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Effects of stressful rearing conditions on the growth performance of Nile tilapia (*Oreochromis niloticus*) in aquaculture

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ABSTRACT

This study aimed to evaluate how simultaneous fluctuations in temperature, salinity, and electrical conductivity, conditions frequently encountered in North African freshwater systems, affect the growth performance of Nile tilapia (*Oreochromis niloticus*). Fry were reared for 75 days under two treatments: a control group maintained at optimal conditions (28 °C; 0‰ salinity), and a stressed group exposed to realistic environmental fluctuations, with temperature ranging from 17.64 to 23.64 °C, salinity from 5.03 to 5.42 ppm, and conductivity from 8.99 to 9.65 mS/cm. Growth was monitored twice weekly, and statistical analyses included two-way ANOVA and principal component analysis (PCA). Stressed fish exhibited markedly reduced growth, reaching final values of 7.97 ± 0.00 g and 7.60 ± 0.00 cm compared with 26.29 ± 0.00 g and 11.03 ± 0.00 cm in the control group. ANOVA revealed significant effects of treatment, exposure duration, and their interactions ($p < 0.05$), while PCA explained 95.7% of the variance and identified body weight as the most sensitive trait to abiotic instability. Although the study was conducted under controlled laboratory conditions and limited to a single salinity level, the results provide quantitative evidence that combined thermal–osmotic instability around 20 °C and 5‰ salinity severely impairs tilapia somatic growth. These findings offer practical reference thresholds for aquaculture operations, particularly in semi-arid regions where environmental fluctuations are common, and highlight the importance of monitoring water mineralization and temperature to minimize chronic stress. The originality of this work lies in its integrated assessment of combined environmental stressors, rather than isolated factors, and in demonstrating the discriminant value of body weight as a biomarker of environmental instability in freshwater tilapia aquaculture.

Keywords: aquaculture stressors, abiotic variability, growth inhibition, environmental management, *Oreochromis niloticus*, fish physiology, temperature fluctuation, salinity stress, aquaculture performance.

INTRODUCTION

Freshwater aquaculture systems are increasingly exposed to environmental instability driven by climatic variability, resource limitations, and anthropogenic pressures. Such fluctuations are particularly critical in semi-arid regions, where temperature extremes, salinity shifts, and water scarcity can disrupt physiological homeostasis in cultured fish, thereby compromising growth performance and overall productivity (FAO, 2022; Mohammed and Al-Amin, 2018). Similar trends have been documented in intensive aquaculture systems, where fluctuations in dissolved oxygen and other abiotic factors induce metabolic stress, oxidative damage, and reduced feed efficiency in cultured fish (Abdel-Tawwab et al., 2014; Abdel-Tawwab et al., 2019; Lushchak, 2011; Menon et al., 2023). In North Africa, environmental constraints are further intensified by recurrent droughts, increased evapotranspiration, and irregular freshwater supplies, which collectively contribute to pronounced fluctuations in temperature, salinity, and electrical conductivity in inland aquaculture systems (Ben Hassen et al., 2025; CRDI, 2023). Several regional studies highlight the chronic instability of Algerian, Tunisian and Moroccan freshwater bodies, where evaporation, groundwater intrusion and climate extremes lead to substantial temporal variability in water quality parameters, with direct implications for fish growth (Hamma et al., 2024; Dhaoui et al., 2021; Belokda et al., 2020). This environmental vulnerability reinforces the importance of developing resilient freshwater aquaculture practices, particularly in Algeria, where tilapia production is identified as a strategic component of the National Blue Economy Strategy 2030 (Ministry of Industry and Fisheries Resources, 2022).

Nile tilapia (*Oreochromis niloticus*) is one of the most widely farmed freshwater fish globally due to its rapid growth, tolerance to high stocking densities, and ability to withstand moderate environmental fluctuations (El Sayed, 2019; Prabu et al., 2019). Despite its robustness, tilapia remains sensitive to abrupt or prolonged exposure to thermal and osmotic stress, which can impair metabolic processes, suppress immune responses, reduce feed efficiency, and ultimately inhibit growth (Dawood et al., 2021; Martins et al., 2022). Numerous studies have examined temperature effects (Santos et al., 2013; Makori et al., 2017) or salinity effects (Iqbal et al., 2012; Hien et al., 2022) in isolation; however, studies investigating their combined influence under controlled conditions remain considerably fewer, despite the fact that fish respond to environmental factors in an interactive and non-linear manner (Li et al., 2024; Abdel-Tawwab and Wafeek, 2017).

