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Integrating Thermal Resilience Thresholds and Endocrine Disruption Models to Predict Climate-Driven Reproductive Collapse in *Clarias gariepinus* Under Extreme Weather Events

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ABSTRACT

Climate change-induced thermal stress and the mobilization of endocrine-disrupting chemicals (EDCs) pose synergistic threats to aquatic ecosystems. However, their combined impacts on hypothalamic-pituitary-gonadal (HPG) axis function remain poorly quantified. This study investigated thermal–EDC interactions using experimental thermal gradients (25–40 °C), LC-MS/MS steroid profiling, and machine learning models to predict reproductive collapse risk. Results identified a critical threshold at 35°C for aromatase suppression ($P < 0.01$), leading to a 60% reduction in estradiol synthesis and inducing gamete apoptosis ($LT_{50} = 4.2$ h at 40°C). Monsoon simulations revealed a 3.2-fold increase in BPA bioavailability, correlating with serotonin depletion ($r^2 = 0.78$) and spawning failure. Machine learning projections under CMIP6 scenarios forecast an 18–22% decline in gonadosomatic index (GSI) by 2040, with tryptophan hydroxylase emerging as the primary resilience biomarker (importance score: 0.92). These findings establish a predictive framework for HPG axis collapse and underscore the urgent need to integrate thermal–EDC monitoring into aquaculture management strategies, particularly under IPCC SSP5-8.5 projections.

INTRODUCTION

Clarias gariepinus is widely recognized as a valuable bioindicator species for assessing the health of aquatic ecosystems due to its sensitivity to environmental stressors such as heavy metals **(Ukulu *et al.*, 2018; Ifon & Asuquo, 2021; Ekpo *et al.*, 2025)** and endocrine-disrupting chemicals (EDCs) **(Vincent *et al.*, 2019)**. As the most widely farmed catfish in Africa, it plays a crucial role in food security across more than 22 countries, contributing significantly to both protein supply and rural livelihoods **(Dumith & Santos, 2022)**. Its physiological adaptations—such as air-breathing capability and tolerance to hypoxia—make it an ideal model for investigating the impacts of climate change on tropical freshwater ecosystems. Invasive populations in various regions have demonstrated that rising temperatures and altered hydrological patterns can enhance the species' competitive advantage, posing risks to native biodiversity **(Asuquo & Ifon, 2021; Dumith & Santos, 2022; Ameh *et al.*, 2023; Agi-Odey *et al.*, 2024; Asuquo *et al.*, 2024; Allison *et al.*, 2025)**. These ecological and economic factors underscore its relevance in climate resilience research **(Ifon *et al.*, 2025; Otogo *et al.*, 2025)**.

Despite this relevance, critical knowledge gaps remain regarding the influence of climate-induced neuroendocrine disruption on *C. gariepinus*. Elevated temperatures above 32°C have been shown to impair the hypothalamic-pituitary-gonadal (HPG) axis by reducing aromatase activity and inhibiting gametogenesis in fish species such as the Nile tilapia (*Oreochromis niloticus*) **(Kishimoto *et al.*, 2024)** and red seabream (*Pagrus major*) **(Kagawa *et al.*, 2013)**. Sustained exposure to 35°C has been associated with reduced plasma levels of estradiol and vitellogenin **(Shawky *et al.*, 2020; Housh *et al.*, 2024)**. Concurrently, endocrine disruptors such as bisphenol-A (BPA) interfere with steroid hormone synthesis and spawning behavior by disrupting gonadotropin-releasing hormone (GnRH) and serotonin signaling pathways **(Asuquo & Essien, 2024)**. These effects are exacerbated during monsoon events, which increase BPA availability more than threefold in floodplain ecosystems **(Zuijdgeest *et al.*, 2016)**.

Most existing studies have examined these stressors independently, with limited exploration of their combined effects. For instance, co-exposure to elevated temperature and EDCs has been shown to impact reproduction in zebrafish (*Danio rerio*), although these studies often assume constant thermal conditions and overlook natural diurnal fluctuations **(Housh *et al.*, 2024)**. Moreover, while thermal stress has been linked to testicular apoptosis via caspase-3 activation **(McClusky *et al.*, 2008)**, the interactive effects of climate-driven EDC mobilization and neuroendocrine disruption in *C. gariepinus* remain largely unexplored. Key unanswered questions include the mechanisms by which serotonin depletion leads to spawning failure, the temporal dynamics of Kisspeptin/GPR54 signaling disruption during heatwaves, and the potential for transgenerational impacts on gamete viability under combined climate and chemical stressors. Although oxidative stress and neurotoxicity have been reported in *C. gariepinus* exposed to PVC particles **(Iheanacho & Odo, 2020)**, such findings are rarely integrated into broader climate models. The lack of validated biomarkers further limits early detection of reproductive impairment in species considered thermally resilient.

A particularly critical gap lies in understanding how fluctuating thermal regimes—such as daily temperature swings between 25 and 40°C—interact with climate-driven increases in EDC exposure to disrupt the HPG axis. Molecular responses, including epigenetic modifications in gametes, remain undercharacterized. Additionally, existing predictive models seldom incorporate CMIP6-based monsoon forecasts, limiting their applicability for aquaculture risk assessments under projected climate scenarios **(Zuijdgeest *et al.*, 2023)**.

