction for achieving ideal aquaculture practices and must be studied intensively to ensure effectiveness and sustainability. This study sought to answer whether extended acclimatization can increase survival rates, eventually leading to improved growth performance, which in turn affects yield. This issue underscores the need for focused research to develop standardized acclimatization protocols that can be broadly applied across aquaculture practices. Such research is not only essential for tilapia aquaculture but may also have relevance for other cultured aquatic species.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Pond and Units

The study was conducted at the Freshwater Aquaculture Center (FAC), Central Luzon State University, Muñoz City, Nueva Ecija, Philippines (see Fig. 1). One experimental pond with an area of 1,000 m² was used, which underwent the standard pond preparation procedures of the institution. Nine experimental units (hapa nets) were utilized, representing three treatments with varying acclimatization periods (Treatment 1: 20 minutes; Treatment 2: 30 minutes; Treatment 3: 40 minutes) and their replicates. The size of each experimental unit was 2.5 x 2 x 1 meters. Each replicate used B-net to prevent predation by birds. The experimental layout followed a Randomized Complete Block Design (RCBD).

### 2.2 Experimental Animal

The Nile tilapia fingerlings, size #22, used in this study were obtained from the pond production facilities of the FAC-CLSU (see Fig. 2). The fingerlings were sex-reversed at the institution prior to the conduct of the study. They were then collected from the nursery hapa and conditioned in a tank for 24 hours before transport. The study used nine plastic bags for transporting the fish, with each bag containing approximately 1,100 fingerlings. The number of fingerlings was estimated by weight (130 grams was approximately 1,100 fingerlings). The fingerlings were packed in the morning and traveled for 4 hours to simulate the usual transport process for customers of the FAC.

