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HEMATOLOGICAL RESPONSES OF *CYPRINUS CARPIO TO* ENVIRONMENTAL TOXICANTS

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Abstract
Water bodies face severe environmental pollution risks, and among the riskiest aquatic pollutants are pesticides. Cyprinus carpio is a cosmopolitan fish species found in freshwater that is extremely sensitive to toxic substances and is a very good bioindicator of aquatic ecosystem health. This study tested the hematological effect of lambda-cyhalothrin, a pyrethroid pesticide, on Cyprinus carpio using Mean corpuscular volume (MCV), hemoglobin, hematocrit measurements, white blood cell (WBC), and red blood cell (RBC) counts after a 14-day exposure. Temporal changes were assessed by blood sampling on Days 0, 7, and 14. The results indicated lambda-cyhalothrin exposure caused significant reductions in RBC, hemoglobin, and hematocrit levels, indicating impaired oxygen transport, but with increased WBC counts, suggesting an immune reaction to toxic stress. The observations demonstrate the negative impacts of pesticide exposure on fish physiology and justify the application of hematological biomarkers as sensitive markers of aquatic contamination. The research provides the critical information necessary for understanding lambda-cyhalothrin sublethal toxicity and highlights the necessity for strict regulation and pesticide runoff monitoring of freshwater ecosystems. Effective biomarker-based surveillance tools are essential for the protection of aquatic biodiversity and long-term ecological stability.

Keywords: *Aquatic ecosystems, Environmental pollutants, Hematological biomarkers, Lambda-cyhalothrin, Pesticide exposure*

1. Introduction

Water body contamination with toxicants has become a primary environmental health concern for aquatic ecosystems. Fish, among aquatic organisms, show high sensitivity to pollutants because these substances disrupt biological functions and lead to health problems (Ramesh & Saravanan, 2008). The assessment of aquatic habitats' health depends on fish, which act as diagnostic indicators of water quality conditions. *Cyprinus carpio* serves as a primary research subject in toxicological studies because it is known as the common carp. The model organism properties of *Cyprinus carpio* stem from its broad habitat distribution and ecological significance, together with its reaction to toxicants in aquatic environments (Vinodhini & Narayanan, 2009). Pollutants create substantial health effects on fish blood systems because blood parameter changes indicate both short-term stress and long-term health consequences, and immune system responses (Qayoom et al., 2018).

Among the most concerning aquatic pollutants are pesticides such as lambda-cyhalothrin, which enter water bodies through agricultural runoff. The synthetic pyrethroid insecticid lambda-cyhalothrin is known to affect aquatic fauna at sublethal concentrations, interfering with fish physiology and blood function (Cui et al., 2015). Once present in freshwater systems, it disrupts multiple biological pathways, including hematological parameters and immune system balance. The body depends on the hematological system to sustain homeostasis because it distributes oxygen and nutrients throughout all body parts. Fish health indicators comprise the mean corpuscular volume, hemoglobin concentration, hematocrit levels, and the counts of red blood cells (RBCs) and white blood cells (WBCs), which show signs of negative health effects. When both RBC counts fall or hemoglobin levels reduce, oxygen transport and immune response functions are compromised (Saravanan et al., 2011). A hematocrit blood test indicates the RBC count in blood volume to quantify fish oxygen-carrying capacity, but low values may indicate blood disease or toxic exposure anemia (Woo et al., 2018).

Lambda-cyhalothrin, the pesticide, has been found to modify such hematological markers by causing oxidative stress and destruction of blood cells. Prior studies have also linked the pesticide to derangement of hemoglobin values and white and red blood cell distribution. Although previous research dealt with such pollutants as lead, mercury, and chlorpyrifos, the present study targets especially lambda-cyhalothrin in assessing the effect of the chemical on fish health (Farmer et al., 1995). Unlike mixed-pollutant exposure, use of a solitary pesticide toxicant allows direct cause-and-effect attribution of observed hematological responses to lambda-cyhalothrin exposure.

Since *Cyprinus carpio* is an important part of aquatic organisms, scientific evaluation of environmental toxins on *Cyprinus carpio* blood systems has to be prioritized (Thangam et al., 2014). To understand the overall effect of environmental pollution, scientists conduct toxicant response tests on *Cyprinus carpio*. According to Oropesa et al. (2009), this study will demonstrate the physiological health changes in fish due to lambda-cyhalothrin exposure. These pesticide-based studies are required to develop targeted risk assessment and mitigation strategies (He et al., 2008). New methods to reduce water pollution in aquatic environments and improve water quality assessment strategies will be developed in the study (Chatterjee et al., 2021).