Furthermore, the integrative studies that do exist tend to focus on extreme salinity levels or stable thermal environments (Qiang et al., 2013; Hien et al., 2022). hey seldom address moderate and fluctuating conditions, which more closely resemble those observed in North African aquaculture facilities. In semi-arid regions of Algeria and Tunisia, several hydrogeochemical studies have shown that inland freshwater resources experience marked spatial and temporal variability in temperature, salinity and mineralization, largely driven by evaporation, groundwater interactions and climate variability (M'nassri et al., 2019, Negm et al., 2020;).

Yet, there is a lack of quantitative data describing how tilapia respond to simultaneous, moderate fluctuations of temperature (~20 °C), salinity (~5‰), and conductivity, despite their relevance for semi-arid aquaculture operations. To date, and to the best of our knowledge, no experimental study has specifically assessed the biological consequences of these combined fluctuations under controlled conditions in a North African context. This represents a significant knowledge gap that limits the development of adaptive management strategies for freshwater aquaculture under increasing climate variability (Maulu et al., 2021; Mugwanya et al., 2022). Understanding the synergistic effects of multiple abiotic stressors is essential, as interactive stress can result in physiological trade-offs not detectable when factors are studied independently (Canosa and Bertucci, 2023; Segurado et al., 2022).

Therefore, this study aims to experimentally evaluate the effects of simultaneous, moderate fluctuations in temperature, salinity and conductivity, representing realistic conditions of North African semi-arid aquaculture, on the growth performance of *Oreochromis niloticus.* We hypothesize that: (i) combined environmental fluctuations will significantly reduce somatic growth due to increased metabolic and osmoregulatory costs; (ii) body weight will respond more strongly than total length, acting as a sensitive biomarker of abiotic instability; and (iii) growth impairment will intensify with prolonged exposure. This work is the first to quantify the biological consequences of moderate, naturally occurring abiotic variability under controlled experimental conditions in a North African context, thereby providing regionally relevant thresholds to improve adaptive aquaculture management.

MATERIALS AND METHODS

Study area

The experiment was conducted at the Aquaculture Division of the National Center for Research and Development in Fisheries and Aquaculture

(CNRDPA), located in Bou Ismaïl (Tipaza, Algeria) (Figure 1). This facility includes indoor controlled-environment rearing rooms, continuous freshwater supply, and standard aquaculture monitoring instruments suitable for growth and environmental experiments. The region is characterized by a Mediterranean semi-arid climate with marked seasonal variations in temperature and water mineralization, conditions known to influence the physiological performance of freshwater fish.

Experimental design

The experimental trial was conducted over a period of 75 days to evaluate the effects of controlled fluctuations in abiotic conditions on the growth performance of *Oreochromis niloticus*. The study consisted of two clearly defined treatments carried out simultaneously under identical physical conditions. (1) A control group was maintained in freshwater at a constant temperature of 28 °C (Figure 2a); (2) A stressed group was exposed to a fixed salinity of 5‰ and to naturally fluctuating laboratory temperatures (Figure 2b).

To ensure strict comparability, both groups were reared in two identical rectangular glass aquaria (40 × 89 × 47 cm), each equipped with its own aeration system. Fish were randomly allocated to the tanks to avoid selection bias: 29 individuals in the control tank and 46 individuals in the stressed tank. Before stocking, all fry were obtained from the CNRDPA hatchery and subjected to a health inspection protocol, including evaluation of swimming behavior, visual examination for lesions, and exclusion of individuals showing signs of external parasites. At the beginning of the experiment (22 February 2024), fish exhibited an average initial weight of 3.35 ± 1.40 g and an average total length of 5.51 ± 0.92 cm.

The experimental workflow followed a fixed measurement schedule, during which abiotic parameters and biometric variables were systematically recorded. This stepwise protocol allowed the monitoring of temporal changes in environmental conditions and their cumulative impact on growth. Biometric data collected throughout the trial were analyzed using a two-way ANOVA (treatment × sampling time). This statistical model, appropriate for unbalanced sample sizes, enabled the assessment of both individual and interactive effects of treatment and exposure duration on fish growth, provided that assumptions of normality and homogeneity of variance were met.

Abiotic variables

The control group was maintained in freshwater (0‰ salinity) at a constant temperature of 28 °C using a calibrated submersible heater to ensure thermal stability throughout the experiment. In contrast, the stressed group was

