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Comparative Evaluation of Tilapia (*Oreochromis niloticus*) Seed Quality in Pond and Recirculating Aquaculture Systems Under Variable Climatic and Water Quality Conditions

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Abstract

Climate variability poses increasing challenges to hatchery performance by altering water quality and larval physiology. This study compared the seed quality of Nile tilapia (*Oreochromis niloticus*) produced in traditional ponds and Recirculatory Aquaculture Systems (RAS) under fluctuating climatic and water-quality conditions. RAS maintained stable conditions (temperature 28.1–29.4°C; DO 6.8–7.4 mg L^{-1}; ammonia≈ 0 mg L^{-1}) compared with pond system (temperature 27.2–32.5°C; DO 5.8–7.0 mg L^{-1}; ammonia 0.02–0.07 mg L^{-1}). Larvae reared in RAS showed higher length gain (2.94±0.658% vs. 1.19±0.207%), greater weight gain (7.24±1.37% vs. 7.04±0.81%), and improved specific growth rate (0.23±0.032% day^{-1}). Fulton's condition factor was also superior in RAS (mean KF 1.97–2.15) compared with pond system (1.45–1.65). Morphometric traits, including total and caudal length, were significantly higher in RAS ($P<0.001$). PCA showed that water-quality variables contributed 42.5% of total variance. GHRH gene expression was markedly upregulated in RAS, indicating enhanced physiological performance. Overall, the stable and controlled environment in RAS improved larval quality, developmental progression, and molecular growth regulation, highlighting its potential as a climate-resilient and sustainable hatchery strategy for tilapia seed production.

Introduction

Aquaculture is one of the fastest-growing food-producing sectors and plays a vital role in global food and nutrition security. With capture fisheries reaching their production limits, aquaculture has become essential for meeting rising global demand for aquatic products (FAO, 2022). Among farmed species, Nile tilapia (Oreochromis niloticus) is widely cultured across more than 120 countries due to its rapid growth, environmental tolerance, and high market demand (Rahman et al., 2021). In Bangladesh, tilapia production has expanded rapidly, supported by the species' ability to thrive under variable water quality, temperature, and salinity conditions in both freshwater and brackish environments.

Seed quality is a key determinant of aquaculture productivity. The timely supply of healthy, robust seed depends on broodstock condition and environmental factors such as temperature, rainfall, and dissolved oxygen (Ogunremi et al., 2020; Bhuyan et al., 2012). However, hatchery operations in Bangladesh remain highly dependent on ambient climatic conditions, making them vulnerable to fluctuations that impair spawning performance, larval survival, and growth (Hossain et al., 2021; Islam et al., 2023). Climate change further intensifies these risks through irregular rainfall, abrupt temperature shifts, and recurring water quality degradation, ultimately reducing hatchery efficiency and seed performance (Ahmed et al., 2020; Wang et al., 2018; Obiero et al., 2024).

Traditional pond-based hatcheries often experience unstable physicochemical conditions, including variations in temperature, pH, and dissolved oxygen, which impose chronic stress on larvae and diminish seed quality. In contrast, Recirculating Aquaculture Systems (RAS) provide a climate-smart alternative by maintaining stable water quality, enhancing biosecurity, and optimizing water reuse (Van Rijn, 2013; Li et al., 2023). Although the production and environmental benefits of RAS are well documented, limited research has assessed how climatic variability and water-quality fluctuations jointly influence seed quality and molecular growth regulation in tropical hatcheries (Sherzada et al., 2023; Aboukila et al., 2021). In particular, the relationship between environmental stability and the expression of growth-regulating genes, such as Growth Hormone-Releasing Hormone (GHRH), remains poorly understood under Bangladesh's variable climatic conditions.

To address this gap, the present study compares the seed quality of Nile tilapia (O. niloticus) reared in traditional ponds and RAS under fluctuating climatic and water-quality conditions. Specifically, it evaluates growth performance, morphometric traits, and GHRH gene expression to clarify how controlled environments influence larval physiology. The findings aim to provide a scientific basis for climate-resilient hatchery management strategies and support the development of sustainable aquaculture systems in Bangladesh and similar tropical regions.

Materials and Methods

Study Area and Experimental Design

The experiment was conducted at the Laboratory of Climate Research for Fishes, Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh-2202 (Supplementary Figure 1). Nile tilapia (*Oreochromis niloticus*) seed performance was compared between a traditional earthen pond (T_1) and a Recirculatory Aquaculture System (RAS) (T_2). Six hundred broodfish obtained from Asia Scientific Hatchery were equally assigned to the two systems. In the RAS, 300 broodfish (mean TL 15.5 cm; 60.47 g; stocking density 3 kg m^{-3}) were held in 500-L circular tanks, while ponds followed standard hatchery management.

The RAS operated as a closed-loop unit with 8–10% daily water exchange, full recirculation every ~5 h, and a combination of solids removal, mechanical filtration, and biofiltration. Solids were removed using a swirl separator and drum filter; ammonia was oxidized via a biofilter containing bio-balls and volcanic rock; dissolved organics were reduced using a protein skimmer and degassing tower. A UV sterilizer ensured microbial control, and DO was maintained above 6 mg L^{-1}. Water temperature was automatically regulated at 29±0.5°C (Faruk et al., 2012). These essential elements ensure proper evaluation of RAS functionality while keeping the description concise.

Fertilized eggs from each system were incubated separately (200 eggs tray^{-1}), and larvae were reared for 30 days. During 1–3 DPH, larvae received egg-yolk suspension followed by a 35% CP powdered diet. Pond larvae were kept in nylon hapas (1.5×1×1 m), whereas RAS larvae were maintained in aerated rearing trays. Three seasonal batches were studied over 12 months.

Measurement of Climatic and Water Quality Parameters

Water temperature, dissolved oxygen (DO), pH, total dissolved solids (TDS), and ammonia were measured daily in both systems using standard hatchery instruments. Temperature was recorded using a SMART Sensor thermometer (Model AR 867; ±0.1°C), DO using a Lutron DO-5509 meter (±0.2 mg L^{-1}), pH with a digital pH meter (Model pH-107; 0.01 resolution), and TDS with a TDS meter (Model TDS-3; ±2%). Ammonia concentration was determined using the API Freshwater Ammonia Test Kit with a detection sensitivity of 0.02 mg L^{-1}. All instruments were calibrated before measurement, and each parameter was measured in triplicate to ensure accuracy. Climatic variables (air temperature, rainfall, humidity, solar intensity) were obtained from the Bangladesh Meteorological Department (BMD), Mymensingh station.

Determination of Larval Growth in Pond and RAS System

Sampling was performed between 11:00 a.m. and 12:00 p.m. to record larval length and weight. Five larvae were randomly collected from each replicate tank or hapa, and the experiment was conducted in triplicate for both the pond and RAS systems (n=15 per treatment). This design follows standard practice in small-scale larval growth assessments (Faruk et al., 2012; Sahami et al., 2020) and ensures adequate replication for statistical reliability. The total length (TL, cm) of each specimen was measured using a DMWiFi portable digital microscope (Android-based, 50× lens). Images were analyzed using ImageJ software (version 2.1.4.7i, Java-based image processing). Individual body weight (g) was determined using a digital balance (Model TANITA KD-160, precision ±0.1 g). The average length and weight of larvae from pond and RAS systems were recorded at stocking and on each sampling date.

Data were pooled across replicates to reduce individual variance. Percent length gain, percent weight gain, and specific growth rate (SGR) were calculated using standard equations (1–3).

$$\text{Percent length gain } (\%) = \left(\frac{Mean\ Final\ Length - Mean\ Initial\ Lengthd}{Mean\ Initial\ Lengthd}\right) \times 100 \quad (1)$$

$$\text{Percent weight gain } (\%) = \left(\frac{Mean\ Final\ Weight - Mean\ Initial\ Weight}{Mean\ Initial\ Weight}\right) \times 100 \quad (2)$$

$$\text{Specific growth rate} (\%/\text{day}) = \left(\frac{\ln W2 - \ln W1}{T2 - T1}\right) \times 100 \quad (3)$$

where W2 is the final weight, W1 is the initial weight, and T2−T1 represents the time difference.

