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Assessment of Embryonic and Larval Development of Nile Tilapia under the Traditional and Re-Circulatory Thermostatic System in Relation to Climatic and Water Quality Variations

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Abstract: Embryonic and larval development of tilapia (*Oreochromis niloticus*) is very vulnerable to climate change. This study was conducted for an assessment of the embryonic and larval development of Nile tilapia in traditional hatchery and re-circulatory thermostatic systems. Daily changes in embryonic and larval development were measured through microscopic observation and image analysis in the laboratory. Climatic data and water quality parameters were measured every day using appropriate devices. Water temperature was varied with room temperature at the traditional hatchery system while it was maintained at 28.50 °C in the re-circulatory thermostatic system. A total of 200 unhatched eggs were stocked in every three trays of both systems. The egg diameters of the gastrula, segmentation, and pharyngula stages were measured at higher ($2261.47 \pm 81.66 \mu\text{m}$, $2646.24 \pm 17.98 \mu\text{m}$, and $2710.90 \pm 16.60 \mu\text{m}$) in the re-circulatory thermostatic system than in the traditional hatchery system ($2261.07 \pm 81.52 \mu\text{m}$, $2645.47 \pm 18.24 \mu\text{m}$, and $2710.01 \pm 16.45 \mu\text{m}$), respectively. For both systems, egg colors, egg size, black pigments, germinal ring, eye shape, tail, and heartbeat were determined through microscopic observation. Higher hatching and survival rates were found under the re-circulatory thermostatic system (95% and 97%) than under the traditional hatchery system (85% and 81%). About 6 h less hatching time was required under the re-circulatory thermostatic system than under the traditional system. At the end of 30 DAH (Days After Hatching), larval length and weight under the re-circulatory thermostatic system were found to be higher ($15.736 \pm 0.424 \text{ mm}$ and $0.0528 \pm 0.004 \text{ g}$) than under the traditional hatchery system ($15.518 \pm 0.415 \text{ mm}$ and $0.050 \pm 0.004 \text{ g}$), respectively. Larval growth patterns for both systems were found to have an exponential trend. PCA analysis revealed that two components were identified, one primarily associated with morphometric characteristics and the other with climatic and water quality parameters. These components showed that there were several interrelationships between the morphometric changes and the climatic and water quality parameters. The characteristic changes of larval development under the re-circulatory thermostatic system and the traditional hatchery system were found to be remarkably similar except for some deformities denoted under the traditional hatchery system. The changes of yolk sac, body pigmentation, dorsal and caudal fin shape, eye size, and head length and width were determined from 1 DAH to 30 DAH. After absorbing the yolk sac, ready-made feed was provided. The water temperature was varied from 30.50 °C to 35.50 °C in the traditional hatchery system. The highest air temperature and humidity were 33.87 °C and 69.94% while the lowest were 29.63 °C and 45.62%, respectively, in the traditional hatchery system. There has been no such comprehensive comparative study on hatchery production in Bangladesh, and therefore, further research might be carried out on broader aspects. This research would be highly beneficial for improving seed production at the tilapia fish hatchery level in the country.



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Keywords: climate change; thermostat; embryonic development; larval development; tilapia; Bangladesh

1. Introduction

Aquaculture is one of the greatest potential sources of fish production in Bangladesh [1,2]. A significant percentage of aquaculture production is dependent on hatchery-based seed production and suppliers [3]. This dependence has been growing day by day because of high demand for aquaculture production and the ever-decreasing trend of high-quality seed collection from natural sources due to ecological and environmental issues [4]. Due to lack of biological expertise and technological knowledge, as well as environmental issues and practicing poor hatchery management protocol, there are still concerns of quality seed production in numerous fish hatcheries in Bangladesh [5].

Cichlids are highly prosperous family of teleost fish [6] that have been successfully raised under culture conditions [7]. Among them, Nile tilapia (*Oreochromis niloticus*) is considered to be a successful intensive aquaculture species in the country due to its relative ease in culture, high market demand, and sustainability to local climate conditions and, consequently, is a successful commercial species worldwide [8,9]. In particular, *O. niloticus* is a scientifically outstanding fish due to its complex social behavior, including parental care, and coloration [6]. It has been an important source of carbohydrates, proteins, fats, vitamins, minerals, iron, and calcium, as well as being noted for its eco-friendly aquaculture [10].

It was observed that tilapia egg production is significantly influenced by water temperature. The egg production of tilapia decreased with the increase of water temperature after the optimum level [5]. Under the ideal temperature, the maximum number of tilapia eggs was produced [11]. The thermo-sensitive period in *O. niloticus* was reported to be 10 to 30 days after fertilization. In their natural environment, *O. niloticus* seeds may be exposed to temperatures that are higher than the optimal temperature for growth [12]. Likely, hatchery-based *O. niloticus* seed production has been reported to be affected by temperature fluctuations in Bangladesh [3]. Habitually, temperature fluctuation is one of the most decisive environmental factors affecting the maturation and embryonic development of *O. niloticus* eggs [8,13].

Determination of the optimal temperatures for egg incubation, as well as for proper embryonic development, are necessary to maximize *O. niloticus* seed production [14]. The development of muscle growth, certain morphological features, hatching rate, and larval behavior are all influenced by temperature [13,15,16]. Changing temperature interferes with various physiological and cellular processes including gonadal maturation, spawning, and subsequent embryonic and larval development [15]. Thus, fluctuating temperature affects spawning and feeding migration, as well as the availability of natural foods, and ultimately invites several pathogenic organisms [17]. All these together result in reduced tilapia seed production in the summer season of Bangladesh [5]. To ensure quality seed production of *O. niloticus* in the hatcheries of Bangladesh, temperature fluctuation should be minimized and adaptive measures towards climate change should be developed to secure and strengthen in intensive aquafarms [8]. Therefore, it might be possible to improve the embryo and larval growth performances of *O. niloticus* by the manipulation of egg incubation temperature using the re-circulatory thermostatic system at the hatchery level in Bangladesh [18].

As the fish is an aquatic animal, it is strongly influenced by fluctuating water temperature [19]. Biologically a fish's life begins with male and female gametes. As quickly as the eggs are fertilized by sperm, the zygote is formed and embryonic development starts and continues up to hatching. Changes of characteristics in embryonic and larval development are very important to understand the biological features of different stages of development [20]. It was reported that important internal and external characteristics of the embryonic and larval development of *O. niloticus* occur under different stages named

zygote, cleavage, blastula, gastrula, segmentation, and pharyngula [6]. The development of the egg in the ovary is maternally derived and predetermined, but the genetic makeup of the egg is decided at the moment of fertilization [21]. Embryonic and larval development is critically affected by temperature, pH, dissolved oxygen, salinity, ammonia, etc. [15,22–25]. Fish embryos and larvae are more sensitive to changes in pH than juveniles and adults [15]. The effect of temperature fluctuation disrupts the immune system of fish which causes various diseases in the fish and ultimately reduces fish production [26,27]. Alongside biological factors, there are physical and chemical parameters known to affect egg development [13]. Some of the constraints against eggs development are still unknown [13]. A number of studies reported that temperature influenced egg development and hatching in *O. niloticus*, *Tilapia zillii*, common carp, *Cyprinus carpio*, and *Gadus morhua* [13]. A previous study stated that temperature is crucial for both the egg production and fry growth of *O. niloticus* in hatchery systems [11]. The impact of water temperature on embryonic, larval, and juvenile growth performances has been documented for many species [13,14,16,25,28,29].

Suitable environmental conditions are required for the best growth performance of *O. niloticus*. Due to excessive temperature, the egg production and larval growth of *O. niloticus* are hampered and, subsequently, hatchery production decreases [11]. With changes in temperature, the feeding and growth rates of *O. niloticus* are reduced at 20 °C or below, and feeding, growth, and egg laying are stopped when the water temperature falls below 19 °C [11]. A study stated that the timing of the embryonic development of *O. niloticus* is directly influenced by egg incubation temperature, which helps to determine the hatching rate [11]. The embryonic development and hatching rate of *O. niloticus* are also sped up at hot temperatures and slowed down at low temperatures [15]. Through a previous study, it was observed that the prolonged exposure of *O. niloticus* to high temperatures (above 35 °C) while undergoing a masculinizing treatment may significantly reduce their survival and growth [29]. The influence of temperature stress persists throughout the life cycle of the eggs and larvae that survive overcoming the temperature impression [30]. Moreover, it has also been reported that the manipulation of incubation temperature at the embryonic, larval, and juvenile stages stimulates growth-related gene expression which results in better growth performance of *Anabas testudineus* [18]. In thermostatic experimental studies, it has been reported that temperature plays an important role on the larval development of *O. niloticus* and the best hatching and larval development was observed at an optimum temperature range of 25–29 °C. Subsequently, an increase in water temperature (from 30–33 °C) significantly decreased the larval development of tilapia [11]. Several studies reported that tilapia stops spawning and physical deformity increases at temperatures lower than 24 °C and higher than 34 °C, respectively [8,31,32]. However, *O. niloticus* larvae reared under the re-circulatory thermostatic system demonstrated better growth and displayed a more rhythmic correlation of digestive enzymes and genes with their meal time than larvae reared at a constant temperature of 28 °C [33].