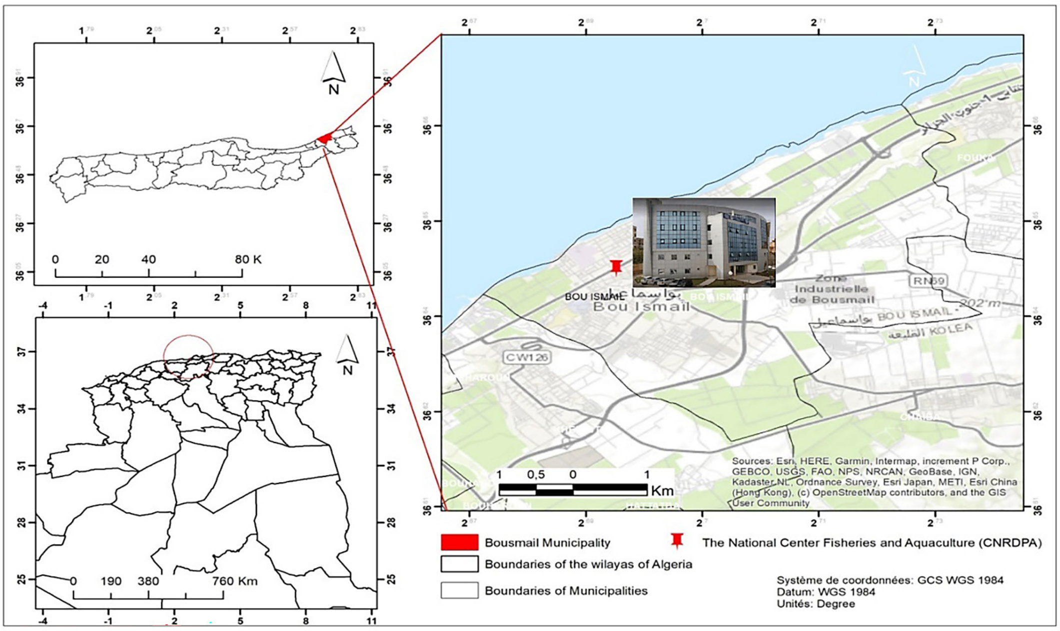

Figure 1. Geographical location of the experimental site: CNRDPA of Bou Ismaïl (Tipaza, Algeria)

Figure 2. (a) Aquarium of the control group equipped with the submersible thermostat used to maintain stable temperature conditions; (b) aquarium of the stressed group showing the multiparameter probe used for monitoring temperature, salinity, and conductivity

subjected to controlled environmental fluctuations: salinity was fixed at 5‰, while temperature followed natural laboratory variations, ranging between 17.64 °C and 23.64 °C. This design simulated realistic instability commonly observed in semi-arid aquaculture environments. Environmental variables were monitored following a standardized measurement protocol. For the stressed group, temperature (T), salinity (S), and electrical conductivity (EC) were recorded three times per day using a Calypso multiparameter probe (Model MP-300). All readings were averaged to obtain daily values. Instrument calibration was performed before each measurement session according to manufacturer guidelines to ensure reliability. Additional water quality parameters, including pH (range 7.71–7.82) and dissolved oxygen (4.05–5.42 mg L^{-1}), were measured twice weekly with a pH meter and a oxygen meter, respectively.

Continuous aeration was provided in both tanks via an air pump and diffuser system to homogenize water mixing and prevent the formation of hypoxic microzones. For the stressed group, salinity was maintained at 5‰ during the first 71 days through periodic additions of filtered seawater (100 µm mesh). The required volume of seawater was determined either directly using the salinity function on the multiparameter probe or by applying the dilution equation:

$$C_1V_1 = C_2V_2 \quad (1)$$

Conductivity measurements were used as a complementary indicator of water mineralization, reflecting ion concentration and total dissolved salts. This parameter provided additional insight into osmotic conditions capable of influencing the physiological responses of *O. niloticus*.

Biotic variables

Biometric monitoring was conducted twice weekly to evaluate individual growth performance under each treatment. At each sampling event, fish were gently captured using a soft mesh net to minimize stress and handling injuries. Total length (L_t) was measured to the nearest millimeter using a standard acrylic ichthyometer, while total weight (P_t) was determined with a KERN precision digital balance (accuracy ±0.01 g). Both instruments were calibrated prior to each measurement session to ensure consistency and accuracy. Each fish was briefly blotted with a damp cloth before weighing to remove excess water, and handling time per individual did not exceed 30 seconds. These morphometric procedures follow established standardized protocols commonly applied in aquaculture and fish biology research (Bwanika et al., 2007; Ricker, 1975), ensuring comparability with prior studies. All biometric measurements were performed by the same trained operator throughout the experiment to reduce inter-observer variability. Water renewal was conducted twice weekly in both tanks using a siphoning method to remove waste and maintain stable environmental conditions, thereby minimizing confounding effects between treatments. Throughout all procedures, fish were handled in accordance with international guidelines for animal welfare in aquaculture research, ensuring minimal stress and preventing behavioral or physiological disturbances that could influence growth patterns.

