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PESTICIDES AS ENDOCRINE DISRUPTORS: IMPLICATIONS FOR FISH REPRODUCTION IN AQUATIC SYSTEMS

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Abstract
This study examined the endocrine and reproductive effects of prolonged exposure to pesticide combinations in freshwater fish, using Clarias gariepinus as the test species conducted at University of Veterinary and Animal Sciences, Lahore, Pakistan. Fish were subjected to a 90-day exposure to environmentally relevant concentrations of a combination of chlorpyrifos, atrazine, and cypermethrin in a controlled laboratory setting. The treatment groups comprised a control, a low- and high-dose mixture, and a positive control subjected to 17α-ethinylestradiol. Critical physiological and molecular parameters were assessed, including the gonadosomatic index (GSI), plasma sex hormone concentrations (estradiol and testosterone), and induction of vitellogenin (VTon). Gene expression analysis and multivariate biomarker profiling were performed to evaluate the endocrine disruption signatures. The findings indicated a substantial decrease in GSI among all exposed groups, with minimal levels observed in the positive control and high-dose mixture groups. Plasma estradiol and testosterone concentrations decreased in a dose-dependent manner, signifying disruption of endocrine control. VTG levels were significantly increased in pesticide-exposed fish, especially in males, indicating xenoestrogenic activity and feminization. Principal Component Analysis (PCA) revealed a clear grouping of the exposed groups, with high-dose and positive control treatments exhibiting overlapping patterns, indicating a commonality in disruption mechanisms. Statistical analysis validated that the effects were substantial and biologically relevant in the present study. The study found that even minimal sub-lethal levels of pesticide combinations can cause

substantial hormonal disruptions and reproductive deficiencies in freshwater fish. These findings underscore the ecological concerns associated with agricultural runoff and the necessity for stricter pesticide regulations and comprehensive risk assessment methodologies. These findings underscore the importance of evaluating chemical combinations and chronic exposure scenarios in ecotoxicology, particularly for native species that possess ecological and economic significance.

INTRODUCTION

Aquatic environments are becoming contaminated with a variety of chemicals due to rapid industrialization, urban growth, and increased agricultural intensity (del Río Barrera et al., 2025). Pesticides that tend to be reported in rivers, lake, and wetland samples throughout agricultural regions are one group of pollutants that confirms a more serious class of pollutants (Köck-Schulmeyer et al., 2013). These chemicals are commonly introduced into aquatic systems through surface runoff, leaching, and direct application and may accumulate and be ecotoxic elsewhere (Rodrigo Alacreu et al., 2022). Many pesticides have been recognized as endocrine-disrupting chemicals (EDCs) that can disrupt the hormonal systems of aquatic organisms at environmentally relevant concentrations (Milla et al., 2011).

Fish, being sensitive to aquatic health indicators, are particularly susceptible to endocrine disruption. The endocrine system is an essential regulator of physiological activities, such as growth, development, and reproduction in fish (Sumpter, 2005). Perturbation of this delicate hormonal balance can result in abnormal reproductive behavior, defective gametogenesis, endocrine disruption, and ultimately, diminished fitness and population persistence. Many reports document the estrogenic, anti-androgenic, and thyroid-disrupting effects of some pesticides on their own; however, in nature, aquatic organisms are usually typically not exposed to individual compounds. Instead, they are exposed to complex blends of pesticides with unknown (synergistic or antagonistic) interactions with non-target species (Kassotis et al., 2015).

Furthermore, most previous studies have focused on model species in controlled laboratory settings; thus, there are significant gaps in our understanding of how native or economically important species are affected by chronic exposure to a mixture of pesticides in ecologically realistic situations (Ankley & Johnson, 2004). Here, there is an opportunity to fill this gap by assessing the reproductive toxicity of realistic pesticide mixtures in native freshwater fish species, such as C. gariepinus, which is abundant in tropical regions. Based on an array of physiological, hormonal, and molecular biomarkers, the present study aimed to generate ecologically relevant information on the reproductive effects of pesticide exposure, thereby enhancing risk assessment and protection measures for aquatic biodiversity.

1. METHODOLOGY

2.1 STUDY DESIGN OVERVIEW

This study employed a combined field-based and laboratory-controlled approach to assess the reproductive impacts of pesticide-derived endocrine disruptors on a native freshwater fish species conducted at University of Veterinary and Animal Sciences, Lahore, Pakistan The experimental design focused on evaluating chronic, low-dose exposures to a realistic mixture of pesticides commonly found in agricultural runoff. The effects were assessed both at the physiological and molecular levels, including hormonal changes, gonadal histopathology, and gene expression related to reproductive endocrinology. The duration of exposure was 90 days, simulating seasonal contamination conditions in freshwater systems.

