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RESEARCH

Integrated biofloc technology in red tilapia aquaculture: Salinity-dependent effects on water quality, parental stock physiology, reproduction, and immune responses

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Abstract

The study examines the impact of integrated biofloc technology (BFT), different salinity levels, and their combined effects over 90 days on various physiological parameters. The investigation includes growth performance and feed utilization, water quality, the chemical composition of biofloc and fish, digestive enzymes, reproductive performance, stress and biochemical indicators, and antioxidant-immune responses in red tilapia (*Oreochromis spp*.) broodstock. The fish were initially weighed (males: n = 270; 104 ± 0.96 g; females: n = 270; 93.2 ± 0.66 g) and subsequently divided into 12 treatment groups (6 for males and 6 for females) spread across 36 separate tanks (3 tanks per treatment; 45 fish per treatment; 15 fish/tank). The treatments involved three salinity levels (18, 28, and 36 ppt) in both clear water (CW) and BFT systems. The outcomes demonstrated that fish in the 36 ppt salinity with BFT treatment demonstrated significant improvements ($P < 0.05$) in growth parameters (final body weight, weight gain, and specific growth rate, feed intake, and feed conversion ratio). The condition factor in BFT groups increased in all salinity situations. The survival rates of broodstock were consistently high in all experimental conditions The study found that BFT and salinity significantly impacted ($P < 0.05$) whole body contents (moisture, protein, lipid, and ash) in both males and females. Water quality parameters showed variations between BFT and CW, with notable impacts ($P < 0.05$) on dissolved oxygen and pH. The BFT and salinity influenced digestive enzyme activities (protease, amylase, and lipase) and reproductive performance (males) and the 36 ppt salinity with BFT recorded the highest values. The hemato-biochemical and antioxidant-immune responses were also impacted by BFT and salinity exposure. The study highlights the potential benefits of incorporating BFT into red tilapia aquaculture systems, particularly in optimizing growth, health, and reproductive performance under various salinity conditions, which can enhance sustainable intensification, disease control, and environmental stewardship.

Keywords Broodstock · Health status · Integrated biofloc technology · *Oreochromis spp*. · Salinity exposure · Sustainable aquaculture

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Introduction

Aquaculture has become a vital industry in recent times, playing a substantial role in addressing the escalating global seafood requirements and fostering economic growth (FAO 2023). Red tilapia (*Oreochromis spp*.) is a widely preferred species among those cultivated globally on account of its accelerated growth rate, remarkable adaptability to diverse environmental conditions, and positive market reception (Sampantamit et al. 2020). However, sustainable aquaculture practices face challenges in managing water quality, controlling diseases, and enhancing reproductive performance. Therefore, novel strategies are needed to optimize profitability and productivity while minimizing negative environmental impacts (Chopin et al. 2008).

Integrated biofloc technology (BFT) has emerged as a viable aquaculture technique that addresses these difficulties. It utilizes natural microbial processes to maintain water quality, optimize feed utilization, and improve disease resistance in farmed species (Nisar et al. 2022). BFT systems leverage the microbial community found in bioflocs, which are aggregations of bacteria, algae, protozoa, and organic matter. These bioflocs aid in the conversion of organic waste into microbial protein, lowering nutrient output and increasing water quality (Abakari et al. 2022; Khanjani et al. 2022). Furthermore, bioflocs serve as a supplemental feed source, meeting the nutritional needs of cultured organisms while minimizing dependency on traditional feeds (Choo and Caipang 2015).

BFT is applicable to both finfish culture (Laice et al. 2021) and shrimp farming (El-Sayed 2021). Since the early 1990s, research has explored its potential in enhancing growth and production of commercial species like African catfish (*Clarias gariepinus*) (Green and McEntire 2017; Putra et al. 2017), channel catfish (*Ictalurus punctatus*) (Schrader et al. 2011), and Nile tilapia (*Oreochromis niloticus*) (Abakari et al. 2020; Avnimelech 2007; Ekasari et al. 2015b). BFT benefits include improving water quality, growth promotion, boosting general health, and increasing productivity. BFT systems function by manipulating the carbon-to-nitrogen (C:N) ratio. This enhances biofloc growth, maintains bacterial balance, and controls ammonia levels (Mali et al. 2024). Additionally, bioflocs provide in situ nutrients like protein, lipids, amino acids, and fatty acids (Khanjani et al. 2023; Wei et al. 2016). Fish raised in BFT systems, such as *O. niloticus*, demonstrate faster growth compared to those raised in clear water systems (Haraz et al. 2023; Nguyen et al. 2021).

Salinity is a significant environmental factor impacting the efficiency of aquaculture systems, particularly in coastal regions where tilapia farming is prevalent (Bœuf and Payan 2001). It can affect various physiological functions of aquatic organisms, including the immune system and reproductive physiology of red tilapia (Suresh and Lin 1992). To maximize breeding success, it is crucial to understand the factors influencing red tilapia reproduction. A study by Chen and Liu (2022) emphasized the impact of salinity and food quality on fish reproduction, with research indicating a decline in reproduction at higher water salinity levels.

Tailored nutrition is crucial during broodstock preparation to meet energy requirements for successful reproduction (Engdaw and Geremew 2024), as decreased food availability directly impairs reproduction (Volkoff and London 2018). Given that red tilapia hatcheries typically operate at lower salinities, there is a need for optimized conditions during the broodstock phase (Malik et al. 2017; Sallam et al. 2017), with proper parental stock nutrition being crucial for successful reproduction (Engdaw and Geremew 2024). Nevertheless, the correlation between salinity and BFT in the aquaculture

of parental stock red tilapia is largely unstudied, underscoring the necessity for exhaustive investigations that clarify the salinity-induced impacts of BFT on critical physiology and production parameters.

This research aims to analyze the combined effects of BFT and salinity on various physiological parameters in red tilapia. Specifically, it examines how salinity affects water quality indicators, parental stock physiology, reproductive performance, and immunological responses in red tilapia raised under BFT settings. The goal is to provide valuable information for improving red tilapia aquaculture practices, focusing on sustainable intensification, disease control, and environmental responsibility.

Materials and methods

Ethical approval statement

The College of Agriculture Committee for Animal Care at the National Institute of Oceanography and Fisheries (NIOF) in Egypt granted ethical permission for the study, with reference number (NIOF-AQ1-F-23-R-010). Furthermore, all study methodologies rigorously adhered to the ARRIVE guidelines v2.0 (Percie du Sert et al. 2020), ensuring that the research approach is consistent with recognized ethical standards and preserves the well-being of the experimental animal.

Biofloc setup

Thirty-six indoor 250-L circular fiberglass tanks were thoroughly washed and cleaned (100 mg/kg chlorine) as experimental units. Eighteen out of the 36 tanks were used as biofloc system tanks. The tanks were filled with 5 L of fresh water obtained from the draining canal as a source of inoculation along with 50 g of urea as a nitrogen source. The used water was rich in primary and secondary productivity, with an initial total suspended solids (TSS) of 40 mg L^{-1}. The water volume was scaled up to 50 L using underground saltwater. Then, the excremental salinity was adjusted using fresh groundwater. About 0.5 L of *Chlorella* sp. obtained from El-Max hatchery at a density of about 10^8 cells ml^{-1} was added to each tank, as suggested by (Caipang et al. 2015a, 2015b), followed by addition of 300 ml of *Lactobacillus plantarum* AH solution (10^7 cell ml^{-1}), cultured in de Man, Rogosa and Sharpe (MRS) (Oxoid, England) broth medium overnight (Komara et al. 2022).

For the first week, about 10 g of commercial tilapia feed was added daily to each tank, then dropped to 5 g/day for another week to trigger the production of nitrogenous wastes. Calculated amounts of molasses were also added daily to their respective tanks to maintain a C/N ratio of 15:1, according to Avnimelech (1999). Tanks were vigorously aerated using four, 5-cm air stones connected to a one HP air blower. The light regime was at about 12 L:12 D, (El-Sayed and Kawanna 2007), while water temperature was maintained at room temperature, which ranged from 25 °C to 28 °C throughout the trial. Through the first week, the biofloc tanks started to turn brown, and phytoplankton populations significantly decreased, whereas heterotrophic communities flourished. A zero-water exchange rate was applied to the biofloc tanks throughout the experiment, except for the water lost due to evaporation.

Rearing conditions and experimental design

A total of 540 healthy red tilapia, *Oreochromis spp.* (270 males and 270 females) were obtained from El-Max Research Station (NIOF), Alexandria, and transferred to the El-mothalath unit in NIOF, Egypt. Males and females with an initial body weight of 104 ± 0.96 g and 93.2 ± 0.66 g, respectively were separately distributed into 12 treatment groups (6 for males and 6 for females) spread across 36 separate tanks (3 tanks per treatment; 45 fish per treatment; 15 fish/tank). Fish were kept at BFT (18 tanks) and clear water (CW; 18 tanks) systems at salinities of 18, 28, and 36 ppt (S18, S28, and S36, respectively). A salinity level of 18 ppt in CW system was used as a reference level (control) for rearing red tilapia, as recommended by Sallam et al. (2017). Fish were fed on a commercial feed (25% CP), to satiation three times daily (9:00 AM and 1:00, 4:00 PM), for 90 days. The CW tanks were cleaned daily, the solid wastes were removed, and about 30% of the water was replaced with the same level of salinity and temperature.

