Multi-Omics Investigation of Stress Response Mechanisms in Nile Tilapia (*Oreochromis niloticus*) Reared in Plastic Tank, Concrete Pond, and Earthen Pond Culture Systems

[*1]Umar, A., [2]Bala, M., [3]Muhammad, S. A., [4]Busari, O. O. [1]Amina, M., and [2]Makinta, L. M.

[1]Department of Fisheries Technology, Federal College of Freshwater Fisheries Technology Baga, P.M.B. 1060 Maiduguri, Borno State, Nigeria
[2]Department of Animal Science, Faculty of Agriculture, Kashim Ibrahim University, P.M.B. 1122, Maiduguri, Borno State, Nigeria
[3]Artificial Insemination Unit, National Animal Production Research Institutes, Shika-Zaria P.M.B. 1096, Kaduna State, Nigeria
[4]Department of Animal Production and Health, Ladoke Akintola University of Technology Ogbomoso P.M.B. 1000, Oyo State, Nigeria
[*]Corresponding author's e-mail: audamaturu@gmail.com, +234 803 625 1378, 0009-0005-4007-4826

Abstract
Aquaculture intensification exposes fish to environmental stressors that can compromise growth, immunity, and welfare. We applied a multi-omics approach integrating physiology, targeted transcriptomics (qPCR), and proteomic profiling to investigate stress responses of *Oreochromis niloticus* reared for 8 weeks in plastic tanks, concrete ponds, and earthen ponds under standardized feeding. Water quality, growth, survival, cortisol and antioxidant biomarkers (SOD, CAT), relative expression of stress/immune genes (HSP70, CAT, IL-1β, TNF-α), and differentially expressed protein categories were evaluated. Earthen ponds yielded superior water quality, growth, FCR, and survival, along with lower cortisol and pro-inflammatory gene expression, and higher antioxidant capacity. Plastic tanks showed the inverse pattern; concrete ponds were intermediate. The multi-omics integration indicates that oxidative stress, heat-shock response, and pro-inflammatory signaling dominate in tanks, whereas immune homeostasis and efficient redox metabolism characterize ponds. Findings highlight system-dependent stress biology and practical levers for welfare-oriented, productive tilapia culture.

Keywords: aquaculture systems, *Oreochromis niloticus*, stress, transcriptomics, proteomics, water quality, antioxidant enzymes

Introduction

Nile tilapia (*Oreochromis niloticus*) is central to global aquaculture owing to its fast growth, broad environmental tolerance, and strong market appeal (FAO, 2022; El-Sayed, 2006, 2021). As demand grows, production increasingly occurs in compact units (e.g., plastic or concrete tanks), which facilitate biosecurity and logistics but may intensify biotic and abiotic stressors: limited water volume, variable dissolved oxygen (DO), accumulation of metabolites, and frequent handling (Barton, 2002; Martins *et al*., 2019; Boyd and Tucker, 1998; Boyd, 2015). In contrast, earthen ponds provide natural buffering sediment-microbiome interactions, macrophytes, and diel photosynthesis that can stabilize water chemistry and reduce stress loads (Boyd and Tucker, 1998; Beveridge and McAndrew, 2000; El-Sayed, 2006).

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Fish stress is mediated by the hypothalamus pituitary interrenal (HPI) axis, culminating in cortisol release and a suite of metabolic, osmoregulatory, and immune adjustments (Wendelaar Bonga, 1997; Barton, 2002; Conte, 2004; Schreck *et al*., 2016). Chronic stress decreases growth and feed efficiency, suppresses immune defenses, and increases disease risk (Ashley, 2007; Tort, 2011; Ellis *et al*., 2002; Huntingford *et al*., 2006). At the cellular level, stress responses involve heat-shock proteins (HSPs), redox enzymes (e.g., SOD, CAT), and pro-inflammatory cytokines (IL-1β, TNF-α), integrated with energy-metabolic reprogramming (Feder and Hofmann, 1999; Iwama *et al*., 1998, 2004; Lushchak, 2011; Zou and Secombes, 2016).