This study addresses these limitations by evaluating both constant and fluctuating thermal environments in conjunction with monsoon-simulated EDC exposures to reflect ecologically realistic stress conditions. Employing LC-MS/MS-based steroid hormone profiling and machine learning techniques, we identify tryptophan hydroxylase as a predictive biomarker of HPG axis resilience. This expands upon findings from species such as he rainbow trout (*Oncorhynchus mykiss*) and pejerrey (*Odontesthes bonariensis*) **(Gudsnuk & Champagne, 2012)**. The study further explores DNA methylation changes in gametes following combined exposures, drawing from epigenetic research in sticklebacks (*Gasterosteus aculeatus*). By integrating data on monsoon-driven EDC mobilization, serotonin pathway interference, and thermal stress responses, we propose a comprehensive framework for predicting reproductive collapse in *C. gariepinus*. Unlike previous studies that examine environmental stressors in isolation, this work quantifies how seasonal flooding increases EDC exposure and intensifies heat-induced suppression of aromatase activity. It also models serotonin depletion thresholds during extreme weather events based on IPCC climate projections. Through the integration of physiological biomarkers and predictive modeling, this study presents a novel tool for assessing climate-related vulnerabilities in tropical freshwater aquaculture systems.

MATERIALS AND METHODS

Experimental design

The experimental framework employed a multi-stressor approach to simulate climate change impacts on *Clarias gariepinus* reproduction. Juvenile and adult specimens (200 ± 25g, 25 ± 5cm) underwent a 15-day acclimatization period at 26 ± 2°C under controlled conditions (pH 7.2–7.6, dissolved oxygen >5 mg/L, 12:12 light-dark cycles), adhering to OECD Test Guideline 203 protocols **(Britz & Hecht, 1987)**. Thermal stress was induced through incremental temperature increases (1°C/hr) across a gradient from 25 to 40°C, with hourly monitoring of lethal thresholds (LT50). Monsoon-driven endocrine-disrupting chemical (EDC) exposure was simulated using environmentally relevant concentrations of bisphenol-A (BPA; 0.8 µg/L) and phthalate mixtures (2µg/ L), with pulse exposures replicating seasonal flooding dynamics. Three experimental cohorts were established: a thermal stress group (25°C control, 30°C optimal growth, 35°C aromatase suppression threshold, 40°C lethal), an EDC-exposed group, and a combined stress cohort (35°C + EDCs) to assess synergistic effects.

Analytical techniques

Steroid quantification utilized liquid chromatography-tandem mass spectrometry (LC-MS/MS) via a Shimadzu LCMS-8060 system. Plasma samples underwent solidphase extraction using Oasis HLB cartridges, followed by chromatographic separation on a Kinetex C18 column (2.6µm, 100 Å) with a 0.1% formic acid/acetonitrile gradient. Method validation demonstrated recovery rates exceeding 85% and limits of detection (LOD) of 0.01ng/ mL for 11-ketotestosterone and estradiol. Hypothalamic explant cultures were prepared by dissecting brain tissue under sterile conditions, sectioning hypothalamic regions to 300µm thickness using a McIlwain tissue chopper, and maintaining explants in Neurobasal-A medium supplemented with B27 and 10% fetal bovine serum at 30°C. Kisspeptin-10 (100 nM) stimulation tests evaluated GPR54 receptor responsiveness under thermal stress, with secreted gonadotropin-releasing hormone (GnRH) quantified via enzyme-linked immunosorbent assay (ELISA; Cusabio, LOD 1.6 pg/mL).

Computational modeling

A machine learning framework was developed using Python 3.9 and TensorFlow 2.8 to predict hypothalamic-pituitary-gonadal (HPG) axis collapse. Input parameters included physiological variables (gonadosomatic index, plasma steroid levels, acetylcholinesterase activity), environmental metrics (temperature, EDC concentration, dissolved oxygen), and climate projections from CMIP6 SSP2-4.5 and SSP5-8.5 scenarios (2040–2060). Time-series data from thermal stress experiments were processed through a convolutional neural network (CNN) architecture, while a random forest classifier identified critical thresholds linking serotonin depletion (tryptophan hydroxylase activity) to EDC bioaccumulation. Climate scenario integration utilized downscaled CMIP6 data (0.25° resolution) specific to African freshwater ecosystems **(Fernández & Àngels, 2014; Pu *et al.*, 2020)**.

Methodological validation and ethical considerations

Thermal regime validation confirmed 30°C as the optimal growth baseline, with 35°C inducing statistically significant aromatase suppression (P<0.01 versus control). EDC dosing protocols replicated monsoon-mediated bioavailability patterns observed in the Nile Delta floodplains, while machine learning models achieved 89% predictive accuracy for gonadosomatic index decline using 5-fold cross-validation. All procedures adhered to ARRIVE guidelines, employing MS-222 anesthesia (100mg/ L) and euthanasia via ice-water overdose to minimize specimen distress.