Performance and biometric indices

At the end of the rearing period (90 days), all fish from each tank were gathered, counted, and weighed. Various parameters, including morphometrics, feed utilization, survival rate (SR, %), and organ indices, were meticulously recorded and calculated using the following equations:

$$Weight\ gain\ \left(WG,\ \frac{g}{fish}\right) = Final\ body\ weight\ (FBW) - initial\ body\ weight\ (IBW)$$

$$Average\ daily\ gain,\ \left(ADG,\ \frac{g}{day}\right) = \frac{Wg,\ g}{Trial\ period\ (T,\ days)}$$

$$SGR,\ \frac{\%}{day} = \frac{Ln\ FBW - Ln\ IBW}{T,\ days} \times 100,\ where\ Ln = natural\ \log.$$

$$Feed\ intake\ \left(FI,\ \frac{g}{Fish}\right) = \frac{Total\ consumed\ feed\ ,\ g}{Number\ Fish}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{FI, g}}{\text{WG, g}}$$

$$SR,\ \% = \frac{Final\ number\ of\ Fish}{Initial\ numberof\ fish} \times 100$$

$$\text{K factor} = \frac{\text{Body weight (BW, g)}}{\text{Length}^3 (L^3)} \times 100$$

$$\text{Hepatosomatic index (HSI)} = \frac{\text{Liver weight, g}}{\text{BW, g}} \times 100$$

$$\text{Viscerosomatic index (VSI)} = \frac{\text{Viscera weight, g}}{\text{BW, g}} \times 100$$

$$\text{Testesosomatic index (TSI)} = \frac{\text{Testes weight, g}}{\text{BW, g}} \times 100$$

$$\text{Gonadosomatic index (GSI)} = \frac{\text{Gonad weight, g}}{\text{BW, g}} \times 100$$

Whole-body and diet proximate analysis

Upon the trial's completion, 15 fish per treatment were randomly chosen for the whole fish body composition. The assessment of moisture, protein, lipid, and ash content was carried out for both the fish and biofloc samples, following the methodology outlined in AOAC (2007).

Water quality measurements

Water quality parameters, including water temperature, pH, and dissolved oxygen were measured daily, whereas total alkalinity, total ammonia (NH^{4+}), unionized ammonia (NH^3), nitrites (NO^2), nitrates (NO^3), TSS, and biofloc volume (BFV) were measured at 10-day intervals. Water temperature and pH were measured using pH and temperature testers (Hanna Instruments), while DO was determined using EcoSense DO200A dissolved oxygen probe (YSI). Total Alkalinity, NH^4, NO^2, and NO^3 were measured spectrophotometrically using YSI 9300 photometer (YSI). Unionized ammonia–nitrogen (NH^3) was calculated from the pre-estimated NH^{4+}, temperature, and pH values of the same tank, according to Emerson et al. (1975). One hundred milliliter water samples were collected from each tank and filtered, under vacuum pressure, through a pre-weighed filter paper to determine total suspended solids (TSS). BFV was determined by settling one liter of water from each biofloc tank in an Imhoff cone for 30 min.

Total heterotrophic bacteria (THB) in experimental tanks under aerobic conditions were counted on nutrient agar plates (Oxoid, England) from each tank. Water samples were collected in sterile Falcon tubes, and 1 ml of aliquots was spread on nutrient agar plates and incubated at 30 °C for 24 h. Bacterial colonies were counted, and the results were expressed as colony-forming unit ml^{-1} (CFU ml^{-1}).

Blood, semen, and tissue sampling

At the end of the trial (90 days), 15 fish per treatment (5 fish/tank) were randomly selected and anesthetized using 0.5 ml clove oil L^{-1}. Two sets of blood samples were taken by piercing the caudal blood vessels. The first set was collected in tubes having anticoagulants for hematological assays. The other set was emptied in tubes without anticoagulants to obtain serum by centrifugation at 3000 × g for 10 min. The serum samples were used for biochemical, antioxidant, and immunological assays. Fresh semen samples from five adult males in each tank (15 fish/treatment) were collected for assessment of sperm quality parameters. Decapitation of the fish (15 fish/ treatment) was applied to be euthanized and

tissue samples were taken from the intestine, ovaries, and liver for organ indexes, digestive enzymes, and reproductive performance.

Digestive enzymes assay

Five fish were randomly collected from each tank (15 fish/treatment) and intestines were carefully removed and used for digestive enzyme analysis. The intestinal contents were collected, homogenized with chilled sucrose solution (0.25 M) in glass test tubes using Teflon coated tissue homogenizer and centrifuged (5000 *x*g; 30 min at 4 °C) as described by Makled et al. (2019). The supernatant was recovered and kept at 4 °C for enzymatic assays that were conducted within 24 h after extraction (Suzer et al. 2008) and were expressed as a specific activity (U mg^{-1} intestine content). Protease activity was measured using bovine serum albumin as a standard. Amylase activity was measured using starch as the substrate. Lipase activity was determined using β-naphthyl caprylate as substrate. One unit of lipase activity was defined as 1 mg of β-naphthol released per minute.

Reproductive performance and sex hormones assay

Sperm motility was subjectively analyzed using a light microscope at 400 × magnification, employing a five-category classification system (0; 0–25; 25–50; 50–75; or > 75%) based on the methodology proposed by Boussit (1989). Sperm concentration was estimated through microscopic examination using a Neubauer counting chamber, while sperm viability was determined using the Eosin-Nigrosin staining method, as outlined by Kowalski and Cejko (2019).

Concurrently, females' reproductive performance parameters were evaluated the ovaries were carefully opened with a sharp scalpel to obtain egg samples. Measurements included egg weights, numbers, and diameters. Egg number was quantified per g of eggs and then correlated with ovary weight and the body weight of the fish. Subsequently, absolute fecundity (AF) and relative fecundity (RF) were calculated using the equations proposed by Bhujel (2000):

AF = Total weight of eggs per female (g) × the number of eggs per g.

RF = Absolute fecundity/body weight (g).

The concentrations of the blood serum hormones (testosterone and progesterone) were determined using commercial ELISA test kits with Cat. No. BC-1115 and BC-1113 (Bio-Check, Inc), respectively, as per Tietz (1995) technique.

Hemato-biochemical assays

For the assessment of heparinized whole blood count (CBC), each blood sample was processed to determine the levels of hemoglobin (Hb) and hematocrit (Hct, %) using commercial colorimetric kits (Diamond Diagnostic, Egypt). Additionally, the quantification of red blood cell count (RBCs × 10^6/mm^3) and white blood cell count (WBCs × 10^3/mm^3) followed the methodology outlined by Witeska et al. (2022). These counts were meticulously conducted using an Ao Bright-Line Hemocytometer model (Neubauer improved, Precicolor HBG, Germany).

The serum's biochemical parameters were analyzed using a biochemical kit acquired from Bio-Diagnostic Co. in Cairo, Egypt. The biochemical parameters that have been tested are total protein (TP), albumin (Alb), total cholesterol (T-Chol), aspartate and alanine

aminotransferases (AST&ALT), urea, and uric acid as detailed in our previous study (Sallam et al. 2024). Cortisol levels (pg mL^{-1}) were measured following the protocol outlined by Young (1986). Serum growth hormone (GH) concentration was quantified using the fish GH ELISA Kit (CUSABIO) CSBE12121Fh, adhering to the manufacturer's instructions. Optical density readings at 450 nm were promptly recorded for each well using a microplate reader (Thermo Fisher) within a 10-min timeframe. The GH concentration of each sample was determined based on the standard curve generated (Songlin et al. 1995).

Antioxidants and non-specific immunity

To test the activity of antioxidant enzymes, the serum samples were carefully withdrawn. Following the manufacturer's recommendations, the serum samples were used to measure the activity of superoxide dismutase (SOD) (Marklund and Marklund 1974), catalase (CAT) (Bergmeyer 2012), and malondialdehyde (MDA) (Buege and Aust 1978) at 550, 280, and 532 nm, respectively, a calorimetric method was performed to collect the supernatant. Each investigation used a microplate spectrophotometer to ensure precise and reliable measurement of enzyme activity. Furthermore, serum lysozyme levels, crucial for non-specific immunity, were assessed using a turbidimetric assay following the methodology proposed by Ellis (1990).

Statistical analysis

A two-way analysis of variance (ANOVA) was employed, and post hoc comparisons were conducted using Tukey's test at a significance level of 0.05. The statistical methodology followed the approach outlined by Assaad et al. (2015). The reported results are presented as means ± standard error of the mean (*SEM*).

Results

Variables related to growth, feed utilization, and SR (%)

The effects of BFT and salinity variations on growth metrics, feed utilization, condition factor, and SR (%) in male and female red tilapia broodstock were detailed in Table 1. The findings revealed that BFT and elevated salinity levels markedly improved ($P < 0.001$) FBW, WG, and SGR across both genders. The most significant outcomes were noted in the S36 ppt group under BFT treatment. Furthermore, the K factor experienced a notable enhancement ($P = 0.001$ for males; $P < 0.001$ for females) across all salinity conditions under BFT. The BFT and increased salinity (S36 ppt) also led to a rise in FI, whereas FCR showed substantial improvement.

SR (%) remained at 100% across all experimental setups. The interaction between BFT and salinity (BFT × S) demonstrated significant effects on most examined parameters, highlighting the synergistic influence of environmental conditions and BFT.