According to the scientific literature, there has been progress in developing high-resolution digital microscopes that replace analog microscopes [34,35]. These digital microscopes come with image-processing features that enable users to capture, store, and manipulate the digital images of objects. They can improve the visualization of biological objects and perform various functions based on user requirements.

In Bangladesh, no thorough study has ever been conducted with a focus in such a way on tilapia hatchery production. As dependency on hatchery-based seed production is increasing day by day, the application of the re-circulatory thermostatic system in the embryonic and larval development of *O. niloticus* might be helpful to increase seed production in adverse climatic situations during summer and even until winter in Bangladesh. That is why the present study broadly aimed at an assessment of the embryonic and larval development of tilapia (*O. niloticus*) in the traditional hatchery system and the re-circulatory thermostatic system for sustainable fish hatchery production.

2. Materials and Methods

2.1. Ethical Statement

This study included sampling of fish from the pond and hatchery, among other activities. Animal scientific procedures were rigorously followed during these activities with prior approval by the Animal Welfare and Ethics Committee of the Bangladesh Agricultural University (BAU) (Ref.no. BAURES/ESRC/FISH-11/2022).

2.2. Study Area

This study was carried out at the indoor laboratory named Laboratory of Climate Research for Fishes and in the pond near the laboratory under BAU's Faculty of Fisheries in Mymensingh, Bangladesh (Figure S1).

2.3. Collection and Domestication of *O. niloticus* for Gonadal Maturation

About 300 mature *O. niloticus* were collected from Asia Scientific Hatchery and Nursery, Dhala, Trishal Upazila of Mymensingh district, Bangladesh, in early June 2021, and they were acclimatized for 24 h in fiber tanks before stocking. Average length, weight, and age of brood fishes were 28.97 cm, 509.33 g, and 2 years, respectively. The fish were then stocked (100 fish of 1:1 sex ratio) at three previously prepared hapas ($2.5 \times 2.5 \times 2.5 \text{ m}^3$ each) placed in the earthen pond ($18 \times 14 \times 1.3 \text{ m}^3$) in front of Aquaculture System Laboratory, BAU, equipped with inlet–outlet and water exchange facilities. In the hapas, they were reared for about 6 months until maturation of the gonad. They were fed with commercial feed containing 35% crude protein, twice a day, at 5% of their body weight. The gravid females of *O. niloticus* in each hapa were monitored twice a week. Water quality parameters of the hapas and pond, such as temperature, DO, pH, and NH_3 , were checked weekly to ensure that they remain within optimum level.

2.4. Collection, Rearing of Eggs and Larvae of *O. niloticus*

Egg spawning and fertilization of tilapia happened naturally in the hapa. The female tilapia in the hapa were monitored twice a week to see if they were carrying fertilized eggs in their mouths. Unhatched eggs were collected from the mouth of the female and then transferred to the traditional hatchery system as treatment one (T_1) and to the re-circulatory thermostatic system as treatment two (T_2) for further incubation and rearing of eggs and larvae. Both systems were previously set up in the Laboratory of Climate Research for Fishes (Figure S2). Each treatment had three replications, such as R_1 , R_2 , and R_3 , respectively. The water temperature in the traditional hatchery system (T_1) was not fixed and varied according to changes in the room temperature. However, the water temperature in the re-circulatory thermostatic system (T_2) was kept constant at $28.5 \text{ }^\circ\text{C}$, which was considered a suitable temperature for tilapia seed production during the entire study period [11]. A flow rate of maximum 8500 L/H was maintained for re-circulating water within the system, and UV light was utilized to decrease the proliferation of undesired organisms. Room temperature in the re-circulatory thermostatic unit was regulated by the air-conditioning system, ensuring that it remained at approximately $25 \text{ }^\circ\text{C}$. Subsequently, embryonic and larval development of *O. niloticus* was examined in both systems up to the end of 30 days. Supplementary Figure S2 represents the design of the traditional and re-circulatory thermostatic systems for the embryonic and larval development of *O. niloticus*. The system was diligently monitored physically at least three times per day to ensure its proper functioning throughout the study period. In addition, indoor operating systems were monitored by closed circuit television (CCTV) camera.

2.5. Observation of Embryonic Developmental Stages of *O. niloticus*

At least ten unhatched eggs were randomly collected from the hatching trays of each treatment and examined immediately under a compound microscope (Olympus CX 43) equipped with a camera (Olympus EP 50). Collected eggs were correctly positioned on the slide, placed under the microscope, and examined at a $4\times$ magnification. Changes

in egg diameters and other physical characteristics, such as egg body color, shape, and the presence of black pigmentation on the inside embryo, were examined and measured every four-hour interval by the image-analyzing software EP view (Olympus Corporation, Tokyo, Japan) until the hatching began. During embryonic development, the present study considered gastrula, segmentation, and pharyngula stages only and the characteristic changes of these stages were described under the embryonic development progress. Each stage was observed under the microscope, and photographs were captured and examined closely for the defining characteristics. If over 50% of these characteristics were present and met the criteria, the corresponding stage was deemed to have been successfully identified.

2.6. Determination of Hatching and Survival Rates of *O. niloticus*

As a way to measure and compare the progress of the entire process, both systems were evaluated in terms of hatching and survival rates. Hatching rate (%) was counted for three trays of both systems separately at the end of a completed hatching. Alongside microscopic observation, the eggs hatching was also observed in a very keen eye observation using a Petri dish. The time required for a complete hatching was measured for both systems. Hatching and survival rates were calculated for each treatment with the following formulae:

$$\text{Hatching rate (\%)} = \text{number of hatched larvae} / \text{number of fertilized eggs} \times 100 \quad (1)$$

$$\text{Survival rate (\%)} = \text{number of live larvae} / \text{number of hatched larvae} \times 100 \quad (2)$$

2.7. Observation of Larval Development and Growth Parameters of *O. niloticus*

After three days of post-hatching (3DPH), larvae were fed with hard boiled chicken egg yolk mixed with water for three days for four times per day. Subsequently, powdered supplementary feed (35% protein) was used to feed the larvae up to 30 days in all treatments. At least 10 larvae were collected from each treatment and kept into 70% ethanol solution for further study. Within one hour after preservation, the next study of larvae was completed under microscope so that any morphological damage did not occur. A similar method for preservation was applied by a previous study for embryonic development [20]. A DM Wifi microscope (Android based portable wireless digital microscope) was used to measure larval development. The weight of every ten larvae was measured using electronic precision balance (FSH, A&D Store, Wood Dale, IL, USA) at a division range of 0.001 g in the laboratory. The length of larvae was measured using “ImageJ” (Java based image-processing program, version 2.1.4.7i1) software similarly used in a previous study [33]. From the first day of larval development, length, weight, and other changes were measured at the same time every day for the next 30 days. The consolidated changes were presented every five days at intervals of larval development because some internal and external changes were not prominently found to be different every day. For each observation, the average length and weight were calculated, as well as the standard error (\pm SE). Percent of Length–Weight gain and specific growth rate were calculated as following formulae:

$$\text{Percent length gain} = (\text{Mean final length} - \text{Mean initial length}) / \text{Mean initial length} \times 100 \quad (3)$$

$$\text{Percent weight gain} = (\text{Mean final weight} - \text{Mean initial weight}) / \text{Time difference} \times 100 \quad (4)$$

$$\text{Specific growth rate} = (\text{Log } W_2 - \text{Log } W_1) / T_2 - T_1 \times 100 \quad (5)$$

where, W_2 is final weight, W_1 is initial weight, and $T_2 - T_1$ is time difference.

2.8. Determination of Climatic Variables and Water Quality Parameters in Traditional Hatchery and Re-Circulatory Thermostatic Systems

Climatic variables like air temperature and humidity were measured using appropriate device (SMART Sensor AR 867) every day under the traditional system. Water quality parameters like water temperature, dissolved oxygen (DO), pH, and ammonia were measured every day using the digital thermometer (SMART Sensor AR 867), DO

meter (Lutron DO-5509), pocket-sized pH meter (pH-107), and ammonia test kit, respectively, for both systems. The daily average values of climatic variables and water quality parameters were measured during the study period. Water temperature was maintained at 28.50 °C as a suitable level for embryonic and larval development in the re-circulatory thermostatic system. The fluctuation of water quality parameters was measured in the traditional hatchery system in relation to the effect of climatic variables during the study period. The climatic variables and water quality parameters were compared and presented based on their highest, lowest, and average values over a period of 30 days.