Determination of Seed Quality Using Length-Weight Relationship

Growth condition and length-weight relationship of fish can be evaluated using several condition indices derived from length-weight relationships. These include the Allometric Condition Factor (K_a), Fulton's Condition Factor (K_F), Relative Condition Factor (K_r), and Relative Weight (W_r) (Faruque & Das, 2024). Each metric provides insights into the physiological status of fish under varying environmental and nutritional conditions. In the present study, K_F was employed to assess larval growth performance, as the growth pattern observed was predominantly isometric (i.e., the exponent *b* in the length-weight relationship was approximately 3). This approach is appropriate when length and weight increase proportionally, making K_F a reliable indicator of fish condition under the culture systems examined (equation 4).

$$\text{Condition Factor K } = \left(\frac{Weight}{Length^3}\right) \times 100 \quad (4)$$

Comparative Morphometric Development

Five morphometric characters were measured daily over 30 days: TL, SL, HL, CL, and ED (Supplementary Figure 2). Measurements followed protocols modified from Sahami et al. (2020). Larvae were photographed using a DMWiFi Microscope (50X lens), and images were analyzed with ImageJ software (v2.1.4.7i).

Determination of the GHRH Gene Expression Level in the Muscle of Tilapia (*O. niloticus*) Larvae

Tilapia (*O. niloticus*) larvae were randomly sampled from conventional ponds and the Recirculatory Aquaculture System (RAS) for molecular analysis. Growth Hormone-Releasing Hormone (GHRH) was selected as a representative upstream regulator of the somatotropic axis because it directly stimulates pituitary growth hormone (GH) secretion and responds to environmental cues. Although growth is controlled by multiple genes (e.g., GH, IGF-1, GHR, Myostatin), GHRH was used here as a key marker to provide an initial molecular insight into growth modulation under different rearing systems.

GHRH sequences of O. niloticus were obtained from the Ensembl Genome Browser and NCBI. Primers for GHRH and the reference gene β-actin were designed using Primer3, and are listed in Table 1. The stability of β-actin across treatments was verified using the ΔCt method to ensure reliable normalization.

Larvae were euthanized, and muscle tissues were preserved in RNAlater at −40°C until processing. Total RNA was extracted (three replicates per treatment), checked for integrity and purity, and treated with DNase I to remove genomic DNA. First-strand cDNA was synthesized from purified RNA using a commercial reverse transcription kit and stored at −20°C.

Quantitative real-time PCR (qRT-PCR) was performed using SYBR Green chemistry on an ABI 7300 platform. Reactions were run in triplicate, using standard cycling conditions followed by melting-curve analysis to confirm primer specificity and the presence of a single amplicon. Gene expression data were analyzed with ABI QuantStudio 1 software using the $2^{-}\Delta\Delta Ct$ method. Fluorescence (Ct) values for GHRH were normalized to β-actin, enabling reliable comparison of relative GHRH expression between pond and RAS larvae.

Statistical Analysis

Data organization and descriptive statistics were performed in Excel and IBM SPSS v21. Normality was checked using Kolmogorov–Smirnov and Shapiro–Wilk tests. A two-way ANOVA was used to evaluate the effects of system (pond vs. RAS) and batch (1–3), followed by Tukey's HSD ($P \leq 0.05$). Independent t-tests were used only for comparing pooled system means. Principal Component Analysis (PCA) was performed in OriginPro 2024 to explore multivariate relationships among climatic, water-quality, and growth variables.

Result

Overview of Climatic Parameters

Monthly variations in air temperature, humidity, rainfall, and solar intensity during the experimental

Table 1. Gene-specific primers used in validation of the growth performance of *O. niloticus*

Gene name	primer	Sequence (5'-3')	Tm (°C)
Beta Actin	Forward	AATGAGAGGTTCCGTTGCC	53.1
	Reverse	TGCTGTTGTAGGTGGTTTCG	55.7
GHRH	Forward	AAATCGCTCCAATGTGTGTG	46.8
	Reverse	AGCTCTCACTTCCATCCCTG	61.8

period are summarized in Supplementary Table 1 and illustrated in Figure 1. Air temperature ranged from 18.5°C to 30.3°C, with the highest values recorded during July–August and the lowest during January–February. Rainfall was irregular, peaking in the monsoon months (June–September). These fluctuations affected the seasonal variation patterns of water quality parameters in the pond system. On the other hand, Recirculating Aquaculture Systems (RAS) maintained tightly regulated conditions for temperature, humidity, and other water parameters. The RAS mitigated the adverse effects of climatic extremes, providing a stable microenvironment that supported improved growth performance and higher survival rates in tilapia culture.

Determination of Water Quality Parameters in the Pond and RAS

Comparative summaries of key water quality parameters between pond and RAS systems are presented in Table 2. The RAS maintained more stable temperature (28.1–29.4°C), pH (7.7–8.1), and dissolved oxygen (6.8–7.4 mg L^{-1}) conditions compared to ponds, where wider fluctuations were observed, particularly in temperature (27.2–32.5°C) and ammonia levels (0.02–0.07 mg L^{-1}) (Supplementary Figure 3). The differences in DO and ammonia were statistically significant ($P<0.05$), indicating the effectiveness of RAS filtration and aeration components in maintaining consistent water quality. Other parameters, including total dissolved solids and alkalinity, showed minor variation between systems ($P>0.05$). Overall, water quality in the RAS remained within optimal ranges for larval tilapia rearing and showed less variability than the pond system, supporting better growth and survival conditions.

Impact of Climatic Factors on Water Quality Parameters in the Pond

Significant relationships were found between environmental and pond parameters (Supplementary Table 2). Air temperature showed a strong positive correlation with pond water temperature (r=0.793,

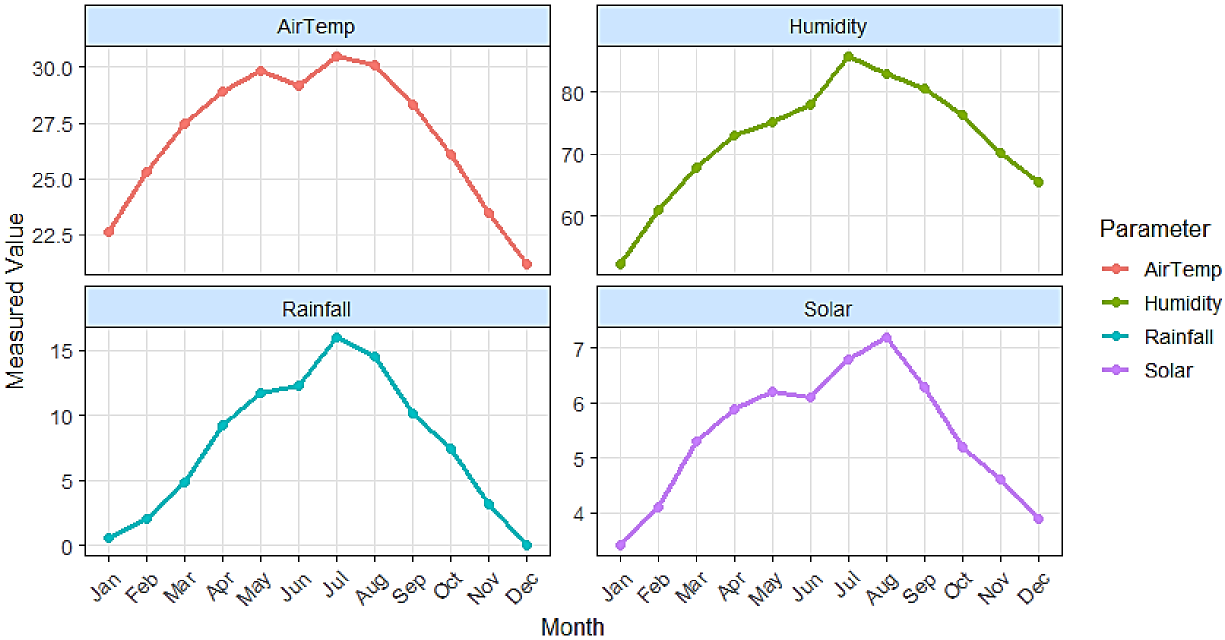

Figure 1. Monthly trends of climatic parameters (air temperature, relative humidity, rainfall, and solar intensity) recorded at the study site from June 2023 to May 2024.