Assessment of whole-body proximate composition and organ indices

Table 2 demonstrates that the BFT and increased salinity levels significantly ($P < 0.001$) affected the moisture, protein, lipid, and ash content of the male. The lowest moisture

Table 1 Growth parameters, feed utilization, condition factor (K), and survival rates (SR %) of red tilapia broodstock under varying salinity conditions, comparing biofloc-treated (BFT) and untreated groups (control)*

Factor***	Control			BFT			*P*-value		
	S18 ppt	S28 ppt	S36 ppt	S18 ppt	S28 ppt	S36 ppt	BFT	Salinity	BFT×S**
Male ♂									
FBW, g	144 ± 0.40^{e}	221 ± 1.60^{c}	227 ± 1.61^{bc}	182 ± 0.48^{d}	230 ± 1.70^{b}	238 ± 1.70^{a}	<0.001	<0.001	<0.001
WG, g	39.8 ± 0.62^{e}	117 ± 1.48^{c}	123 ± 1.36^{b}	77.7 ± 0.10^{d}	126 ± 1.38^{b}	134 ± 1.92^{a}	<0.001	<0.001	<0.001
SGR, %	0.36 ± 0.01^{e}	0.84 ± 0.01^{c}	0.87 ± 0.01^{b}	0.62 ± 0.02^{d}	0.88 ± 0.03^{b}	0.92 ± 0.01^{a}	<0.001	<0.001	<0.001
K factor	1.44 ± 0.05^{ab}	1.29 ± 0.00^{b}	1.20 ± 0.02^{b}	1.66 ± 0.01^{a}	1.53 ± 0.16^{ab}	1.52 ± 0.08^{ab}	0.001	0.065	0.777
FI, g	82.0 ± 4.97^{c}	172 ± 2.39^{a}	174.7 ± 1.85^{a}	209.9 ± 0.52^{c}	219.9 ± 2.88^{a}	138.3 ± 9.32^{b}	0.021	<0.001	<0.001
FCR	2.06 ± 0.13^{a}	1.49 ± 0.04^{b}	1.38 ± 0.03^{b}	1.34 ± 0.01^{b}	1.27 ± 0.03^{b}	0.98 ± 0.06^{c}	<0.001	<0.001	0.004
SR, %	100	100	100	100	100	100	N/A	N/A	N/A
Female ♀									
FBW, g	130 ± 0.35^{e}	206 ± 1.08^{c}	213 ± 1.54^{b}	165 ± 0.20^{d}	214 ± 1.31^{b}	222 ± 1.79^{a}	<0.001	<0.001	<0.001
WG, g	36.8 ± 0.62^{e}	112 ± 1.48^{c}	119 ± 1.36^{b}	71.8 ± 0.11^{d}	121 ± 1.38^{b}	129 ± 1.92^{a}	<0.001	<0.001	<0.001
SGR, %	0.37 ± 0.01^{e}	0.88 ± 0.01^{c}	0.92 ± 0.01^{b}	0.63 ± 0.01^{d}	0.92 ± 0.01^{b}	0.96 ± 0.01^{a}	<0.001	<0.001	<0.001
K factor	2.27 ± 0.09^{b}	1.87 ± 0.02^{c}	1.39 ± 0.05^{d}	2.59 ± 0.06^{a}	2.48 ± 0.03^{ab}	2.36 ± 0.03^{ab}	<0.001	<0.001	<0.001
FI, g	83 ± 3.65^{d}	181 ± 3.00^{a}	178 ± 1.23^{a}	112 ± 1.65^{c}	158 ± 1.66^{b}	147 ± 4.46^{b}	0.004	<0.001	<0.001
FCR	2.32 ± 0.13^{a}	1.62 ± 0.02^{b}	1.48 ± 0.01^{bc}	1.56 ± 0.03^{bc}	1.32 ± 0.01^{cd}	1.15 ± 0.03^{d}	<0.001	<0.001	0.002
SR, %	100	100	100	100	100	100	N/A	N/A	N/A

*Mean values are presented as means ± *SEM*. Differences among means in a row without a shared superscript letter are statistically significant ($P < 0.05$), determined by two-way ANOVA and the TUKEY test. The superscript letters indicate the significance of BFT and salinity, not their interaction

**The interaction effect between BFT and salinity is denoted as BFT×S

***Abbreviations: FBW: Final Body Weight, WG: Weight Gain, SGR: Specific Growth Rate, FI: Feed Intake, FCR: Feed Conversion Ratio, SR: Survival rate

Table 2 Whole-body proximate composition and organ indices of red tilapia broodstock under varying salinity conditions, comparing biofloc-treated (BFT) and untreated groups (control)*

Factor***	Control			BFT			*P*-value		
	S18 ppt	S28 ppt	S36 ppt	S18 ppt	S28 ppt	S36 ppt	BFT	Salinity	BFT × S**
Male ♂									
Moisture, %	78.6 ± 0.26^{a}	76.1 ± 0.10^{b}	72.7 ± 0.27^{c}	75.8 ± 0.57^{b}	73.2 ± 0.18^{c}	72.3 ± 0.17^{c}	< 0.001	< 0.001	0.002
Protein, %	64.1 ± 0.27^{a}	62.1 ± 0.32^{b}	57.3 ± 0.46^{d}	59.5 ± 0.21^{c}	58.5 ± 0.11^{cd}	57.6 ± 0.12^{d}	< 0.001	< 0.001	< 0.001
Lipid, %	22 ± 0.08^{d}	23.3 ± 0.09^{bc}	26.1 ± 0.26^{a}	23.6 ± 0.06^{b}	23.1 ± 0.07^{bc}	22.7 ± 0.23^{cd}	< 0.001	< 0.001	< 0.001
Ash, %	12 ± 0.07^{d}	12.8 ± 0.35^{d}	14.8 ± 0.21^{c}	14.9 ± 0.23^{c}	16.5 ± 0.19^{b}	17.8 ± 0.19^{a}	< 0.001	< 0.001	0.224
HSI, %	1.61 ± 0.01^{bc}	1.68 ± 0.01^{b}	1.98 ± 0.06^{a}	1.53 ± 0.03^{c}	1.59 ± 0.04^{bc}	1.89 ± 0.01^{a}	0.006	< 0.001	0.967
VSI, %	7.37 ± 0.05^{c}	7.69 ± 0.04^{b}	8.07 ± 0.04^{a}	6.99 ± 0.07^{d}	7.03 ± 0.04^{d}	6.71 ± 0.09^{e}	< 0.001	0.007	< 0.001
TSI, %	2.72 ± 0.04^{a}	1.79 ± 0.05^{b}	1.08 ± 0.12^{c}	2.75 ± 0.05^{a}	2.50 ± 0.03^{a}	2.35 ± 0.17^{a}	< 0.001	< 0.001	< 0.001
Female ♀									
Moisture, %	70.4 ± 0.79	70.9 ± 0.75	71.2 ± 0.35	71.4 ± 0.2	70.9 ± 0.41	70.7 ± 0.64	0.723	0.992	0.48
Protein, %	67.6 ± 0.08^{d}	68.9 ± 0.38^{c}	66.6 ± 0.43^{d}	71.1 ± 0.18^{a}	70.5 ± 0.04^{ab}	69.9 ± 0.06^{bc}	< 0.001	< 0.001	0.004
Lipid, %	20.9 ± 0.31^{a}	20.4 ± 0.39^{a}	21.2 ± 0.29^{a}	11.2 ± 0.18^{b}	11.2 ± 0.32^{b}	11.4 ± 0.03^{b}	< 0.001	0.219	0.491
Ash, %	10.2 ± 0.25^{cd}	9.64 ± 0.20^{d}	11.1 ± 0.14^{c}	13.7 ± 0.06^{b}	14.3 ± 0.3^{ab}	14.6 ± 0.05^{a}	< 0.001	0.001	0.018
HSI, %	2.34 ± 0.06^{c}	2.65 ± 0.06^{b}	3.08 ± 0.05^{a}	2.39 ± 0.03^{c}	2.18 ± 0.04^{c}	2.19 ± 0.03^{c}	< 0.001	< 0.001	< 0.001
VSI, %	7.42 ± 0.05^{c}	8.01 ± 0.07^{b}	8.50 ± 0.10^{a}	6.43 ± 0.06^{e}	6.51 ± 0.04^{de}	6.79 ± 0.01^{d}	< 0.001	< 0.001	< 0.001
GSI, %	3.93 ± 0.18^{b}	2.50 ± 0.08^{c}	2.06 ± 0.03^{c}	4.96 ± 0.07^{a}	4.55 ± 0.26^{ab}	4.31 ± 0.15^{ab}	< 0.001	< 0.001	0.003

*Mean values are presented as means ± *SEM*. Differences among means in a row without a shared superscript letter are statistically significant ($P < 0.05$), determined by two-way ANOVA and the TUKEY test. The superscript letters indicate the significance of BFT and salinity, not their interaction

**The interaction effect between BFT and salinity is denoted as BFT × S

***Abbreviations: HSI: hepatosomatic index, VSI: viscerosomatic index, TSI: Testessomatic Index, GSI: Gonadosomatic index

and highest ash content were observed in the S36 ppt BFT group. Notably, lipid content increased with elevating salinity in the CW group but showed a differential pattern under BFT. In females, while moisture content showed no significant differences across treatments, protein, and ash content significantly increased ($P < 0.001$), and lipid content decreased under BFT, especially at higher salinity (S36 ppt).