2.9. Relationship of Climatic Variables, Water Quality Parameters and Morphometric Change of *O. niloticus* Larvae under Traditional Hatchery System

The relationships among the climatic variables, water quality parameters, and morphometric changes of tilapia larvae from 5 DAH to 30 DAH were analyzed under the traditional hatchery system. Due to the fluctuation of both climatic variables and water quality parameters in the traditional system during the study period, the objective of this analysis was to assess the extent of the relationships between these variables, as well as their correlation with morphometric characteristics of tilapia. The images of tilapia larvae were captured using the DMWifi microscope to determine and observe what changes occurred in larval development under the traditional system. Under this system, ten larvae were randomly collected from three replications each day. The typical Truss Morphometric Network (TMN) system was applied to determine morphometric characteristics [36] using analysis (Figure S3). Eleven morphometric characteristics, namely total length (ad), standard length (ac), body weight, body width (ef), head length (ag), head width (gh), caudal fin length (cd), caudal fin width (jl), dorsal fin back to caudal top (kl), anal fin to caudal bottom (ij), and eye area were measured daily during the study period (Figure S3). Principal component analysis (PCA) was performed to categorize the morphometric characteristics and to what extent the characteristics are changed due to water quality parameters as well as by climatic factors. The PCA equation is as follows:

$$PC_i = a_{1i}V_1 + a_{2i}V_2 + \dots + a_{ni}V_n \quad (6)$$

where PC_i is the principal component i , and a_{ni} ($n = 1 \dots n$) is the loading (correlation coefficient) of the original variable V_n [37].

A degree of subjectivity regarding the number of components was present in the PCA extraction. The standard stopping rule for determining the number of components is to stop when the eigen value drops below 1 because eigen values have standardized variances with mean 0 and standard deviation 1. Thus, components with an eigen value < 1 are considered unimportant and were therefore excluded; however, components with an eigen value > 1 are considered important and were therefore retained [38]. This analysis was performed using MS excel 2016, SPSS (Statistical Package for Social Science) version 23 (IBM SPSS Statistics 23) and Minitab (version 19).

2.10. Statistical Analysis

All the data were compiled and saved in a computer on a daily basis during the study. The means of every significant calculation are presented with Standard Error (\pm SE). Different statistical tests were applied to establish the inferential statements. After analysis, the results were presented by different parameters and key aspects of the experiments. A descriptive statistical analysis was carried out to explain every stage of embryonic and larval development and measuring climatic variables and water quality during the study. All the statistical analyses were carried out using MS Excel 2016, SPSS (Statistical Package for Social Science) version 23 (IBM SPSS Statistics 23), Minitab 19 and "ImageJ" software (Java based image-processing program, version 2.1.4.7i1).

3. Results

3.1. Observation of Embryonic Development Stages of *O. niloticus*

The average egg diameters of the gastrula stage under the traditional hatchery system and the re-circulatory thermostatic system were $2261.07 \pm 81.52 \mu\text{m}$ and $2261.47 \pm 81.66 \mu\text{m}$, respectively (Figure S4). Initially, the eggs were yellowish in color and oval in shape (Figure 1a(os)) and gradually became greenish color to a hue on the outside (Figure 1b(gcl)). The quantity of black pigments was increased (Figure 1b,c(bp)). Over the yolk sac, a thick layer of blastoderm or germinal ring occupied three-quarters of the total area (Figure 1c(gr)). The average egg diameter of the segmentation stage under the traditional and the control system were $2645.47 \pm 18.24 \mu\text{m}$ and 2646.24 ± 17.98 , respectively (Figure S4). Over the yolk sac, the embryo had a nearly round shape with a distinct head (h) and tail (t) (Figure 1d,e(t, h)). Eye shape (es) was observed on one end of it (Figure 1f(es)). The average egg diameter of the pharyngula stage under the traditional and the control system were $2710.01 \pm 16.45 \mu\text{m}$ and $2710.90 \pm 16.60 \mu\text{m}$, respectively (Figure S4). The tail region straightened out, and the trunk-posterior tail's (pt) end elongated and separated (Figure 1g–i(pt)). A heart beat (hb) was detected in this stage also (Figure 1g–i(hb)).

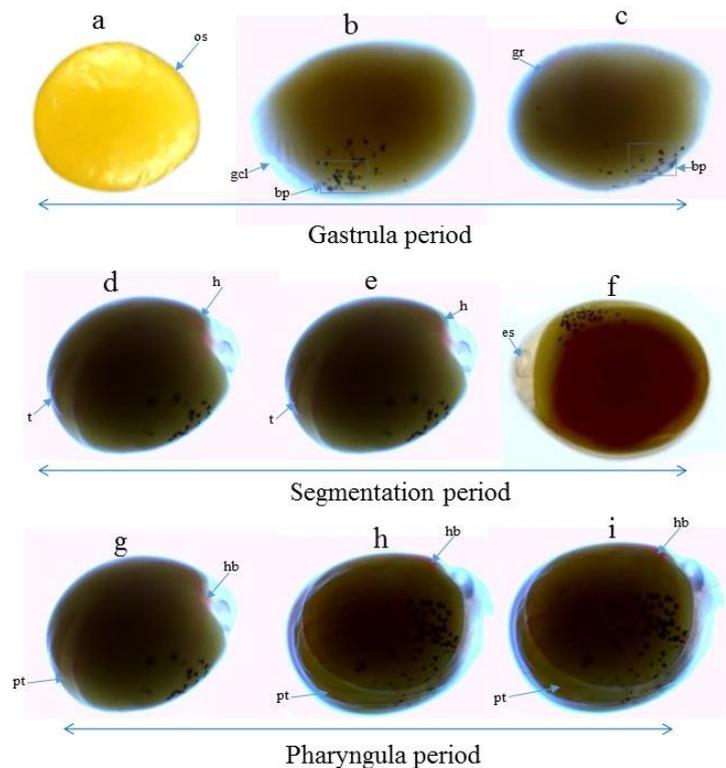


Figure 1. The characteristic changes of embryonic development in the re-circulatory thermostatic system (T_2). Gastrula period: (a) os—oval shape, (b) gcl—greenish color layer, bp—black pigments, (c) gr—germinalring, bp—black pigments. Segmentation period: (d,e) h—head, t—tail, (f) es—eye shape. Pharyngula period: (g–i) hb—heartbeat, pt—posterior tail.

3.2. Hatching and Survival Rate of *O. niloticus*

The hatching and survival rate of tilapia (*O. niloticus*) in the traditional system were 85% and 81% while in control system they were 95% and 97%, respectively. The development of the gastrula, segmentation, and pharyngula were ended at 15 h, 29 h, and 35 h, respectively, in the re-circulatory thermostatic system, while 16 h, 32 h, and 39 h, respectively, were required the in traditional system. Hatching time for the traditional system required 42 h, while the control system required 36 h which was about 6 h less than that of the re-circulatory thermostatic system. A comparison of hatching rate, survival rate,

and hatching time for both the traditional and re-circulatory thermostatic system are shown in Figure 2a,b.

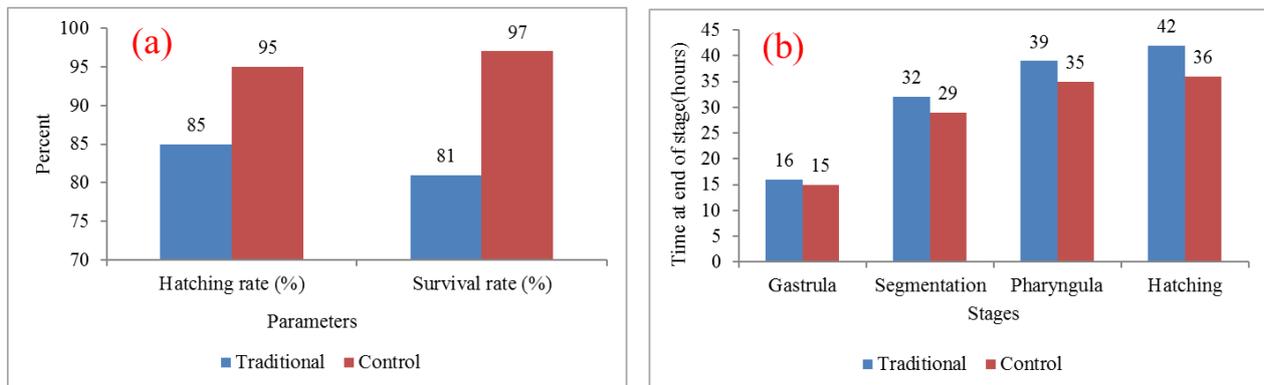


Figure 2. A comparison of hatching rate, survival rate, and hatching time in the traditional hatchery system and the re-circulatory thermostatic system for tilapia larvae; (a) hatching and survival rate in the traditional hatchery system and the re-circulatory thermostatic system, (b) hatching time (hours) required in the traditional hatchery system and the re-circulatory thermostatic system.