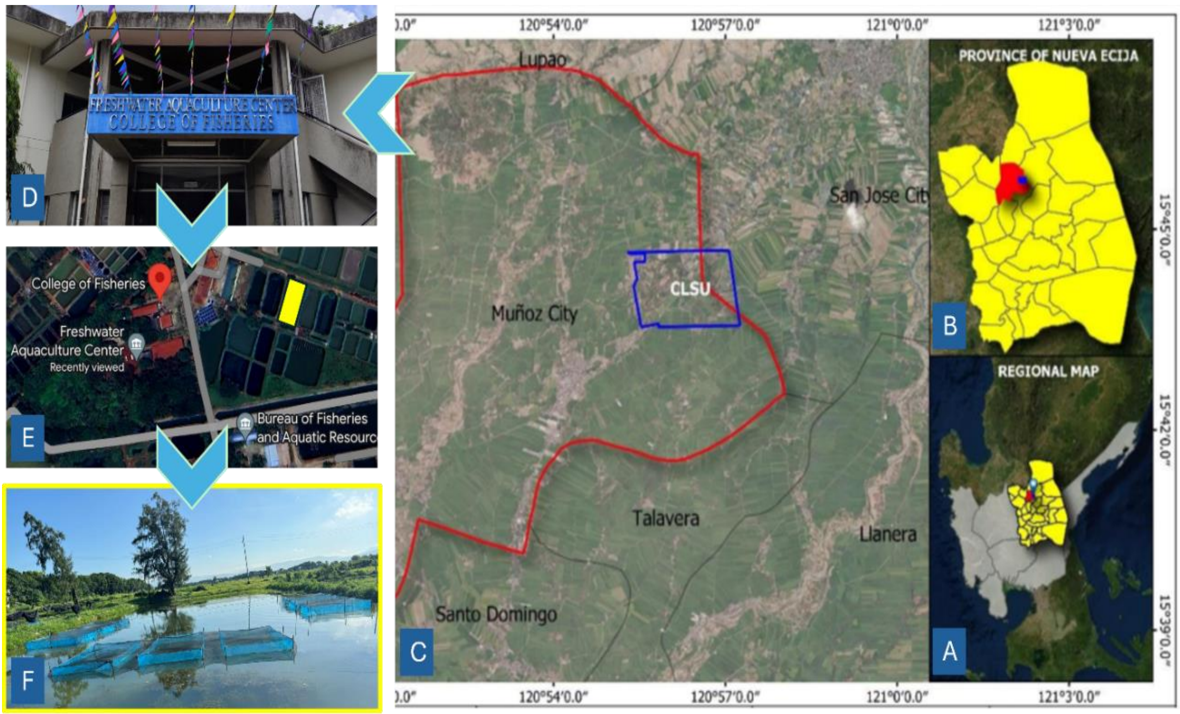


**Fig. 1. Location of the study site, illustrating the following: (A) the position of Nueva Ecija on the regional map, (B) the location of Muñoz City within Nueva Ecija, (C) the site of CLSU within Muñoz City, (D) the Freshwater Aquaculture Center within CLSU, (E) the specific experimental pond within the Center, and the (F) arrangement of experimental units within the pond**

**Fig. 2. Sex-reversed Nile tilapia fingerlings, size #22 during weighing prior to packing (left) and after packing (right)**

### 2.3 Stocking and Acclimatization Procedure

The time of stocking was 3:00-4:00 p.m. Water quality parameters such as temperature, dissolved oxygen (DO), and pH were recorded prior to stocking. One plastic bag was designated for each of the nine experimental units. The sealed plastic bags were allowed to float in the water for 15 minutes (Treatment 1), 25 minutes (Treatment 2), and 35 minutes (Treatment 3), respectively. The bags were then opened, and a cup of water was added to each bag every minute for the remaining five minutes of acclimatization.

### 2.4 Monitoring and Feeding

The tilapia fingerlings were fed and monitored for 15 days. Water quality parameters were recorded twice a day—once in the morning and once in the afternoon. Feeding practices adhered to those established by the FAC. This study exclusively used commercially available feed.

### 2.5 Fish Sampling

After 15 days, the fingerlings were collected from each experimental unit using a bamboo pole to lift the bottom, concentrating all the fingerlings at one end of the unit. They were then collected using a strainer and placed in small basins for weighing. The total weight of fingerlings in each experimental unit was recorded and manually counted for the computation of the survival rate. From each experimental unit, 50 representatives were collected for individual weighing and measurement of size for the Length-Weight Relationship analysis. A digital weighing scale was used for the weight measurements and a caliper for the measurement of total length. The following parameters were used to evaluate the growth performance of the fingerlings, feed conversion ratio, and survival rate:

Mean Weight Gain (g) = Final weight (g) – Initial weight (g)

Mean Length Gain (cm) = Final length (cm) – Initial length (cm)

Specific Growth Rate (%) = {(ln Final weight (g) – ln Initial weight (g)/Culture Days)}x100

Average Daily Growth (g/d) = Mean weight gain / Culture Days

Feed Conversion Ratio (FCR) = Total amount of feed given (g)/ Total Weight gain (g)

Survival rate (%) = (No. of Stocks Harvested/ Initial No. of Stocks) x100

As for the condition factor of the fish, it was estimated using Fulton's condition factor formula:

Condition Factor (K) = $(100^*W)/L^3$

Where, W is the weight in grams, and

L is the total length in cm

### 2.6 Water Quality Analysis

Water samples were collected from five different parts of the experimental pond before and after the rearing period. The water quality analyses conducted included measurements of alkalinity, hardness, nitrite, Total Ammonia Nitrogen (TAN), and phosphorus.

### 2.7 Statistical Analysis

The data on the different growth parameters, feed conversion ratio, survival rate, and condition factor were analyzed using Analysis of Variance (ANOVA). Comparisons of means for the water quality analyses before and after the rearing period were done using a paired two-sample t-test. Microsoft Excel's Analysis Toolpak was used for all the analyses at a 5% level of significance. Furthermore, this Microsoft Excel add-in was used for the determination of the *b* value for the length-weight relationship analysis.

## 3. RESULTS

### 3.1 Growth Performance

In Table 1, different growth parameters of the sex-reversed Nile tilapia fingerlings from the three treatments, such as mean weight gain, mean length gain, specific growth rate, average daily growth, and condition factor, were statistically compared. Length-weight relationship data, including the *b* values that reveal the growth behavior of the fish, were also presented. Moreover, the feed conversion ratio and survival rate in each treatment were presented.