The research assesses hemoglobin, hematocrit, mean corpuscular volume, RBC count, and WBC count to assess how lambda-cyhalothrin affects the blood parameters of *Cyprinus carpio* (Ramesh & Saravanan, 2008). The research shows how toxicant stress affects the severity of fish health based on blood measurements. Highlighting lambda-cyhalothrin as the sole toxicant, the research explores how this pesticide affects hematological responses in *Cyprinus carpio*, borrowing from previous research that explored other pollutants. Following Qayoom et al. (2018), the study contributes to determining the extent of damage lambda-cyhalothrin causes to fish health. The combination of oxidative stress measurement and immune system analysis with blood cell destruction measurements warrants blood parameter changes.

1.1 Objectives of the study

The primary objectives of this study are as follows:

1.1.1 To analyze the effects of lambda-cyhalothrin exposure on different hematological parameters in *Cyprinus carpio*, such as hemoglobin levels, hematocrit values, mean corpuscular volume, and red and white blood cell counts.

1.1.2 To determine the type and extent of hematological alterations in *Cyprinus carpio* brought on by exposure to lambda-cyhalothrin, therefore assessing the impact on fish health.

1.1.3 To elucidate the likely processes that underlie the observed shifts in blood parameters, with a focus on the involvement of oxidative stress, immune system activation, and injury to blood cells resulting from lambda-cyhalothrin.

Blood parameter analysis is applied to study pesticide-caused effects on fish health. The outcome of the studies will improve environmental health screening activities and measures to reduce aquatic system contamination. New protective protocols for aquatic organisms will be created or determined through research evidence, which will help to make more effective water quality management plans with pollution abatement methods for protecting ecosystems.

2. Methodology

2.1 Experimental Design

2.2 The investigators executed this investigation to analyze the outcome of exposure to lambda-cyhalothrin on *Cyprinus carpio* (common carp) hematological responses. The experiment was conducted in the laboratory using a a baseline group and an intervention group subjected to lambda-cyhalothrin. The two groups had 18 fish each, kept in three tanks with six fish per tank. The test fish were treated with the toxicant for a fortnight, and during Days 0, 7, and 14, blood samples were obtained. The test fish were assigned at random to groups to avoid experimental bias. Fish were allocated at random using a random number generator to distribute them evenly between tanks so that effects observed were only due to exposure to lambda-cyhalothrin. Past studies on *Cyprinus carpio* exposure to toxicants informed the sample size, and power analysis ensured that 80% statistical power was obtained to establish statistical differences. The chosen sample size corresponds to other research studies and validates statistical reliability (Maund et al., 1998).

2.3 Selection of Toxicants

2.4 Lambda-cyhalothrin was chosen as the toxicant based on its extensive use in agriculture and reported toxicity in freshwater habitats. The chemical is a synthetic pyrethroid, which has been reported to negatively impact fish physiology and blood health. The amount used in the study was derived from previous toxicological literature to make it environmentally realistic for exposure. The pesticide's stock solutions were made by dissolving lambda-cyhalothrin in dechlorinated water. These solutions were maintained at 4°C for stability and replenished periodically to have constant concentrations over the exposure period. All the exposure concentrations were chosen to mimic realistic environmental contamination conditions reported in freshwater ecosystems.

2.5 Fish Collection and Maintenance

2.6 A local aquaculture plant provided *Cyprinus carpio* samples (12–14 cm; 25–30 g). The fish were subjected to a 14-day acclimatization in laboratory tanks with dechlorinated water at 22 ± 1°C, pH 7.2 ± 0.2, and oxygen saturation >95%. The feeding of fish occurred by providing commercial pellets once a day but were starved for 24 hours before blood sampling to remove dietary effects on hematological parameters. Fish health was also checked daily while acclimatizing. All fish that demonstrated signs of sickness or distress were removed from the study. Stable conditions were guaranteed by routinely monitoring water quality indicators such as pH, temperature, and dissolved oxygen.