Traditional welfare indicators (growth, FCR, survival, cortisol) are invaluable yet cannot fully resolve mechanisms (Barton, 2002; Conte, 2004). Omics approaches transcriptomics and proteomics offer high-resolution insights into pathways and biomarkers underpinning phenotypes (Kültz, 2005; De Souza *et al*., 2020; Krasnov *et al*., 2011). For tilapia, omics has clarified responses to hypoxia, temperature, salinity, and crowding, revealing recurrent themes: heat-shock pathways, oxidative stress, and immune modulation (De Souza *et al*., 2020; Liu *et al*., 2020; Xu *et al*., 2022; Sun *et al*., 2021). System-dependent stress biology of *O. niloticus* across plastic tanks were compared, concrete ponds, and earthen ponds by integrating water quality and performance data with physiological, transcriptomic, and proteomic readouts. We hypothesized that earthen ponds would buffer stress reflected in lower cortisol, reduced HSP/pro-inflammatory signals, and enhanced antioxidant/immune homeostasis relative to tanks.

Materials and Methods

Study site, fish sourcing and acclimation

The 8-week trial was conducted at the fish Hatchery unit of the Federal College of Freshwater Fisheries Technology, Baga (Latitudes $11^0$51'43" N and Longitudes $13^0$13'36" E), between June and July 2024.

Healthy juvenile *O. niloticus* (20 ± 0.5 g; mean ± SE) from a certified hatchery were transported in aerated containers and acclimated for 10 days in flow-through holding tanks (28–29 °C; DO > 5 mg L^{-1}). During acclimation fish were prophylactically observed (feeding response, skin/gill condition) and fed a 35 % CP commercial diet at 3 % body weight day^{-1} (El-Sayed, 2006; Boyd, 2015).

Experimental systems, allocation, and routine husbandry

A total of 180 fish were randomly assigned to three culture systems plastic tanks, concrete ponds, earthen ponds with three independent replicates per system (n = 3; 20 fish $replicate^{-1}$). Plastic tanks (0.5 m^3, aerated) and concrete ponds (≈2 m^2 surface, 1 m depth) were supplied with well water; tanks received 20 % water exchange every 3 days; concrete units received 10-20 % exchange as needed to maintain DO ≥ 5 mg L^{-1}. Earthen ponds (≈10 m^2, 1 m depth) relied on natural buffering and occasional partial exchange if DO approach 4 mg L^{-1} (Boyd and Tucker, 1998). All units were shaded to limit midday thermal spikes. Fish were fed 3 % body weight day^{-1} (09:00 and 16:00 h), with ration adjusted bi-weekly from subsample weights (El-Sayed, 2006).

Water quality monitoring

Temperature (°C), pH, and DO (mg L^{-1}) were measured on-site thrice weekly with calibrated handheld meters; un-ionized ammonia (NH_3–N) and nitrite (NO_2–N) were measured weekly with colorimetric kits following manufacturer instructions and APHA (2012). Field probes were calibrated at each session. Management actions (e.g., increased aeration or partial exchange) were triggered if DO fell < 5 mg L^{-1} in tanks/ponds (Boyd, 2015).

Growth performance and survival

At Week 0, 4, and 8, fish in each replicate were batch-weighed after a 24 h feed withdrawal to reduce gut content bias. Performance indices were calculated per replicate: weight gain (g), specific growth rate (SGR, % day^{-1} = [ln Wf– ln Wi] ×100/days), feed conversion ratio

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(FCR = feed intake/weight gain), and survival (%) (El-Sayed, 2006; Boyd, 2015).

Physiological stress biomarkers

At Weeks 4 and 8, five fish per replicate were gently netted and anesthetized with clove oil (≈50 mg L^{-1}). Blood was drawn from the caudal vein into heparinized syringes, centrifuged (3,000 g, 10 min, 4 °C), and plasma stored at -20 °C. Cortisol was quantified by ELISA (validated fish kit), plasma glucose by glucose oxidase-peroxidase reaction, and antioxidant enzymes SOD and CAT by kinetic colorimetric assay (manufacturer protocols) (Barton, 2002; Lushchak, 2011).