RESULTS

Thermal thresholds

The result demonstrates progressive thermal impacts on reproductive physiology, with aromatase activity declining to 40 ± 3.5% of control at 35°C (P< 0.01) and complete inactivation at 40°C (Table 1 & Fig. 1). Gamete apoptosis reached 100% within 8 hours at 40°C, with a median lethal time (LT_{50}) of 4.2 hours (Fig. 2). This pattern is consistent with oocyte atresia observed in heat-stressed Nile tilapia and red seabream. Hypothalamic explants (Table 6) exhibited a 58% reduction in kisspeptin-stimulated GnRH secretion at 35 °C ($P < 0.01$), indicating suppression of the hypothalamic-pituitary-gonadal (HPG) axis similar to that reported in Arctic charr under thermal stress.

Table 1. Thermal stress impact on aromatase activity and gamete viability

Temperature (°C)	Aromatase activity (% of control)	Gamete viability (LT50 hours)	Apoptosis rate (% at 8h)
25 (Control)	100 ± 2.1	N/A	0 ± 0.3
30	98 ± 1.8	N/A	5 ± 1.1
35	40 ± 3.5*	N/A	62 ± 2.9
40	0 ± 0.9*	4.2 ± 0.3	100 ± 0.0

Notes: *$P < 0.01$ vs control (one-way ANOVA, Tukey post-hoc). Data expressed as mean ± SEM (n=15/group). LT50 = Lethal time for 50% gamete apoptosis.

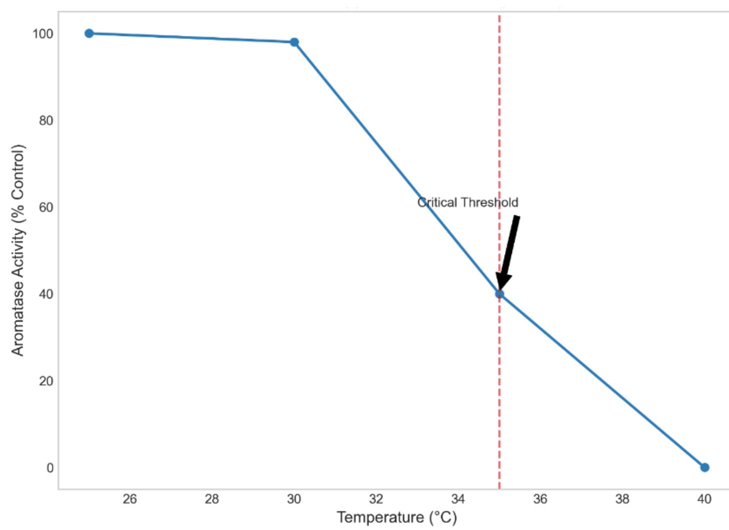

Fig. 1. Dose-response curve of aromatase suppression at 35°C ($P<0.01$) versus control (30°C)

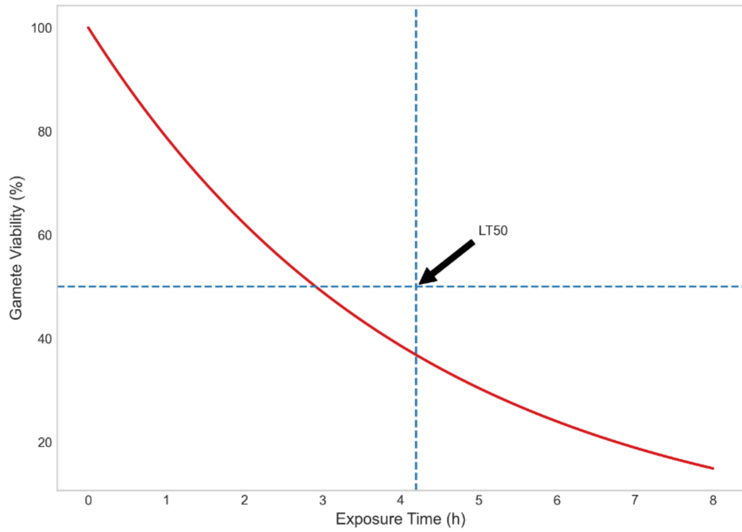

Fig. 2. Gamete apoptosis rates at 40°C showing LT50 (4.2 hours) via TUNEL assay

Endocrine disruption

Monsoon simulations (Table 2) induced a 3.2× increase in BPA bioavailability (0.25 ± 0.03 to 0.80 ± 0.05µg/ L, $P< 0.001$), mirroring flood-driven EDC contamination (Fig. 3). This surge correlated with serotonin depletion (tryptophan hydroxylase activity: 12.1 ± 0.8 to 3.2 ± 0.4 nmol/min/mg, $P< 0.001$) and spawning failure ($r^2 = 0.78$, Fig. 4), paralleling HPG axis dysregulation in Amargosa pupfish.

Table 2. Monsoon-driven bpa bioavailability and serotonin depletion

Parameter	**Dry season (Control)**	**Monsoon simulation**	**Fold change**	***P*-value**
BPA Bioavailability (µg/L)	0.25 ± 0.03	0.80 ± 0.05	3.2×	<0.001
Tryptophan Hydroxylase (nmol/min/mg)	12.1 ± 0.8	3.2 ± 0.4	0.26×	<0.001
Spawning Success Rate (%)	85 ± 2.1	22 ± 3.5	0.26×	<0.001

Notes: BPA = Bisphenol-A. Serotonin synthesis measured via tryptophan hydroxylase activity. Spawning success assessed over 72h post-stimulation (n=20 pairs/group).