3.3. Larval Development of *O. niloticus* during Study Period

The highest average length (5.484 ± 0.329 mm) and weight (0.002 ± 0.000 g) of one-day-old larvae of *O. niloticus* were found in the T₂ (re-circulatory thermostatic system), followed by length (4.834 ± 0.369 mm) and weight (0.002 ± 0.000 g) in the T₁ (traditional hatchery system) (Figure 3(day-1)). The larvae had a clear straight body (sb) and a greenish yolk sac (ys) observed (Figure 3(day-1)). The density of pigmentation was found to be increased. Due to the large yolk sac, no free-swimming ability was observed at this stage as indicated in Figure 3(day-1, d, ysd, e, ys, f). Five-day-old larvae had the highest average length (9.630 ± 0.166 mm) and weight (0.007 ± 0.001 g) in T₂ followed by length (7.354 ± 0.209 mm) and weight (0.007 ± 0.001 g) in T₁, respectively (Figure 3(day-5)). The number of pigments (melanophores) increased on the surface of the head (dp) and appeared just below the dorsal side (dp) and over the yolk sac (Figure 3(day-5, dp)). The larvae eyes (be) became black in color (Figure 3(day-5, be)). Subsequently, the vertebral column and tail fin became visible (Figure 3(day-5)). The gut cavity and the dorsal surface of the head were covered with melanophores as also noticed in Figure 3(day-5) and Figure 4j(dp). Ten-day-old larvae had the highest average length (10.888 ± 0.066 mm) and weight (0.013 ± 0.001 g) in T₂ followed by length (10.550 ± 0.142 mm) and weight (0.012 ± 0.001 g) in T₁, respectively (Figure 3(day-10)). The number of pigments (dp) on the head surface was increased and also found on the underside of the surface (dp) and on the yolk sac (Figure 3(day-10, dp) and Figure 4e(ys)). Black spots and melanophores were seen and scattered on the dorsal fin and trunk tail region (Figure 4j(dp)), respectively. The yolk sac was absorbed and reduced (Figure 4e(ys)). The swim bladder was inflated and the larvae begin to swim as shown in Figure 3(day-10) and Figure 4j(dp). The highest average length (12.026 ± 0.155 mm) and weight (0.016 ± 0.001 g) of fifteen-day-old larvae were found in T₂ followed by length (10.950 ± 0.149 mm) and weight (0.015 ± 0.001 g) in T₁, respectively (Figure 3(day-15)). The yolk sac had been completely absorbed (nys), and the swim bladder had developed to full capacity and the fish could float (Figure 3(day-15, nys)). The melanophores were increased in number and more distinct than in the previous stages. Black spot on the dorsal fin became more distinct (Figure 3(day-15, dp)). Melanophores for the tilapia mark were more pronounced than in the previous stage as indicated in Figure 3(day-15) and Figure 4i-k as well. The highest average length (13.101 ± 0.474 mm) and weight (0.027 ± 0.003 g) of twenty-day-old larvae were found in T₂ followed by length (12.933 ± 0.4729 mm) and weight (0.025 ± 0.003 g) in T₁, respectively (Figure 3(day-20)). The dorsal fin became enlarged

with faint rays. The swim bladder and tail (TS) were more advanced and developed than in the previous stage (Figure 4i(TS)). The black spot on the dorsal fin became more visible. The melanophores (dp) were more distinct at this stage compared to the earlier ones as represented in Figure 3(day-20) and Figure 4i(TS),j(dp),k(df). The twenty-five-day-old larvae of *O. niloticus* showed the highest average length (14.540 ± 0.322 mm) and weight (0.036 ± 0.002 g) in T₂ followed by length (14.225 ± 0.398 mm) and weight (0.033 ± 0.002 g) in T₁, respectively (Figure 3(day-25)). Pigmentation on the dorsal region was increased in number. The caudal fin's fork shape was widened. The eyes were increased in size and pigmented. The shape of the tail had evolved gradually, noted in Figure 3(day-25) and Figure 4i-l. The highest average length (15.736 ± 0.424 mm) and weight (0.053 ± 0.004 g) of thirty-day-old larvae were found in T₂ followed by length (15.518 ± 0.415 mm) and weight (0.050 ± 0.004 g) in T₁, respectively (Figure 3(day-30)). On the body, the vertical band (vb) became more prominent. In comparison to the previous stage, the dorsal fin shape (ds) and caudal shapes (cs) were more developed (Figure 3(day-30, ds, cs) and Figure 4k(df)). On the sides of the larvae, vertical bands of melanophores were prominently noted in Figure 3(day-30) and Figure 4h-l as well. The exponential growth trend was found for both the traditional and the re-circulatory thermostatic system (Figure 5a-d). Length-weight was found strongly correlated for the traditional hatchery system ($R^2 = 0.821$) and the re-circulatory thermostatic system ($R^2 = 0.846$) from 1 DAH to 30 DAH (Figure 5e,f).

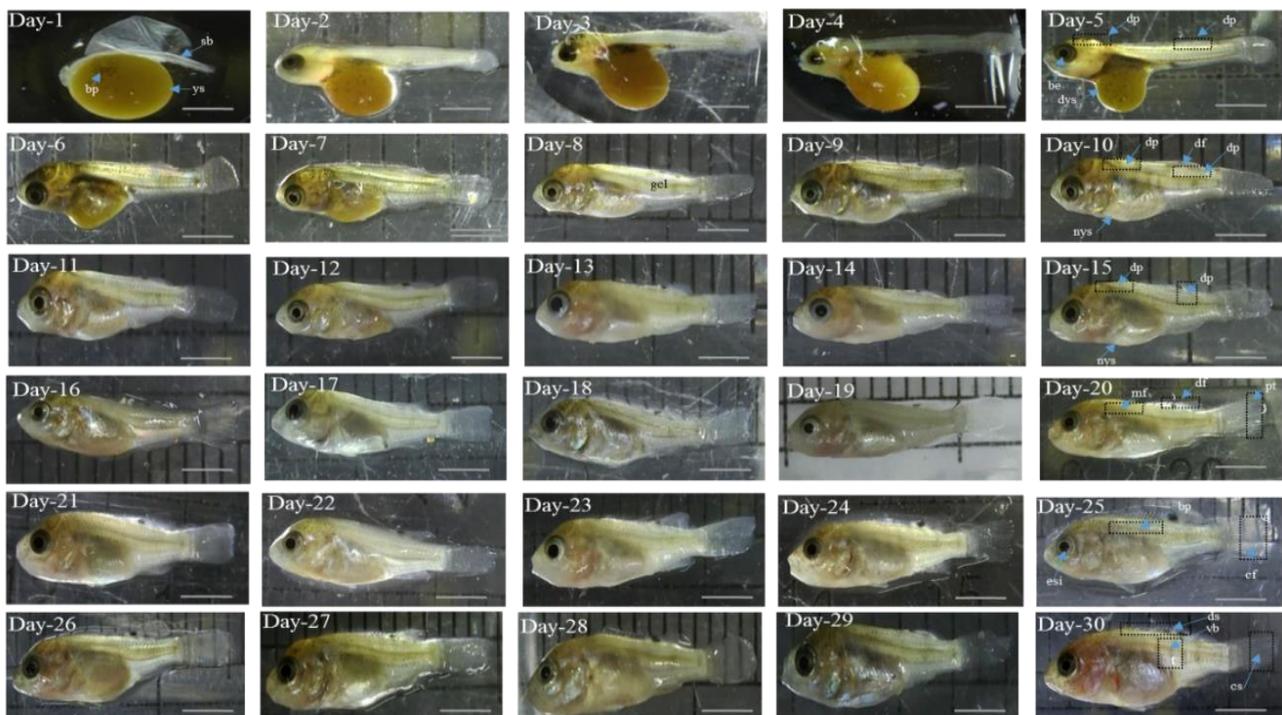


Figure 3. The characteristic changes in larval development of tilapia from 1 DAH to 30 DAH in the re-circulatory thermostatic system; Day-1: sb—straight body, ys—yolk sac, bp—black pigments. Day-5: dp—dorsal pigmentation, be—black eye, dys—decreasing yolk sac. Day-10: dp—dorsal pigmentation, df—dorsal fin, nys—no yolk sac. Day-15: dp—dorsal pigmentation, nys—no yolk sac. Day-20: mf—melanophores, df—dorsal fin, pt—posterior tail. Day-25: bp—black pigments, cf—caudal fin, esi—eye shape increase. Day-30: df—dorsal fin shape, vb—vertical band, cs—caudal shape. But under the traditional system, continuous conspicuous external traits in larval development were not seen during microscopic observation.