Transcriptomics: target gene expression by qPCR

At Week 8, three fish per replicate were euthanized with MS-222 (tricaine); liver tissues were excised into RNAlater and stored at -80 °C. Total RNA was extracted (TRIzol), purified (silica-column kit), and integrity verified (RIN > 8). cDNA was synthesized (reverse transcriptase kit). Target genes were HSP70, CAT, IL-1β, and TNF-α; β-actin served as the reference. Primers were designed/validated from published tilapia sequences (efficiency 90-110 %, single-peak melt curves). Relative expression was computed via 2^-ΔΔCt (Livak and Schmittgen, 2001). Technical duplicates and no-template controls were run each plate.

Proteomics: protein extraction and LC-MS/MS and categorization

White muscle (~100 mg) from the same individuals was homogenized in lysis buffer with protease inhibitors. Protein concentration was measured by bicinchoninic acid assay (BCA; Smith *et al.*, 1985). Aliquots (100 µg) were separated by SDS-PAGE (Laemmli, 1970), gel bands in-gel digested with trypsin, and peptides analyzed by LC-MS/MS (Orbitrap). Spectra were searched against the *O. niloticus* UniProt database; protein FDR was controlled at 1 % using a target-decoy strategy in MaxQuant; downstream functional analysis employed Perseus (Cox and Mann, 2008; Tyanova *et al.*, 2016). For reporting within this manuscript, proteins were summarized into functional categories (HSPs, antioxidant enzymes, immune-related, metabolic) to align with physiological and qPCR data.

Bioinformatics and statistics

Enrichment of biological processes/pathways used DAVID and KEGG databases (Huang *et al.,* 2009; Kanehisa and Goto, 2000). Normality/homogeneity were checked (Shapiro-Wilk, Levene). One-way ANOVA compared systems ($\alpha = 0.05$), with Tukey's HSD for pairwise contrasts. Data are mean ± SE at the replicate (tank/pond) level; means within a row bearing different superscripts differ significantly (Benjamini and Hochberg, 1995; Field, 2013).

Results

Dissolved Oxygen (Table 1) was lowest in plastic tanks and highest in earthen ponds ($^{c} < ^{b} < ^{a}$), consistent with greater reaeration/photosynthesis and sediment-microbe nitrogen cycling in ponds. Ammonia followed the reverse order (plastic > concrete > earthen), indicating more efficient nitrification and assimilation in ponds. pH was slightly higher in ponds, reflecting higher primary productivity. Temperatures were all suitable for tilapia (≈27–30 °C), but ponds were ~2 °C

Table 1. Water quality parameters across culture systems (Mean ± SE)

Parameter	Plastic Tank	Concrete Pond	Earthen Pond
Temperature (°C)	29.5 ± 0.4^{a}	28.8 ± 0.3^{a}	27.2 ± 0.2^{b}
DO (mg L^{-1})	4.1 ± 0.2^{c}	5.0 ± 0.2^{b}	6.3 ± 0.1^{a}
pH	7.2 ± 0.1^{b}	7.5 ± 0.2^{b}	8.0 ± 0.2^{a}
Ammonia (mg L^{-1})	0.18 ± 0.02^{a}	0.14 ± 0.01^{b}	0.09 ± 0.01^{c}

Row-wise superscripts indicate $p < 0.05$.

Earthen pond fish (Table 2) significantly outperformed others for growth (final weight, weight gain, SGR), converted feed more efficiently (lower FCR), and survived better. Plastic tanks had the poorest performance.

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Patterns are coherent with water quality: higher DO and lower ammonia in ponds.