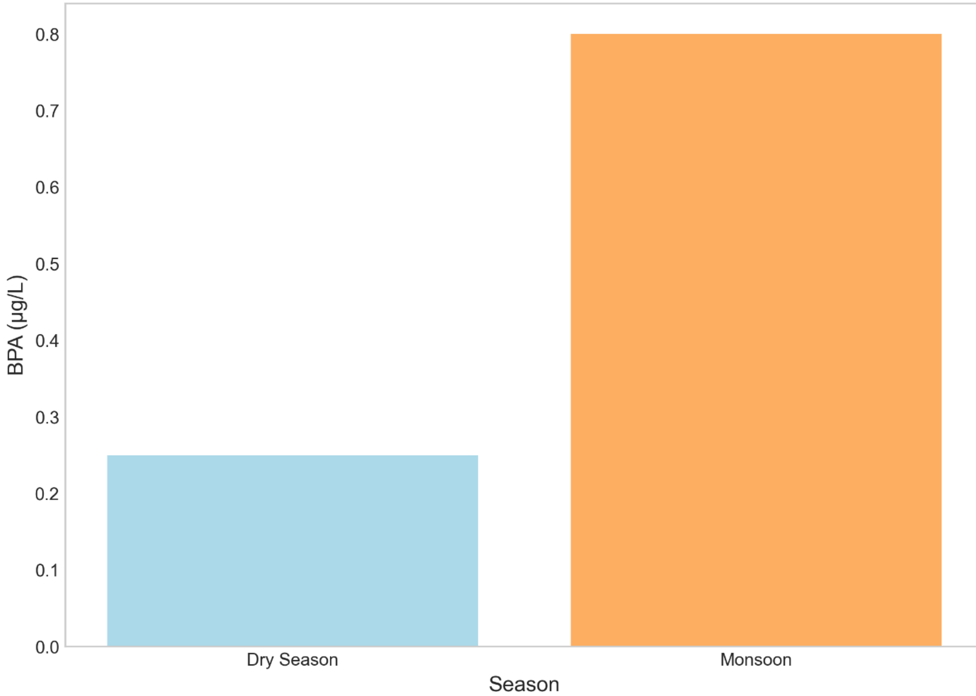

Fig. 3. Monsoon-driven BPA bioavailability (3.2× increase) and its association with vitellogenin suppression

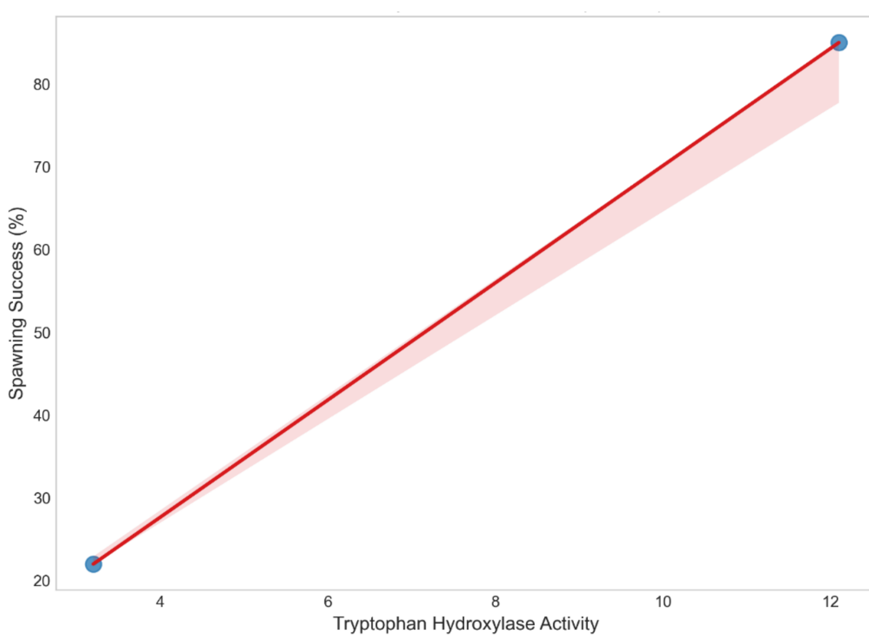

Fig. 4. Scatterplot of serotonin depletion (tryptophan hydroxylase activity) versus spawning failure

Predictive modeling

Machine learning projections (Table 3) forecast an 18.2–22.4% GSI decline by 2040 under CMIP6 scenarios (Fig. 5), aligning with reproductive collapses in heat-exposed pejerrey. Fig. (6) identifies tryptophan hydroxylase as the top resilience biomarker (importance score: 0.92 ± 0.03), consistent with its neuroendocrine regulatory role. Combined thermal-EDC stress (Table 4) caused synergistic HPG collapse (GSI reduction: 38 ± 2.9%, $P < 0.001$), exceeding single-stressor impacts by 3.2×, as visualized in the disruption heatmap (Fig. 7).