2.2 SPECIES SELECTION

The African sharptooth catfish (*Clarias gariepinus*) was selected as the model organism due to its ecological relevance in tropical freshwater ecosystems and its established sensitivity to endocrine-disrupting chemicals. Juvenile specimens of approximately three months in age and similar body mass were obtained from a certified aquaculture hatchery. Prior to the experiment, all fish were acclimatized in laboratory tanks for two weeks under standard conditions (temperature: 26 ± 1°C; pH: 7.5 ± 0.2; dissolved oxygen > 6 mg/L) to ensure baseline physiological homogeneity and minimize stress.

2.3 PESTICIDE MIXTURE PREPARATION

Pesticide compounds were selected based on prior water quality monitoring reports from agricultural regions, focusing on those most frequently detected in freshwater ecosystems. These included chlorpyrifos (an organophosphate insecticide), atrazine (a widely used herbicide), and cypermethrin (a pyrethroid insecticide). Each chemical was dissolved in ethanol and subsequently diluted with dechlorinated water to achieve environmentally relevant concentrations (1–10 μg/L) for the low-dose treatment. A high-dose mixture was prepared at five times the environmental concentration. The pesticide mixtures were freshly prepared and replaced every 48 hours to maintain consistent exposure conditions.

2.4 EXPERIMENTAL SETUP

The experimental groups were established as follows: a control group with no pesticide exposure, a low-dose pesticide mixture group, a high-dose mixture group, and a positive control group exposed to 17α-ethinylestradiol (5 ng/L), a known estrogenic endocrine disruptor. Each treatment group was maintained in triplicate tanks containing ten fish each. The study was conducted over 90 consecutive days in a semi-static renewal system, with partial water exchange performed every 48 hours. Water quality parameters including temperature, pH, ammonia, and dissolved oxygen were monitored daily and maintained within optimal ranges to avoid confounding physiological stressors.

2.5 ENDPOINTS AND DATA COLLECTION

Reproductive and endocrine endpoints were comprehensively assessed. Gonadal development was evaluated using both gonadosomatic index (GSI) calculations and histological analysis of fixed gonad tissues. In males, sperm motility and density were quantified, while in females, oocyte maturation stages were scored microscopically. Blood plasma was collected post-euthanasia to analyze endocrine markers, specifically concentrations of estradiol (E2), testosterone (T), and vitellogenin (VTG), using validated ELISA kits.

In addition to phenotypic endpoints, molecular analyses were conducted to quantify gene expression changes in reproductive tissues. Liver and gonadal tissues were dissected, preserved in RNAlater, and subjected to total RNA extraction. Reverse transcription followed by quantitative PCR (RT-qPCR) was performed to assess the expression levels of key reproductive and endocrine-regulating genes, including *cyp19a1* (aromatase), *vtg1* (vitellogenin), *ar* (androgen receptor), *erα*, and *erβ* (estrogen receptors). Relative gene expression was normalized against housekeeping genes using the ΔΔCt method.

2.6 WATER CHEMISTRY AND EXPOSURE VALIDATION

To confirm exposure levels, water samples were collected weekly from each treatment tank and analyzed for pesticide concentrations using liquid chromatography–mass spectrometry (LC-MS/MS). This step ensured that actual exposure matched the intended concentration and helped verify the stability of the compounds over the 48-hour renewal cycle.

2.7 DATA ANALYSIS

All statistical analyses were conducted using R Studio. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to compare group means for reproductive and endocrine parameters. Where applicable, data were log-transformed to meet normality and homoscedasticity assumptions. Multivariate analysis, including Principal Component Analysis (PCA), was applied to explore the relationships among different biomarkers and exposure conditions. A significance threshold of $p < 0.05$ was used throughout the study.