Feeding regime

Fry were fed four times per day at regular intervals to ensure adequate nutrient intake and support optimal growth. A commercial pelleted diet formulated for tilapia fry was used throughout the experiment. The proximate composition of the feed is presented in Table 1. The protein content of this diet (33%) is in accordance with recommended nutritional requirements for tilapia fry, which range from 35–40% for fish weighing 0.5–10 g, 30–35% for 10–35 g, and 25–30% for broodstock (Jauncey and Ross, 1982).

Feed was distributed manually to prevent overfeeding and ensure uniform access to feed among individuals within each tank. Uneaten feed and residues were removed during routine maintenance to maintain stable water quality.

Statistical analysis

All statistical analyses were performed using RStudio (version 4.4.2). Results are illustrated using boxplots to visualize the distribution and variability of growth responses. Differences between the control and stressed groups were assessed using a two-way ANOVA (sampling duration × abiotic conditions), after verifying the assumptions of the model. Normality of residuals was tested using the Shapiro–Wilk test, and homogeneity of variances was evaluated using Levene's test.

When significant effects were detected, Tukey's HSD post-hoc test was applied to identify pairwise differences between treatment groups.

Table 1. Proximate composition of the commercial feed used for Nile tilapia fry

Component	Percentage (%)
Crude protein	33
Crude fat	6
Crude fiber	4.1
Crude ash	11.4
Phosphorus	1.2
Calcium	0.4
Sodium	0.1

A principal component analysis (PCA) was also conducted to differentiate the two groups based on abiotic and biometric variables and to explore multivariate relationships between environmental fluctuations and growth performance.

RESULTS AND DISCUSSION

Abiotic parameters

The abiotic parameters recorded in the stressed treatment are presented in Figure 3. Temperature, salinity, and electrical conductivity exhibited pronounced temporal fluctuations throughout the experimental period, with temperature ranging from 17.64 °C to 23.64 °C, salinity from 5.03

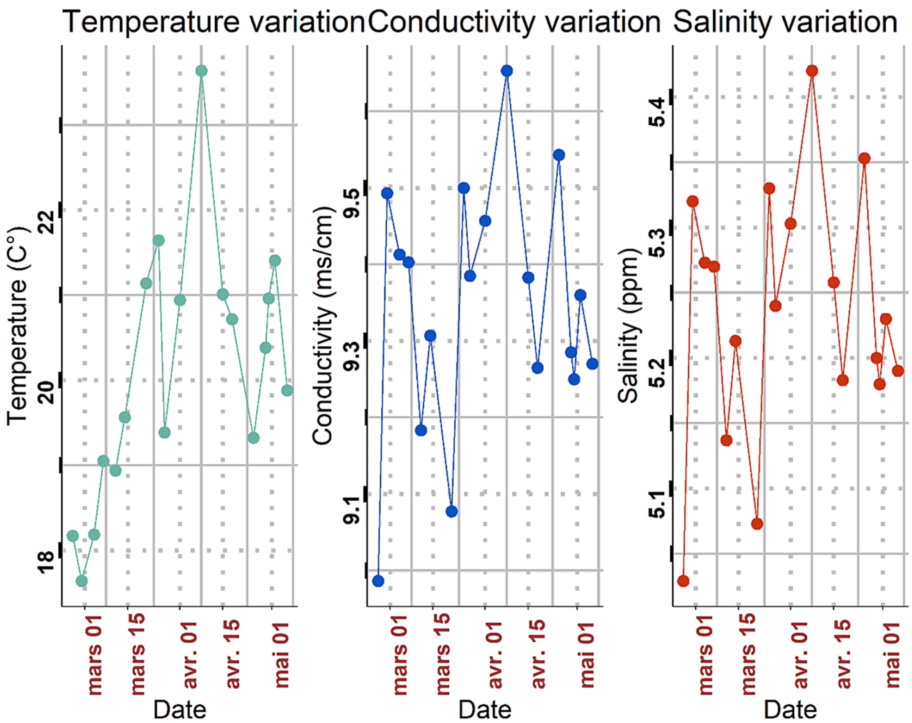

Figure 3. Correlogram of abiotic factors in the stressed sample

to 5.42 ppm, and conductivity from 8.99 to 9.65 $mS \cdot cm^{-1}$. The correlogram (Figure 3) reveals a strong correlation between temperature, salinity, and conductivity in the stressed group, indicating that these parameters varied synchronously. This pattern is consistent with physicochemical principles, as conductivity reflects water mineralization and increases proportionally with dissolved ion concentrations (Mustapha et al., 2017; Verma et al., 2022). In our experiment, the combination of elevated salinity (5‰) and pronounced thermal fluctuations suggests the onset of a combined osmoregulatory stress, likely increasing the metabolic cost required to maintain ionic homeostasis.