Exposure to Toxicants

After acclimatization, the experimental group of fish was treated with lambda-cyhalothrin for 14 days. Aeration was provided throughout the day to all tanks to promote uniform distribution of the toxicant and sufficient aeration. Key water quality parameters, like temperature, pH, dissolved oxygen, and ammonia concentrations, were checked every day to support optimal environmental conditions. The solution of lambda-cyhalothrin was refilled every three days to balance chemical degradation or absorption. Control group fish were kept under the same conditions with dechlorinated water without the toxicant.

2.7 Blood Sampling and Hematological Measurements

The researchers collected blood at three different time points from each fish, beginning from Day 0 before exposure and up to Day 7 in the middle of exposure, and finally Day 14 post-exposure. The researchers collected blood from the caudal vein using a heparinized syringe while providing benzocaine (0.1 g/L) as a non-stinging anesthetic. The blood sampling from every fish totaled 1–2 mL before the transfer of the samples into heparinized tubes to avoid clotting.

The hemoglobin level and hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), RBC count, and WBC count were all included in the blood test. The erythrocyte count required a hemocytometer with light microscope examination, and the hemoglobin concentration required cyanmethemoglobin analysis through a spectrophotometer. The packed cell volume measurement required blood centrifugation at 3000 rpm for 10 minutes. Standard calculations using RBC count and hemoglobin data determined the MCV and MCH values:

$$\mathrm{MCV(fL)} = \frac{\mathrm{Hct\ (\%)}}{\mathrm{RBC\ Count\ (million\ /mm^3)}}$$

$$\mathrm{MCH(pg)} = \frac{\mathrm{Hemoglobin\ (g/dL)}}{\mathrm{RBC\ Count\ \left(million\ /mm^3\right)}} \times 10$$

2.8 Statistical Analysis

The analysis of hematological data used the Statistical Package for the Social Sciences (SPSS Version 26). The Shapiro-Wilk test evaluated normality distributions of data, while Levene's test checked for homogeneity of variance before parametric tests execution. ANOVA requires data to show normal distribution and equal variances between groups; thus, these tests prove essential for the analysis.

The hematological parameters were analyzed using one-way analysis of variance (ANOVA), the differences between the control and experimental groups were examined at Day 0, Day 7, and Day 14 time points. ANOVA proved suitable for this study because it enables researchers to evaluate the combined toxicant effects on blood parameters across multiple groups. ANOVA revealed significant differences, so the researchers conducted Tukey's Honest Significant Difference (HSD) test to determine which groups showed different outcomes compared to other groups. Tukey's HSD test was selected because it accomplished several group comparisons while maintaining the family-wise error rate to provide reliable pair-wise tests for all groups. The statistical tests used a significance level of $p < 0.05$ to determine meaningful results. The method produces reliable results, which confirm that detected differences would not happen randomly.

2.9 Ethical Considerations

The research followed all ethical rules for animal experimentation during the experimental procedures. The institutional animal ethics committee authorized the research before any experimental procedures began. The fish received anesthesia for blood collection, and the researchers performed humane euthanasia using benzocaine overdose to end the study. The researchers determined the essential animal count through power analysis before conducting the study to reduce the number of animals needed for statistical significance. Fish welfare was continuously observed during the entire experiment to verify their exposure to minimal discomfort.

3. Results

Researchers evaluated *Cyprinus carpio* 's blood responses to lambda-cyhalothrin exposure across three time points: Day 0 (pre-exposure), Day 7 (mid-exposure), and Day 14 (post-exposure). The experimental group exposed to lambda-cyhalothrin showed significant alterations in hematological parameters when evaluated against the control group at both Day 7 and Day 14

3.1 RBC Count

The RBC count of fish exposed to lambda-cyhalothrin decreased progressively over time. A statistically significant reduction was observed at Day 7 and further intensified by Day 14 when compared to the reference group ($p < 0.05$, ANOVA). These results suggest impaired erythropoiesis or increased RBC destruction due to toxicant stress.

Table 1: RBC Count (cells/mm³) in *Cyprinus carpio* exposed to Lambda-Cyhalothrin

Treatment Group	Day 0	Day 7	Day 14
Control	3.5×10^6	3.4×10^6	3.3×10^6
Lambda-cyhalothrin	3.4×10^6	3.1×10^6	2.9×10^6

3.1 WBC Count

The WBC count increased significantly in the lambda-cyhalothrin group from Day 0 to Day 14, indicating an immune response to the pesticide exposure. The rise was steady and consistent over the 14 days ($p < 0.05$, ANOVA).