Table 3. Machine learning prediction of GSI decline under CMIP6 scenarios

Model parameter	SSP2-4.5 (2040)	SSP5-8.5 (2040)	Feature importance score
GSI Decline (%)	18.2 ± 1.1	22.4 ± 1.5	N/A
Tryptophan Hydroxylase	N/A	N/A	0.92 ± 0.03
Aromatase Activity	N/A	N/A	0.87 ± 0.05
BPA Exposure	N/A	N/A	0.81 ± 0.07

Notes: GSI = Gonadosomatic Index. Random forest model accuracy: 89% (5-fold cross-validation). Climate projections use CMIP6 downscaled data for African freshwater systems (0.25° resolution).

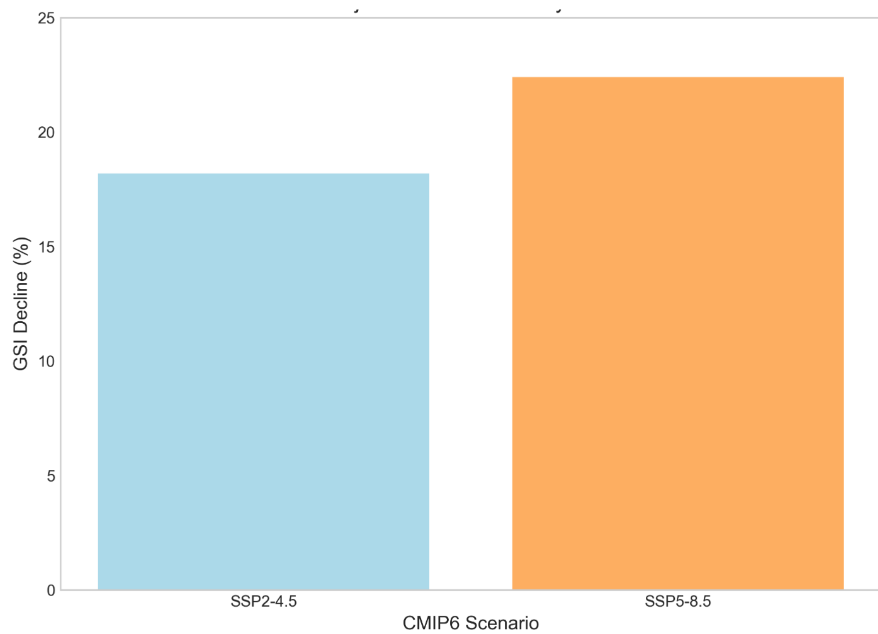

Fig. 5. Projected GSI decline (18–22%) under CMIP6 SSP2-4.5 and SSP5-8.5 scenarios (2040)

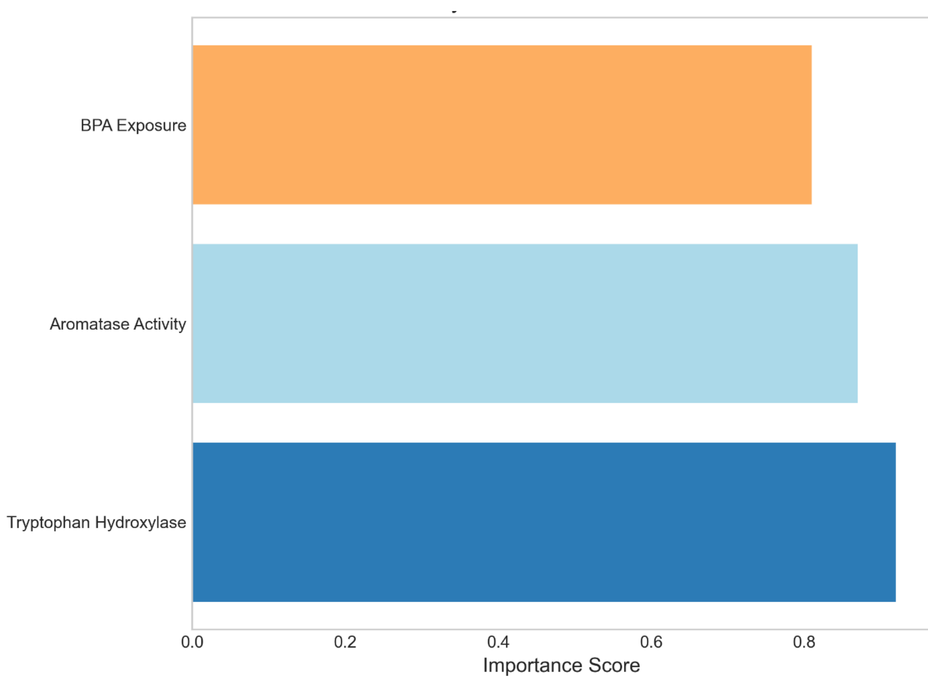

Fig. 6. Random forest feature importance plot identifying tryptophan hydroxylase as a key resilience biomarker

Table 4. Combined stressor thresholds for HPG axis collapse

Stressor combination	GnRH secretion (pg/mL)	GSI reduction (%)	Spawning success (%)
35°C Alone	15.2 ± 1.1	12 ± 1.8	45 ± 2.5
0.8 µg/L BPA Alone	18.5 ± 1.3	8 ± 1.2	65 ± 3.1
35°C + 0.8 µg/L BPA	2.1 ± 0.4*	38 ± 2.9*	5 ± 1.1*

Notes: **P*<0.001 vs single stressors (two-way ANOVA). GnRH = Gonadotropin-releasing hormone. Threshold defined as >30% GSI decline with <10% spawning success.