Growth in length and weight

The evolution of total length (L_t) and total weight (W_t) in the control and stressed groups is presented in Figure 4. The two-factor ANOVA showed that both L_t and W_t were significantly affected by environmental conditions. Fish in the control group displayed markedly higher growth values than those in the stressed group. Sampling time also had a significant effect on growth, and a significant interaction between abiotic conditions and time was detected for L_t, indicating that the impact of environmental stress on body size varied over the course of the experiment. Overall, these results demonstrate that combined thermal and osmotic fluctuations strongly reduced growth in *O. niloticus*. These results are consistent with previous studies showing that temperature and salinity are major determinants of tilapia growth (Hien et al., 2022; Bhatt et al., 2026). Chronic exposure to suboptimal abiotic conditions can suppress both linear and weight growth by altering metabolic rates, feed efficiency, and endocrine function (Dawood et al., 2021; Li et al., 2024).

The significant interaction between sampling date and environmental conditions indicates that the negative effects of stress intensified over time, a pattern also noted in long-term stress studies on Nile tilapia (Elnady et al., 2021; Iqbal et al., 2012). The growth parameters of the control group are summarized in Table 2.

Temporal patterns in length and weight differences

The pairwise comparisons (95% family-wise confidence intervals) performed on log-transformed weight data and raw length data are presented in Figure 5.

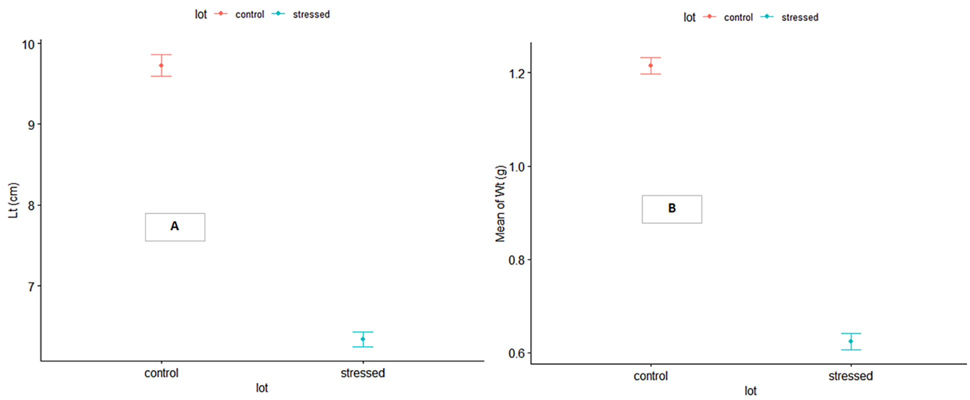

Figure 4. Mean total length (A) and total weight (B) of *Oreochromis niloticus* in control and stressed groups. Error bars represent standard errors

Table 2. Growth parameters of the control group (*Oreochromis niloticus*)

Daily feeding ration (g)	11.75		16.843		24.545		30.496	
	W_t (g)	L_t (cm)	W_t (g)	L_t (cm)	W_t (g)	L_t (cm)	W_t (g)	L_t (cm)
Sample size	29		29		29		29	
Maximum	16.95	9.5	20.95	15	33.87	12.2	42.84	13
Minimum	7.17	7.1	9.1	8	12.61	8.5	13.61	8.9
Mean	10.13	8.09	14.52	9.39	21.16	10.4	26.29	11.03
Standard deviation	2.31	0.57	3.33	1.258	5.411	0.93	7.50	0.95

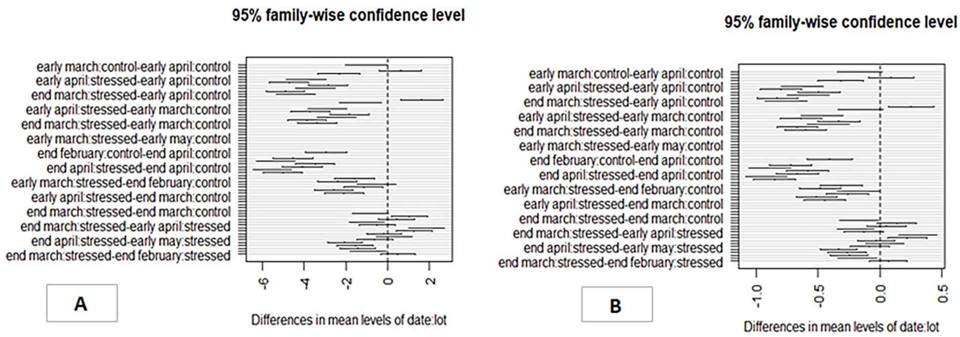

Figure 5. Pairwise comparisons (95% family-wise confidence intervals) for weight (A) and length (B) between sampling dates within control and stressed groups of *Oreochromis niloticus* during the study period

The results show that fish in the control group maintained significantly higher body weight and total length than those in the stressed group throughout the experimental period. The most pronounced differences occurred between late March and late April, when weight differences approached nearly 10 g and length differences reached approximately 0.5 cm. These trends indicate a cumulative negative effect of prolonged exposure to stressful abiotic conditions, particularly fluctuations in temperature, salinity, and conductivity, on the growth performance of *O. niloticu.* The growth parameters of the stressed group are summarized in Table 3.

