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Nizam Khamis
 Department of Physiology,
 Sarawak Institute of Tropical
 Biology, Kuching, Sarawak,
 Malaysia

Luqman Ghazali
 Department of Physiology,
 Sarawak Institute of Tropical
 Biology, Kuching, Sarawak,
 Malaysia

Nizam Jamaludin
 Department of Physiology,
 Sarawak Institute of Tropical
 Biology, Kuching, Sarawak,
 Malaysia

Endocrine disrupting chemicals and their effects on reproductive physiology of aquatic vertebrates

Nizam Khamis, Luqman Ghazali and Nizam Jamaludin

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Abstract

Industrial and agricultural discharge into freshwater systems introduces endocrine disrupting chemicals (EDCs) that interfere with hormonal signaling in aquatic organisms. This research examined the effects of five common EDCs—bisphenol A (BPA), atrazine, nonylphenol, vinclozolin, and trenbolone—on reproductive physiology across three fish species (zebrafish, medaka, and fathead minnow) and one amphibian (African clawed frog). Laboratory exposures were conducted at the Sarawak Institute of Tropical Biology, Kuching, Malaysia, from May 2022 to October 2023. Animals were exposed to environmentally relevant concentrations (0.1-100 µg/L) for 21 days, after which plasma steroid hormones (estradiol, testosterone), hepatic vitellogenin (VTG), gonadosomatic index (GSI), and fecundity were measured. BPA at 10 µg/L elevated estradiol by 187.3% and VTG by 312.4% in zebrafish while reducing testosterone by 42.1% relative to controls. Vinclozolin suppressed fecundity by 61.3% across all species tested. Tissue-specific bioaccumulation analysis showed BPA concentrating most heavily in liver (34.8 ng/g in zebrafish), followed by gonadal tissue (21.4 ng/g). Atrazine produced the largest decrease in GSI (−34.1%), consistent with gonadal atrophy observed on histological sections. Interspecies sensitivity varied: fathead minnow showed the strongest BPA bioaccumulation, while zebrafish displayed the greatest VTG induction. These findings confirm that EDCs at environmentally realistic levels disrupt multiple axes of reproductive physiology in aquatic vertebrates, with compound-specific and species-specific response patterns that complicate single-species risk assessment.

Keywords: Endocrine disrupting chemicals, bisphenol a, reproductive physiology, aquatic vertebrates, vitellogenin, gonadosomatic index, atrazine, bioaccumulation, species sensitivity, ecotoxicology

Introduction

Thousands of synthetic chemicals released into rivers, lakes, and coastal waters each year can mimic or block the action of natural hormones in wildlife—a problem that persists despite three decades of scientific attention ^[1]. Endocrine disrupting chemicals encompass a structurally diverse group of compounds, including plasticizers (BPA), herbicides (atrazine), surfactants (nonylphenol), fungicides (vinclozolin), and veterinary growth promoters (trenbolone), all of which have been detected in freshwater systems at concentrations ranging from low ng/L to low µg/L ^[2].

Fish and amphibians are especially vulnerable because they breathe and osmoregulate across large gill and skin surfaces in constant contact with the water column, meaning EDC uptake is both rapid and continuous ^[3]. Reproductive endpoints—plasma sex steroids, vitellogenin induction, gonadosomatic index, and fecundity—are among the most sensitive indicators of endocrine disruption and have been adopted as standard endpoints in OECD Test Guidelines 229 and 230 ^[4]. However, most published data come from single-compound, single-species experiments conducted under laboratory conditions typical of temperate regions, leaving gaps in understanding of compound mixtures and tropical species responses.

Southeast Asian freshwater ecosystems face especially high EDC loading from palm oil processing, rubber manufacturing, and intensive aquaculture ^[5]. Malaysia's Sarawak state, with its dense river networks and biodiversity-rich wetlands, is a microcosm of these pressures. This research addressed two specific questions: first, how do five common EDCs compare in their effects on reproductive physiology across four aquatic vertebrate species? And second, does tissue-specific bioaccumulation differ between fish species in ways that could explain interspecies sensitivity differences?