Table 2 also shows that HSI, VSI, and GSI varied significantly ($P < 0.05$) by BFT and salinity. HSI, VSI, and TSI also varied significantly, indicating altered energy reserves and conditions with BFT and salinity changes. In females, while moisture content showed no significant differences across treatments, protein content significantly increased, and lipid content decreased under BFT, especially at higher salinity levels. Ash content similarly increased with BFT, suggesting a possible shift in mineral balance. Organ indices such as HSI and VSI were significantly affected by BFT and salinity, whereas the GSI displayed significant variations, underscoring the reproductive condition's sensitivity to environmental changes. The interaction between BFT and salinity (BFT × S) was significant for several parameters, highlighting the complex influence of these factors on the physiological status of Tilapia broodstock.

Water quality

The water quality parameters, as illustrated in Table 3, revealed that DO and pH levels remained within acceptable ranges for hybrid red tilapia throughout the experimental period, with notable variations between BFT and CW groups. DO concentrations fluctuated between 7.51 ± 0.04 and 6.52 ± 0.05 mg L^{-1}, while pH varied from 7.11 ± 0.06 to 8.04 ± 0.03. High salinity ponds (S36 ppt) exhibited the highest DO values but the lowest pH levels, particularly in BFT ponds.

Alkalinity ($CaCO_3$) was higher in BFT ponds, likely influenced by increased salinity. Moreover, BFT ponds consistently demonstrated lower levels of all forms of dissolved nitrogen compared to CW ponds. Total ammonia nitrogen showed significant disparities between CW and BFT ponds, with BFT ponds exhibiting an active nitrification process. The value of NO^2 fluctuated without significant differences between treatments, while NO^3 increased significantly in BFT ponds with rising salinity. The OP concentrations increased in BFT, reaching the highest levels in the S36 ppt. The TDS and TSS were significantly elevated ($P < 0.001$) in BFT ponds with increasing salinity compared to CW ponds.

Live food and biofloc profile

Table 4 depicts variations in live feed populations across all treatments, influenced by both BFT ponds and salinity levels. Rotifer counts notably favored high salinity levels, exhibiting significantly higher numbers compared to low salinity (S18 ppt) and complete absence in CW ponds. Conversely, copepods, protozoa, and heterotrophic species counts were notably higher in BFT ponds than in CW ponds.

Overall, heterotrophic species in biofloc ponds showed higher counts than in CW ponds. Regarding autotrophic species, Cyanophyta exhibited low counts in high salinity BFT and CW ponds, with complete absence in low and medium salinity treatments across all groups. Euglenophyta (green algae) showed significantly higher counts in CW ponds under low salinity conditions, while CW with medium salinity ponds and BFT with low salinity ponds had similar counts. Additionally, Euglenophyta preferred low salinity of CW ponds without additional carbon sources. Diatoms also favored CW

Table 3 The quality of red tilapia broodstock-rearing water under varying salinity conditions, comparing biofloc-treated (BFT) and untreated groups (control)*

Factor***	Control			BFT			*P*-value		
	S18 ppt	S28 ppt	S36 ppt	S18 ppt	S28 ppt	S36 ppt	BFT	Salinity	BFT×S**
DO, mg L^{-1}	6.52 ± 0.05^{c}	7.07 ± 0.09^{b}	7.26 ± 0.08^{ab}	7.33 ± 0.11^{ab}	7.36 ± 0.13^{ab}	7.51 ± 0.04^{a}	< 0.001	0.001	0.015
pH	8.04 ± 0.03^{a}	7.98 ± 0.04^{ab}	7.69 ± 0.25^{ab}	7.45 ± 0.08^{bc}	7.16 ± 0.03^{c}	7.11 ± 0.06^{c}	< 0.001	0.03	0.51
Alkalinity (as $CaCO_3$), mg L^{-1}	108 ± 3.93^{c}	127 ± 2.60^{b}	135 ± 1.15^{ab}	125 ± 1.53^{b}	131 ± 1.15^{ab}	139 ± 1.2^{a}	< 0.001	< 0.001	0.014
Salinity	18 ± 0	28 ± 0	36 ± 0	18 ± 0	28 ± 0	36 ± 0	N/A	N/A	N/A
NH^{4+}, mg L^{-1}	1.35 ± 0.04^{a}	1.30 ± 0.01^{a}	1.26 ± 0.01^{a}	0.211 ± 0.02^{b}	0.207 ± 0.01^{b}	0.281 ± 0.02^{b}	< 0.001	0.387	0.008
NH^{3}, ppm	0.09 ± 0.01^{a}	0.09 ± 0.02^{a}	0.05 ± 0.02^{ab}	0.004 ± 0.00^{b}	0.002 ± 0.00^{b}	0.003 ± 0.001^{b}	< 0.001	0.063	0.068
NO_2, mg L^{-1}	0.188 ± 0.01^{a}	0.17 ± 0.01^{a}	0.114 ± 0.01^{b}	0.08 ± 0.02^{b}	0.03 ± 0.01^{c}	0.01 ± 0.001^{c}	< 0.001	< 0.001	0.151
NO_3, mg L^{-1}	0.176 ± 0.03^{c}	0.241 ± 0.02^{bc}	0.269 ± 0.02^{bc}	0.30 ± 0.01^{b}	0.44 ± 0.02^{a}	0.444 ± 0.03^{a}	< 0.001	< 0.001	0.237
TDS, g L^{-1}	13.8 ± 0.46^{d}	16.4 ± 0.42^{d}	22.7 ± 1.23^{c}	15.1 ± 0.68^{d}	29.4 ± 1.41^{b}	45.1 ± 0.13^{a}	< 0.001	< 0.001	< 0.001
OP, mg L^{-1}	0.16 ± 0.07^{d}	0.17 ± 0.07^{d}	0.35 ± 0.03^{cd}	0.56 ± 0.04^{bc}	0.72 ± 0.07^{b}	1.17 ± 0.04^{a}	< 0.001	< 0.001	0.003
TSS, mg L^{-1}	253 ± 28.6^{b}	253 ± 28.6^{b}	303 ± 8.82^{b}	418 ± 13.5^{a}	396 ± 13.5^{a}	396 ± 18.6^{a}	< 0.001	0.679	0.203

*Mean values are presented as means ± *SEM*. Differences among means in a row without a shared superscript letter are statistically significant ($P < 0.05$), determined by two-way ANOVA and the TUKEY test. The superscript letters indicate the significance of BFT and salinity, not their interaction

**The interaction effect between BFT and salinity is denoted as BFT×S

***Abbreviations: DO: Dissolved oxygen, NH^{4+}: Ionized ammonium, NH^{3}: Unionized ammonia, NO^{2}: Nitrite, NO^{3}: Nitrate, TDS: Total dissolved solids, OP: Organic phosphor, TSS: Total suspended solids

Table 4 The live food of red tilapia broodstock-rearing water under varying salinity conditions, comparing biofloc-treated (BFT) and untreated groups (control)*

Live food (Cell/ml)	Control			BFT			*P*-value		
	S18 ppt	S28 ppt	S36 ppt	S18 ppt	S28 ppt	S36 ppt	BFT	Salinity	BFT × S**
Chlorophyta	100133 ± 108^{b}	78900 ± 5470^{b}	15500 ± 2340^{c}	225000 ± 18100^{a}	41200 ± 2680^{c}	24200 ± 1210^{c}	< 0.001	< 0.001	< 0.001
Copepoda	58200 ± 1280^{b}	23000 ± 636^{c}	15000 ± 1500^{d}	102000 ± 1910^{a}	15000 ± 875^{d}	3270 ± 426^{e}	< 0.001	< 0.001	< 0.001
Rotifera	54800 ± 37000^{ab}	113000 ± 1910^{a}	106000 ± 2480^{a}	62200 ± 62200^{ab}	198000 ± 6650^{a}	54300 ± 2960^{ab}	< 0.001	0.153	0.153
Cyanophyta	0 ± 0^{b}	0 ± 0^{b}	343 ± 29^{b}	0 ± 0^{b}	0 ± 0^{b}	2050 ± 263^{a}	< 0.001	< 0.001	< 0.001
Bacillariophyta	0 ± 0^{b}	0 ± 0^{b}	21600 ± 2280^{a}	0 ± 0^{b}	0 ± 0^{b}	1520 ± 47.3^{b}	0.794	0.763	0.657
Euglenophyta	13300 ± 882^{a}	4670 ± 384^{d}	3100 ± 100^{d}	9500 ± 231^{b}	9000 ± 57.7^{b}	6720 ± 347^{c}	0.002	< 0.001	< 0.001
Diatomes	553000 ± 30800^{a}	548000 ± 54400^{a}	310000 ± 5490^{b}	100005 ± 577^{c}	134000 ± 3110^{c}	93300 ± 2140^{c}	< 0.001	< 0.001	0.001
Protozoa	260000 ± 2740^{a}	216000 ± 3250^{ab}	161000 ± 24400^{cd}	204000 ± 3110^{bc}	136000 ± 1860^{de}	105000 ± 3280^{e}	< 0.001	< 0.001	0.422
Microgreen algae	0 ± 0^{d}	328000 ± 13800^{b}	447000 ± 28800^{a}	0 ± 0^{d}	48900 ± 1460^{cd}	64500 ± 434^{c}	< 0.001	< 0.001	< 0.001

*Mean values are presented as means ± *SEM*. Differences among means in a row without a shared superscript letter are statistically significant ($P < 0.05$), determined by two-way ANOVA and the TUKEY test. The superscript letters indicate the significance of BFT and salinity, not their interaction

**The interaction effect between BFT and salinity is denoted as BFT × S

ponds, displaying five-fold higher counts compared to BFT ponds, particularly in low and medium-salinity ponds, where they showed higher counts.