Table 2. Comparative water quality parameters between pond and RAS systems during the experimental period

Parameter	Pond (Mean±SD)	RAS (Mean±SD)	Range (Pond)	Range (RAS)	Remarks / Observation
Dissolved Oxygen (mg L^{-1})	7.03±0.12	8.45±0.12	6.43–7.57	7.54–8.90	Significantly higher DO in RAS due to aeration and water circulation (P<0.05)
pH	7.72±0.10	7.71±0.09	7.15–8.37	7.30–8.21	pH values remained stable and similar between systems (P>0.05)
Total Dissolved Solids (mg L^{-1})	235.24±1.48	247.28±2.47	227.87–244.67	230.07–255.70	Slightly higher in RAS due to recirculation and mineral concentration (P>0.05)
Temperature (°C)	27.61±1.23	28.34±0.18	19.13–33.70	27.00–29.00	RAS maintained stable temperature within optimal rearing range (P<0.05)
Turbidity (NTU)	20.53±0.71	–	17.68–27.32	–	Slightly variable in ponds due to organic load; RAS remained clear
Ammonia (mg L^{-1})	0.12±0.01	0.00±0.00	0.04–0.18	0.00–0.00	Ammonia negligible in RAS due to effective biofiltration (P<0.05)

P<0.01), indicating that pond temperature fluctuates with environmental changes. A moderate positive correlation was observed between air temperature and rainfall (r=0.681, p=0.015), suggesting warmer periods may bring increased rainfall due to higher moisture retention in warm air. The humidity had a moderate positive correlation with dissolved oxygen (r=0.629, p=0.029) and pond temperature (r=0.754, p=0.005), indicating that higher humidity increases these parameters. Rainfall correlated moderately with dissolved oxygen (r=0.625, p=0.03), likely due to water agitation during rainfall, and negatively with pond pH (r=-0.58, p=0.048), suggesting rainfall dilutes pond water and lowers pH. The findings (Figure 2) highlight the influence of climatic factors, such as rainfall and humidity, on critical pond water quality parameters. Variations in pH, dissolved oxygen, and temperature due to changing climate conditions may affect the growth environment, potentially influencing fish seed health and development.

Comparative Assessment of Larval Growth

Larval growth performance, including mean length, mean weight, percentage length gain, percentage weight gain, and specific growth rate (SGR), was evaluated for both culture systems, with comparative data presented in Table 3 and illustrated in Supplementary Figure 4. In the RAS system, Batch 1 achieved a length gain of 2.02%, Batch 2 reached the highest at 2.94%, and Batch 3 maintained 2.01%. In contrast, the pond system showed consistently lower length gains across all batches, averaging around 1.19%. While the SGR of Batch 1 was nearly identical in both

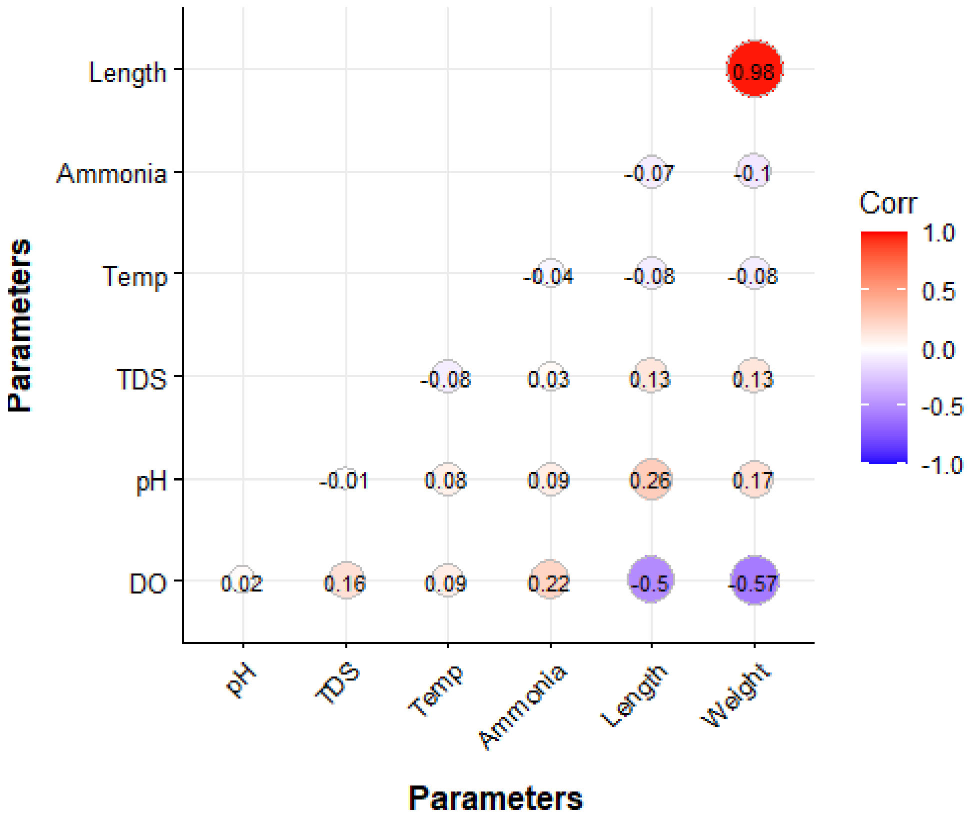

Figure 2. Correlation heatmap showing relationships between climatic factors and pond water quality parameters (June 2023-May 2024).

Table 3. Comparative growth performance of *O. niloticus* larvae reared in pond and RAS systems across three batches, including measurements of total length, total weight, percentage length gain, percentage weight gain, and SGR. Values are expressed as mean±SD

System	Batch	Total Length (mm)	Total Weight (g)	% Length Gain	% Weight Gain	SGR (%/day)
Pond	1	11.891±1.780	0.031±0.017	1.190±0.207	7.420±1.131	0.230±0.063
	2	14.135±2.829	0.043±0.021	1.191±0.207	7.420±1.131	0.199±0.025
	3	11.495±1.245	0.025±0.012	1.190±0.363	7.044±0.810	0.224±0.025
RAS	1	12.122±1.880	0.038±0.020	2.016±0.235	7.400±1.120	0.230±0.032
	2	12.330±2.294	0.043±0.019	2.940±0.658	6.770±0.925	0.215±0.027
	3	12.634±1.964	0.043±0.019	2.010±0.136	7.240±1.370	0.226±0.038

systems (0.23% per day), the RAS showed a slight advantage in subsequent batches, with Batch 2 reaching 0.215% per day compared to 0.199% in the pond, and Batch 3 showing 0.226% and 0.224%, respectively. Overall, the RAS system showed a trend of improved growth performance compared to pond systems, rather than a consistently superior outcome. Two-way ANOVA revealed a significant effect of culture system on percentage length gain and SGR ($P<0.05$), while batch and interaction effects were nonsignificant, confirming that the observed differences were moderate yet consistent across batches.

Larval Development of *O. niloticus* in Pond and RAS Condition

Larval growth was stable and more clearly defined in the RAS. During the gastrula stage, eggs averaged a diameter of 2035 to 2820µm, initially appearing yellow and oval-shaped. As development progressed, the egg color gradually changed to a greenish hue, with an increase in black pigmentation. A thick blastoderm layer, or germinal ring, occupied approximately three-quarter of the yolk sac area, marking significant developmental changes.