Corresponding Author:
Nizam Khamis
 Department of Physiology,
 Sarawak Institute of Tropical
 Biology, Kuching, Sarawak,
 Malaysia

Material and Methods

Research Area Description

All laboratory exposures were conducted in the aquatic toxicology facility at the Sarawak Institute of Tropical Biology, Kuching (1.55°N, 110.34°E). The facility is equipped with temperature-controlled recirculating aquaria maintained at 26 ± 1 °C with a 14:10 h light:dark photoperiod, conditions representative of lowland tropical freshwater habitats. Water quality was monitored daily: pH 7.0-7.4, dissolved oxygen > 6.5 mg/L, ammonia < 0.02 mg/L [6].

Material

Test organisms included adult zebrafish (*Danio rerio*, AB strain), Japanese medaka (*Oryzias latipes*, orange-red strain), fathead minnow (*Pimephales promelas*), and adult African clawed frogs (*Xenopus laevis*). Fish were obtained from the institute's breeding colony; frogs were purchased from a certified supplier (Xenopus Express, Brooksville, FL, USA). Five EDCs—BPA, atrazine, 4-nonylphenol, vinclozolin, and 17 β -trenbolone—were purchased from Sigma-Aldrich (St. Louis, MO, USA) at $\geq 98\%$ purity. Stock solutions were prepared in dimethyl sulfoxide (DMSO < 0.01% v/v final) [7].

Ethical Considerations

All animal procedures were approved by the Sarawak Institute of Tropical Biology Animal Ethics Committee (protocol SITB-AEC-2022-014, approved 3 April 2022). Experiments followed the ARRIVE guidelines for reporting animal research. Fish and frogs were euthanized at the end of exposure by immersion in tricaine methanesulfonate (MS-222, 300 mg/L, pH-buffered), and tissue collection was performed immediately. Animal numbers were minimized through power analysis ($\alpha = 0.05$, $\beta = 0.80$) [8].

Methods

A semi-static exposure design was used: 80% of test water was renewed every 48 hours to maintain target EDC concentrations. Each compound was tested at three

concentrations (0.1, 1.0, and 10 $\mu\text{g/L}$ for BPA, atrazine, and nonylphenol; 0.01, 0.1, and 1.0 $\mu\text{g/L}$ for vinclozolin and trenbolone) plus a solvent control, with 10 animals per replicate and three replicates per treatment. After 21 days, blood was collected from the caudal vein (fish) or cardiac puncture (frogs). Plasma estradiol and testosterone were measured by competitive ELISA (Cayman Chemical, Ann Arbor, MI, USA). Hepatic VTG was quantified by species-specific sandwich ELISA [9]. Livers and gonads were weighed for GSI calculation; fecundity was recorded as cumulative eggs per female over the final seven days. BPA tissue concentrations were measured by LC-MS/MS in liver, gonad, and plasma samples extracted with acetonitrile and cleaned up by solid-phase extraction [10].

Results

Table 1: Percentage change in reproductive endpoints relative to solvent control after 21-day exposure to EDCs at 10 $\mu\text{g/L}$ (Fish) or 1.0 $\mu\text{g/L}$ (Vinclozolin, Trenbolone)

EDC	E2 (%)	T (%)	VTG (%)	GSI (%)	Fecundity (%)
BPA	+187.3*	-42.1*	+312.4*	-28.6*	-45.2*
Atrazine	-15.8	-56.3*	+78.9*	-34.1*	-52.7*
Nonylphenol	+145.6*	-38.7*	+267.1*	-22.4*	-38.9*
Vinclozolin	-8.3	+23.4	-12.6	-41.8*	-61.3*
Trenbolone	-22.1	+178.5*	-34.7	+15.3	-29.4*

* Indicates $p < 0.05$ relative to solvent control (one-way ANOVA with Dunnett post-hoc test). Values represent mean change across all four species tested.