The mean weight gain (MWG) of Nile tilapia fingerlings varied little between acclimatization periods. The MWG for T1 (20 minutes) was 0.58±0.01g, T2 (30 minutes) was 0.60±0.07g, and T3 (40 minutes) was 0.55±0.02g. These deviations were not significant, according to the statistical analysis, despite the slight differences.

There were slight variations in the mean length gain (MLG) between the three treatments. T2 had a gain of 0.74±0.13 cm, T3 had the lowest gain at 0.66±0.01 cm, and T1 reported an MLG of 0.72±0.05 cm. But like with MWG, the statistical analysis revealed that these variations were not statistically significant.

The specific growth rate (SGR) represents the fingerlings' daily growth efficiency. The daily SGR for T1 was 11.48±0.12%, for T2 it was a little lower at 11.15±0.45%, and for T3 it was somewhat higher at 11.32±0.23%. There were no appreciable variations in the SGR levels among the various treatments.

The average daily growth (ADG) showed very little change between the treatments. An ADG of 0.030±0.001 g/day was recorded by T1, 0.032±0.005 g/day by T2, and 0.029±0.001 g/day by T3. Based on a statistical analysis, it was concluded that there were no notable differences between the treatments.

### 3.2 Feed Conversion Ratio

For T1, the feed conversion ratio (FCR) was 1.10 ± 0.02; this means that, on average, 1.10 units of feed are needed by the fish to gain one unit of body weight. The best feed efficiency was indicated by the lowest FCR of the three treatments (see Table 1). In T2, the FCR marginally rose to 1.17±0.10. Since more feed is needed to gain the same weight as T1, this higher figure suggests a minor decline in feed efficiency. For T3, its FCR was 1.14±0.05, which was somewhat lower than T2 but higher than T1. Although FCR values varied numerically, these variations were not great enough to be deemed statistically significant in this study.

**Table 1. Summary of the growth parameters, food conversion ratio, condition factor, and survival rate (±SD) of sex-reversed Nile tilapia fingerlings acclimatized for 20, 30, and 40 minutes, and reared for 15 days**

| Parameters | Acclimatization Periods | | |
|---|---|---|---|
| | **T1 - 20 minutes (control)** | **T2 - 30 minutes** | **T3 - 40 minutes** |
| MWG (g) | 0.58±0.01$^a$ | 0.60±0.07$^a$ | 0.55±0.02$^a$ |
| MLG (cm) | 0.72±0.05$^a$ | 0.74±0.13$^a$ | 0.66±0.01$^a$ |
| SGR (% / day) | 11.48±0.12$^a$ | 11.15±0.45$^a$ | 11.32±0.23$^a$ |
| ADG (g / day) | 0.030±0.001$^a$ | 0.032±0.005$^a$ | 0.029±0.001$^a$ |
| *b* value | 2.71 | 2.43 | 2.66 |
| Growth Behaviour | negative allometry | negative allometry | negative allometry |
| $R^2$ value | 0.78 | 0.75 | 0.81 |
| K value | 1.66±0.04$^a$ | 1.72±0.15$^a$ | 1.69±0.04$^a$ |
| FCR | 1.10±0.02$^a$ | 1.17±0.10$^a$ | 1.14±0.05$^a$ |
| SR (%) | 86±2.75$^a$ | 83±4.86$^a$ | 84±3.23$^a$ |

**Values sharing the same superscript are not significantly different at P <0.05. Data are represented as means ± standard deviation*

### 3.3 Length-Weight Relationship and Condition Factor

In research on fish growth, the *b* value denotes the exponent in the length-weight relationship analysis and offers information about the fish's growth pattern [13]. The *b* values in this study were 2.71 for T1, 2.43 for T2, and 2.66 for T3 (may also refer to Fig. 3). When a fish exhibits isometric growth, its weight rises in direct proportion to its length (*b* value of 3) [14]. Negative allometry, defined as values less than 3, indicates that the fish is growing longer as it grows [15].