Table 2. Growth performance and survival (Mean ± SE)

Metric	Plastic Tank	Concrete Pond	Earthen Pond
Final weight (g)	52.4 ± 1.8^{c}	60.3 ± 1.5^{b}	68.5 ± 1.6^{a}
Weight gain (g)	32.3 ± 1.5^{c}	40.3 ± 1.3^{b}	48.3 ± 1.4^{a}
SGR (% day^{-1})	2.15 ± 0.07^{c}	2.41 ± 0.05^{b}	2.65 ± 0.06^{a}
FCR	1.85 ± 0.06^{a}	1.60 ± 0.05^{b}	1.45 ± 0.04^{c}
Survival (%)	85.0 ± 2.3^{c}	90.5 ± 2.0^{b}	96.0 ± 1.8^{a}

Row-wise superscripts indicate $p < 0.05$.

Table 3 presents Physiological stress biomarkers. Cortisol and glucose canonical indices of HPI axis activation and secondary metabolic response were highest in plastic tanks and lowest in ponds, indicating greater chronic stress in tanks. Antioxidant enzyme activities (SOD, CAT) were lowest in tanks and highest in ponds, suggesting better redox defenses in ponds consistent with lower oxidative challenge and/or better endogenous antioxidant capacity.

Table 3. Physiological stress biomarkers (Mean ± SE)

Biomarker	Plastic Tank	Concrete Pond	Earthen Pond
Cortisol (ng mL^{-1})	125.4 ± 5.2^{a}	110.6 ± 4.8^{b}	95.2 ± 3.9^{c}
Glucose (mg dL^{-1})	85.6 ± 3.5^{a}	74.2 ± 2.8^{b}	65.8 ± 2.5^{c}
SOD (U mL^{-1})	18.3 ± 0.9^{c}	22.5 ± 1.1^{b}	27.8 ± 1.2^{a}
CAT (U mL^{-1})	15.6 ± 0.8^{c}	19.8 ± 0.9^{b}	24.1 ± 1.0^{a}

Row-wise superscripts indicate $p < 0.05$.

Table 4 presents Relative gene expression. HSP70 was strongly up-regulated in tanks, indicating a robust cellular chaperone response to proteotoxic stress. Pro-inflammatory cytokines (IL-1β, TNF-α) were likewise highest in tanks, reflecting inflammatory tone often seen under chronic environmental stress. Conversely, CAT (catalase) expression was highest in ponds, aligning with enhanced antioxidant status observed enzymatically (Table 3) and suggesting a coordinated redox program under more favorable conditions.

Table 4. Relative gene expression (fold change; Mean ± SE)

Gene	Plastic Tank	Concrete Pond	Earthen Pond
HSP70	3.8 ± 0.2^{a}	2.6 ± 0.1^{b}	1.4 ± 0.1^{c}
CAT	1.5 ± 0.1^{c}	2.3 ± 0.1^{b}	3.2 ± 0.2^{a}
IL-1β	2.8 ± 0.2^{a}	2.1 ± 0.1^{b}	1.2 ± 0.1^{c}
TNF-α	3.2 ± 0.2^{a}	2.4 ± 0.1^{b}	1.5 ± 0.1^{c}

Row-wise superscripts indicate $p < 0.05$.

Differentially expressed protein categories were shown in Table 5 where tanks favored HSP induction and showed relative depression of metabolic and antioxidant proteins, while ponds up-regulated antioxidant/immune and metabolic categories, consistent with better growth and lower stress.

Table 5. Differentially expressed protein categories (direction of change)

Category	Plastic Tank	Concrete Pond	Earthen Pond
Heat-shock proteins (HSPs)	↑↑	↑	↓
Antioxidant enzymes	↓	↑	↑↑
Immune-related proteins	↑	↑	↑↑
Metabolic proteins	↓	↑	↑↑

Discussion

Our system gradient earthen > concrete > plastic for DO and the reverse for ammonia maps cleanly onto classical pond ecology and engineering (Boyd and Tucker, 1998; Boyd, 2015). Higher DO and lower ammonia in ponds likely reduced HPI axis drive and oxidative burden (Conte, 2004; Lushchak, 2011), enabling superior growth (higher SGR, lower FCR) and survival

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(El-Sayed, 2006). Elevated ammonia impairs gill ionoregulation and raises energy costs, aggravating cortisol responses (Ip and Chew, 2010; Barton, 2002). Similar water-quality-linked growth advantages in earthen tilapia ponds versus tanks have been observed by Abdel-Tawwab *et al*. (2019) and Martins *et al*. (2019).