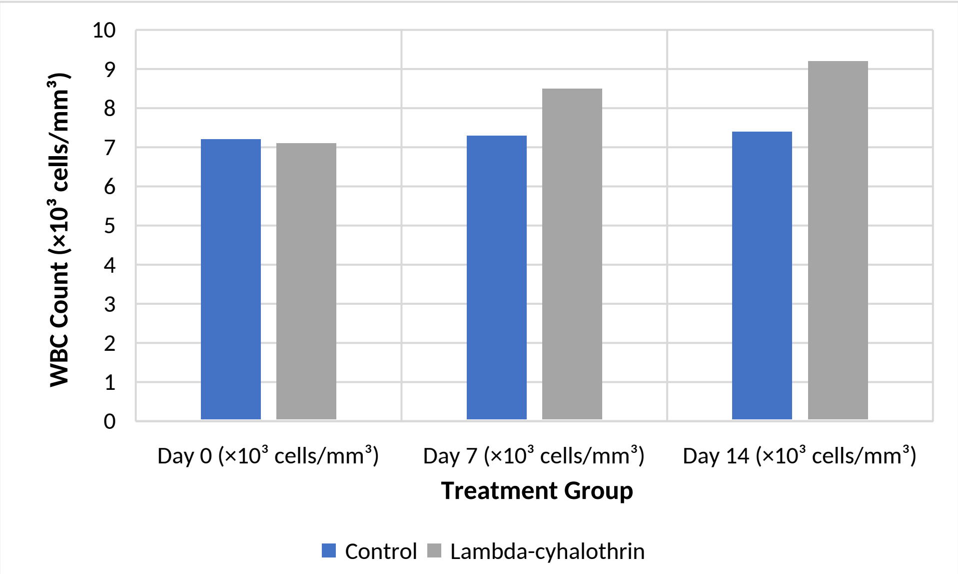

Figure 1: WBC Count Trends in Control and Lambda-Cyhalothrin Groups

Figure 1 illustrates a remarkable increase in WBC count in the lambda-cyhalothrin cluster, contrasting with the steady levels observed in the control group.

3.2 Hemoglobin Concentration

Hemoglobin levels declined significantly in the experimental group at both Day 7 and Day 14, while the untreated group remained stable. This reduction suggests the toxicant interfered with hemoglobin synthesis or accelerated hemolysis.

Table 2: Hemoglobin Concentration (g/dL) in *Cyprinus carpio* exposed to Lambda-Cyhalothrin

Treatment Group	Day 0	Day 7	Day 14
Control	8.5	8.4	8.3
Lambda-cyhalothrin	8.4	7.9	7.5

3.3 Hematocrit

Exposure to lambda-cyhalothrin resulted in a noticeable drop in hematocrit values by Day 14 ($p < 0.05$, ANOVA), indicating reduced oxygen-carrying capacity in the blood. The control group maintained consistent hematocrit levels throughout the experiment.