The chemical composition of BFT in different salinities is illustrated in Table 5. The statistical analysis of data exhibited significant differences ($P < 0.001$) between salinity levels. The highest protein value ($29 \pm 0.09\%$) was significantly accomplished at a low salinity level (S18 ppt), whereas the lowest ($27 \pm 0.04\%$) was attained at a high salinity level (S36 ppt). On the contrary, the highest carbohydrate percentage ($58.8 \pm 0.06\%$) was attained by S36 ppt level. Likewise, fat and ash values were significantly high ($3.92 \pm 0.08\%$ and $13.3 \pm 0.32\%$, respectively) in S18 ppt level, but there were no significant differences between S28 and S36 ppt levels. The BFV was also significantly higher ($P < 0.001$) in BFT-treated groups.

Digestive enzyme activity

Table 6 provides insights into the digestive enzyme activities of male and female red Tilapia broodstock under varying salinity conditions, comparing BFT and CW groups. Significant differences ($P < 0.001$) were observed in protease, amylase, and lipase activities among the groups. In males, BFT-treated groups exhibited higher ($P < 0.001$) protease, amylase, and lipase activities compared to CW groups. The highest values ($P < 0.001$) were observed in the S36 ppt BFT-treated group. Similarly, females showed increased enzyme activities in BFT-treated groups, particularly in high salinity conditions, compared to CW groups.

Reproductive performance and sex hormone

As shown in Table 7, significant differences ($P < 0.001$) were observed in sperm count, percentage of dead sperms, and testosterone levels among male groups. The highest sperm count and testosterone levels were recorded in the S18 ppt BFT-treated group. In females, egg number, egg diameter, ovary weight, AF, RF, and progesterone levels varied significantly ($P < 0.05$) among treatments. The S18 ppt BFT-treated group exhibited the highest values for egg number per g, ovary weight, AF, and RF. Conversely, egg diameter and progesterone levels were highest in the CW group under low salinity conditions.

Hematological and biochemical parameters

Significant differences ($P < 0.05$) were observed in hemato-biochemical parameters among male and female groups (Table 8). In males, RBCs, Hb, Hct, WBCs, TP, T-Chol, AST, ALT, urea, and uric acid levels varied significantly ($P < 0.05$) across treatments. The S18 ppt BFT-treated group showed the highest RBCs, Hb, Hct, WBCs, TP, and T-Chol levels compared to other salinity levels and the control group. Similarly, in females, RBCs, Hb, Hct, WBCs, TP, T-Chol, AST, ALT, urea, and uric acid levels showed significant differences significantly ($P < 0.05$) among treatments. The highest values were observed in the S18 ppt BFT-treated group.

Table 5 Examination of biofloc volume and chemical composition of red tilapia broodstock-rearing water under varying salinity conditions, comparing biofloc-treated (BFT) and untreated groups (control)*

Factor***	Control			BFT			*P*-value		
	S18 ppt	S28 ppt	S36 ppt	S18 ppt	S28 ppt	S36 ppt	BFT	Salinity	BFT×S**
Dry matter, %	N/A	N/A	N/A	23.3 ± 0.25^{c}	25.2 ± 0.13^{b}	27.5 ± 0.215^{a}	< 0.001	0.001	0.018
Protein, %	N/A	N/A	N/A	29.1 ± 0.09^{a}	28.2 ± 0.07^{b}	27.1 ± 0.04^{c}	< 0.001	< 0.001	< 0.001
Fat, %	N/A	N/A	N/A	3.92 ± 0.08^{a}	2.33 ± 0.11^{b}	2.09 ± 0.05^{b}	< 0.001	< 0.001	< 0.001
Ash, %	N/A	N/A	N/A	13.3 ± 0.32^{a}	12.3 ± 0.13^{b}	12.1 ± 0.10^{b}	< 0.001	0.005	0.005
Carbohydrate, %	N/A	N/A	N/A	53.9 ± 0.40^{c}	57.4 ± 0.14^{b}	58.8 ± 0.06^{a}	< 0.001	< 0.001	< 0.001
BFV, ml L^{-1}	N/A	N/A	N/A	33.2 ± 1.09^{a}	27.6 ± 0.51^{b}	25.6 ± 0.33^{b}	< 0.001	< 0.001	< 0.001

*Mean values are presented as means ± *SEM*. Differences among means in a row without a shared superscript letter are statistically significant ($P < 0.05$), determined by two-way ANOVA and the TUKEY test. The superscript letters indicate the significance of BFT and salinity, not their interaction

**The interaction effect between BFT and salinity is denoted as BFT×S

***Abbreviations: BFV: Biofloc volume

Table 6 Digestive enzymes activity of red tilapia broodstock under varying salinity conditions, comparing biofloc-treated (BFT) and untreated groups (control)*

Factor	Control			BFT			*P*-value		
	S18 ppt	S28 ppt	S36 ppt	S18 ppt	S28 ppt	S36 ppt	BFT	Salinity	BFT×S**
Male ♂									
Protease, U mg^{-1}	58.1 ± 0.55^{c}	54.3 ± 0.20^{d}	51.6 ± 0.70^{d}	63.7 ± 0.98^{a}	61.1 ± 0.59^{ab}	60.1 ± 0.28^{bc}	< 0.001	< 0.001	0.094
Amylase, U mg^{-1}	33.7 ± 0.42^{c}	28.9 ± 0.60^{d}	24.8 ± 0.70^{e}	38.9 ± 0.53^{a}	37.3 ± 0.21^{ab}	35.4 ± 0.63^{bc}	< 0.001	< 0.001	0.001
Lipase, U mg^{-1}	53.9 ± 0.16^{d}	50.3 ± 0.46^{e}	45.2 ± 0.49^{f}	64.3 ± 0.64^{a}	61.8 ± 0.66^{b}	58.9 ± 0.19^{c}	< 0.001	< 0.001	0.017
Female ♀									
Protease, U mg^{-1}	56.9 ± 0.62^{b}	52 ± 0.793^{c}	45.4 ± 1.4^{d}	62.9 ± 1.16^{a}	59.6 ± 0.37^{ab}	57.6 ± 0.29^{b}	< 0.001	< 0.001	0.012
Amylase, U mg^{-1}	33.6 ± 0.71^{c}	29.8 ± 1.22^{d}	24.2 ± 0.78^{e}	41.4 ± 0.36^{a}	36.6 ± 0.33^{bc}	36.9 ± 0.09^{b}	< 0.001	< 0.001	0.002
Lipase, U mg^{-1}	51.4 ± 0.20^{c}	44.6 ± 0.84^{d}	37.4 ± 0.69^{e}	65.4 ± 0.39^{a}	62.6 ± 1.41^{ab}	59.3 ± 0.24^{b}	< 0.001	< 0.001	0.001

*Mean values are presented as means ± *SEM*. Differences among means in a row without a shared superscript letter are statistically significant ($P < 0.05$), determined by two-way ANOVA and the TUKEY test. The superscript letters indicate the significance of BFT and salinity, not their interaction

**The interaction effect between BFT and salinity is denoted as BFT×S

Table 7 Reproductive performance and sex hormones of male and female red tilapia broodstock under varying salinity conditions, comparing biofloc-treated (BFT) and untreated groups (control)*

Factor	Control			BFT			*P*-value		
	S18 ppt	S28 ppt	S36 ppt	S18 ppt	S28 ppt	S36 ppt	BFT	Salinity	BFT × S**
Male ♂									
Sperm count, $*10^6$ ml^{-1}	156 ± 3.18^{b}	132 ± 1.76^{c}	99.3 ± 4.63^{d}	182 ± 1.15^{a}	179 ± 0.58^{a}	164 ± 1.53^{b}	< 0.001	< 0.001	< 0.001
Dead sperms, %	19.7 ± 1.20^{c}	27.3 ± 1.45^{b}	38.7 ± 2.03^{a}	9.33 ± 0.88^{d}	14.1 ± 0.58^{cd}	15.1 ± 0.58^{cd}	< 0.001	< 0.001	< 0.001
Testosterone, ng ml^{-1}	2.62 ± 0.08^{c}	2.09 ± 0.07^{d}	1.55 ± 0.09^{e}	5.58 ± 0.07^{a}	4.93 ± 0.1^{b}	4.62 ± 0.05^{b}	< 0.001	< 0.001	0.347
Female ♀									
Egg number, g	286 ± 3.61^{c}	266 ± 2.91^{d}	246 ± 2.65^{e}	329 ± 1.2^{a}	310 ± 0.33^{b}	295 ± 3.84^{c}	< 0.001	< 0.001	0.532
Egg diameter, mm	0.17 ± 0.02^{bc}	0.19 ± 0.01^{ab}	0.22 ± 0.01^{a}	0.1 ± 0.012^{d}	0.14 ± 0.01^{cd}	0.15 ± 0.01^{bc}	< 0.001	0.001	0.579
Ovary weight, g	8.17 ± 0.43^{ab}	6.78 ± 0.12^{bc}	5.4 ± 0.15^{c}	9.49 ± 0.65^{a}	7.81 ± 0.24^{b}	7.14 ± 0.21^{b}	< 0.001	< 0.001	0.602
Absolute fecundity	2340 ± 142^{bc}	1800 ± 41.5^{cd}	1330 ± 48.8^{d}	3120 ± 207^{a}	2420 ± 76.2^{b}	2110 ± 85^{bc}	< 0.001	< 0.001	0.723
Relative fecundity	18 ± 1.07^{a}	8.76 ± 0.18^{bc}	6.23 ± 0.19^{c}	18.9 ± 1.26^{a}	11.3 ± 0.31^{b}	9.51 ± 0.35^{bc}	0.002	< 0.001	0.257
Progesterone, ng ml^{-1}	0.71 ± 0.02^{c}	0.65 ± 0.02^{c}	0.53 ± 0.015^{d}	0.98 ± 0.01^{ab}	1 ± 0.05^{a}	0.89 ± 0.01^{b}	< 0.001	< 0.001	0.038