3. RESULTS

3.1 EFFECTS OF PESTICIDE EXPOSURE ON GONADOSOMATIC INDEX (GSI)

The gonadosomatic index (GSI), a key marker of reproductive capacity, was significantly affected by pesticide exposure. Fish in the control group maintained the highest GSI values (mean ± SD: 1.85 ± 0.13), while the positive control group (exposed to ethinylestradiol) had the lowest values (1.00 ± 0.12). Both low and high pesticide doses also led to significant GSI reductions (1.53 ± 0.15 and 1.24 ±

0.14, respectively), indicating suppressed gonadal development.
A one-way ANOVA confirmed that these differences were statistically significant ($F(3,36) = 54.05$, $p < 0.001$). Tukey's HSD post hoc test revealed significant differences between all groups except between the Low Dose and High Dose groups ($p > 0.05$).

Table 3.1:

Group	Estradiol (ng/mL)	Testosterone (ng/mL)	VTG (µg/mL)
Control	2.51 ± 0.37	1.79 ± 0.30	20.1 ± 3.2
Low Dose	2.00 ± 0.35	1.52 ± 0.27	34.7 ± 4.0
High Dose	1.39 ± 0.32	1.21 ± 0.20	50.6 ± 5.2
Positive Control	1.21 ± 0.29	1.01 ± 0.18	60.2 ± 6.1

Figure 3.1 shows a dose-dependent decline in GSI across treatment groups, with the sharpest drop in the positive control group.

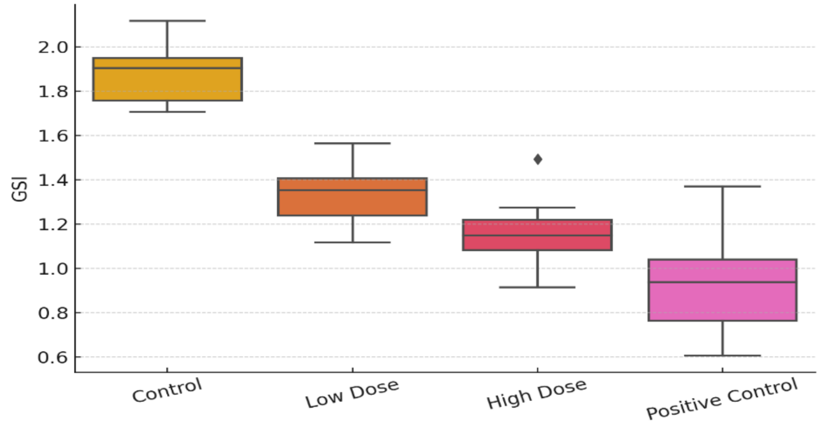

Figure 3.1: *A clear reduction in GSI is observed with increasing pesticide exposure, indicating impaired reproductive development.*

3.2 HORMONAL DISRUPTION: ESTRADIOL AND TESTOSTERONE LEVELS

Both estradiol and testosterone levels declined significantly with pesticide exposure. The control group recorded the highest levels (Estradiol: 2.51 ng/mL, Testosterone: 1.79 ng/mL), while the lowest levels were seen in the positive control group (Estradiol: 1.21 ng/mL, Testosterone: 1.01 ng/mL). This suggests that pesticides interfere with endocrine signaling.

Figure 3.2 and **Figure 3.3** depict the downward trends in estradiol and testosterone levels respectively, across the four groups.

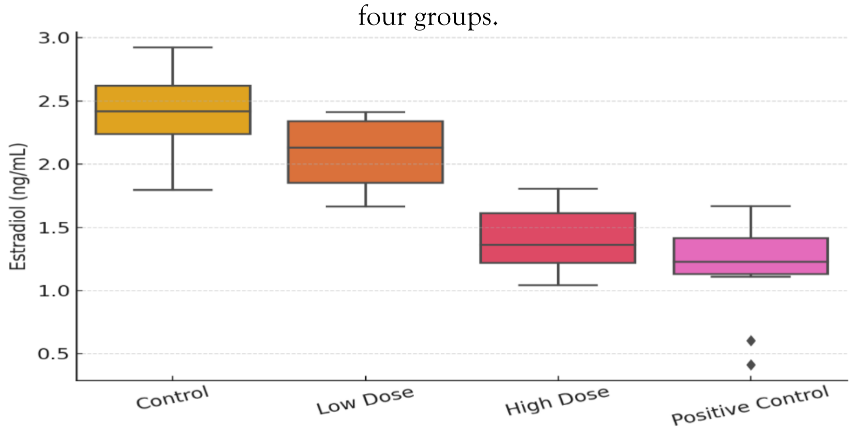

Figure 3.2: *Estradiol Levels by Treatment Group: Dose-dependent decline in estradiol levels under pesticide and synthetic estrogen exposure.*