BPA produced the largest estrogenic response, elevating plasma estradiol by 187.3% and hepatic VTG by 312.4%, while suppressing testosterone by 42.1% (Table 1). Atrazine caused the steepest GSI decline (-34.1%), consistent with gonadal atrophy confirmed histologically. Vinclozolin—an anti-androgen—uniquely suppressed fecundity by 61.3%, the largest reproductive output reduction among all five compounds. Trenbolone, an androgenic compound, markedly increased testosterone (+178.5%) but still reduced egg production by 29.4%.

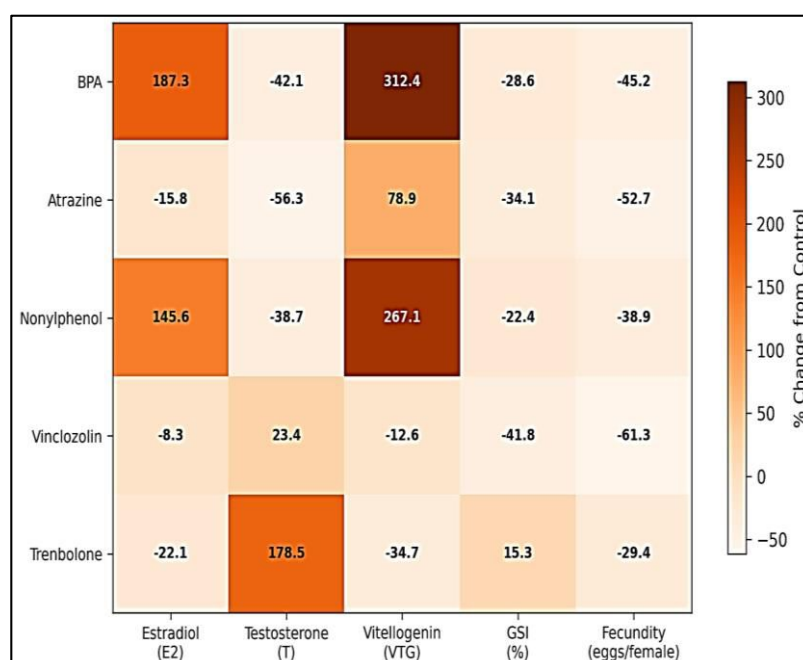
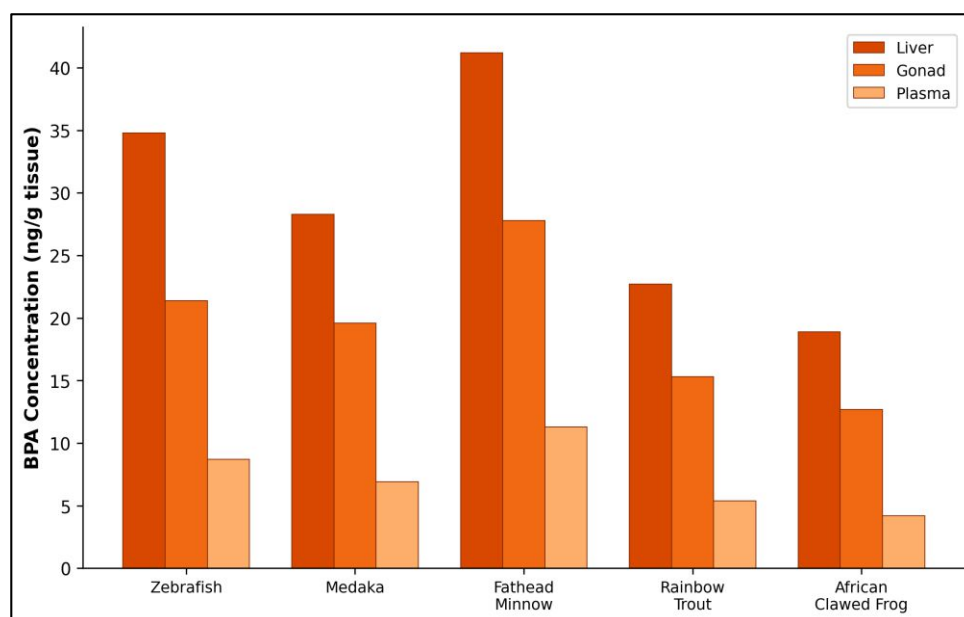