The stepwise decrease in cortisol/glucose from plastic → concrete → earthen mirrors a chronic stress gradient (Wendelaar Bonga, 1997; Barton, 2002; Schreck *et al*., 2016). Elevated cortisol mobilizes glucose but, chronically, suppresses immunity and growth matching our lower survival and poorer FCR in tanks (Ashley, 2007; Tort, 2011; Ellis *et al.*, 2002). Antioxidant enzyme activities (SOD, CAT) were lowest in tanks, signaling either enzyme inhibition or overwhelming ROS production (Lushchak, 2011; Livingstone, 2001). Comparable patterns high cortisol/low antioxidant capacity under crowding/poor water quality have been reported in tilapia and carp (Abdel-Tawwab *et al*., 2019; Dawood *et al.*, 2020; Hoseinifar *et al*., 2018).

HSP70 up-regulation in tanks indicates proteostasis stress (Feder and Hofmann, 1999; Iwama *et al*., 2004), frequently observed under heat, hypoxia, and metabolite stress (Liu *et al*., 2020; Sun *et al*., 2021). Concurrent elevation of IL-1β and TNF-α suggests activation of innate inflammatory cascades during chronic environmental challenge (Zou and Secombes, 2016; Tort, 2011). In ponds, lower HSP70 and cytokine transcripts, with higher CAT, reflect a milieu closer to homeostasis, consistent with our enzymatic data and improved performance. Similar antagonistic patterns (↑HSP/↑pro-inflammatory vs. ↑antioxidant/immune homeostasis) are recurrent in multi-omics tilapia studies under stress gradients (De Souza *et al*., 2020; Xu *et al.*, 2022).

Proteomic categorization aligned with physiology and transcriptomics: tanks favored HSP pathways and showed depressed metabolic/antioxidant profiles, whereas ponds showed the opposite. KEGG enrichment for protein processing in the endoplasmic reticulum and oxidative stress modules in tanks, and for complement/immune regulation and energy metabolism modules in ponds, mirror fish stress biology (Kültz, 2005; Kanehisa and Goto, 2000). Similar proteome-level shifts have been noted in finfish exposed to crowding or low DO (Krasnov *et al*., 2011; Palstra *et al*., 2010).

The congruence among water quality, performance, cortisol/glucose, antioxidant enzymes, and HSP/cytokine transcripts plus proteomic categories builds a coherent picture: tanks impose a chronic stress load that elevates HPI activity and proteotoxic/oxidative stress, tipping immunity toward pro-inflammation; ponds buffer stress, enabling antioxidant and immune-homeostatic programs and better energy allocation to growth (Wendelaar Bonga, 1997; Ashley, 2007; Kültz, 2005). This integrative view supports using a small panel (e.g., cortisol, SOD, CAT, HSP70, IL-1β) as operational biomarkers to audit system welfare (Barton, 2002; De Souza *et al*., 2020; Dawood *et al*., 2020).

Our findings resonate with studies showing that husbandry improvements (aeration, water exchange, biofilters, substrates) and biological interventions (e.g., probiotics, functional feeds) can mitigate tank-associated stress reducing cortisol and inflammatory markers while improving growth (Verschuere *et al.*, 2000; Merrifield and Carnevali, 2014; Ringø *et al*., 2010; Newaj-Fyzul and Austin, 2015; Dawood *et al*., 2020; Hoseinifar *et al.*, 2018).

Conclusion

Culture system profoundly shapes tilapia stress biology. Earthen ponds provided the most favorable combination of water quality, performance, and molecular signatures of low stress; plastic tanks showed the opposite, with concrete ponds intermediate. The agreement among physiology, targeted gene expression, and proteomic categories underscores the value of multi-omics for practical welfare assessment and system optimization. Where tanks are necessary, engineering choices that emulate pond stability (aeration, exchange, biofiltration, substrates) and targeted biotic interventions can narrow the welfare/performance gap.

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