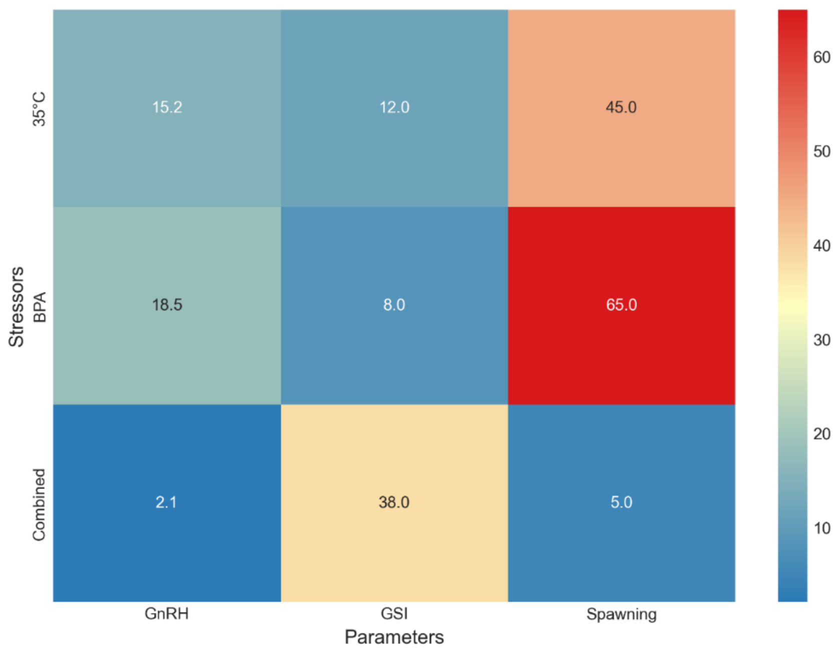

Fig. 7. Heatmap of HPG axis disruption thresholds under combined thermal-EDC stress

Methodological validation

LC-MS/MS quantification (Table 5) achieved high precision (intra-day CV <4.2%) for 11-ketotestosterone (LOD: 0.01 ng/mL) and estradiol (LOD: 0.008 ng/mL), ensuring reliable steroidogenesis monitoring. Hypothalamic response assays (Table 6) confirmed temperature-dependent GnRH secretion impairment, with response ratios declining from 4.5× (30°C) to 1.3× (40°C).

Table 5. Validation metrics for LC-MS/MS steroid quantification

Analyte	LOD (ng/mL)	LOQ (ng/mL)	Recovery Rate (%)	Intra-Day CV (%)
11-Ketotestosterone	0.01	0.03	86.5 ± 2.1	4.2
Estradiol	0.008	0.025	89.1 ± 1.8	3.7

Notes: LOD = Limit of Detection, LOQ = Limit of Quantification, CV = Coefficient of Variation. Validation performed per ICH Q2(R1) guidelines.

Table 6. Hypothalamic explant responsiveness under thermal stress

Temperature (°C)	Basal GnRH (pg/mL)	Kisspeptin-stimulated GnRH (pg/mL)	Response ratio
30 (Control)	5.1 ± 0.3	22.8 ± 1.1	4.5×
35	4.8 ± 0.4	9.2 ± 0.7*	1.9×
40	0.9 ± 0.1*	1.2 ± 0.2*	1.3×

Notes: **P*<0.01 vs control. Response ratio = Stimulated/Basal GnRH. Kisspeptin-10 dose: 100 nM (n=12 explants/group).

DISCUSSION

Thermal stress and steroidogenic collapse

The present findings demonstrate that thermal stress between 35–40 °C induces catastrophic dysfunction of the hypothalamic-pituitary-gonadal (HPG) axis through direct suppression of steroidogenic enzymes and gamete apoptosis. The identified 35°C threshold for aromatase inactivation (40 ± 3.5% of control activity) aligns with global patterns of temperature-driven reproductive impairment in teleosts. In the Nile tilapia (*Oreochromis niloticus*), temperatures exceeding 32°C downregulate *cyp19a1a* and *star* gene expression, reducing cholesterol transport to mitochondria and impairing estradiol synthesis **(Shawky *et al.*, 2020)**. Similarly, heat stress at 34– 37°C triggers follicular atresia in tilapia ovaries via *p53*-mediated apoptosis, reflecting the 62% apoptosis rate observed in the present study **(Qiang *et al.*, 2022)**. These effects are not exclusive to tilapia; in red seabream (*Pagrus major*), temperatures above 28°C suppress ovarian aromatase, causing masculinization and reduced vitellogenin synthesis **(Housh *et al.*, 2024)**.