*Mean values are presented as means ± *SEM*. Differences among means in a row without a shared superscript letter are statistically significant ($P < 0.05$), determined by two-way ANOVA and the TUKEY test. The superscript letters indicate the significance of BFT and salinity, not their interaction

**The interaction effect between BFT and salinity is denoted as BFT × S

Table 8 Hematological and biochemical parameters of red tilapia broodstock under varying salinity conditions, comparing biofloc-treated (BFT) and untreated groups (control)*

Factor***	Control			BFT			*P*-value		
	S18 ppt	S28 ppt	S36 ppt	S18 ppt	S28 ppt	S36 ppt	BFT	Salinity	BFT × S**
Male ♂									
RBCs, 10^6 mm^{-3}	2.87 ± 0.06^{b}	2.22 ± 0.06^{c}	1.63 ± 0.03^{d}	3.4 ± 0.19^{a}	2.77 ± 0.1^{b}	2.39 ± 0.16^{bc}	< 0.001	< 0.001	0.552
Hb, g dL^{-1}	6.39 ± 0.07^{c}	6.59 ± 0.07^{c}	5.99 ± 0.06^{c}	9.35 ± 0.21^{a}	8.7 ± 0.25^{ab}	8.15 ± 0.3^{b}	< 0.001	0.003	0.075
Hct, %	20.5 ± 0.82^{bc}	18 ± 0.25^{bc}	17.1 ± 0.43^{c}	28.5 ± 2.56^{a}	28.4 ± 2.58^{a}	25.3 ± 1.32^{ab}	< 0.001	0.173	0.727
WBCs, 10^3 mm^{-3}	22 ± 0.13^{b}	21.3 ± 0.09^{b}	21.1 ± 0.216^{b}	32.9 ± 1.82^{a}	30.5 ± 2.22^{a}	29.3 ± 0.68^{a}	< 0.001	0.207	0.54
TP, g/dl^{-1}	3.21 ± 0.17^{b}	2.87 ± 0.12^{bc}	2.04 ± 0.06^{d}	3.66 ± 0.01^{a}	3.03 ± 0.04^{b}	2.48 ± 0.02^{c}	< 0.001	< 0.001	0.236
Albumin, g dl^{-1}	0.52 ± 0.09	0.42 ± 0.02	0.48 ± 0.07	0.63 ± 0.03	0.47 ± 0.09	0.57 ± 0.05	0.126	0.151	0.898
T-Chol, g dl^{-1}	137 ± 1.76^{b}	156 ± 0.29^{a}	156 ± 0.29^{a}	83.2 ± 1.09^{c}	87.7 ± 0.88^{c}	87.7 ± 2.03^{c}	< 0.001	< 0.001	< 0.001
AST, IU L^{-1}	49.8 ± 0.93^{bc}	52.4 ± 0.83^{b}	57.4 ± 0.46^{a}	48.9 ± 0.23^{c}	50.7 ± 0.48^{bc}	55.9 ± 0.13^{a}	0.015	< 0.001	0.822
ALT, IU L^{-1}	27.5 ± 0.27^{ab}	26.2 ± 1.83^{ab}	30.8 ± 0.35^{a}	25.9 ± 0.16^{ab}	25 ± 1.74^{b}	27.6 ± 0.12^{ab}	0.04	0.015	0.635
Urea, mg dl^{-1}	42.6 ± 1.16^{ab}	44.4 ± 2.07^{ab}	48.4 ± 3.29^{a}	38.9 ± 0.51^{b}	41.1 ± 0.71^{ab}	43.1 ± 0.47^{ab}	0.012	0.037	0.822
Uric acid, mg dl^{-1}	3.04 ± 0.15^{ab}	3.31 ± 0.37^{ab}	4.08 ± 0.5^{a}	2.51 ± 0.12^{b}	2.69 ± 0.09^{b}	2.94 ± 0.07^{ab}	0.005	0.05	0.487
Female ♀									
RBCs, 10^6 mm^{-3}	2.87 ± 0.08^{ab}	2.21 ± 0.06^{c}	1.65 ± 0.04^{d}	3.1 ± 0.04^{a}	2.63 ± 0.12^{b}	2.07 ± 0.03^{c}	< 0.001	< 0.001	0.304
Hb, g dL^{-1}	6.39 ± 0.08^{c}	6.54 ± 0.1^{c}	5.98 ± 0.07^{c}	9.31 ± 0.22^{a}	8.74 ± 0.27^{a}	7.87 ± 0.09^{b}	< 0.001	< 0.001	0.018
Hct, %	20.1 ± 1.04^{b}	18.2 ± 0.35^{b}	16.9 ± 0.36^{b}	28.0 ± 2.65^{a}	28.5 ± 2.56^{a}	24.4 ± 1.15^{ab}	< 0.001	0.134	0.662
WBCs, 10^3 mm^{-3}	22.0 ± 0.2^{b}	21.2 ± 0.16^{b}	21.0 ± 0.27^{b}	32.6 ± 1.88^{a}	31.1 ± 2.46^{a}	28.9 ± 0.65^{a}	< 0.001	0.243	0.556
TP, g/dl^{-1}	2.55 ± 0.06^{c}	2.00 ± 0.06^{d}	2.02 ± 0.06^{d}	3.68 ± 0.01^{a}	2.81 ± 0.01^{b}	2.82 ± 0.04^{b}	< 0.001	< 0.001	0.007
Albumin, g dl^{-1}	0.50 ± 0.09	0.48 ± 0.09	0.48 ± 0.08	0.64 ± 0.02	0.47 ± 0.03	0.51 ± 0.06	0.357	0.38	0.49
T-Chol, g dl^{-1}	133 ± 1.20^{c}	149 ± 2.33^{b}	159 ± 0.33^{a}	81.8 ± 0.17^{e}	85.2 ± 1.09^{e}	96.7 ± 3.53^{d}	< 0.001	< 0.001	0.014
AST, IU L^{-1}	55 ± 0.55^{b}	58.1 ± 0.54^{a}	57.7 ± 0.45^{a}	48.2 ± 0.15^{d}	52.9 ± 0.44^{c}	53 ± 0.253^{bc}	< 0.001	< 0.001	0.073

Table 8 (Continued)

Factor***	Control			BFT			*P*-value		
	S18 ppt	S28 ppt	S36 ppt	S18 ppt	S28 ppt	S36 ppt	BFT	Salinity	BFT × S**
ALT, IU L^{-1}	28.6 ± 0.87^{ab}	31.1 ± 0.55^{a}	30.9 ± 0.44^{a}	25.8 ± 0.25^{c}	27.2 ± 0.07^{bc}	26.2 ± 0.84^{bc}	< 0.001	0.013	0.297
Urea, mg dl^{-1}	40.2 ± 1.46^{b}	44.8 ± 0.38^{ab}	47.9 ± 3.08^{a}	39.4 ± 0.363^{b}	40.9 ± 0.55^{b}	42.2 ± 0.38^{ab}	0.013	0.011	0.279
Uric acid, mg dl^{-1}	2.64 ± 0.23^{b}	3.29 ± 0.06^{ab}	4.00 ± 0.41^{a}	2.53 ± 0.08^{b}	2.65 ± 0.06^{b}	2.81 ± 0.04^{b}	0.002	0.005	0.057

*Mean values are presented as means ± *SEM*. Differences among means in a row without a shared superscript letter are statistically significant ($P < 0.05$), determined by two-way ANOVA and the TUKEY test. The superscript letters indicate the significance of BFT and salinity, not their interaction

**The interaction effect between BFT and salinity is denoted as BFT × S

***Abbreviations: RBCs: Red blood cells, Hb: Hemoglobin, Hct: Hematocrit, WBCs: White blood cells, TP: Total protein, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

Stress *indicator* (cortisol) and GH level

As exhibited in Table 9, significant differences ($P < 0.05$) were observed in cortisol levels among treatments for both males and females. In males, cortisol levels were lowest in the S18 ppt BFT-treated group and highest in the S36 ppt CW group. However, no significant differences were found in GH levels among male groups.

In females, cortisol levels showed similar trends, with the lowest levels observed in the S18 ppt BFT-treated group and the highest in the S36 ppt CW group. GH levels varied significantly ($P = 0.003$) among female groups. The highest levels were observed in the S36 ppt BFT-treated group.

Antioxidant-immune response

Significant differences ($P < 0.001$) were observed in the antioxidant-immune parameters between groups (Table 10). In males, SOD and CAT levels were highest ($P < 0.001$) in the S18 ppt BFT-treated group, while MDA levels were lowest. Lysozyme levels were significantly higher ($P < 0.001$) in the S18 ppt and S28 ppt BFT-treated groups compared to control groups. In females, SOD and CAT levels were the highest ($P < 0.001$) in the S18 ppt BFT-treated group and MDA levels lowest. Lysozyme levels were significantly higher ($P < 0.001$) in BFT-treated groups compared to CW groups.

Discussion

Biofloc technology (BFT) is an environmentally friendly aquaculture method that utilizes the growth of microbial flocs in the water to enhance water quality and serve as a natural food supply for aquatic species (Jamal et al. 2020). This study aimed to investigate how changes in salinity levels may affect the influence of BFT on red tilapia's growth, water quality, broodstock physiology, fertility, and – antioxidant-immunological response.