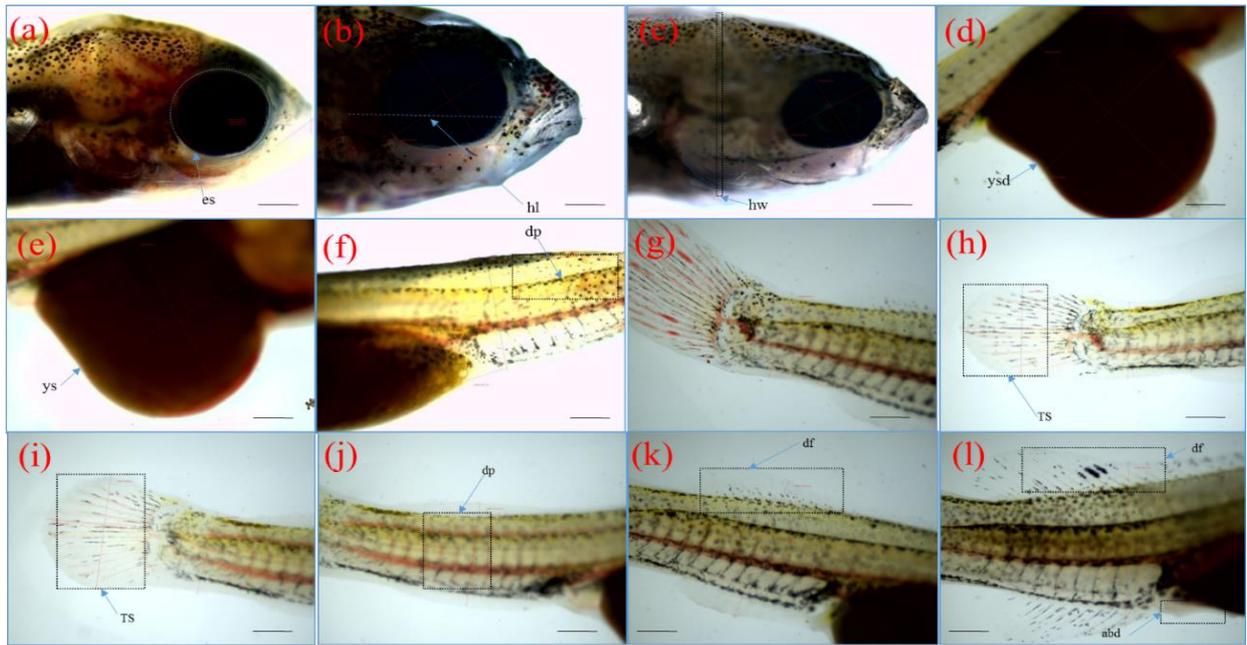
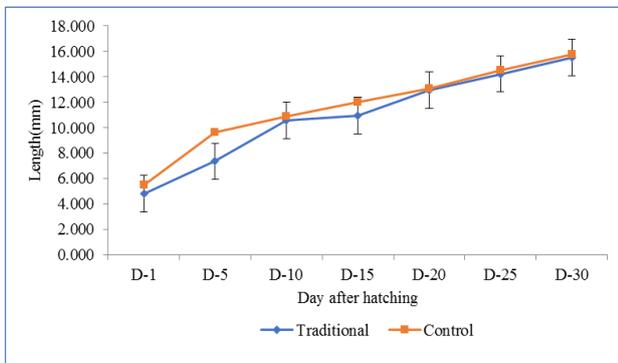
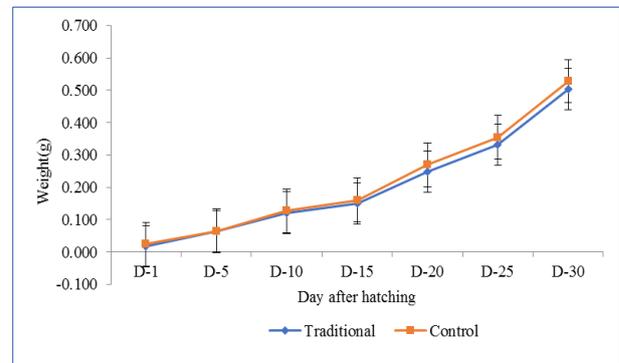


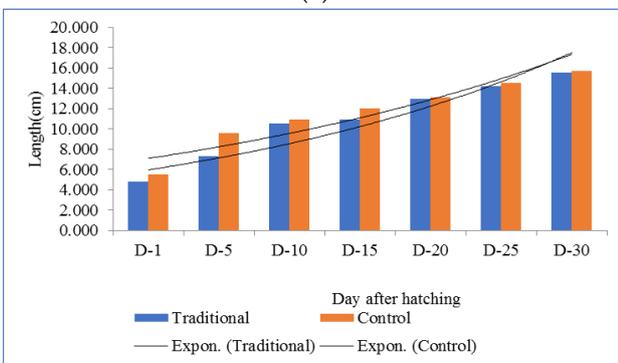
Figure 4. Particular morphometric changes of tilapia larvae (a–l) under the re-circulatory thermostatic system; (a) es—eye shape, (b) hl—head length, (c) hw—head width, (d) ysd—yolk sac decreasing, (e) ys—yolk sac, (f) dp—dorsal pigments, (h,i) TS—tail shape, (j) dp—dorsal pigments, (k) df—dorsal fin, (l) df—dorsal fin, abd—abdominal distance. Under the traditional system, continuous conspicuous external traits in larval development were not seen during microscopic observation.



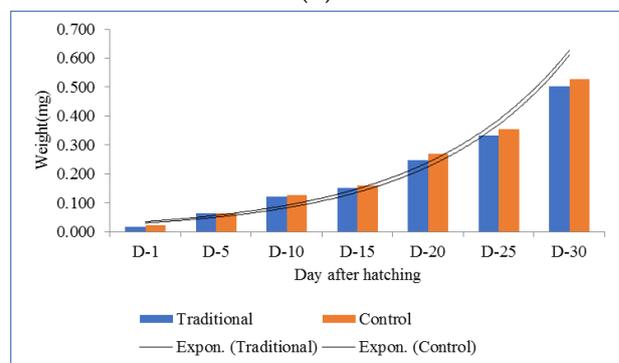
(a)



(b)



(c)



(d)

Figure 5. Cont.

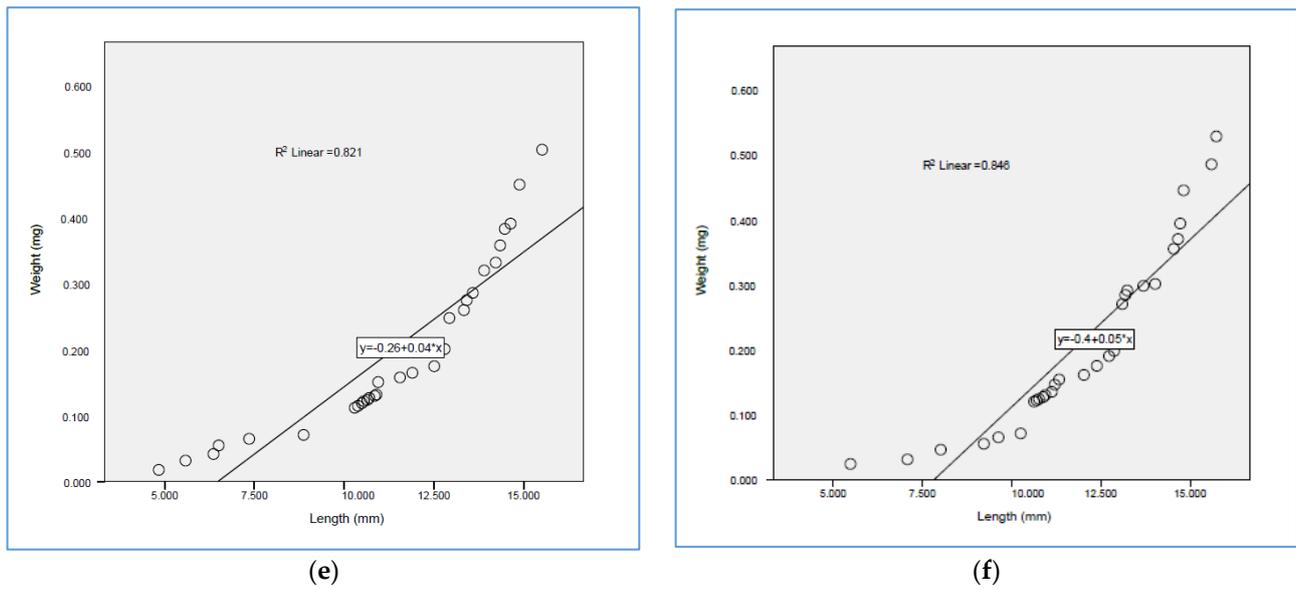


Figure 5. Comparative growth of larvae in the traditional and the control system; (a) length increase, (b) weight increase, (c) length increase trend—exponential, (d) growth increase trend—exponential, (e) strong length–weight relationship exists in the traditional system where $R^2 = 0.821$, (f) strong length–weight relationship exists in the re-circulatory thermostatic system where $R^2 = 0.846$.