Across all treatments, the *b* values were less than 3, suggesting that the fish exhibited negative allometric growth. While T2 showed the lowest *b* value, indicating a more pronounced negative allometric growth compared to T1 and T3, the variances in *b* values throughout the treatments suggest small variability in growth patterns.

Furthermore, the strength of the correlation between the fish's length and weight is indicated by the $R^2$ value of the length-weight relationship analysis in this study. T1, T2, and T3 all had $R^2$ values of 0.78, 0.75, and 0.81, respectively (may also refer to Fig. 3).

For the condition factor, values were 1.66±0.04 (T1), 1.72±0.15 (T2), and 1.69±0.04 (T3). The statistical analysis revealed that there were no appreciable differences between these values as they were generally close to one another.

### 3.4 Water Quality

#### 3.4.1 Dissolved oxygen, temperature, and pH

The physicochemical parameters (dissolved oxygen, temperature, and pH) of water before stocking and during the conduct of the study are shown in Tables 2 and 3 to investigate if these parameters were within the optimal range of growth for Nile tilapia. Before the stocking of Nile tilapia fingerlings, dissolved oxygen (DO), water temperature, and pH were monitored. The DO levels in the three treatments have means of 11.53±0.015 mg/L for Treatment 1, 11.49±0.025 mg/L for Treatment 2, and 11.45±0.040 mg/L for Treatment 3. The DO levels are slightly different among the treatments but remain within a close range. The temperature level for the three treatments were also very close suggesting uniform thermal conditions across the treatments, with means of 34.5±0.012°C for Treatment 1, 34.3±0.064°C for Treatment 2, and 34.8±0.115°C for Treatment 3. The pH levels are slightly higher in Treatment 3, but all treatments are within a narrow range.

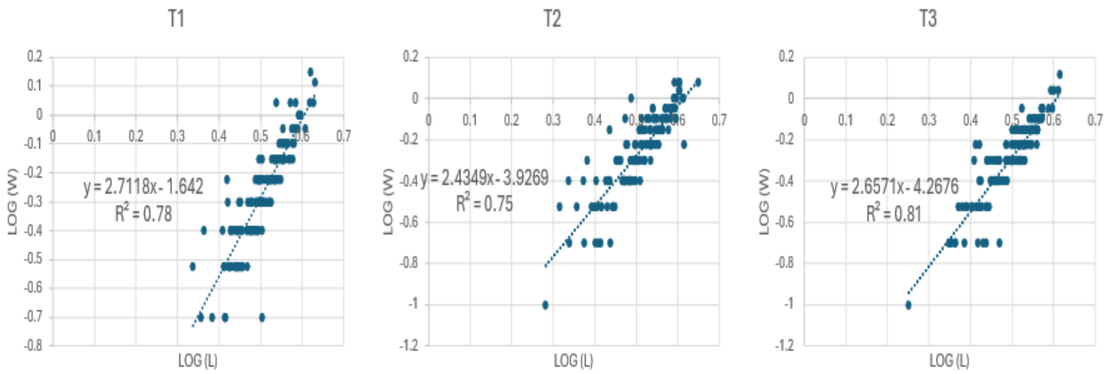


**Fig. 3. Linear regression of length-weight analysis of sex-reversed Nile tilapia fingerlings acclimatized for 20, 30, and 40 minutes, and reared for 15 days**

**Table 2. Summary on average (±SD) readings of dissolved oxygen, temperature, and pH before stocking the Nile tilapia fingerlings**

| Treatment | Dissolved Oxygen (mg/L) | Temperature (°C) | pH |
|---|---|---|---|
| 1 | 11.53±0.015 | 34.5±0.012 | 9.21±0.015 |
| 2 | 11.49±0.025 | 34.3±0.064 | 9.32±0.015 |
| 3 | 11.45±0.040 | 34.8±0.115 | 9.40±0.017 |