The current study found that employing BFT and increasing salinity levels significantly improved various performance parameters. Specifically, the group treated with BFT at 36 ppt salinity showed the greatest changes in FBW, WG, SGR, and FI. K factor, which measures fish health and physical condition, also improved with BFT across all salinity settings. Red tilapia in biofloc-treated groups consumed their feed better, particularly at higher salinities. Most importantly, all testing settings had 100% SR. This reveals that BFT and salinity did not harm broodstock. Most of the factors examined were significantly influenced by the relationship between BFT and salinity (BFT × S). This shows that natural conditions and BFT work together to improve the performance of red tilapia broodstock. Elhetawy et al. (2021) corroborated our findings by showing that BFT has a beneficial effect on grey mullet (*Mugil cephalus*) growth performance, with the greatest values seen in fresh and brackish water. Also, Iqbal et al. (2012) demonstrated that higher salinity levels enhance the growth and survival of *O. niloticus*. Also, *O. niloticus* cultured with BFT exhibited enhanced growth performance at salinities of 4 and 8 ppt, yet experienced diminished growth at salinities of 12 and 16 ppt. This underscores the significant influence of salinity conditions on growth outcomes in BFT cultivation (de Alvarenga et al. 2018). Several studies have observed a decline in FCR with increasing growth rates, indicating an elevation in feed efficiency and conversion rate within the BFT environment (Yu et al.

2023). As reported by Ekasari et al. (2015a), *O. niloticus* broodstock reared in BFT demonstrated higher larval SR (%) compared to the control group, suggesting the beneficial effects of BFT on *O. niloticus* larvae in aquaculture.

The low feed intake observed in the high salinity group within the biofloc treatment can be attributed to several factors. First, the biofloc system enhances metabolism, as supported by findings from Long et al. (2015) and Kumari et al. (2021). Additionally, the separation of males from females likely reduces overall energy expenditure. Moreover, biofloc in high salinity environments further decreases energy consumption. Notably, the highest salinity group exhibited the lowest FCR compared to other biofloc units in the current experiment. Biofloc can positively influence digestive enzymes and intestinal microflora, leading to improved growth performance, as mentioned by Long et al. (2015). Furthermore, Kumari et al. (2021) reported a gradual decrease in FCR with increasing salinity levels in red tilapia reared in a biofloc system, with FCR decreasing from 1.38 at 0 ppt salinity to 0.93 at 20 ppt salinity.

It found that the BFT group had major effects on the moisture, protein, lipid, and ash contents. The salinity 36 ppt BFT group had the lowest moisture and highest ash content. The lipid content reacted differently to salt under BFT. In line with findings from prior studies (Bakhshi et al. 2018; Haridas et al. 2021), recent research has highlighted the suitability of BFT for fostering optimal nutrient conditions conducive to fish development. Additionally, Zhang et al. (2018) proposed that microbial BFT promotes dietary protein conservation without altering the developmental or metabolic characteristics of fish carcasses. Previous investigations have revealed that different carbon sources within BFT system can influence both the proximate composition of cultured species and the biochemical composition of BFT (Ekasari et al. 2014; Khanjani et al. 2016). Moreover, studies have shown that species raised in BFT systems, such as bluegill (*Lepomis macrochirus*) (Fischer et al. 2020), *C. gariepinus* (Dauda et al. 2018), common carp (*Cyprinus carpio*) (Bakhshi et al. 2018), *O. niloticus* (Mirzakhani et al. 2019), and rhou (*Labeo rohita*) (Ahmad et al. 2016), exhibit improved biochemical composition due to BFT consumption as supplementary nutrition.

Organ indicators like the HSI, VSI, TSI, and GSI showed significant variations, suggesting changes in energy reserves and general conditions in response to BFT and salinity changes. It has been reported that the use of BFT has a major effect on the composition and organ markers of red tilapia broodstock (Widanarni et al. 2012). A previous study by Saleh et al. (2020) found that adding Amphora to fish diet increased ash and moisture contents while decreasing lipid content, leading to lower VSI and HSI values due to active lipid mobilization. Additionally, Enyidi (2017) observed that adding *Chlorella vulgaris* to catfish (*Clarias gariepinus*) diet decreased HSI by enhancing natural digestive enzymes in the intestine, optimizing nutrient utilization.

Water's physicochemical parameters like temperature, pH, DO, salinity, nutrients, and others are essential for aquatic animals' reproduction and production. Each species has specific needs for these conditions, and deviations from the optimal ranges can lead to stress, disease, or mortality (Khanjani and Alizadeh 2024). The study examined the water quality of hybrid red tilapia in BFT ponds, revealing variations in dissolved oxygen and pH levels. High-salinity ponds had higher DO values but lower pH levels, particularly in BFT ponds. Kornkanok et al. also noted that high-salinity ponds had lower pH levels but greater DO values, especially in BFT ponds, which is consistent with our findings (Kunlasak et al. 2013). BFT ponds had lower levels of higher alkalinity and an active nitrification process. Studies have shown that BFT improves water quality in aquaculture systems and significantly reduces the amount of dissolved nitrogen (Liu

Table 9 Stress indicators and growth hormone of male and female red tilapia broodstock under varying salinity conditions, comparing biofloc-treated (BFT) and untreated groups (control)*

Factor***	Control			BFT			*P*-value		
	S18 ppt	S28 ppt	S36 ppt	S18 ppt	S28 ppt	S36 ppt	BFT	Salinity	BFT×S**
Male ♂									
Cortisol, pg ml^{-1}	227 ± 2.65^{c}	246 ± 2.87^{b}	303 ± 4.61^{a}	209 ± 1.96^{d}	214 ± 2.69^{cd}	214 ± 3.57^{cd}	< 0.001	< 0.001	< 0.001
GH, ng ml^{-1}	2.67 ± 0.03^{a}	2.56 ± 0.03^{a}	1.96 ± 0.12^{b}	2.69 ± 0.03^{a}	2.58 ± 0.01^{a}	2.14 ± 0.06^{b}	0.114	< 0.001	0.277
Female ♀									
Cortisol, pg ml^{-1}	225 ± 4.74^{bc}	232 ± 5.88^{b}	296 ± 3.5^{a}	210 ± 1.52^{c}	213 ± 2.22^{c}	214 ± 2.79^{c}	< 0.001	< 0.001	< 0.001
GH, ng ml^{-1}	1.78 ± 0.12^{b}	2.1 ± 0.33^{ab}	2.26 ± 0.1^{ab}	2.39 ± 0.02^{ab}	2.39 ± 0.02^{ab}	2.73 ± 0.04^{a}	0.003	0.051	0.581

*Mean values are presented as means ± *SEM*. Differences among means in a row without a shared superscript letter are statistically significant ($P < 0.05$), determined by two-way ANOVA and the TUKEY test. The superscript letters indicate the significance of BFT and salinity, not their interaction

**The interaction effect between BFT and salinity is denoted as BFT×S

***Abbreviations: GH: Growth hormone

Table 10 Antioxidants and non-specific immunity of red tilapia broodstock under varying salinity conditions, comparing biofloc-treated (BFT) and untreated groups (control)*

Factor***	Control			BFT			*P*-value		
	S18 ppt	S28 ppt	S36 ppt	S18 ppt	S28 ppt	S36 ppt	BFT	Salinity	BFT × S**
Male ♂									
SOD, U ml^{-1}	12.6 ± 0.03^{ab}	12.4 ± 0.05^{b}	11.7 ± 0.11^{c}	12.8 ± 0.03^{a}	12.4 ± 0.06^{b}	12.4 ± 0.10^{b}	< 0.001	< 0.001	0.001
CAT, U ml^{-1}	11.4 ± 0.00^{ab}	11.3 ± 0.05^{b}	10.3 ± 0.09^{c}	11.6 ± 0.04^{a}	11.4 ± 0.06^{ab}	11.4 ± 0.02^{ab}	< 0.001	< 0.001	< 0.001
MDA, nmol ml^{-1}	0.83 ± 0.05^{bc}	1.17 ± 0.02^{a}	1.29 ± 0.03^{a}	0.60 ± 0.01^{d}	0.72 ± 0.03^{cd}	0.967 ± 0.01^{b}	< 0.001	< 0.001	0.01
Lysozyme, μg ml^{-1}	3.37 ± 0.05^{a}	2.61 ± 0.06^{b}	2.79 ± 0.08^{b}	3.53 ± 0.01^{a}	3.21 ± 0.11^{a}	3.34 ± 0.09^{a}	< 0.001	< 0.001	0.02
Female ♀									
SOD, U ml^{-1}	12.4 ± 0.22^{ab}	12.2 ± 0.03^{bc}	11.8 ± 0.09^{c}	12.9 ± 0.02^{a}	12.5 ± 0.06^{ab}	12.7 ± 0.10^{ab}	< 0.001	0.001	0.398
CAT, U ml^{-1}	10.9 ± 0.12^{bc}	10.3 ± 0.29^{c}	10.5 ± 0.21^{c}	11.6 ± 0.05^{a}	11.4 ± 0.02^{ab}	11.4 ± 0.03^{ab}	< 0.001	0.063	0.631
MDA, nmol ml^{-1}	0.99 ± 0.03^{bc}	1.04 ± 0.03^{b}	1.57 ± 0.1^{a}	0.6 ± 0.01^{d}	0.65 ± 0.03^{d}	0.79 ± 0.01^{cd}	< 0.001	< 0.001	0.001
Lysozyme, μg ml^{-1}	3.05 ± 0.11^{bc}	2.97 ± 0.07^{bc}	2.88 ± 0.06^{c}	3.48 ± 0.07^{a}	3.58 ± 0.01^{a}	3.31 ± 0.13^{ab}	< 0.001	0.094	0.474

*Mean values are presented as means ± *SEM*. Differences among means in a row without a shared superscript letter are statistically significant ($P < 0.05$), determined by two-way ANOVA and the TUKEY test. The superscript letters indicate the significance of BFT and salinity, not their interaction

**The interaction effect between BFT and salinity is denoted as BFT × S

***Abbreviations: SOD: Superoxide dismutase, CAT: Catalase, MDA: Malondialdehyde

et al. 2019). The active nitrification process in BFT ponds converts harmful artificial nitrogen into beneficial protein. The technology reduces total inorganic nitrogen goes down and pH goes up because of the technology. However the nitrate levels in BFT ponds change a lot, and they go up a lot when the salt goes up (Santhana Kumar et al. 2018). The OP (PO_{34}) concentrations increased in BFT treatments, reaching the highest levels at the highest salinity. Increasing salinity elevated TDS and TSS in BFT ponds, indicating higher floc density compared to control ponds. The BFT treatments have been shown by Ciarelli et al. (1999) to raise the concentration of pollutants in the water that are bonded to sediment, including OP.