3.4. Determination of Climatic Variables and Water Quality Parameters in the Traditional Hatchery System and the Re-Circulatory Thermostatic System

The water temperature was fluctuated during the study period under the traditional system (Figure S5). The highest and lowest water temperatures were 35.5 °C and 30.5 °C in traditional system, respectively. The highest levels of pH and dissolved oxygen were 8.6 and 9.75 mg/L while the lowest were 7.5 and 7.2 mg/L, respectively, in the traditional system during study period (Table 1 and Figure S5). Air temperature and humidity were varied during the study period. The highest air temperature and humidity were 33.87 °C and 69.94% while the lowest were 29.63 °C and 45.62%, respectively, in the traditional hatchery system (Table 1 and Figure S5). The trend of air temperature and water temperature variation during the larval study period in the traditional system is shown in the graph (Figure S5). On the other hand, water temperature (28.50 °C) was maintained in the re-circulatory thermostatic system. The highest pH and dissolved oxygen levels were 8.4 mg/L and 9.0 mg/L while the lowest were 7.5 and 7.1 mg/L, respectively, in the re-circulatory thermostatic system. Ammonia levels were quite low (0 mg/L) in both systems. The summary findings of water quality parameters are presented in the Table 1.

Table 1. The highest, lowest, and average values of climatic variables for the traditional system, and water quality parameters in both the traditional and the re-circulatory hatchery system.

Parameter	Traditional Hatchery System			Re-Circulatory Thermostatic System		
	Highest	Lowest	Average	Highest	Lowest	Average
Air temperature (°C)	33.87	29.63	31.52 ± 0.21	-	-	-
Humidity (%)	69.94	45.62	58.72 ± 1.47	-	-	-
Water temperature (°C)	35.50	30.50	32.71 ± 0.22	28.50	28.50	28.50
pH	8.60	7.50	7.99 ± 0.06	8.40	7.20	7.96 ± 0.04
DO (mg/L)	9.75	7.20	8.01 ± 0.11	9.00	7.10	8.06 ± 0.09
Ammonia (mg/L)	0	0	0	0	0	0

3.5. Analysis of the Relationship of Climatic Variables, Water Quality Parameters, and Morphometric Change of *O. niloticus* Larvae under the Traditional Hatchery System

The PCA results including climatic variables, water quality parameters, and morphometric change are summarized in Table 2. The eigen values of PCA with climatic variables like air temperature and humidity including water quality parameters like water temperature, pH, dissolved oxygen (DO), and 11 morphometric characteristics of tilapia were presented. A total of 16 variables resulted in two PCs with eigen values greater than one, explaining 85.706% of the total variance (Table 3). The variance of the first PC was 74.36% while the second PC was 11.346%. Figure 6 denotes the scree plot of all principal components, obtaining an elbow shape and giving a visual expression of redundancy for the next consideration. Moreover, this elbow in the scree plot (Figure 6) confirms the eigen value (>1) (Table 3). These two components showed a logical interpretation based on the values of the corresponding loadings shown in Table 2. Examining the variables with factor loadings value 0.5 or more were considered in order to interpret the content of each major component (Table 2 and Figure 7). Moreover, the component plots in rotated space were corresponded to the principal components (Figure 7).

The PCA for the analysis of the morphometric characteristics of tilapia larvae consists of two components in which component one is populated with morphometric characteristics. In the first component (morphometric features), a large number of morphometric variables collectively denoted the positive correlation among them. Body weight (0.951) contributed the highest factor loading to this component, followed by body width (0.943), dorsal fin back to caudal top (0.932), eye area (0.926), standard length (0.922), head width (0.922), total length (0.921), caudal fin width (0.899), head length (0.883), anal fin to caudal bottom (0.865), pH (0.761), humidity (0.626), and caudal fin length (0.55) (Table 2 and Figure 7). The second component is populated by climatic and water quality parameters where air temperature (0.555), humidity (−0.879), and DO (0.669) contributed the highest factor loading (Table 2 and Figure 7).

Table 2. Rotated component matrix and correlation coefficient of different variables with significant components for the morphometric characteristics of fishes.

Rotated Component Matrix ^a		
	Component	
	1	2
Total length	0.921	0.317
Body weight	0.951	0.203
Standard length	0.922	0.365
Body width	0.943	0.320
Head length	0.883	0.437
Head width	0.922	0.366
Caudal fin length	0.550	0.462
Caudal fin width	0.899	0.413
Dorsal fin back to caudal top	0.932	0.318
Anal fin to caudal bottom	0.865	0.449
Eye area	0.926	0.338
Air temperature	−0.278	−0.876
Humidity	0.626	0.555
Water temperature	−0.189	−0.879
pH	0.761	−0.319
DO	0.151	0.669

Rotation Method: Varimax with Kaiser Normalization ^a. Rotation converged in 3 iterations ^a.

Table 3. Eigen values of PCA of the correlation matrix.

Principal Component	Initial Eigenvalues		
	Total	% of Variance	Cumulative %
1	11.898	74.360	74.360
2	1.815	11.346	85.706
3	0.783	4.894	90.600
4	0.655	4.094	94.694
5	0.420	2.624	97.318
6	0.207	1.296	98.614
7	0.092	0.573	99.187
8	0.058	0.360	99.546
9	0.032	0.199	99.745
10	0.017	0.109	99.854
11	0.011	0.066	99.921
12	0.006	0.037	99.958
13	0.003	0.019	99.977
14	0.003	0.017	99.994
15	0.001	0.004	99.998
16	0.000	0.002	100.000

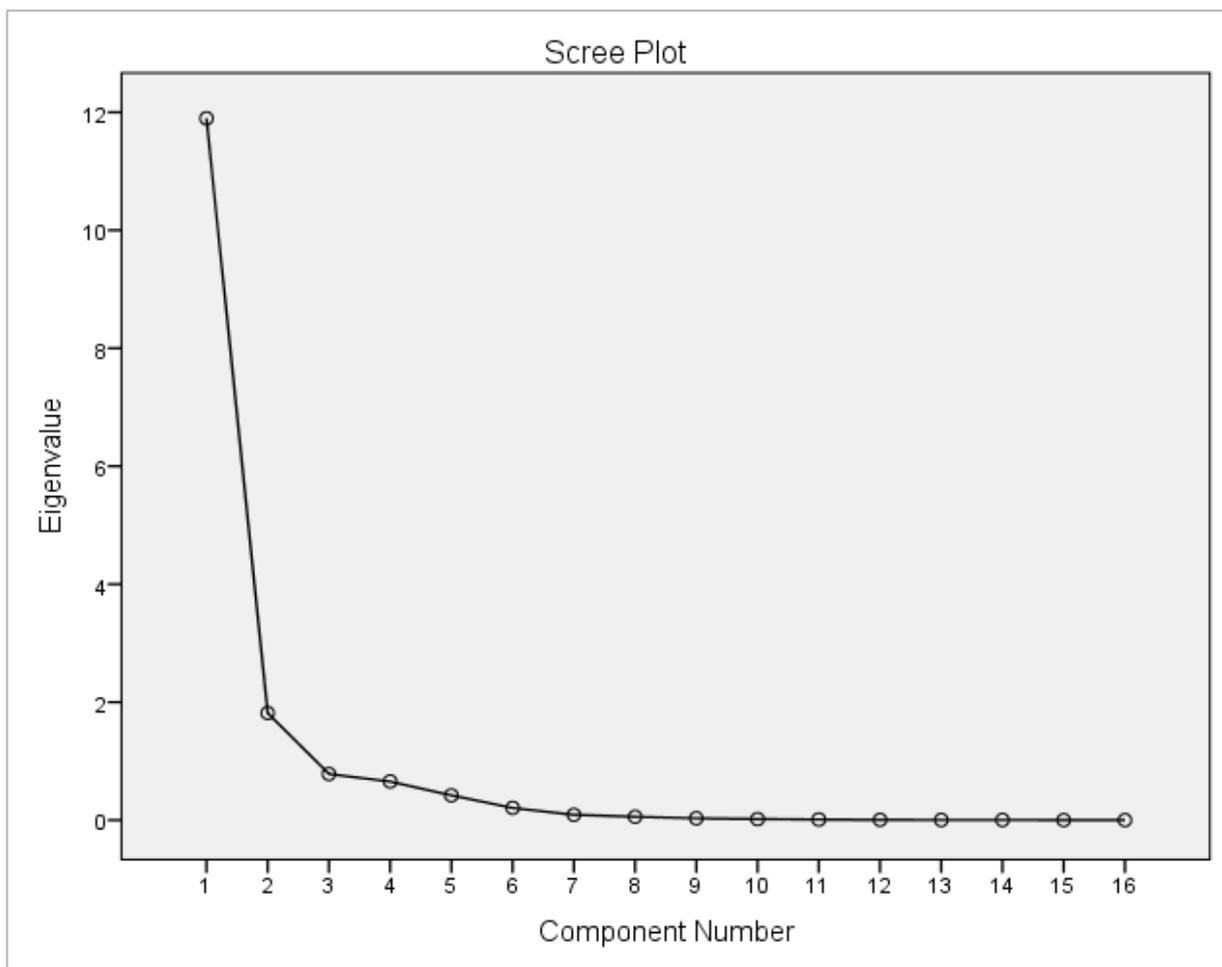


Figure 6. Scree plot (explained variance of each principal component) calculated for the factors.