**Table 3. Summary on average (±SD) readings of daily recorded water quality parameters for 15 days**

| Treatment | Dissolved Oxygen (mg/L) | Temperature (°C) | pH |
|---|---|---|---|
| **AM** | | | |
| 1 | 7.52±1.16 | 31.04±0.99 | 9.47±0.23 |
| 2 | 7.40±1.15 | 31.10±0.99 | 9.40±0.32 |
| 3 | 7.43±1.14 | 31.15±1.00 | 9.43±0.31 |
| **PM** | | | |
| 1 | 9.38±0.49 | 32.40±1.32 | 9.31±0.44 |
| 2 | 9.36±0.49 | 32.39±1.29 | 9.32±0.43 |
| 3 | 9.35±0.49 | 32.35±1.26 | 9.30±0.47 |

During the conduct of the study, the DO levels ranged from 7.40±1.15 to 9.38±0.49 mg/L which shows increasing DO levels as the day progresses, the same can be observed for the temperature which recorded from 31.04±0.99 to 32.40±1.32°C. The pH was high during the conduct of the study ranging from 9.30±0.47 to 9.47±0.23 which was within the same range before the conduct of the study.

#### 3.4.2 Other physicochemical parameters recorded

The alkalinity, hardness, nitrite, TAN, and phosphorus investigated before and after the conduct of the study are listed in Table 4. The total alkalinity was significantly lower before the conduct of the study the same can be observed in the TAN and phosphorous. Total hardness has significantly lower values after the conduct of the study. The recorded nitrite is not statistically significant before and after the conduct of the study.

### 3.5 Survival Rate

With 86±2.75%, the control group (T1), acclimatized for 20 minutes, had the highest survival rate. The group that had a 40-minute acclimatization time (T3) had a survival rate of 84±3.23%, whereas the group that underwent a 30-minute acclimatization period (T2) had the lowest survival rate at 83±4.86%. Although there were slight differences in the survival rates of sex-reversed Nile tilapia fingerlings throughout various acclimatization times, they were not statistically significant ($P < 0.05$).

## 4. DISCUSSION

### 4.1 Growth Performance

No significant differences were observed in the different growth parameters (MWG, MLG, SGR, ADG) measured among the treatments. This result could be due to the exposure of the tilapia fingerlings to the same environment and water quality parameters. Several studies have shown that culturing tilapia in varying environments with different water quality parameters can yield different growth performances. For example, in the study by Moses et al. [16], significant differences in growth performance were observed in tilapia reared in fresh and brackish waters. Moreover, Santos et al. [17] stated that tilapia strains raised in varying temperatures exhibit different growth performances.

The results on growth performance could be supported by the findings on the feed conversion ratio (FCR). The FCRs of the treatments in this study showed little variation, indicating that feed efficiency did not greatly differ among the treatments. Similarly, Rahman and Arifuzzaman [18] highlighted in their study that a lower FCR was associated with better growth performance in fish. Rodde et al. [19] also stated that faster-growing fish had a better (lower) FCR. Additionally, the obtained FCR values were very low or very close to 1.00 (ranging from 1.10±0.02 to 1.17±0.10), which is lower than the expected FCR values of 1.5 to 2.0 for tilapia as stated by Watanabe et al. [20].

### 4.2 Length-Weight Relationship and Condition Factor

Since *b* values of the treatments were less than 3 (T1: 2.71, T2: 2.43, T3: 2.66), these results show that the fingerlings had negative allometric growth. This means that the fish becomes slender as it increases in weight [15]. These results coincide with the study by Saura et al. [21], which obtained a *b* value of 2.603 from Nile tilapia reared in ponds. Negative allometric growth of Nile tilapia, with *b* values ranging from 2.88 to 2.99, was also recorded in the study of

Wainaina et al. [22]. Moreover, $R^2$ values (T1: 0.78, T2: 0.75, T3: 0.81) obtained in the linear regression analysis (Fig. 3) indicate that weight and length have a strong positive correlation. This type of correlation was also reported in the study of Saura et al. [21] wherein $R^2$ values of 0.60 and 0.88 were obtained.