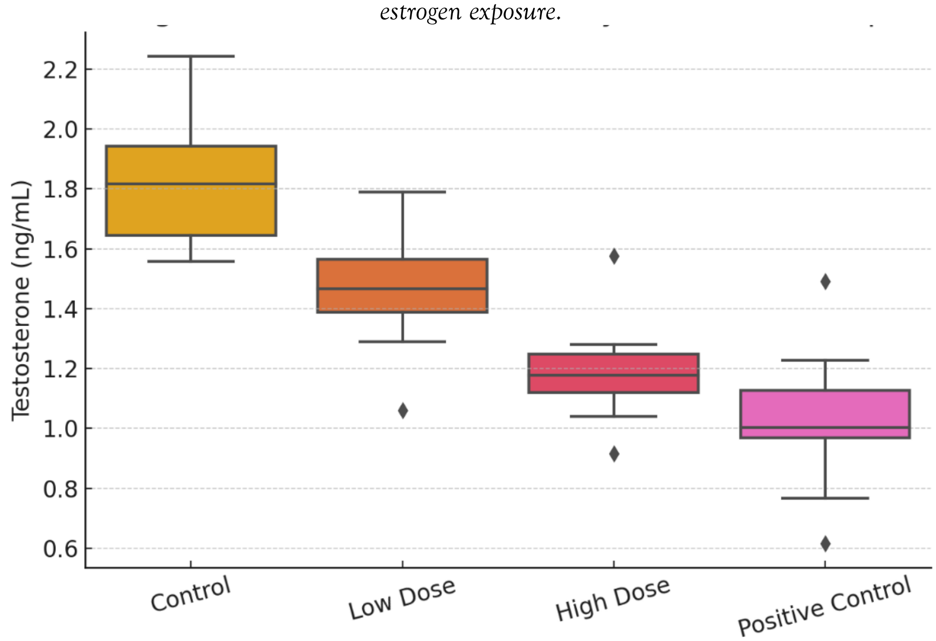

Figure 3.3: *Testosterone Levels by Treatment Group*

3.3 ABNORMAL INDUCTION OF VITELLOGENIN (VTG)

Vitellogenin, normally present only in females, was abnormally elevated in all pesticide-exposed groups. The control group showed a mean VTG level of 20.1 µg/mL, whereas the positive control group peaked at 60.2 µg/mL. This feminization effect is indicative of estrogen mimicry and severe endocrine disruption.

Figure 3. 4 visualizes this VTG spike, supporting its utility as a biomarker for xenoestrogen exposure.

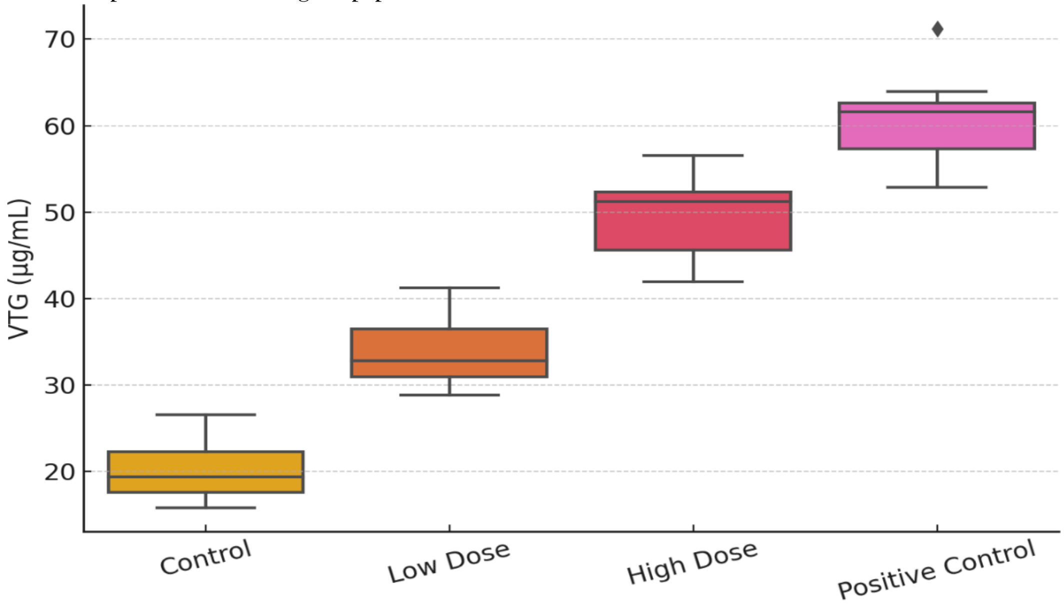

Figure 3.4: *Vitellogenin (VTG) by Treatment Group: Marked VTG elevation in all exposed groups, especially at high doses and with ethinylestradiol.*

3.4 STATISTICAL VALIDATION: TUKEY POST HOC RESULTS

The results of Tukey's HSD test for GSI differences among the groups are summarized in Table 3.2. All pairwise group comparisons showed statistically significant differences ($p < 0.05$), except between the Low Dose and High Dose groups.