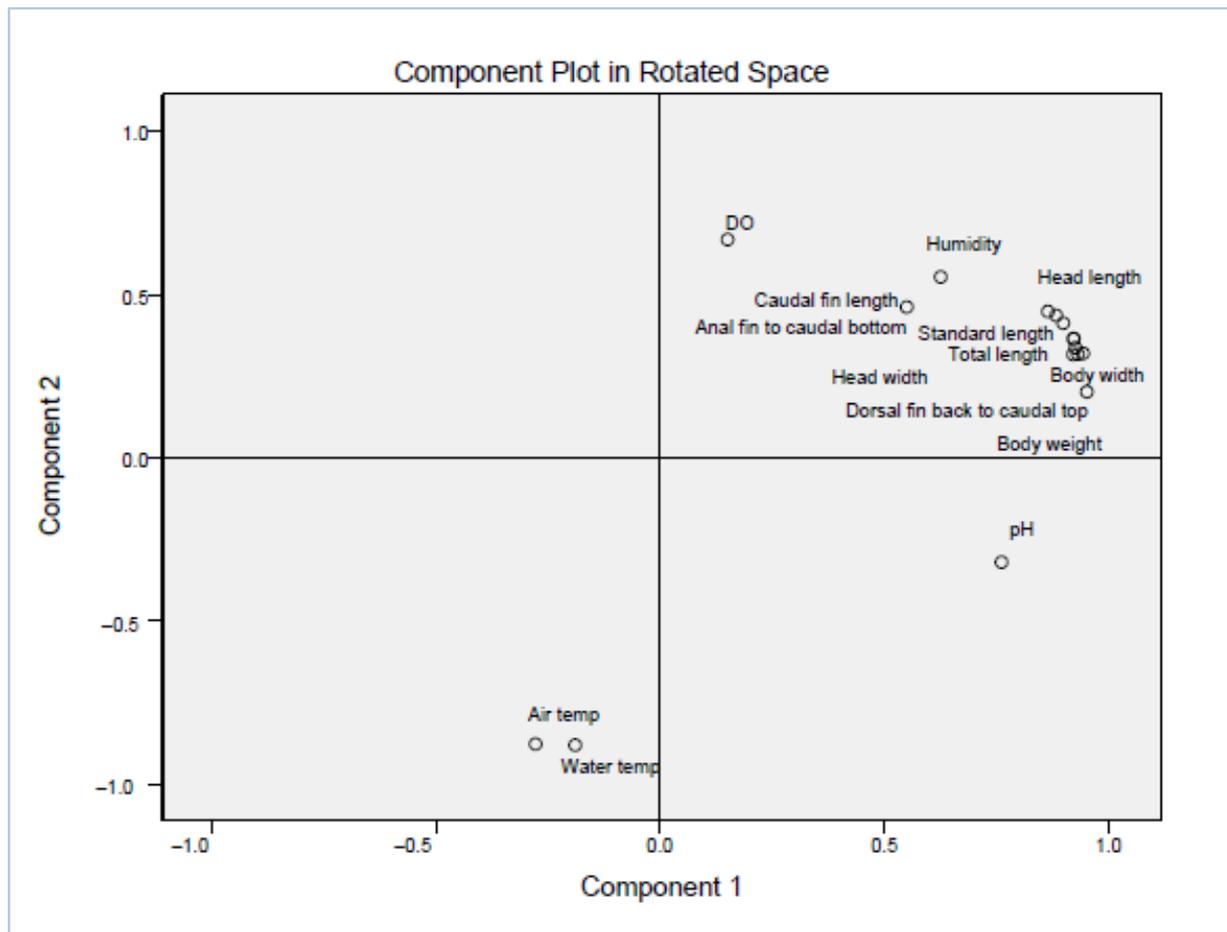


Figure 7. Loading component plots corresponding to the principal components.

4. Discussion

4.1. Observation of Embryonic Development Stages of *O. niloticus*

During each observation, egg diameter including the changes in internal and external characteristics of the gastrula, segmentation, and pharyngula were noted during the embryonic development stage. The gastrula stage was observed with egg diameters ($2261.07 \pm 81.52 \mu\text{m}$) and ($2261.47 \pm 81.66 \mu\text{m}$) in the traditional hatchery system and in the re-circulatory thermostatic system, respectively, and it was continued until just before the segmentation stage. The eggs were found almost oval in shape and yellow in color. To the outside, this color was gradually turning to a greenish hue. Black pigments were observed on the eggs' bodies, which gradually formed over the next phases of development. Three-quarters of the entire area of the egg included a thick layer called blastoderm or germinal ring. The segmentation stage was stated with egg diameters ($2645.47 \pm 18.24 \mu\text{m}$) and ($2646.24 \pm 17.98 \mu\text{m}$) in the traditional hatchery and in the re-circulatory thermostatic system, respectively, and it progressed until just before the pharyngula stage. Under this stage, the embryo had a virtually spherical form over the yolk sac, with a definite head and tail. On one end, the shape of an eye was clearly detected. The pharyngula stage was observed with egg diameters ($2710.01 \pm 16.45 \mu\text{m}$) and ($2710.90 \pm 16.60 \mu\text{m}$) in the traditional hatchery system and in the re-circulatory thermostatic system, respectively, and it was continued until just before hatching. The trunk-posterior tail end extended and separated, and the tail area straightened out. The egg diameter was found to be slightly higher in the re-circulatory thermostatic system than in the traditional hatchery system. The present findings on the characteristic changes of the embryonic development of tilapia are almost positively consistent with the findings of several previous studies [6,13,20,39].

On the other hand, there are some studies that observed almost similar characteristics in embryonic development [40–42].

4.2. Hatching and Survival Rates of *Tilapia*, *O. niloticus*

The hatching and survival rates of *O. niloticus* under the traditional hatchery system were 85% and 81% while they were 95% and 97% in the re-circulatory thermostatic system, respectively. The findings indicate that the re-circulatory thermostatic system is more effective than any other traditional system. For hatching, about 6 h less time was required under the re-circulatory thermostatic system than under the traditional hatchery system. The survival rate up to 30 days was found to be 81% in the traditional hatchery system while it was 97% in the re-circulatory thermostatic system, denoting a comparative performance of the re-circulatory thermostatic system. In natural aquatic systems, temperature along with other climate factors degrade the ecological balance and negatively impact hatching performance; that is why natural resources have been gradually declining [43,44]. In the traditional hatchery system, hatching rate is severely impacted by temperature fluctuation, which leads to increases in the mortality of yolk sac larvae [14,19,41,45]. Larval fishes are more sensitive to environmental changes and more vulnerable to climate change than adults. Temperature influences hatching, larval development, and survival rate [19]. The hatching and survival rate is performed very well if the temperature and other physico-chemical parameters are properly managed [13,19,46–48]. Too low or too high temperatures result in the malformation of larvae, which increases the mortality of larvae at the hatchery level [8].