Throughout the 30-day rearing period, tilapia larvae in both RAS and pond systems showed steady increases in length and weight, although morphological development occurred earlier in the RAS. At the beginning, RAS larvae measured 9.05±0.23 mm and weighed 0.00942±0.00054 g, with a visible greenish yolk sac and limited movement. Pond larvae, although slightly larger (9.12±0.21 mm; 0.00958±0.00049 g), displayed similar early-stage features. By day 3, approximately 45% of yolk absorption was completed in the RAS, compared with only 25% in the pond. At day 6, swim bladder inflation and early swimming activity were observed in RAS (10.54±0.27mm; 0.0144±0.00072g), whereas pond larvae at this point exhibited only early pigmentation. By day 9, RAS larvae had completed yolk absorption (≈95%) and reached 10.74±0.34 mm, while pond larvae showed only partial absorption (~70%). At day 12, clearer body features, fin development, and increased pigmentation were noted in RAS larvae (11.3±0.25 mm; 0.03014±0.00124g), while pond larvae reached full yolk absorption. Fin-ray formation progressed rapidly in RAS larvae, with 70–80% of fin rays segmented by day 18, compared with similar progress around day 21 in the pond.

By the final week, RAS larvae exhibited well-defined dorsal, anal, and caudal fins with visible rays, dark body bands, and fully developed opercula (16.05±0.45 mm; 0.0736±0.0022 g). Pond larvae attained similar sizes (16.62±0.47 mm; 0.0778±0.0024 g) but reached these morphological stages 2–3 days later. These observations indicate that RAS-reared larvae progressed through developmental stages approximately two to three days earlier than those in pond conditions, suggesting enhanced growth performance and morphological advancement under RAS. These developmental patterns are visually depicted in Figure 3, which presents a day-by-day photographic sequence of RAS larvae from 1 to 30 days after hatching (DAH), showcasing key morphological transitions including yolk sac absorption, pigmentation, fin ray formation, and operculum development.

Supplementary Figure 5 summarizes quantitative morphometric increments, including yolk absorption (%) and fin-ray formation rate, with photographic evidence of key developmental milestones. Pigmentation developed progressively across the head, eyes, abdomen, and caudal regions (Supp. Figure 5a). Caudal-fin rays and pigmentation formed rapidly as larvae matured (Supp. Figure 5b), while eye pigmentation appeared within the first week (Supp.Figure 5c). Fin differentiation (dorsal, anal, pelvic, pectoral) followed yolk absorption, enabling active swimming (Supp. Figure 5d). Skeletal ossification was slower, completing by 30–32 days (Supp.Figure 5e), and the feeding apparatus (jaw, teeth) evolved gradually for exogenous feeding (Supp. Figure 5f). In contrast, pond larvae showed delayed and inconsistent development, reflecting environmental fluctuations in temperature, DO, and pH.

Morphometric Growth of tilapia (*O. niloticus*) Larvae in the Pond and RAS

A comparative study of morphometric traits between pond and RAS systems shows noticeable differences (Supplementary Table 3 and Figure 4). In Batch 1, TL was slightly higher in RAS (12.12±1.88 cm) compared to the pond (11.89±1.78 cm), while SL was higher in the pond (10.17±1.26 cm) than in RAS (9.73±1.33 cm). Other parameters, such as CL, HL, and ED, were consistently higher in RAS, with CL at 2.39±0.60 cm compared to 1.72±0.57 cm in the pond. In Batch 2, TL and SL were higher in the pond (14.14±2.83 cm and 11.32±1.88 cm, respectively) than in RAS (12.33±2.29 cm and 9.52±1.35 cm, respectively). However, CL was nearly the same between the pond (2.81±0.97 cm) and RAS (2.81±0.97 cm), while HL and ED were higher in the pond. For Batch 3, RAS performed better for most parameters, with TL at 12.63±1.96 cm compared to 11.50±1.24 cm in the pond, and CL at 2.78±0.98 cm versus 1.96±0.53 cm in the pond. However, this morphometric study indicates a treatment-specific influence on growth parameters.

Seed Quality Assessment Based on Fulton's Condition Factor (K_F)

As presented in Table 4, larvae reared in the RAS demonstrated consistently higher mean K_F values across all batches compared to those reared in pond systems. The highest mean K_F was recorded in RAS Batch 2 (2.149±0.210), followed by Batch 3 (1.967±0.194) and Batch 1 (1.904±0.468). In contrast, the pond system exhibited lower condition factors, with the lowest value

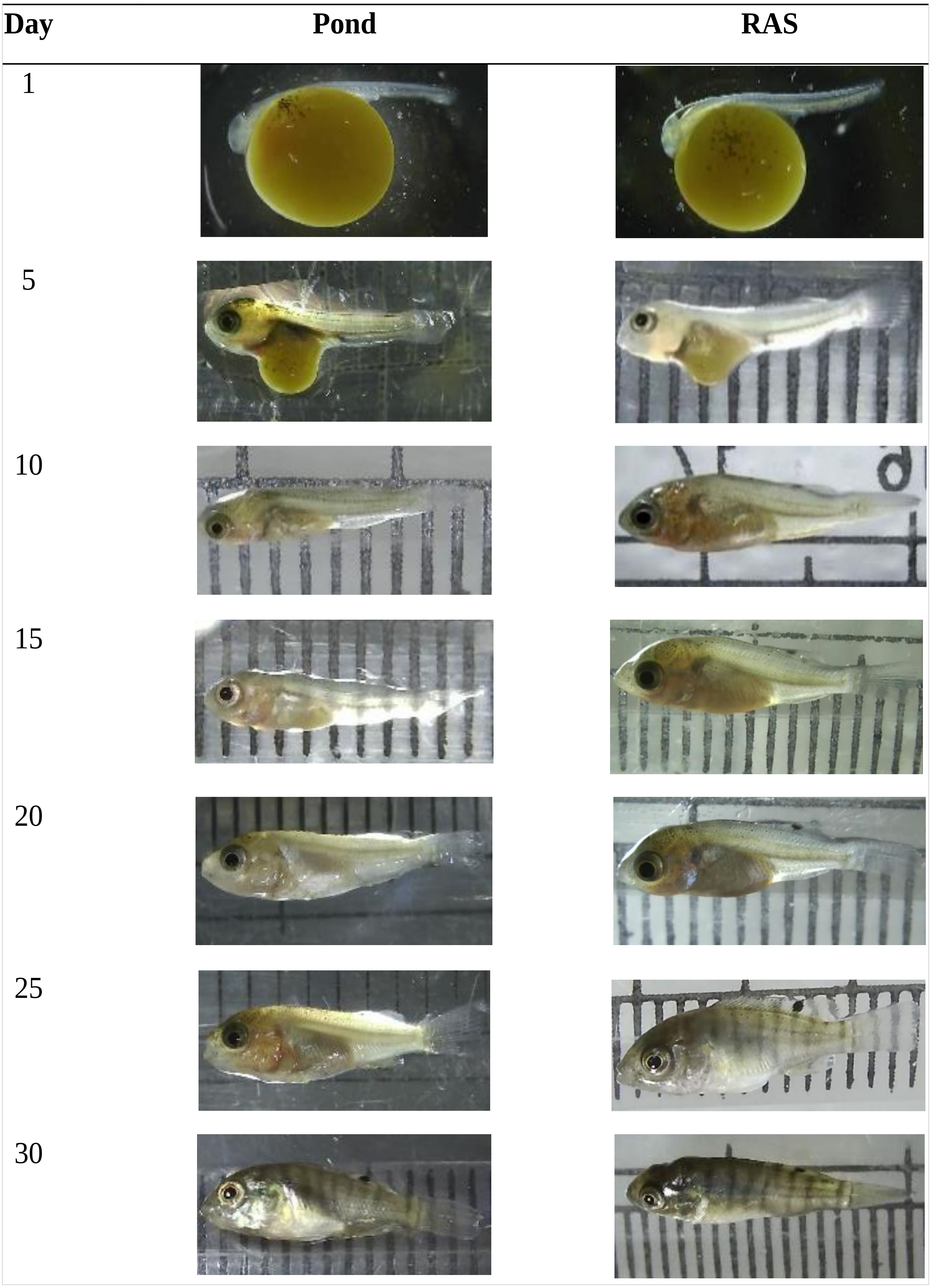

Figure 3. The characteristics change in larval development of tilapia from 1 DAH to 30 DAH in the Recirculatory Aquaculture System (RAS); Day 1: straight body (sb), yolk sac (ys), black pigments (bp). Day 5: dorsal pigmentation (dp), black eye (be), decreasing yolk sac (dys). Day 10: dorsal pigmentation (dp), dorsal fin (df), no yolk sac (nys). Day 15: dorsal pigmentation (dp), no yolk sac (nys). Day 20: melanophores (mf), dorsal fin (df), posterior tail (pt). Day 25: black pigments (bp), caudal fin (cf), eye shape increase (esi). Day 30: dorsal fin shape (df), vertical band (vb), caudal shape (cs)).

observed in Pond Batch 2 (1.454±0.192), followed by Batch 3 (1.508±0.264) and Batch 1 (1.654±0.331). The Supplementary Figure 6 indicates that RAS provides a more favorable environment for larval development and overall growth efficiency.