Differences in the obtainable *b* value may be due to different ecological conditions [14]. The negative allometric growth in this study may not be attributed to feeding because proper feeding management was strictly observed, following the practice done by the FAC, and obtained FCR values were low (better). One possible factor to consider was space. The fingerlings were reared in hapa nets that possibly became smaller as they grew during the 15-day rearing period. This assumption is supported by Diallo et al. [23], wherein it was stated that fish growth can be adversely affected by social interactions, such as competition for food and/or space. Consequently, larger stocking densities raise stress levels, which in turn raises energy needs and lowers growth rates and food consumption. Another thing to consider was the findings from Li et al. [24], wherein it was determined that most omnivorous fish, which includes tilapia, tend to have a growth that falls somewhere between herbivorous fish (tends to be shorter and fatter) and carnivorous fish (tends to be longer and thinner).

Condition factor is a recorded measure of fish welfare in their natural habitat (wild fisheries and aquaculture), according to several investigators [25-31]. Put differently, Nehemia et al. [14] suggested that it serves as a gauge of many biological and ecological aspects concerning their feeding circumstances. Furthermore, according to Jisr et al. [32], the good growth condition of the fish is deduced when the K value is equal to or greater than 1, while the organism is in poor growth condition when the K value is less than 1.

Since K values in this study were more than 1.00, it can be concluded that the fingerlings were in good condition. It can also be assumed from these values that the fingerlings made good use of their feeding source as reported by Bagenal and Tesch [33].

## 4.3 Water Quality

### 4.3.1 Dissolved oxygen, temperature, and pH

The dissolved oxygen in the pond before the conduct of the study was greater than 10 mg/L which is high or the pond is supersaturated as stated by Boyd [34]. The average dissolved oxygen (DO) levels of all the treatments range from 7.40±1.15 mg/L to 9.38±0.49 mg/L. According to Riche and Garling [35], the ideal DO for the optimum growth of tilapia is above 5 mg/L. Fish growth and yields are higher in ponds with high DO concentrations as stated by Green [36]. In the current study, the pond DO was above the optimum DO levels required for Nile tilapia growth.

The temperature was high before the conduct of the study which ranged from 34.3±0.064 to 34.8±0.115°C, which was beyond the optimal range of temperature range according to Leonard and Skov [37] which they concluded to be 20.2 to 31.7°C although other literature states that the preferred temperature ranges of Nile tilapia are from 31 to 36 °C [38,39]. While Ngugi et al. [40] gave a range of between 20 and 35 °C as ideal for tilapia culture, the recorded temperature in the study is within this range. In the study conducted by Pandit and Nakamura [41] they concluded that water temperatures ranging from 27-32°C seemed to be the most effective for rearing Nile tilapia juveniles and fries, and higher temperatures (>32°C) resulted in slow growth, reduced feeding efficiency, and increased mortality. This study also demonstrated the possible impact of global warming on natural fishery resources. The study conducted recorded a 31.04±0.99 to 32.40±1.32°C temperature range during the 15-day duration of the study. The upper limit was recorded at noon time which may still be tolerable for Nile tilapia as stated in different literature above.

**Table 4. Mean of water analyses (±SD) on alkalinity, hardness, nitrite, TAN, and phosphorus before and after the conduct of the study**

| Period | Total Alkalinity (mg/L) | Total Hardness (mg/L) | Nitrite (mg/L) | TAN (mg/L) | Phosphorus (mg/L) |
|---|---|---|---|---|---|
| Before | 139.60±3.05$^a$ | 90.89±6.73$^a$ | 0.02±0.001$^a$ | 0.16±0.04$^a$ | 0.017±0.003$^a$ |
| After | 126.20±2.59$^b$ | 83.28±2.69$^b$ | 0.05±0.022$^a$ | 0.32±0.03$^b$ | 0.021±0.001$^b$ |

**Values having different superscripts are significantly different at P <0.05. Data are represented as means ± standard deviation*

The recorded average pH in the study ranges from 9.30±0.47 to 9.47±0.23 which is within the ideal range for fish culture as stated by Santhosh and Singh [42] in which the suitable pH range for fish culture ranges between 6.7 and 9.5. The study by Bryan et al. [43] states that most fish would do better in ponds with a pH near 7.0 and that ponds with a pH less than 6.0 may result in stunting or reduced fish production.