4.3. Larval Development of *O. niloticus* during the Study Period

The first observation of larval development was made shortly after hatching, and the larvae's initial length (5.484 ± 0.329 mm) and weight (0.0024 ± 0.000 g) were recorded in T₂ (re-circulatory thermostatic system) followed by length (4.834 ± 0.369 mm) and weight (0.0018 ± 0.0002 g) in T₁ (traditional hatchery system). At the end 30 days, the length (15.736 ± 0.424 mm) and weight (0.0528 ± 0.0040 g) of larvae were recorded in T₂ followed by length (15.518 ± 0.415 mm) and weight (0.0503 ± 0.0041 g) in T₁, respectively. In the re-circulatory thermostatic system, growth was found to be a little higher than in the traditional hatchery system. The regression analysis for both systems revealed that the length and weight of larvae showed a strong correlation ($R^2 = 0.762$ and $b = 0.05$ for the traditional, whereas $R^2 = 0.788$ and $b = 0.05$ for the re-circulatory thermostatic system) which indicated positive allometric growth. The growth pattern of tilapia larvae showed an exponential trend, which was consistent with the findings of a previous study [49], where an absolute exponential growth pattern in Juvenile Nile tilapia (*O. niloticus*) was observed as modeling the impact of water temperature. There was no available established research in Bangladesh using the re-circulatory thermostatic system. As a result, comparing the current findings to those of others in Bangladesh and elsewhere was difficult. But the findings of the present study were partially supported with several previous studies [16,23]. The characteristics of larval development for tilapia under the present study support the findings of a few previous studies on the embryonic, larval, and early juvenile periods of *O. niloticus* [6]. According to an earlier study, the daily growth rate of larvae showed a change in average weight for 28 days, meaning that the average weight of cultured fish was increased with the increasing of maintenance time [16]. In a study, it was reported that the average length of newly hatched larvae was about 7.3 ± 0.7 mm at the first day of hatching, which was almost near to the findings of the present study [50]. In the present study, yolk sac absorption was almost complete after seven days of hatching, which was consistent with the findings of a previous study [50]. Similarly, the characteristic changes in larvae at aged 7–20 days were almost identical to the previous findings [50].

4.4. Determination of Climatic Variables and Water Quality Parameters in the Traditional Hatchery System and the Re-Circulatory Thermostatic System

For both systems, no critical values were detected for pH, dissolved oxygen, and ammonia. Water temperature was varied from 30.5 °C to 35.5 °C in the traditional hatchery system. On the other hand, water temperature was maintained at 28.50 °C in the re-circulatory thermostatic system, which was suitable for the embryonic and larval development of *O. niloticus*. Specific water temperature is a pre-requirement to the progression of embryonic and larval development. The critical variation of water temperatures affects fertilization, hatching rates, and survival rates. In this regard, a very significant study showed that fertilization and hatching rates of *O. niloticus* were significantly higher at 28 °C and 30 °C in compared to 26 °C and 32 °C, accordingly [46]. According to canonical correlation analysis, there was a strong correlation (92%) showing in the first function while moderate correlation (50%) existed in the second correlation between climatic variables and water quality parameters. The contributions of all parameters under climatic variables and water quality parameters showed as an individual percent of variance alongside the collective loading percent of variance. Under the first canonical correlation, air temperature and water temperature showed negative correlation as higher loading variance while pH and DO showed an opposite relationship as minimum loading of variance. In the second canonical correlation, air temperature (0.129) and humidity (0.713) and water temperature (0.129), pH (0.781), and DO (0.499) showed a positive moderate correlation where a very minimum percent loading of variance existed. Overall, correlation analysis of the present study indicates that climatic variables and water quality parameters have positive, negative, and opposite correlation. No correlation was not detected in the case of any function during the study period. Two previous studies noticed a relationship between air temperature, relative humidity, pH, DO, electrical conductivity, and TDS during a water quality study in Lake Manzala, Egypt [51]. According to another study, the growth performance of *O. niloticus* was decreased after 34 °C of water temperature [8]. If other conditions like temperature and pH are still suitable, *O. niloticus* may tolerate a low level of oxygen below 2.3 mg/L [8].

4.5. The Relationship of Climatic Variables, Water Quality Parameters, and Morphometric Changes of *O. niloticus* Larvae under the Traditional Hatchery System

A wide range of relationships between climatic variables like air temperature and humidity, water quality parameters such as water temperature, pH, and DO, and the morphometric characteristics of tilapia were determined under the traditional hatchery system. PCA explained cumulatively 85.706% of the total variance, expressed as strong correlation among the variables. It indicates that PCA successfully made redundant all the variables in the two principal components by forming the clearly denoted elbow shape, explaining more than 85% of those variables, where the first component explained 74.36% of the variance while 11.34% of variance was explained by the second component. Under the first PC, body weight showed the highest factor loading as the relationship to this component, followed by caudal fin length as the lowest loading factor in morphometric cases. On the other hand, humidity, pH, and DO showed positive correlation among themselves but air temperature and water temperature showed negative loading factors under the components. It is clearly indicated that pH and DO have strong positive correlation among all morphometric variables which could be influenced by pH and DO. In contrast, both air temperature and water temperature have opposite correlation with all morphometric variables which could be negatively impacted by air temperature and water temperature. According to a previous study, *O. niloticus* fry and juveniles raised well in temperatures between 27–32 °C [52]. When temperatures rise beyond 32 °C, growth becomes slow, feeding efficiency is reduced, and eventually mortality occurs [52]. Under the second PC, humidity and DO showed positive loading factors but air temperature, water temperature, and pH showed negative loading factors. It denoted that morphometric characteristics were moderately influenced by the climatic variables and water quality parameters. A

previous study under an intensive re-circulating aquaculture system with a low water exchange found that the specific growth rate of *O. niloticus* fingerlings was influenced by the variation of DO and that ultimately explained 44% and 47% of weight variation [53]. According to an empirical study, the most significant factor affecting the production of fish seeds was temperature, followed by rainfall, humidity, and sunshine intensity, and these changes impact the metabolic and developmental processes of fishes [5]. Other studies reported that changes in temperature and climatic factors have an impact on embryonic & larval development, feeding rate, DO uptake, pH level, other water quality parameters and those would then affect a variety of fish health indicators [54,55].

5. Conclusions

This study on the embryonic and larval development of *O. niloticus* addressed the current needs of fish hatcheries in Bangladesh. Many technical aspects of the embryonic and larval growth of *O. niloticus* are difficult for hatchery operators to understand and most hatcheries lack the facilities to explore these technical matters. As a result, embryonic and larval mortality occur, which leads to financial losses at the hatchery level. The characteristic changes of the embryonic and larval development of *O. niloticus* were measured by image analysis using compound and DMWifi android portable microscopes which performed very effectively. In comparison to the traditional hatchery system, the re-circulatory thermostatic system required about 6 h less time for embryonic development. During the production cycle, this could save enough time and resources to help increase hatchery profits. The hatching and survival rates under the re-circulatory thermostatic system were found to be significantly higher than under the traditional hatchery system. Larval growth of *O. niloticus* was reported slightly higher in the re-circulatory thermostatic system than in the traditional system, which could be acceptable and beneficial for fish hatchery operators in Bangladesh for sustainable aquaculture production. Relationships between all chosen morphometric characteristics and climatic factors under the traditional system were determined using PCA. All the changes were found to be nearly identical to those discovered by others for *O. niloticus* and a few other relevant species. Variations of water temperature were determined in traditional hatchery systems, which could have an effect on embryonic and larval development. Water temperature was found to be a very influential factor which promoted other variables. It was shown that hatching and survival rates performed very well if the temperature and other physico-chemical parameters were managed properly. Therefore, the re-circulatory thermostatic system might be an effective strategy for more seed production at the fish hatchery level, which could contribute greatly to the aquaculture sector for sustainable fish production.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/aquacj3020008/s1>, Figure S1. Study area; (a) study area into the country map; (b) study area into the district map, (c) broodfish pond, (d) Laboratory of Climate Research for Fishes. Figure S2. Experimental set up in the Laboratory of Climate Research for Fishes with (T₁) traditional mini type hatchery with three replications, and (T₂) automated re-circulatory thermostatic system with three replications. Figure S3. Morphometric characteristics of tilapia in the traditional system; total length (ad), standard length (ac), body weight, body width (ef), head length (ag), head width (gh), caudal fin length (cd), caudal fin width (jl), dorsal fin back to caudal top (kl), anal fin to caudal bottom (ij), and eye area. Figure S4. Egg diameter during embryonic development under the traditional and control systems; (a) the trend of egg diameter increase in the traditional system (T₁), (b) the trend of egg diameter increase in the control system (T₂). Figure S5. Air and water temperature variation during the larval study period in the traditional system (T₁) from 1 DAH to 30 DAH; the three highest temperatures of 35.5 °C, 34.6 °C, and 34.5 °C were recorded in 8 DAH, 7 DAH, and 10 DAH, respectively, while the three lowest temperatures of 30.5 °C, 30.6 °C, and 31.7 °C were recorded in 22 DAH, 16 DAH, and 17 DAH, respectively.

Author Contributions: M.A.B.S.: Overall data analysis & presentation and writing the full original draft; B.M.: Image analysis and assisting with writing the original draft; M.M.H. (Mohammad Mahfujul Haque): Concept development, supervision, investigation, validation, and editing the draft; A.B.: Editing the draft; M.M.H. (Md. Mahmudul Hasan): Editing the draft; M.H.S.: Data collection and assisting with data analysis; M.M.N.T.: Sampling, data collection, image capturing, and data entry; J.C.B.: Funding acquisition, investigation, project administration, and editing the draft; A.K.S.A.: Overall research facilitation, methodology development, supervision, editing the draft, project administration, and funding implementation. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: The authors disclosed no conflict of interest to anybody or any organization.

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