These findings are consistent with earlier studies reporting reductions in tilapia growth under combined thermal and saline stress. Bhatt et al. (2025) documented substantial declines in weight gain in GIFT tilapia subjected to elevated temperatures and salinity, while Iqbal et al. (2012) showed that increasing salinity significantly reduces both weight and length in Nile tilapia due to the high energetic cost of osmoregulation. Similarly, Santos et al. (2013) demonstrated that optimal growth in *O. niloticus* occurs near 30 °C, with exposure to higher temperatures intensifying metabolic demands and lowering feed conversion efficiency.

In the present study, the mean total length of stressed fish (7.60 cm; range 4.7–10.1 cm) closely matches the values reported by Makila et al. (2020), supporting the growth suppression observed under stressful environmental conditions. Together, these results indicate that the impact of environmental stress intensified over time and underscore the importance of maintaining stable water quality parameters to avoid chronic physiological strain and sustain optimal growth in tilapia aquaculture.

Principal components analysis (PCA)

The multivariate relationships among biometric variables from the stressed and control groups are presented in Figure 6.

The PCA results summarized in Figure 6 show a clear multivariate separation between the stressed and control conditions. In panel A, *stressed-length* and *stressed-weight* display the strongest contributions to Dim1 (49.6%), whereas *controlled-length* and *controlled-weight* contribute primarily to Dim2 (46.1%). This structure indicates that biometric traits of stressed fish follow a coherent internal growth gradient distinct from that of the control group.

Table 3. Growth parameters of the stressed group (Oreochromis niloticus)

Daily feeding ration (g)	6.164 g		5.904 g		6.368 g		8.580 g		9.937 g		12.433 g	
Weighing number	Weighing No. 1		Weighing No. 2		Weighing No. 3		Weighing No. 4		Weighing No. 5		Weighing No. 6	
	W_t (g)	L_t (cm)	W_t (g)	L_t (cm)	W_t (g)	L_t (cm)	W_t (g)	L_t (cm)	W_t (g)	L_t (cm)	W_t (g)	L_t (cm)
Sample size	46		40		40		39		39		39	
Maximum	6.47	7	6.3	7.1	7.14	7.7	10.7	8.8	13.02	9.3	18.15	10.1
Minimum	0.99	3.1	0.65	3.2	1.21	4.1	1.44	4.3	1.53	4.5	1.71	4.7
Mean	3.35	5.51	3.69	5.7	3.98	6	5.5	6.5	6.37	6.9	7.97	7.6
Standard deviation	1.39	0.91	1.66	1.02	1.77	1,05	3.30	2.27	4.32	2.62	4.62	1.68