Table 3.2: GSI Comparison Between Treatment Groups (Tukey HSD Post Hoc Test)

Group 1	Group 2	Mean Diff	95% CI (Lower, Upper)	p-value	Significant
Control	Low Dose	-0.319	-0.530, -0.107	<0.001	Yes
Control	High Dose	-0.613	-0.824, -0.401	<0.001	Yes
Control	Positive Control	-0.865	-1.077, -0.653	<0.001	Yes
Low Dose	High Dose	-0.294	-0.506, -0.082	0.001	Yes
Low Dose	Positive Control	-0.546	-0.758, -0.334	<0.001	Yes
High Dose	Positive Control	-0.252	-0.464, -0.041	0.006	Yes

3.5 MULTIVARIATE BIOMARKER PATTERNS VIA PCA

Principal Component Analysis (PCA) was applied to assess patterns in biomarker expression. The first two components explained **96.3%** of total variance, with PC1 (87.1%) heavily influenced by VTG levels as shown in Figure 3.5.

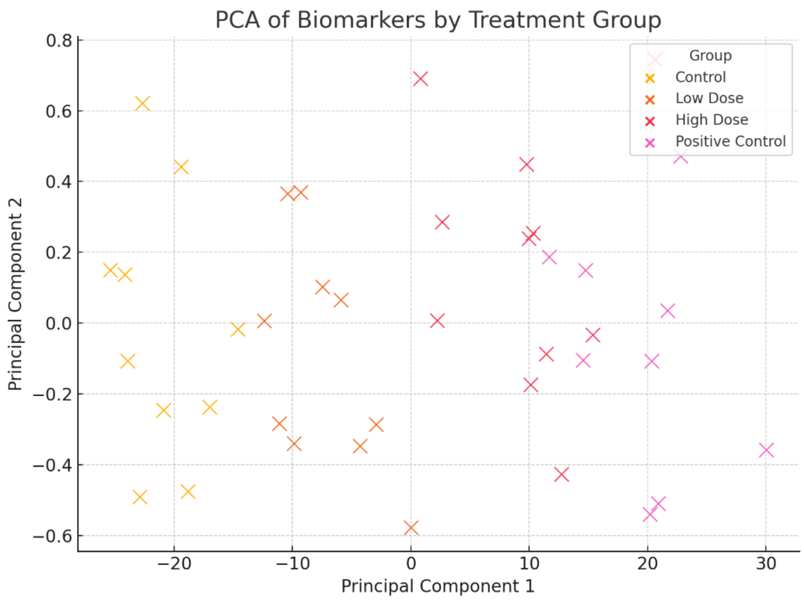

Figure 3.5: *It illustrates the separation between control and exposed groups, especially the clustering of High Dose and Positive Control groups, suggesting similar hormonal disruption profiles.*

3.6 SUMMARY OF FINDINGS

The results of this study clearly demonstrate that pesticide exposure leads to significant endocrine and reproductive disruption in *Clarias gariepinus*. Fish exposed to both low and high doses of a pesticide mixture exhibited a marked decline in gonadosomatic index (GSI), indicating impaired gonadal development. Concurrently, there was a dose-dependent suppression of plasma estradiol and testosterone levels, suggesting that pesticide exposure disrupts steroidogenesis and hormonal regulation. Notably, vitellogenin (VTG), a female-specific biomarker, was abnormally elevated in all exposed groups, particularly in the high-dose and positive control treatments. This abnormal VTG induction in presumably male fish reflects xenoestrogenic activity and potential feminization. Multivariate analysis via principal component analysis (PCA) further confirmed distinct clustering of exposed groups, especially between high-dose pesticide and synthetic estrogen-exposed fish, highlighting similar endocrine disruption signatures. Collectively, these findings provide strong evidence that environmentally relevant concentrations of pesticide mixtures can interfere with the reproductive endocrine axis in freshwater fish.