The results of the water quality analysis in this study were found to be within ideal and acceptable ranges, except for pH. According to Das [44], fish are highly sensitive to environmental changes, and any alterations can induce stress. The greater and more sudden the changes, the more significant the stress on the fish. Thus, the pH levels in this study might have contributed to fish mortality. Water with a pH of 4 to 6.5 or 9 to 11 makes fish more stressed and practically guaranteed to die; therefore, a pH of 7 to 8.5 is generally appropriate for biological productivity [45]. In addition to this, it was also studied that a pH of 9 significantly reduces the mean body weight gain and SGR of Nile tilapia fingerlings compared to pH levels of 6 and 7 [46].

Observations showed that the pH level in the experimental pond was consistently around 9.5, a level also recorded in other nearby ponds. Researchers suspect that this could be related to pond preparation. At higher pH levels, where removing plants and microalgae is not feasible [47], adding small amounts of easily decomposable organic matter may serve as an effective preventative measure. The decomposition of this organic matter produces carbon dioxide, which can help lower and stabilize the pH over time.

#### 4.3.2 Other physicochemical parameters recorded

The water quality parameters—total alkalinity, total hardness, nitrite, Total Ammonia Nitrogen (TAN), and phosphorus—were measured in the laboratory both before stocking Nile tilapia fingerlings and after the final data gathering.

The total concentration of bases in pond water, comprising carbonates, bicarbonates, hydroxides, phosphates, borates, dissolved calcium, magnesium, and other substances, is measured as alkalinity, which represents the water's resistance to pH changes [48]. It is determined by the quantity of acid (hydrogen ion) that the water can neutralize (buffer) before reaching a specific pH level, referred to as the "buffering capacity" [49]. Furthermore, the water's total alkalinity indicates how much inorganic carbon is present. Since inorganic carbon is required for photosynthesis, alkalinity directly impacts primary production and fish supply [50]. In the present study, the values of alkalinity obtained before and after the study were 139.60±3.05 to 126.20±2.59, which were within the acceptable range for fish growth. Studies have shown that total alkalinity between 75 to 200 mg/L is the acceptable range while 25-100 mg/L is the desirable range [48]. Bhatnagar et al. [51] noted that an alkalinity level of 300 ppm is undesirable due to the non-availability of $CO_2$.

Water hardness is an important factor in fish culture as it measures the concentration of calcium and magnesium in water samples [52]. The water hardness readings which were 90.89±6.73 before stocking and 83.28±2.69 after the rearing period were found to be within the recommended range of 30 to 180 mg/L [60]. According to Bhatnagar et al. [51], hardness levels below 20 ppm may stress fish, while between 75 and 150 ppm is ideal for fish culture, and over 300 ppm is fatal to fish life since it raises pH and prevents fish from getting nutrients.

Nitrite, an intermediate product in the oxidation of ammonia nitrogen to nitrate by nitrifying bacteria in soil and water, is substantially more hazardous than nitrate [53-54]. Exposure to nitrite inhibits respiration, producing gill lesions and edema in fish's skeletal muscles [55]. Rebouças et al. [56] reported that the optimum concentration of nitrite for tilapia in freshwater is 0.3 mg/L. Similarly, Stone & Thomforde [57] suggested that nitrite concentration ranging from 0-1 mg/L in aquaculture is ideal. Further, a range below 4 mg/L is still acceptable. Higher levels may significantly reduce the growth performance of a fish [58]. The mean nitrite of the site before and after fish stocking was 0.02±0.001 mg/L and 0.05±0.022 mg/L, respectively. Although the results showed no significant difference ($P<0.05$), the slight increase in the amount of nitrite may be caused by the pond's oxygen level gradually dropping as a result of growing fish and increasing stocking density. The nitrogen-containing organic matter thus starts to break down, releasing ammoniacal and nitrite nitrogen, which will then undergo oxidation to produce nitrate-nitrogen [59] [60].