GHRH Gene Expression for Seed Quality Evaluation

The melt curve plot (Figure 5) is typically used in qPCR (quantitative Polymerase Chain Reaction) analysis to assess the specificity of PCR products. A distinct peak around 80°C confirms the amplification of specific target products. The amplification plot illustrates the real-time accumulation of PCR products. The amplification curves of target genes across various treatments demonstrate successful qPCR amplification, as seen by the increase in fluorescence (ΔRn) over cycles. Different treatments exhibit slightly varied cycle threshold (Ct) values, indicating different initial quantities of target RNA or DNA. Faster amplification, reflected by lower Ct values, suggests a higher initial concentration of the target genetic material in these samples. The absence of fluorescence in early cycles confirms that non-specific amplifications were effectively minimized by the reagents and protocols used. The qPCR analysis indicated distinct patterns of GHRH gene expression across the different treatments (Figure 6). Notably, GHRH gene expression levels were higher in the RAS treatment, which showed a significant upregulation compared to the pond treatment. This finding suggests that the treatments substantially impact GHRH expression levels. The increased mRNA expression in the RAS highlights the superior quality of seeds produced in the RAS compared to those from the traditional pond system.

Larval Growth Concerning Water Quality Parameters

The correlation analysis between water-quality parameters and larval growth metrics revealed distinct patterns between the RAS and pond systems (Figure 7).

In the RAS heat map (Figure 7a), water temperature showed a moderate positive correlation with pH (0.53) and DO (0.36), indicating that higher

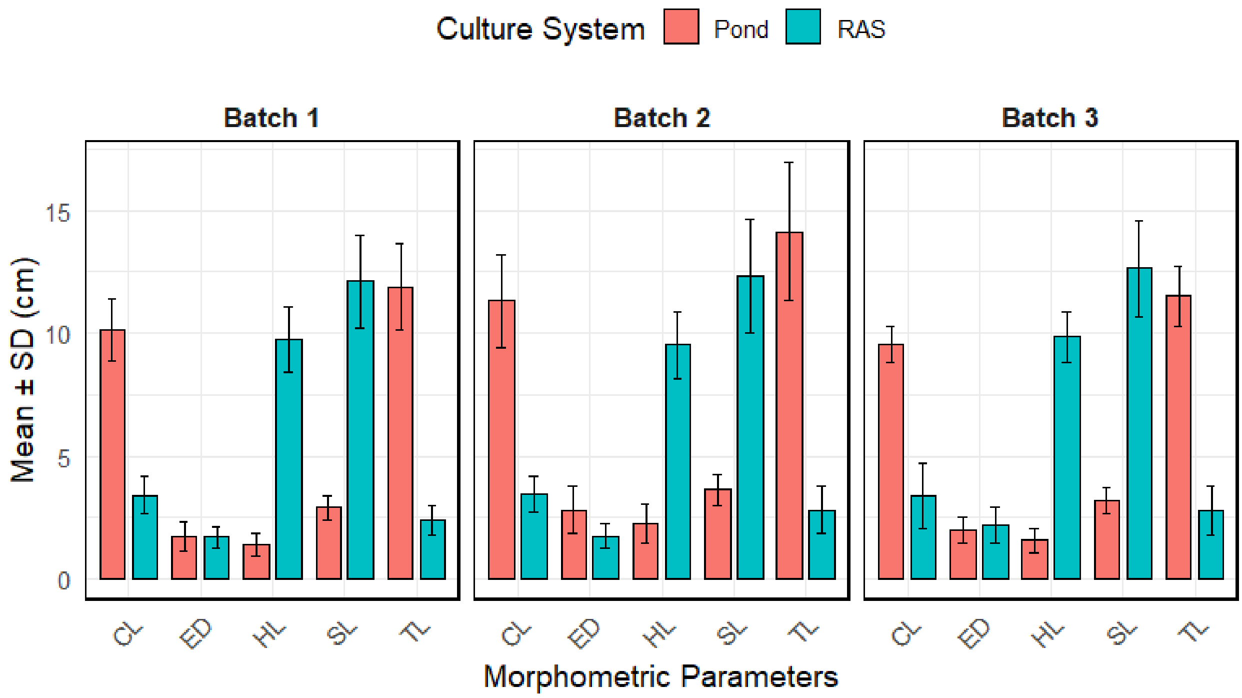

Figure 4. Morphometric comparison of *O. niloticus* larvae reared in pond and RAS systems across three batches, based on total length (TL), standard length (SL), head length (HL), caudal length (CL), and eye diameter (ED).

Table 4. Fulton's Condition Factor (KF) of *O. niloticus* larvae reared in pond and RAS systems across three batches. Data include minimum, maximum, 95% confidence intervals (CI), mean±standard deviation (SD), and corresponding health status assessments

System	Batch	Min	Max	95% CI of K_F	Mean ± SD	Health Status
Pond	1	1.123	2.108	1.654±0.124	1.654±0.331	Good
	2	1.162	1.871	1.454±0.072	1.454±0.192	Good
	3	1.079	1.983	1.508±0.099	1.508±0.264	Good
RAS	1	0.972	2.413	1.904±0.175	1.904±0.468	Excellent
	2	1.714	2.912	2.149±0.078	2.149±0.210	Excellent
	3	1.237	2.150	1.967±0.072	1.967±0.194	Excellent

temperatures were generally accompanied by slight increases in these parameters. Conversely, temperature exhibited weak negative correlations with both length (−0.13) and weight (−0.13), suggesting a minimal inhibitory effect on growth. Dissolved oxygen had a moderate negative correlation with TDS (−0.44), implying that elevated oxygen levels coincided with reduced total dissolved solids. Other parameters, including pH, ammonia, and TDS, displayed very weak correlations with growth ($|r|<0.20$), indicating limited influence on larval performance. The strong positive correlation between length and weight ($r= 1.00$) reflected expected proportional growth trends. Overall, these weak interrelationships suggest that environmental fluctuations within the RAS had minimal and inconsistent effects on growth, reflecting a stable and buffered system.

In the traditional pond system (Figure 7b), water temperature showed a strong negative correlation with length (−0.63) and weight (−0.67), implying that elevated temperatures may have adversely affected larval growth under fluctuating pond conditions. Dissolved oxygen correlated slightly positively with both length (0.17) and weight (0.12), suggesting limited benefits of higher DO levels. pH, ammonia, and TDS exhibited weak correlations with growth variables ($|r| < 0.15$), indicating minimal impact on size or biomass accumulation. The high length–weight correlation ($r = 0.96$) again confirmed internal consistency of the growth dataset.