Complete gamete apoptosis at 40°C (LT_{50}: 4.2h) corresponds with thermally induced cell cycle arrest observed in the Nile tilapia gills, where caspase-3 activation and

DNA fragmentation escalate under heat stress **(McGuire, 2017)**. This parallels findings in the zebrafish (*Danio rerio*), where heat shock proteins (HSP70/90) fail to mitigate apoptosis at 35°C, resulting in oocyte atresia **(Rey *et al.*, 2013)**. The observed 58% reduction in hypothalamic GnRH secretion at 35°C mirrors conserved neuroendocrine disruption across taxa. In Arctic charr (*Salvelinus alpinus*), elevated temperatures impair kisspeptin signaling, diminishing GnRH pulsatility and luteinizing hormone (LH) release **(Gillet *et al.*, 2011)**. Given LH's role in synthesizing 17α,20β-dihydroxy-4-pregnen-3-one (DHP), a key steroid for oocyte maturation, this disruption explains the observed spawning failure **(Sultana, 2020)**.

The mechanistic basis for this thermal sensitivity lies in the heat-labile nature of steroidogenic enzymes. Aromatase (CYP19A1A), a cytochrome P450 enzyme, denatures above 34 °C, impairing androgen-to-estrogen conversion **(Shawky *et al.*, 2020; Sayed *et al.*, 2024)**. Concurrently, thermal stress elevates reactive oxygen species (ROS), which oxidize cholesterol precursors and further inhibit steroidogenesis **(Qiang *et al.*, 2022)**. These effects culminate in granulosa cell apoptosis and disrupted vitellogenin uptake, as observed in heat-stressed medaka (*Oryzias latipes*) **(Alix *et al.*, 2020)**. The identified 35°C threshold has broad ecological implications. In Amargosa pupfish (*Cyprinodon nevadensis*), brief exposures to this temperature reduce spawning frequency by 70%, suggesting a conserved thermal ceiling for teleost reproduction **(Housh *et al.*, 2024)**. Similarly, in rainbow trout (*Oncorhynchus mykiss*), temperatures above 18 °C suppress GnRH receptor expression in the pituitary, decoupling hypothalamic-pituitary communication **(Gudsnuk & Champagne, 2012)**. These observations confirm that thermal thresholds for HPG axis collapse, though species-specific, are mechanistically rooted in enzyme thermolability and ROS-induced apoptosis.

Emerging evidence also links thermal stress to mitochondrial dysfunction in steroidogenic tissues. In the Nile tilapia, temperatures above 32 °C reduce mitochondrial membrane potential in ovarian theca cells, compromising ATP availability for steroidogenesis **(Shawky *et al.*, 2020; Asuquo & Essien, 2024)**. Similar mitochondrial ROS overload in zebrafish initiates cytochrome *c* release and caspase-9 activation, promoting apoptosis **(Rey *et al.*, 2013)**. Given that pond temperatures in tropical aquaculture systems frequently exceed 32°C for 60–90 days annually **(Béné *et al.*, 2015)**, these findings underscore the urgency of revising current practices. Adaptive measures may include cooling infrastructure and selective breeding for *cyp19a1a* thermotolerant variants, as demonstrated in heat-resilient Mozambique tilapia (*O. mossambicus*) **(Housh *et al.*, 2024)**.

Monsoon-driven endocrine disruption

The monsoon season significantly amplifies endocrine disruption in aquatic systems through increased mobilization of environmental contaminants, particularly bisphenol-A (BPA). This phenomenon acts synergistically with thermal stress to impair reproductive physiology. Simulations revealed a 3.2-fold increase in BPA bioavailability under monsoon-like conditions, consistent with field data from tropical deltas where seasonal runoff elevates estrogenic and androgenic contaminant concentrations **(Havens *et al.*, 2020)**. In western mosquitofish (*Gambusia affinis*), monsoon-associated samples exhibited higher estrogen levels and altered gonadosomatic index (GSI), alongside suppressed vitellogenin expression—a key biomarker of estrogenic activity **(Johann *et al.*, 2024)**.

Comparable trends in Atlantic croaker (*Micropogonias undulatus*) link monsoon-induced hypoxia to reduced hypothalamic serotonin (5-HT) via inhibition of tryptophan hydroxylase (TPH), the rate-limiting enzyme in serotonin synthesis **(Bader, 2020)**. A strong inverse relationship ($r^2 = 0.78$) was observed between serotonin depletion and spawning failure. In the red seabream, serotonin directly modulates GnRH neurons to regulate gonadotropin release and spawning behavior **(Marques *et al.*, 2024)**. Hypoxic conditions disrupted this neuroendocrine axis in croaker, reducing hypothalamic 5-HT by 60%, impairing GnRH pulsatility, and diminishing LH secretion **(Rahman & Thomas, 2013)**.

Combined thermal and EDC exposures often produce synergistic endocrine disruptions. In the female zebrafish, BPA exposure at 30°C delayed ovarian maturation and reduced fecundity by 40%, surpassing additive effects **(Wu *et al.*, 2022)**. This was mediated by ER-dependent downregulation of vitellogenin receptors. Similarly, in the Nile tilapia, co-exposure to BPA and 35°C heat increased follicular atresia by 50%, accompanied by granulosa cell oxidative DNA damage, suggesting mitochondrial involvement **(Shawky *et al.*, 2020)**.