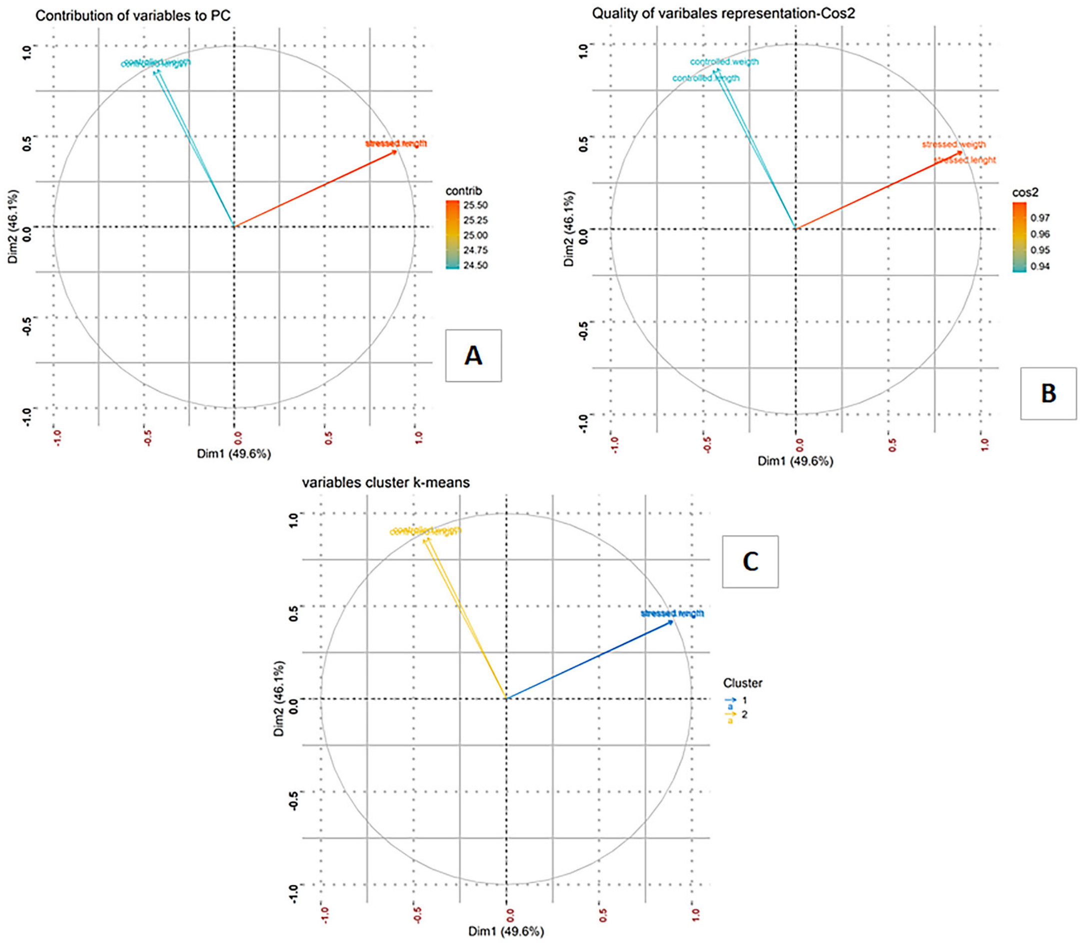

Figure 6. Correlation circle from PCA analysis of "stressed-length," "stressed-weight," "controlled-length," and "controlled-weight." A: contribution of variables to the formation of principal axes; B: quality of variable representation on the principal axes (Cos²); C: k-means clustering of variables based on PCA coordinates

Panel B shows that all variables exhibit high Cos² values (> 0.94), indicating excellent projection quality and confirming that Dim1 and Dim2 reliably capture the multivariate structure. Together, these two components explain 95.7% of the total variance, reflecting the strong covariation between length and weight across conditions. In panel C, k-means clustering applied to PCA coordinates further reinforces this separation: stressed variables cluster together, while control variables form a distinct group.

This pattern suggests that environmental stress modifies the magnitude of growth but does not disrupt the internal proportionality between biometric traits. Such internal consistency agrees with findings showing that Nile tilapia maintain stable biometric relationships across different environmental settings (Asmamaw et al., 2019; La Rosa et al., 2025). Other studies similarly report that even when growth is reduced under stressors such as hypoxia, the structural relationship between biometric traits remains preserved (Yu et al., 2021). Overall, the PCA results indicate that *O. niloticus* maintains coordinated growth patterns, with stress affecting growth intensity rather than the organization of biometric covariation, consistent with physiological resilience previously documented in tilapia (El-Sayed, 2020; Mengistu et al., 2022).

PCA with qualitative variable

The multivariate structure integrating biometric variables from the control and stressed groups, based on a PCA combining quantitative and qualitative factors, is presented in Figure 7.

The PCA performed using total length, total weight, and the qualitative variable "sample type" (control vs. stressed) revealed a clear separation between the two groups. The first axis (Dim1) explained 96.2% of the total variance,