Overall, the predominance of weak or poor correlations indicates that changes in individual water-quality parameters exerted minimal or inconsistent influence on others, signifying either biological buffering under RAS or environmental instability in pond

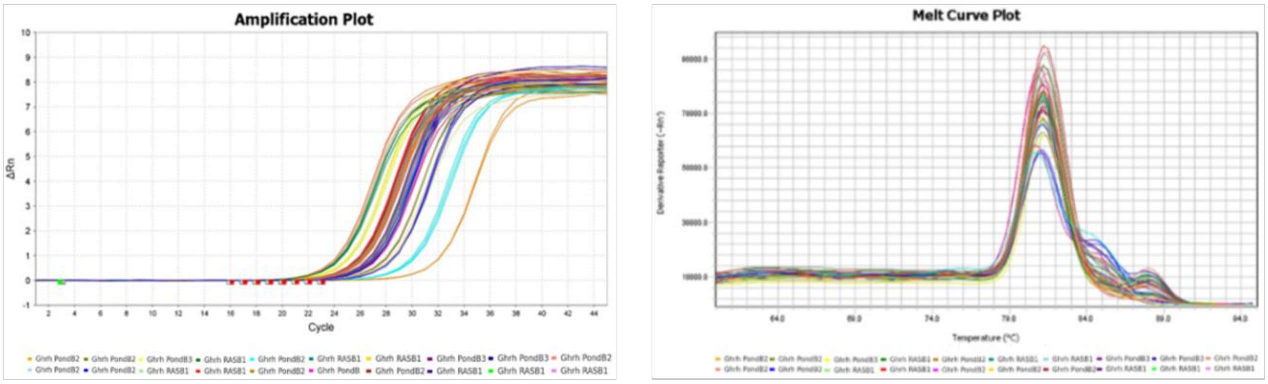

Figure 5. Amplification plot and melt curve plot of Growth Hormone Releasing Hormone (GHRH) gene.

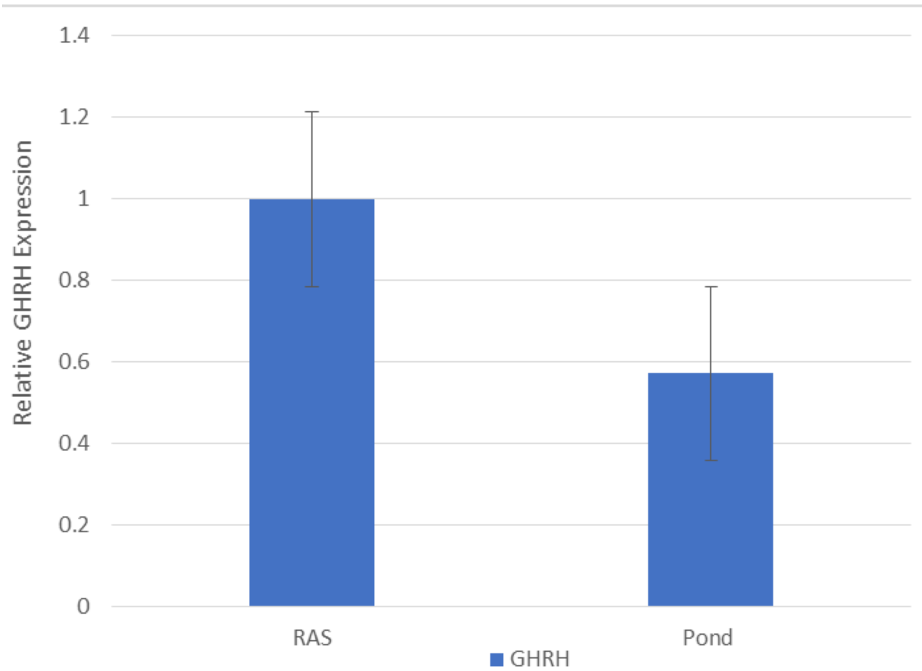

Figure 6. Relative expression (means±SE) of Growth Hormone Releasing Hormone (GHRH) in pond and RAS.

conditions. Such results align with previous observations that system stability, rather than individual parameter fluctuation, governs growth performance in controlled aquaculture environments (Boyd et al., 2022; Lembang et al., 2025; Siddique et al., 2025).

Morphometric Characters of *O. niloticus* Under Climatic and Water Quality Parameters

The influence of water quality parameters on the morphometric traits of *O. niloticus* larvae was evaluated through correlation analysis (Supplementary Table 4). A strong negative correlation was observed between water temperature and all measured morphometric parameters TL, SL, HL, CL, and ED indicating that higher water temperatures were associated with reduced larval body dimensions (P<0.01 for all traits). Conversely, the morphometric traits were all strongly and positively interrelated (P<0.01), suggesting synchronized and consistent growth across body parts during larval development (Figure 8). Among the water quality variables, DO, pH, TDS, and ammonia exhibited weak or no significant correlations with morphometric traits. For instance, DO showed a weak positive relationship with TL (r= 0.173) and SL (r= 0.196), while pH and TDS had negligible or slightly negative

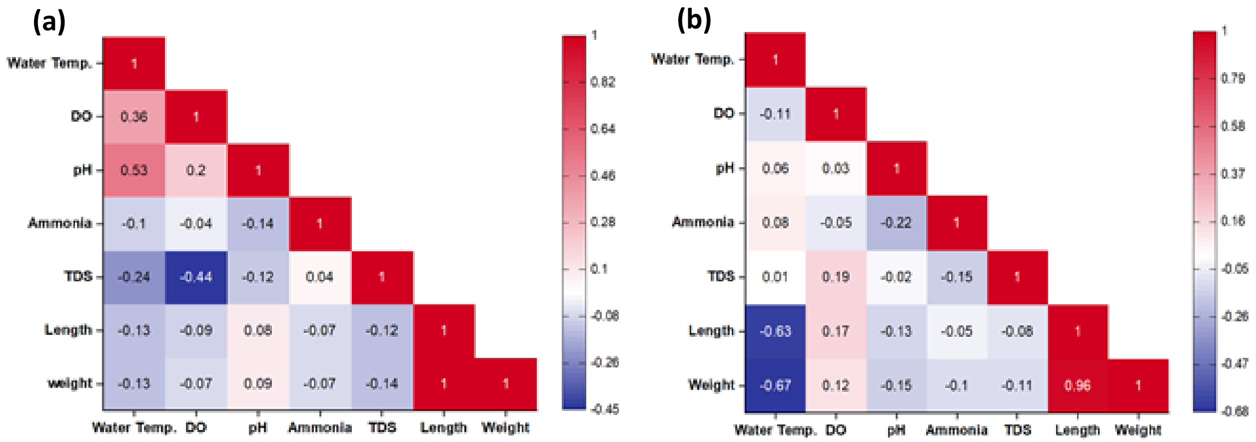

Figure 7. Correlation heatmaps showing relationships between water quality parameters and fish growth metrics in (a) a recirculating aquaculture system (RAS) and (b) a conventional pond system, September 2023..