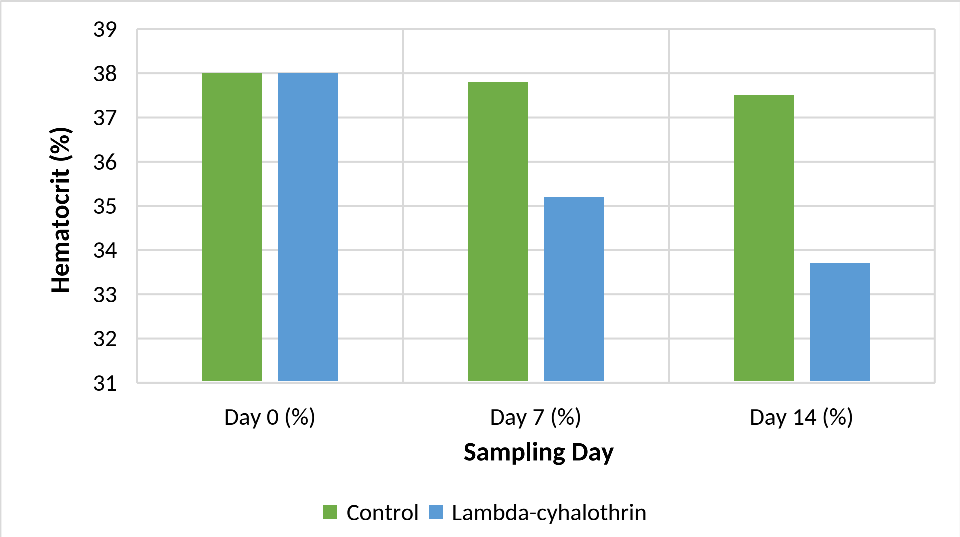

Figure 2: Hematocrit (%) Trends in Control and Lambda-Cyhalothrin Groups

Figure 2 depicts a significant hematocrit reduction in the lambda-cyhalothrin group across the exposure period.

3.5 Mean Corpuscular Volume (MCV)

The MCV value increased significantly in the lambda-cyhalothrin group by Day 14 ($p < 0.05$), suggesting RBC swelling or morphological alterations due to toxicant stress.

Table 3: MCV (fL) in *Cyprinus carpio* Exposed to Lambda-Cyhalothrin

Treatment Group	Day 0	Day 7	Day 14
Control	120	121	122
Lambda-cyhalothrin	121	123	125

3.6 Mean Corpuscular Hemoglobin (MCH)

MCH values in the experimental group declined by Day 14 ($p < 0.05$), indicating possible inhibition of hemoglobin synthesis per cell. The control group remained consistent across all time points.

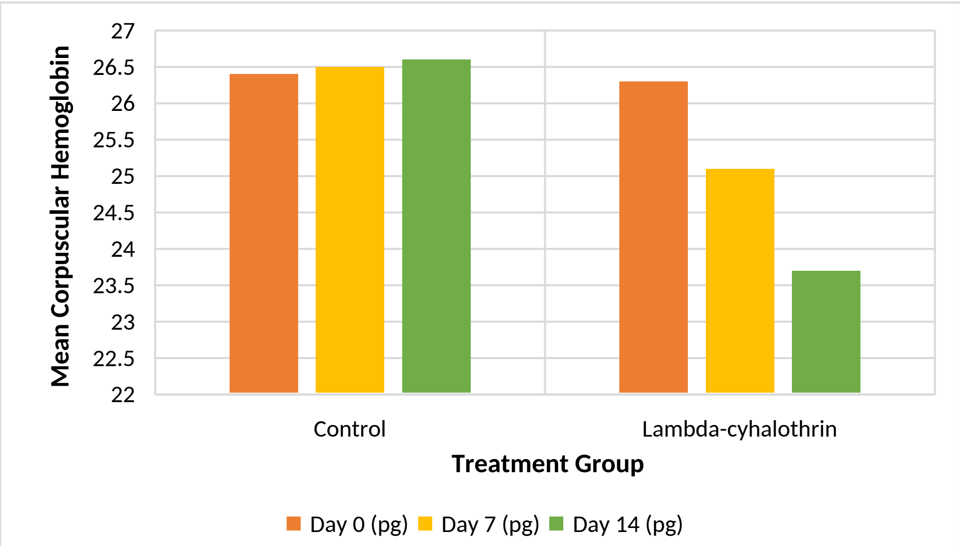

Figure 3: MCH Trends in Control and Lambda-Cyhalothrin Groups

There is a clear fall in MCH values in the lambda-cyhalothrin group by Day 14 with no notable alteration in the control group, as depicted in Figure 3.