The major sources of ammonia in aquaculture ponds are fertilizers and feeds, and problems with high ammonia are most common in feed-based aquaculture [61], as the current study used a pond without prior fish cultivation it does not have the source needed to acquire high ammonia levels, this indicates that when the fish was placed in the pond it caused a slight increase in the ammonia levels of the pond. El-Sherif and El-Feky [62] cited that, at concentrations of 7.1 mg/L, ammonia is toxic to tilapia, while at concentrations as low as 0.1 mg/L, it has the opposite effect. It was calculated that the optimal concentrations are less than 0.05 mg/L. The TAN values were 0.16±0.04 mg/L before stocking and 0.32±0.03 mg/L after the rearing period. Throughout the culture period, the readings were constant, as evidenced by the small standard deviations.

Fish require phosphorus, an element that is necessary for all living things, for optimal growth, feed efficiency, bone development, and the maintenance of acid-base homeostasis [63]. It can be found in waterbodies in both dissolved and particulate forms. However, high phosphorus release from aquaculture operations can cause water eutrophication, algal blooms, and other detrimental effects on aquaculture farms [64]. The phosphorus concentrations of the site before and after the stocking were 0.017±0.003 mg/L and 0.021±0.001 mg/L, respectively. According to Boyd [65], the typical range of phosphorus for surface waters is 0.005 to 0.5 mg/l. It can be observed that the phosphorus concentrations in the fishpond in the study were at acceptable levels and the reduced standard deviation following the rearing period suggested that the phosphorus levels were more stable.

### 4.4 Survival Rate

The survival rate of fingerlings in this study is similar to Saad & Habashy [66], and Riberio et al. [67], where the survival and growth rates for tilapia at early stages usually fall between 70% and 90%. This means that the methods used in this study seem to be effective, as they produce survival rates comparable to the previous studies conducted.

The results of the water quality analysis in this study were found to be within ideal and acceptable ranges, except for pH. According to Das [44], fish are highly sensitive to environmental changes, and any alterations can induce stress. The greater and more sudden the changes, the more significant the stress on the fish. Thus, the pH levels in this study might have contributed to fish mortality. Observations showed that the pH level in the experimental pond was consistently around 9.5, a level also recorded in other nearby ponds. Researchers suspect that this could be related to pond preparation. At higher pH levels, where removing plants and microalgae is not feasible [68], adding small amounts of easily decomposable organic matter may serve as an effective preventative measure. The decomposition of this organic matter produces carbon dioxide, which can help lower and stabilize the pH over time.

When 14 different species of freshwater fish were experimentally exposed to naturally alkaline lakes (pH 8.5–10.8) in Nebraska, USA, it was discovered that none of the species could survive for longer than 24 days, and the majority could only survive for 4–22 hours [69]. A similar study conducted by Sahu & Datta [39] showed that the pH of water above 8.2 and below 5.71 may cause serious stress to fish (*Trichlgaster lalius*) in captive conditions and mortality may cross 50%.

Optimal acclimatization enhances their physiological responses to changing temperatures, as highlighted by Leonard & Skov [37]. High survival rates of fingerlings during the study were supported by research showing that survival rates can exceed 96% in low-salinity biofloc systems with stocking densities of up to 1000 fish/m³ [69]. This indicates that high survival rates are achievable under optimal conditions and proper acclimatization practices. Additionally, Nehemia et al. [70] found that during acclimatization in freshwater fishponds, Tilapia zillii and *Oreochromis urolepis* fingerlings showed survival rates ranging from 89% to 100%, respectively. It was also indicated in their study that this high yield may be because of the environmental parameters being suitable for rearing the two species.

Various factors may contribute to the causes of mortality. Since fingerlings are vulnerable, their physiological and physical requirements during travel are quite important [71]. Obirikorang et al. [72] found that post-transport mortality was significantly higher in fish transported over rough roads, highlighting mechanical stress as one factor.

Also, mortalities during the study may be attributed to the observed presence of predators like birds which prey on fish. The presence of these predators reduces the survival of the stock.

## 5. CONCLUSION

Sex-reversed Nile tilapia fingerlings could be acclimatized from 20 to 40 minutes without significant differences in growth performance and survival rates. However, to reduce time consumption, 20 minutes is still a good option. The application of this result can also be considered for other aquaculture species aside from tilapia. This finding is particularly beneficial for commercial aquaculture operations, where time efficiency and cost reduction are crucial.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during the writing or editing of manuscripts.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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