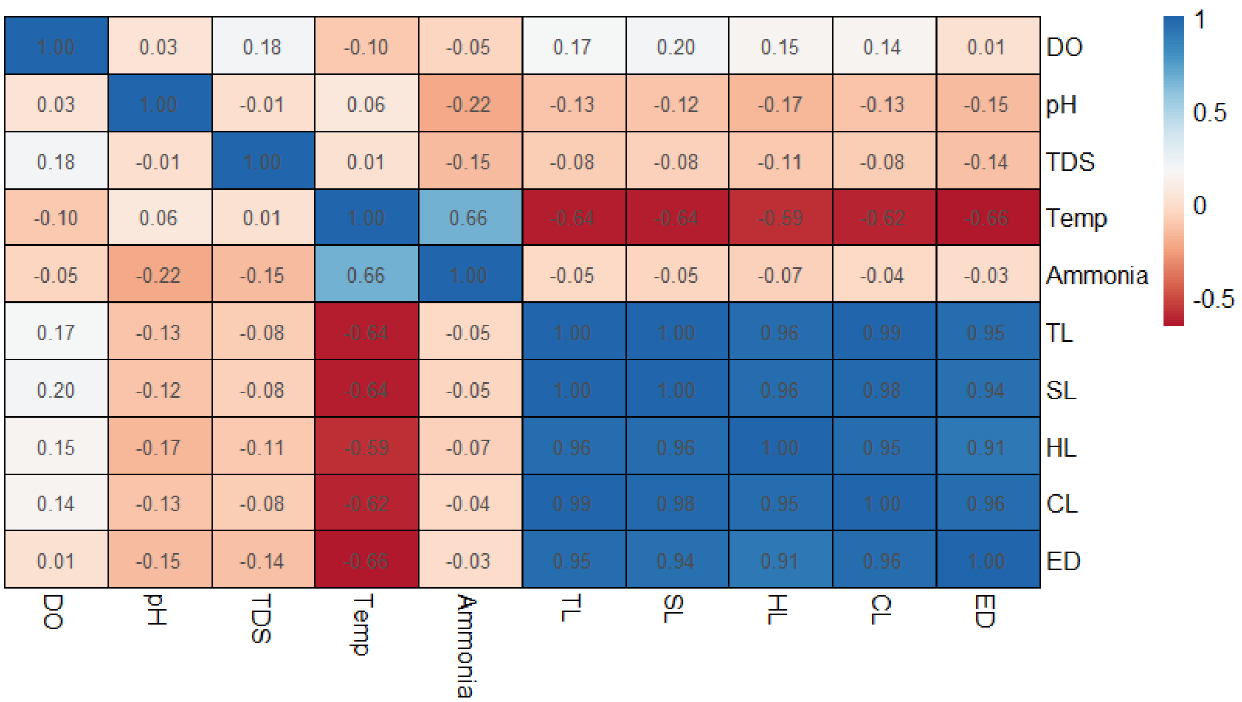

Figure 8. Heatmap showing the Pearson correlation coefficients among morphometric traits and water quality parameters of *O. niloticus*. Correlation values range from -1 to +1, with color intensity representing strength and direction of relationships. (Derived from Table 8).

associations with most morphometric parameters. To further explore these relationships, a PCA was conducted (Figure 9; Supplementary Table 5). The PCA identified two major components explaining most of the variability in the dataset. PC1, with an eigenvalue of 3.19, accounted for the largest proportion of variance and was predominantly influenced by water quality parameters specifically TDS (loading= 0.82), water temperature (0.80), and ammonia (0.76). In contrast, DO and pH exhibited negative loadings, indicating inverse relationships with PC1. The second PC2, with an eigenvalue of 1.28, was more strongly associated with larval morphometric traits, especially Standard Length (loading= 0.72) and Eye Diameter (0.60). This component highlights the secondary yet distinct contribution of larval morphology to overall variation, independent of the water quality-driven trends observed in PC1. These findings underscore the dominant role of water quality particularly temperature, TDS, and ammonia in shaping larval morphometric outcomes, while the morphometric parameters themselves maintained a highly coordinated growth pattern across culture conditions (Figure 9; Supplementary Table 5).

Seed Quality Comparison

This study compares the effects of conventional pond systems and RAS on key parameters influencing tilapia seed quality and growth. Parameters assessed include larval length, weight, development stages, morphometric traits, and gene expression. Findings indicate that RAS provides a stable environment, resulting in higher seed quality and improved growth performance. Additionally, gene expression and developmental consistency were better controlled in RAS, with precise thermal regulation allowing for a clear assessment of stress response. Seed quality varies in the pond due to environmental factors, whereas RAS provides higher and more consistent seed quality, benefiting from stable, controlled conditions (Table 5).

Discussion

The study compared traditional pond conditions with a Recirculatory Aquaculture System (RAS) for *O. niloticus* larvae, focusing on seed quality, growth metrics, morphometric traits, and GHRH gene expression. The findings revealed consistent and significant advantages for RAS over pond systems, underscoring the critical role of water quality stability and environmental control in larval development and health. However, while RAS demonstrated positive outcomes, these results should be interpreted cautiously, considering the experimental scale and controlled rearing environment, which may not fully capture field-level variability.

Fish larval development is highly sensitive to climate and water quality variations, making it essential to adopt climate-resilient systems. Climate change impacts such as elevated water temperatures, erratic rainfall, and salinity fluctuations directly affect fish metabolism, reproduction, and survival (Macusi et al., 2015; Singh et al., 2015; Adhikari & Mandal, 2019; Maulu et al., 2021; Wang et al., 2018). In Bangladesh, pond-based aquaculture is particularly vulnerable to such fluctuations, which compromise seed quality and larval survival (Ahmed et al., 2020). Water parameters such as temperature, dissolved oxygen (DO), pH, total dissolved solids (TDS), and ammonia significantly impact seed performance. In this study, RAS provided stable temperature (29°C), optimal DO (>6 mg/L), and

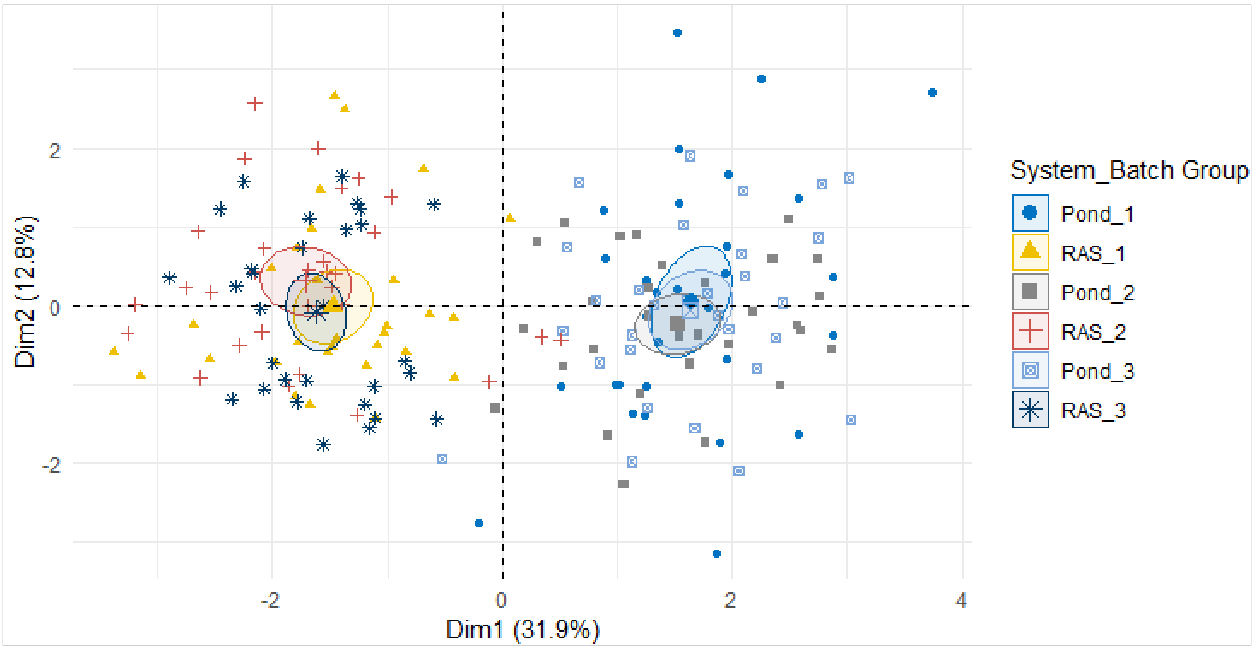

Figure 9. PCA of Water Quality Influence on Larval Morphometrics Across Aquaculture Systems and Batches..

negligible ammonia, whereas the pond system exhibited fluctuations (temperature: 19.13–33.7°C; DO often <5 mg/L; ammonia ~0.118 mg/L) that negatively affected larval growth and health (Stone & Thomforde, 2004; Morrow, 2009; Refaey et al., 2025). Optimal pH (7–8) was maintained in RAS, aligning with El-Sherif and El-Feky (2009), who noted reduced growth efficiency at pH 6 and 9 in Nile tilapia. Similarly, temperature stability in RAS supported faster growth and lower mortality, in agreement with Kaneshima et al. (2022). High ammonia in ponds can inhibit respiration and growth (Riche & Garling, 2003; Abdel-Tawwab et al., 2019), while DO levels below 3 mg/L impair feeding and cause mortality (Mallya, 2007). The principal component analysis (PCA) confirmed that water quality accounted for over 42% of growth variability, consistent with Verma et al. (2022) and Mannan et al. (2012), highlighting the need to manage physicochemical parameters.