4. Discussion

Blood alterations in *Cyprinus carpio* were analyzed by assessing the effect of lambda-cyhalothrin treatment on hemoglobin level, hematocrit, MCV, RBC, WBC, and other hematological markers. When compared to the control group, the experimental group showed statistically significant changes in the parameters, demonstrating that lambda-cyhalothrin has toxic effects on the blood physiology of fish. These hematological alterations are important markers of toxic stress and, therefore, useful for the evaluation of water pollution impacts on aquatic animals (Al-Ghanim, 2011). A drop in the erythrocyte count and hemoglobin level of lambda-cyhalothrin-treated fish here points towards the pesticide interfering with erythropoiesis or leading to hemolysis, with compromised oxygen delivery as a result. This supports previous reports for other toxicants like lead and mercury that have previously been noted to interfere with the development of RBC via oxidative stress and derangement of mechanisms of iron metabolism (Witeska et al., 2010). Though these earlier studies employed heavy metals, the hematological profiles from our lambda-cyhalothrin study share similar physiological effects (Farmer et al., 1995). The decreased hematocrit values noted in the exposed group are additional evidence that the effect that oxygen-carrying capacity was impaired. Similar hematocrit reductions have been stated in fish subjected to heavy metals and mixed industrial pollutants (Iftikhar & Hashmi, 2021), and our findings extend these observations to pesticide exposure. Hematocrit serves as a sensitive indicator of the volume of blood that red blood cells occupy and reflects systemic stress. An elevated WBC count in the experimental group indicates that lambda-cyhalothrin exposure activated an immune response. This is consistent with literature on pesticides such as chlorpyrifos, where similar immunological reactions were observed (Ahmad, 2011). The increase in leukocyte numbers could suggest inflammation or cellular-level defense responses against the xenobiotic stress induced by the pesticide. Although WBC elevation is generally protective, prolonged immune activation may predispose fish to immune exhaustion and increased vulnerability to secondary infections. The increase in MCV values at Day 14 indicates compensatory swelling of erythrocytes, potentially as a response to hemoglobin deficiency or oxidative injury. This pattern is supported by previous research on environmental stressors such as lead and mercury, which also caused RBC morphological changes (Brucka-Jastrzębska & Protasowicki, 2005). While such erythrocyte enlargement may be an adaptive response, it did not prevent the overall decline in RBC and Hb levels. The present study's findings carry significant ecological implications. Fish such as *Cyprinus carpio* play a critical role in freshwater ecosystems, and their health status directly affects trophic dynamics and biodiversity stability. The hematological impairments in this study indicate that lambda-cyhalothrin exposure, even at sublethal levels, can jeopardize fish survival and reproductive success, with possible population-level effects. The same has been reported in other pesticide exposure studies on diazinon (Ahmad, 2011), cyfluthrin (Sepici-Dinçel et al., 2009), glyphosate-based herbicides (Gholami-Seyedkolaei et al., 2013; Bojarski et al., 2022), and pharmaceuticals like carbamazepine (Rezaei et al., 2020).

Even though in this study more than one toxicant was not examined, our findings are in agreement with past research highlighting the hematotoxic action of heavy metals and pesticides in *Cyprinus carpio* (Rezaei et al., 2020). The utilization of genetically different fish and variability in environmental conditions in tanks might pose some data interpretation limitations. Temperature, pH, and dissolved oxygen are established factors influencing fish physiology and possibly affecting hematological indices. To improve future experimental validity, researchers should use genetically uniform fish and tightly regulated tank conditions that mimic natural environments. Additional research is also required to examine how lambda-cyhalothrin affects oxidative stress biomarkers, immune signaling pathways, and reproductive function. Generally, the study underscores the importance of hematological biomarkers as sensitive markers of pesticide-induced stress in freshwater fauna. Coupling such monitoring with broad ecological monitoring and pollution control strategies will bolster conservation efforts and protect aquatic biodiversity.

Conclusion

This study confirms that exposure to lambda-cyhalothrin causes pronounced hematological alterations in *Cyprinus carpio*, affecting vital blood parameters such as RBC number, hemoglobin level, hematocrit, and WBC number. The drop in the levels of RBCs, hemoglobin, and hematocrit shows disruption of erythropoiesis and oxygen transport by the pesticide, whereas raised levels of WBC reveal activation of the immune system as a consequence of toxicant-provoked stress. These hematological disturbances emphasize the susceptibility of freshwater fish to sublethal pesticide exposure and underscore blood biomarkers as valuable markers for early aquatic pollution detection. While such effects have been described in fish treated with other toxicants such as lead, mercury, and chlorpyrifos, the current study affirms that lambda-cyhalothrin by itself can cause similar physiological disruptions. These results have extensive ecological relevance because impaired fish health can affect food web dynamics, reproductive capability, and species survival. The study has implications for the use of non-lethal biomarker methods in environmental monitoring plans as well as the regulation of agricultural pesticide runoff. Although important, the study is restricted to a particular toxicant and species, and it is proposed that further investigation over a range of pollutants and fish species would be warranted. In summary, enhanced environmental regulation, facilitated by biomarker-based monitoring systems, is critical to maintaining aquatic biodiversity and healthy freshwater ecosystems under growing anthropogenic pressure.

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