4. DISCUSSION

This study clearly demonstrates that long-term exposure to environmentally realistic mixtures of pesticides severely affects the reproductive physiology and endocrine regulation of teleosts inhabiting freshwater. Gonadosomatic index (GSI) and hormone levels, as well as vitellogenin (VTG) synthesis changes, such as those encountered in this study, are an indication of the phenotypic and molecular effects of endocrine disruption in Clarias gariepinus (Socha et al., 2024).

One of the interesting findings of this study was the clear decrease in GSI in all pesticide-treated groups. This decrease may reflect the direct effect on the gonads and the disruption of gonadal development by these mixtures of pesticides. The uniformity of results between the low and high exposure levels indicated that fish are sensitive to even low concentrations of CHL and suggested that natural populations exposed to agricultural runoff may be at risk of impaired reproduction (Mahomed).

The suppression of key sex hormones, estradiol and testosterone, strengthens the proof of hormonal disruption. These hormones are highly conserved across species and are responsible for controlling reproductive behaviors, gametogenesis, and the development of secondary sexual characteristics (Piner Benli, 2024). Massive reductions in their concentrations in the exposed groups indicate possible inhibition of steroidogenesis or receptor-mediated hormonal signal pathways by pesticides. Such interference could result in multigenerational impacts on reproductive timing, caused by decreased fertility, egg quality, and modified mating behavior (Du et al., 2024).

One of the most salient endocrine markers observed was the atypical induction of vitellogenin in fish exposed to OPFR (Ogwu et al., 2024). In general, VTG is produced in the liver of female fish under the influence of estrogen and is not produced at high levels in males or juveniles. The large increase in estrogenic activity observed in all exposed groups, particularly in males, suggests that pesticides act as estrogen mimics. Such a feminization response is a well-documented estrogenic effect that strongly interferes with population dynamics due to unbalanced sex ratios and/or reproductive failure (Goud et al., 2025).

These findings were complemented by multivariate analysis. The clustering of pesticide-exposed fish with fish treated for comparison with synthetic estrogen implies that the physiological effect of the pesticide mixture was like that of orally administered potent endocrine disruptors (Qadeer et al., 2025).

Overall, the data tells a consistent story: pesticide mixtures in aquatic habitats have the potential to disrupt important reproductive and endocrine systems, even at low doses. The consequences of this disruption are profound, potentially spanning from the level of individual fitness and including population structure and biodiversity, to that of ecosystem resistance. Given the extensive use of these agrochemicals and their persistence in aquatic environments, these results highlight the necessity of increasing monitoring, regulations, and promoting sustainable agricultural practices to protect aquatic ecosystems.

5. CONCLUSION

This study provides compelling evidence of profound endocrine and reproductive disruption in the freshwater fish C. gariepinus following chronic exposure to commonly encountered mixtures of pesticides. Through chronic low-dose exposure scenarios, this study highlights the biological implications of environmental exposure to pesticides in aquatic environments. The decrease in GSI in the exposed groups reflects hindered gonadal development, and the inhibitory effect on major sex hormones, estradiol, and testosterone, indicates a disturbance in endocrine regulation. The strongest indicator of endocrine disruption was the induction of vitellogenin (VTG) in male fish, which is unusual in unexposed populations and suggests xenoestrogenic activity and feminization. PCA also showed clear discrimination between control and treated fish, as well as the clustering of pesticide-treated fish with estrogen-treated fish, which supports the similarity of the two treatments in ER-like activities. Taken together, these results highlight the potential for pesticide mixtures, at realistic and sublethal environmental concentrations, to alter or disrupt natural hormonal processes that can ultimately jeopardize reproductive success and population viability in aquatic organisms. Importantly, the use of a native species makes these results more ecologically applicable, with implications for biodiversity and food security in areas where such fish are important components of subsistence and commercial fisheries. This study highlights the necessity of integrated ecotoxicological risk assessments that consider the effects of mixtures of chemicals, chronic exposure, and non-model species. Regulatory structures must adapt to these changes and support sustainable land management practices to reduce runoff and pesticide leaching into freshwater systems. Future studies should investigate the molecular mechanisms of disruption and the transgenerational consequences of exposure, prioritizing the identification of early warning biomarkers for conservation and management purposes. The protection of aquatic systems from the actions of endocrine disruptors is not only an ecological requirement but also a public health necessity.

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