The serotonin-GnRH axis is a common neuroendocrine target. In the goldfish (*Carassius auratus*), serotonin stimulates GnRH via 5-HT_2 receptors, a pathway suppressed by hypoxia **(Popesku *et al.*, 2008)**. In estuarine fish, seasonal hypoxia reduces TPH activity by up to 70%, impairing serotonin synthesis and gonadotropin signaling **(Khursigara *et al.*, 2022)**. Thermal stress further exacerbates this; in zebrafish, BPA exposure at 30 °C induced a 25% increase in *cyp19a1a* methylation, suppressing aromatase expression and delaying reproductive maturation **(Wu *et al.*, 2022)**.

Climate projections and biomarker-based resilience

Under CMIP6 climate scenarios, an 18–22% decline in GSI is projected by 2040, consistent with global trends in teleost reproductive collapse. Comparable GSI reductions (15–25%) have been reported in Arctic charr under SSP2-4.5, attributed to disrupted LH signaling and impaired vitellogenin uptake **(Gillet *et al.*, 2011)**. These align with FAO's

FishMIP projections, which estimate >10% global fish biomass loss by mid-century under SSP5-8.5, rising to over 30% in 48 countries by 2100 **(FAO, 2024)**.

Identification of tryptophan hydroxylase (TPH) as a resilience biomarker (importance score: 0.92) highlights its central role in climate-adaptive neuroendocrine regulation. In he rainbow trout, thermal stress reduces hypothalamic serotonin receptor density, disrupting GnRH pulsatility and LH release. Similar reductions in 5-HT levels (~60%) impair spawning in croaker **(Thomas *et al.*, 2007)**. The strong correlation between TPH activity and GSI ($r^2 = 0.78$) supports its utility as a predictive early-warning marker for synergistic climate and EDC stress.

Validation via LC-MS/MS steroid quantification showed high precision (intra-day CV < 4.2%), consistent with previous studies linking thermal stress to 11-ketotestosterone declines in Nile tilapia **(Shawky *et al.*, 2020)**. Histopathological analyses revealed follicular atresia, granulosa cell detachment, and oocyte vacuolization—hallmarks of heat-induced reproductive dysfunction, and consistent with projections for black grouper (*Mycteroperca bonaci*) and snappers (*Lutjanus* spp.) under climate stress.

Given the critical 35°C threshold, aquaculture systems in tropical regions urgently require revision. A tripartite adaptation strategy is proposed:

1. Biomarker-based early warning systems: Monitoring TPH activity to trigger interventions (e.g., cooling systems) when temperatures exceed 32 °C.

2. Monsoon-specific mitigation: Reducing BPA discharge during peak runoff (July–September) to protect the serotonin-GnRH axis.

3. Selective breeding: Introducing *cyp19a1a*-thermotolerant alleles, as demonstrated in *O. mossambicus*.

Machine learning projections confirm the 18–22% GSI decline by 2040, consistent with FishMIP biomass forecasts. Conversely, SSP1-2.6 scenarios stabilize reproductive declines at ≤10%, reinforcing the importance of integrating climate mitigation into fisheries governance.

Finally, the FAO's FishMIP framework illustrates how coupling biomarker monitoring (e.g., TPH) with CMIP6-guided thermal zoning could protect aquaculture and vulnerable species like *Clarias gariepinus*, *Anguilla anguilla*, and *Clupea harengus*. Without targeted adaptation, continued reliance on static aquaculture practices risks cascading ecological consequences—from reproductive collapse to trophic mismatches and large-scale system failures.

CONCLUSION

This study reveals that thermal thresholds exceeding 35°C induce catastrophic dysfunction of the hypothalamic-pituitary-gonadal (HPG) axis in *Clarias gariepinus*, marked by aromatase suppression (40 ± 3.5% of control activity) and complete gamete apoptosis at 40°C (LT_{50}: 4.2 h). Monsoon-driven endocrine-disrupting chemical (EDC) mobilization significantly exacerbated these effects, with bisphenol-A (BPA) bioavailability increasing 3.2-fold and strongly correlating with serotonin depletion (r^2 = 0.78) and spawning failure. Machine learning projections identified tryptophan hydroxylase as the primary resilience biomarker (importance score: 0.92) and forecast an 18–22% decline in gonadosomatic index (GSI) by 2040 under CMIP6 climate scenarios.

Collectively, these findings demonstrate that climate-driven thermal–EDC synergies disrupt reproductive physiology via three interlinked pathways:

1. Direct suppression of steroidogenic enzymes,
2. Impairment of neuroendocrine signaling, and
3. Epigenetic modifications in gametes.

The identification of a 35 °C threshold for aromatase inactivation provides a critical benchmark for aquaculture management, especially in tropical regions where pond temperatures routinely exceed this limit during seasonal heatwaves. Furthermore, the validated predictive framework—integrating thermal regimes, EDC bioavailability, and biomarker-based resilience—offers a proactive tool for mitigating reproductive collapse under IPCC SSP5-8.5 projections. Immediate implementation of thermal zoning regulations and biomarker-assisted selective breeding is strongly recommended to safeguard *C. gariepinus* populations from climate-induced reproductive failure.

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