Growth analysis revealed better performance in RAS across all three batches. Condition factor (KF), an indicator of fish health (Christophe et al., 2015; Asadi et al., 2017), was significantly higher in RAS (KF= 2.149) compared to pond systems, indicating better physiological status (Urbanski et al., 2023). Morphometric traits total length, head length, caudal length, and eye diameter showed less variability in RAS, affirming the stabilizing influence of controlled environments (Wimberger, 1992; Kováč et al., 1999; Carpenter, 1996). Larvae in RAS also progressed through defined developmental stages yolk sac absorption, pigmentation, and fin development whereas pond larvae showed developmental delays, often attributable to stress and heavy metal exposure (Taslima et al., 2022). Nevertheless, it should be noted that the sample size per batch was relatively small, which may limit the statistical power and increase the risk of underestimating within-treatment variability. Batch-to-batch variation in spawning and larval quality could also have influenced some observed differences.

GHRH gene expression was markedly higher in RAS-reared larvae, suggesting enhanced growth hormone signaling and better seed quality. GHRH regulates somatic growth and immune function (Vance, 1990; Nam et al., 2011), and its upregulation reflects reduced environmental stress in RAS. Aboukila et al. (2021) and Sherzada et al. (2023) observed similar trends, where better environmental conditions elevated expression of growth-related genes. In contrast, pond-reared larvae showed suppressed GHRH levels, likely due to suboptimal water quality and climate-induced stressors. While qPCR remains a gold standard for gene-specific expression (Smith & Osborn, 2008), the current study analyzed only one growth-related gene, which provides a limited molecular perspective. Inclusion of additional genes or broader transcriptomic approaches would strengthen interpretation of growth-regulatory pathways. Correlation analyses showed that growth in RAS was largely unaffected by minor fluctuations in temperature or DO, underscoring system stability. However, in the pond system, elevated temperature showed a moderate negative correlation with length (r= -0.63), and rainfall negatively impacted growth. These patterns mirror findings by Smoliński & Mirny (2017), Ekubo & Abowei (2011), and Huang et al. (2020), where unstable environments hampered growth and increased mortality. Adoption of Recirculating Aquaculture Systems (RAS) offers a practical solution to mitigate environmental stress. RAS supports optimal DO levels, lowers ammonia, and ensures biosecurity (Van Rijn, 2013; Li et al., 2023; Wei et al., 2019). However, its high setup cost and energy requirements remain major barriers to adoption, especially for smallholder farmers. To address these challenges, the revised discussion proposes several practical strategies. Integrating solar-

Table 5. Comparison of seed quality traits in *O. niloticus* reared in pond and RAS systems, highlighting differences in egg characteristics, growth, development, morphometrics, and gene expression influenced by environmental control and system conditions

SL	Parameters	Pond System	RAS System	Remarks
1	Egg color	Natural pigmentation. Egg color varied due to environmental conditions	Natural pigmentation mostly. But can be white due to lack of direct light exposure.	-
2	Egg size	The egg diameter is comparatively lower than the RAS	The larger egg quality over the batches	Indicates better egg quality
3	General Growth performance	Slower and influenced by seasonal changes	Enhanced growth performance, as environmental factors can be tightly managed and optimized	-
4	Stages of development	Comparatively slower, temperature-dependent	More consistent, uniform growth due to controlled conditions	Development stages were not well defined in the pond system
5	Morphometric study (TL, SL, HL, CL, ED)	Larger variation due to environmental factors	Consistent measurements yield more uniform morphometric data due to stable conditions	More variable pond environments.
6	Gene expression	Influenced by natural environmental stressors	Potentially optimized gene expression due to a controlled environment	The overall gene expression level was netter in RAS

powered aeration and water-recirculation units can substantially reduce operational energy costs and enhance sustainability in rural settings. The incorporation of biofloc or periphyton-based nutrient recycling systems can further improve water quality while lowering feed costs and waste discharge (Avnimelech, 2015; Hossain et al., 2023). Additionally, establishing community-based hatchery cooperatives could distribute initial investment costs and maintenance responsibilities among groups of small-scale farmers, improving financial feasibility and technical support (Hasan et al., 2021). Still, these correlations should be interpreted with caution, as the relationships observed were weak to moderate in strength, suggesting that growth responses may be influenced by other unmeasured factors such as feed intake, microclimate, or management variability. Furthermore, challenges like energy demand and cost (Mehrim & Refaey, 2023; Lal et al., 2024) must be addressed through renewable integration and operational innovations (Seginer & Mozes, 2012).

As Bangladesh faces increasing climate risks, the shift to RAS and similar controlled systems is essential for ensuring high-quality fish seed, maintaining biodiversity (Allendorf & Leary, 1988), and supporting sustainable aquaculture development (Thilsted et al., 2016; Arthur et al., 2010). Length-weight condition indices such as KF are effective for rapid seed quality assessments in varied environmental contexts (Alphonse et al., 2017; Ravikumar et al., 2023). Nonetheless, while RAS demonstrated consistent positive trends, these findings represent early-stage evidence from a controlled study. Large-scale validation, longer-term monitoring, and inclusion of economic feasibility analyses are necessary before generalizing the observed benefits.

Overall, this study highlights that RAS not only supports superior growth, morphology, and gene expression in Nile tilapia larvae but also provides a replicable model for climate-resilient aquaculture. However, given the small sample size, batch variability, and limited gene selection, the findings should be interpreted as indicative rather than conclusive. Despite the promising results, this study has certain limitations. It was conducted under controlled experimental conditions that may not reflect large-scale practices. Only one growth-related gene (GHRH) was analyzed, offering limited molecular insight. Economic feasibility was not assessed, and long-term performance outcomes such as survivability and reproductive maturity remain unexamined. Future studies should consider multi-gene or transcriptomic approaches (e.g., RNA-Seq) to capture a comprehensive view of stress and growth regulatory mechanisms. Additionally, integrating RAS with renewable energy sources could help mitigate operational costs. Although RAS buffers most external climatic fluctuations, future work should focus on optimizing internal environmental controls such as photoperiod regulation, temperature-regime planning, and improved management of mechanical and biological filtration, UV sterilization, ozone treatment, and protein skimming—to further enhance system stability. Evaluating hybrid or flow-through RAS configurations and assessing economic feasibility will also be essential for practical adoption in resource-limited hatcheries. Moreover, stage-specific integration, where RAS is used for broodstock conditioning, larval rearing, and early fry nursing while ponds support later juvenile phases, may offer a more cost-effective pathway. Therefore, although RAS provides clear advantages in stabilizing water quality and improving seed performance, these benefits must be balanced with operational costs and validated across diverse production settings before large-scale implementation.

Conclusion

This study compared the seed production performance of O. niloticus in traditional ponds and Recirculatory Aquaculture Systems (RAS). As expected, RAS-reared larvae exhibited higher growth, condition, and GHRH gene expression, reflecting the advantages of stable water quality and controlled rearing conditions. However, the experiment was conducted on a limited scale, and small sample size, batch variation, and short study duration warrant cautious interpretation. Based on the current findings, we recommend using RAS primarily for broodstock conditioning, spawning, egg incubation, and early larval and fry nursing, when fish are most sensitive to environmental fluctuations. Once fry reach a more resilient juvenile stage, transfer to flow-through systems or earthen ponds may offer a cost-effective strategy for grow-out. Future studies should explore strategies for managing fry exposure to pathogens common in flow-through systems, assess the economic trade-offs between RAS and pond-based production, and investigate the operational considerations involved in scaling RAS for commercial tilapia hatcheries.

Ethical Statement

The study, including sample collection, experimentation, and sacrifice, met the ethical guidelines, including adherence to the legal requirements of the study country. The study was approved by the Ethical Standard of Research Committee of Bangladesh Agricultural University (BAU) with the reference code BAURES/ESRC/FISH-11/2022.

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Author Contribution

Safiara Nusrat Nova: Overall data analysis & presentation and writing the original draft; Mohammad Abu Baker Siddique: Overall technical analysis & presentation and writing the original draft; Ilias Ahmed: Overall data analysis & presentation and Editing the draft; Imran Bin Younos: Editing the draft; Md Shiekh Tauhiduzzaman Shimul: Editing the draft; Mohammad Mahfujul Haque: Review & editing the draft; Mariom: Review & editing the draft; A. K. Shakur Ahammad: Concept development, validation, overall supervision and editing the draft.

Conflict of Interest

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

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