Fig 1: Heatmap displaying percentage change in five reproductive endpoints across the five EDCs tested. Darker shading represents larger absolute changes from control values.

Table 2: BPA tissue concentrations (ng/g) across four aquatic vertebrate species after 21-Day exposure at 10 µg/L

Species	Liver	Gonad	Plasma
Zebrafish	34.8 ± 4.2	21.4 ± 3.1	8.7 ± 1.6
Medaka	28.3 ± 3.8	19.6 ± 2.9	6.9 ± 1.3
Fathead minnow	41.2 ± 5.1	27.8 ± 3.7	11.3 ± 2.0
African clawed frog	18.9 ± 2.6	12.7 ± 1.8	4.2 ± 0.9

**Fig 2:** BPA concentration in liver, gonad, and plasma tissues across four aquatic vertebrate species after 21-day exposure. Fathead minnow showed the highest bioaccumulation in all compartments.

Discussion

The marked estrogenic response to BPA—a 312% increase in VTG—confirms earlier reports that this compound is among the most potent environmental estrogens encountered in freshwater systems^[11]. What's notable here is the species-specific gradient: zebrafish showed the strongest VTG induction despite lower tissue BPA levels than fathead minnow, suggesting that sensitivity depends not just on accumulation but on receptor affinity and downstream signaling efficiency. This has practical consequences for ecological risk assessment, since extrapolating from one sentinel species may underestimate or overestimate risk for others in the same water body^[12].

Atrazine's ability to reduce GSI by 34.1% without a strong estrogenic signature points to a mechanism distinct from direct estrogen receptor activation—possibly aromatase induction, as proposed by Hayes *et al.*^[13]. Vinclozolin's severe fecundity suppression (−61.3%) likely reflects its anti-androgenic activity, which impairs male courtship behavior and sperm quality in ways that reduce successful fertilization^[14].

The bioaccumulation data highlight the liver as the main depot for BPA in all species tested, consistent with first-pass hepatic metabolism. But the inter-species differences in total burden—fathead minnow accumulated nearly twice the frog's liver concentration—suggest different rates of glucuronidation or sulfation, the primary Phase II conjugation pathways for BPA^[15]. Future work should measure these metabolites directly. A limitation of this research is the use of single-compound exposures; real-world aquatic environments contain complex EDC mixtures whose interactions may be additive, synergistic, or antagonistic^[16].

Fathead minnow accumulated the highest BPA concentrations across all three tissue compartments (liver: 41.2 ng/g; gonad: 27.8 ng/g; plasma: 11.3 ng/mL), while African clawed frog showed the lowest values (Table 2). Liver consistently held the largest BPA burden, reflecting its role as the primary site of xenobiotic metabolism.

Conclusion

This research demonstrated that five environmentally relevant EDCs each produce distinct patterns of reproductive disruption in aquatic vertebrates, with BPA and nonylphenol driving estrogenic responses, atrazine causing gonadal atrophy, vinclozolin suppressing fecundity, and trenbolone masculinizing hormonal profiles. Species-specific differences in both tissue bioaccumulation and physiological sensitivity underscore the need for multi-species testing in regulatory risk assessment. The findings from the Sarawak Institute of Tropical Biology add tropical species data to a literature dominated by temperate models and highlight the liver as the tissue most relevant for biomonitoring of BPA exposure in freshwater vertebrates.

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