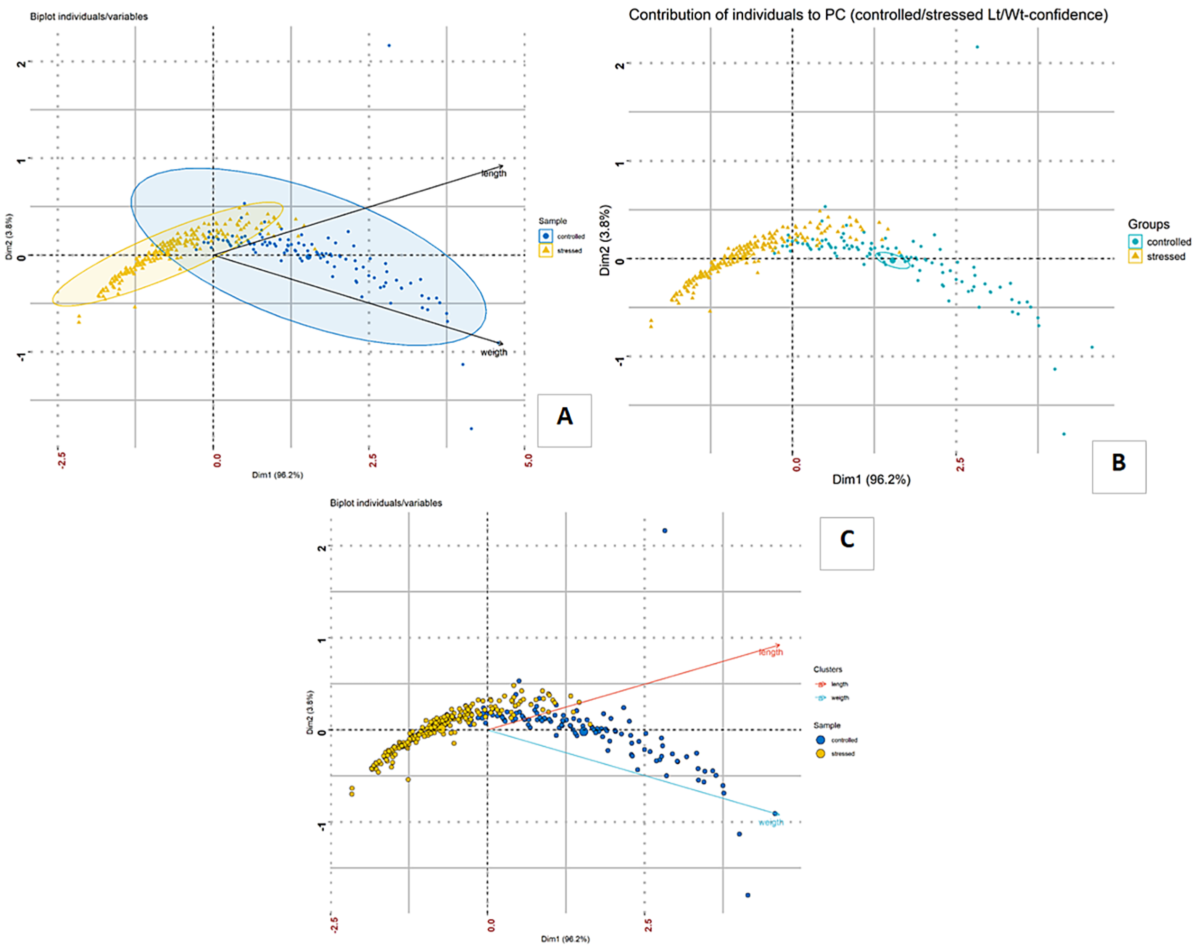

Figure 7. PCA analysis based on two quantitative variables ("length" and "weight") and one qualitative variable ("control–stressed"). A: Biplot of individuals and variables grouped by sample type. B: Contribution of individuals to the principal components. C: Biplot of individuals and variables grouped by sample type and cluster

while the second axis (Dim2) accounted for 3.8%, indicating that the main biometric structure of the dataset is concentrated on Dim1. Individuals from the control group aligned strongly with the length and weight vectors (Figure 7A), showing that the heaviest and longest fish consistently belonged to this group. This pattern aligns with previous work demonstrating the positive effects of optimal environmental conditions on somatic growth in Nile tilapia (Makori et al., 2017; Prabu et al., 2019).

Conversely, stressed individuals clustered toward the negative side of Dim1 (Figure 7B–C), reflecting reduced performance in both size and body mass. This pattern aligns with previous findings demonstrating that abiotic stressors, such as suboptimal water quality, elevated ammonia, and increased metabolic load, primarily suppress body weight and, to a lesser extent, linear growth in Nile tilapia (Bwanika et al., 2007; Abdel-Tawwab and Wafeek, 2017).

The projection of stressed fish away from the biometric vectors supports documented evidence that tilapia body mass is highly sensitive to environmental constraints, leading to reduced growth potential under stressful rearing conditions (Santi et al., 2023). Overall, this PCA reveals a clear multivariate distinction between control and stressed fish, indicating that differences in growth originate directly from the imposed abiotic constraints. Body weight emerged as the most discriminant variable, confirming its relevance as a sensitive indicator of environmental quality in aquaculture production systems.

CONCLUSIONS

Stressful abiotic conditions, combining a salinity of 5‰ with marked temperature fluctuations around 20 °C, significantly reduced the growth performance of Nile tilapia. Fish exposed to these conditions exhibited lower total length and weight throughout the experiment compared to those reared under optimal parameters. Despite this reduction in growth, no external lesions or clinical signs of disease were observed, indicating that the species maintained overall physiological stability under the imposed conditions. These findings highlight the measurable impact of suboptimal environmental parameters on somatic development while confirming the inherent capacity of Nile tilapia to withstand moderate environmental stress.

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