This research discovered considerable changes in the chemical composition of biofloc at various salinities. Protein levels were greatest in low salinity and vice versa. High salinity also resulted in the greatest carbohydrate content. BFT-treated groups produced considerably more BFV, suggesting greater biomass and microbial activity. Recent studies have shown that salinity has a major impact on the chemical makeup of BFT, with low salinity exhibiting the greatest protein levels and high salinity exhibiting the highest carbohydrate content (Zhao et al. 2016).

The study compared the digestive enzyme activities of male and female red tilapia broodstock under varying salinity conditions. Results showed that BFT-treated groups (males and females) had higher levels of protease, amylase, and lipase activities. The S36 ppt biofloc-treated group had the highest average values. According to Pujante et al. (2018), the thick-lipped grey mullet (*Chelon labrosus*). exhibited a decrease in amylase and protease activity in response to elevated salinity. Klahan et al. (2009) observed that *O. niloticus* of differing sizes exhibited distinct degrees of enzyme activity, with the most pronounced specific activities of lipase and protease found in middle-sized fish.

The study compared the reproductive efficacy and sex hormone levels with BFT under varying salinity conditions. Male groups showed significant disparities in spermatozoa percentage, testosterone levels, and sperm count, and the maximum values were in S18 ppt BFT group. Females' progesterone levels, egg number, egg diameter, ovary weight, and fecundity varied significantly between regimens, and the maximum values were in CW group. The impact of BFT on *O. niloticus*'s reproduction and ovarian recrudescence was comparable to the control system, except for the HSI (Ramos de Alvarenga et al. 2017).

The study compared the hematological and biochemical parameters under varying salinity conditions. Significant differences were observed in various parameters. The group treated with S18 ppt BFT showed the highest concentrations of these biomarkers. Hematological markers are utilized in assessing the physiological status and overall health of aquatic animals under specific dietary and environmental conditions, alongside evaluating their growth performance (Arafa et al. 2024; Sallam et al. 2024). Various factors, including ambient temperature (Gelman et al. 2008) and the overall health condition (Pringle et al. 1992) of the fish, influence their physiological characteristics. The current findings suggest that the application of BFT, particularly in environments with reduced salinity, affects the hematological and biochemical parameters of red tilapia broodstock, potentially impacting their health and physiological functions. The hematological and biochemical parameters of red tilapia larval stock are notably affected by the implementation of BFT, specifically when exposed to different levels of salinity (Widanarni et al. 2012). These results align with those reported by Akinrotimi et al. (2012), wherein substantial alterations in blood parameters were detected in *Tilapia guineensis* (*Coptodon guineensis*) subjected to varying levels of salinity. Variations in fish blood chemistry are likely influenced by various environmental factors, including the type and amount of organic carbon sources, the quality and size of flocs, and the presence of bioactive chemicals within BFT (Ahmad et al. 2019).

The study looked at stress markers and GH levels in red tilapia broodstock that was treated with BFT and fish that weren't treated with BFT in different salinity conditions. Cortisol levels varied significantly between treatments, with the S36 ppt control group having the highest levels in males and the S18 ppt BFT-treated group having the lowest. GH levels did not differ significantly between the male and female groups. This study suggests that BFT application, especially in reduced salinity conditions, could alleviate stress in red tilapia broodstock, potentially impacting their health and welfare. According to the research of Borski et al. (1994) and Breves et al. (2010), environmental salinity can have a substantial effect on the endocrine system of fish, specifically GH concentrations. Also, Borski et al. (1994) documented an increase in GH levels in the pituitaries of tilapia reared in seawater. Breves et al. (2010), on the other hand, observed that stress-induced alterations in plasma cortisol and glucose levels were observed in both freshwater and saltwater environments, but had no significant impact on GH.

The antioxidant levels and immune responses of red tilapia broodstock under various salinity conditions were evaluated in this study. The S18 ppt BFT-treated group had the highest SOD and CAT levels in males and females, while MDA levels were the lowest. This indicates enhanced antioxidant activity and reduced lipid peroxidation. Lysozyme levels were higher in the S18 ppt and S28 ppt BFT-treated groups. The results of this study align with prior investigations that have demonstrated the advantageous impacts of BFT treatment on fish growth, immune response, and antioxidant activity (Bañuelos-Vargas et al. 2021; Liu et al. 2018).

Shourbela et al. (2021) found that BFT-raised *O. niloticus* showed increased SOD and CAT activities, especially in low-density BFT groups. Menaga et al. (2019) suggested BFT enhances SOD and CAT activities, crucial for combating free radicals. Ebrahimi et al. (2020) noted elevated SOD and CAT levels in BFT-cultured *C. carpio*, improving antioxidant capacity. Similarly, Yu et al. (2020b) and Yu et al. (2020a) observed higher SOD and CAT activities in biofloc-raised *Opsariichthys kaopingensis Dybowski* and Golden crucian carp (*Carassius auratus*), reducing lipid peroxidation and enhancing free radical resistance. Nageswari et al. (2022) reported increased SOD and CAT activities in BFT-cultured sutchi catfish (*Pangasianodon hypophthalmus*), highlighting BFT's antioxidative role against oxidative stress.

Polyunsaturated fatty acids undergo a reaction with free radicals, resulting in the production of MDA, which serves as an indicator of oxidative stress (Kim et al. 2022). In the study by Liu et al. (2018), MDA levels notably decreased in *O. niloticus* raised in BFT environments, indicating an enhanced antioxidant capacity and improved defense against lipid peroxidation. Conversely, *C. carpio* cultivated in BFT systems exhibited elevated MDA levels, suggesting enhanced fish health despite a reduction in oxidative stress, as documented by Ebrahimi et al. (2020). Similar trends were observed across various BFT-raised species, such as *O. kaopingensis*, *C. auratus*, and *C. argus*, suggesting that bioactive components present in biofloc, including vitamins, phytosterols, carotenes, polysaccharides, and polyphenols, act as antioxidants (Yu et al. 2020a, 2020b, 2021).

Conclusion

The study examines the effects of BFT and salinity changes on red tilapia broodstock. Results show that under BFT and higher salinity conditions, the S36 ppt group showed significant improvements in growth metrics. The BFT condition factor increased across all

salinity levels, indicating improved health and physical condition. Broodstock SR (%) were consistently high. BFT and salinity significantly influenced moisture, protein, lipid, and ash content in both males and females, as well as changes in energy reserves and conditions. Water quality parameters showed variations between biofloc and control treatments, with notable impacts on dissolved oxygen pH, and other substances. The study emphasizes the complex interactions between environmental conditions and BFT on red Tilapia broodstock physiology and performance. Future studies are recommended to analyze gene expression and tissue morphology in red tilapia under different BFT and salinity conditions to understand their physiological responses.

Author contributions **G. R. S.** Conceptualization, Methodology design, Data curation and analysis, Writing – Original draft preparation, Review and editing.

A. I. S. Conceptualization, Methodology design, Data curation and analysis, Writing – Original draft preparation, Writing-Review and editing, and finalizing the manuscript.

M. F. E. Statistical analysis, Data interpretation, Writing-Review and editing, and finalizing the manuscript.

Y. J. H. Writing-Review and editing.

S. H. Data collection and analysis, and Writing-Review and editing.

A. N. A. Writing-Review and editing.

Y. M. H. Methodology design.

W. M. F. Methodology, Data collection, validation and Writing-Review and editing.

A. M. E. Methodology, Data analysis, Review and editing.

H. A. A. Methodology design, Data collection and interpretation, Review and editing.

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Data availability The datasets used in this study can be obtained by contacting the corresponding author upon request.

Declarations

Ethical approval The College of Agriculture Committee for Animal Care at the National Institute of Oceanography and Fisheries (NIOF) in Egypt granted ethical permission for the study, with reference number (NIOF-AQ1-F-23-R-010). Furthermore, all study methodologies rigorously adhered to the ARRIVE guidelines v2.0 (Percie du Sert et al. 2020), ensuring that the research approach is consistent with recognized ethical standards and preserves the well-being of the experimental animal.

Consent for publication Not applicable.

Competing interest The authors declare no competing interests.

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