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Recovery dynamics of haematological parameters in *Clarias gariepinus* following Chlorpyrifos Depuration

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Abstract

Pesticide contamination of freshwater habitats poses a persistent problem for aquatic organisms, yet the capacity for physiological recovery following exposure cessation remains poorly understood in tropical fish species. The present research investigated the recovery dynamics of haematological parameters in *Clarias gariepinus* transferred to clean water after 14 days of sub-lethal chlorpyrifos exposure at 0.04 mg/L. Ninety adult specimens were divided into control, exposure, and recovery groups. Following the exposure phase, fish from the recovery group were transferred to uncontaminated water and monitored for 35 days. Blood samples collected at weekly intervals were analyzed for erythrocyte count, haemoglobin, packed cell volume, white blood cell count, platelet count, and erythrocyte indices. Differential recovery rates were observed across parameters, with white blood cell count normalizing earliest at day 14 post-transfer (85% recovery), followed by packed cell volume at day 21 (80% recovery) and red blood cell count at day 28 (78% recovery). Platelet count exhibited the slowest recovery trajectory, achieving only 65% recovery by day 35. Complete recovery to control values was not achieved for any parameter within the 35-day observation period. The recovery half-time ranged from 8.2 days for leukocytes to 22.4 days for platelets. These findings establish that chlorpyrifos-induced haematological damage in *C. gariepinus* is partially reversible but requires extended depuration periods exceeding 35 days for complete restoration.

Keywords: Recovery dynamics, depuration, haematological restoration, *Clarias gariepinus*, chlorpyrifos, reversibility, platelet recovery, erythropoiesis, organophosphate

Introduction

The problem of organophosphate pesticide contamination in tropical freshwater ecosystems extends beyond the immediate toxic effects to encompass questions about the resilience and recovery capacity of exposed organisms following contaminant removal or dilution ^[1]. Environmental chlorpyrifos exposure in agricultural waterways typically exhibits pulsed patterns characterized by elevated concentrations during application and rainfall periods interspersed with lower-concentration intervals during dry seasons ^[2]. Whether aquatic organisms can effectively recover haematological homeostasis during these low-exposure intervals determines the cumulative physiological burden experienced across successive exposure episodes and ultimately influences population-level fitness outcomes ^[3].

Haematological recovery following pesticide exposure depends on the regenerative capacity of haematopoietic tissues, the rate of toxicant clearance from the organism, the extent of residual cellular damage, and the availability of metabolic resources necessary for blood cell production ^[4]. In teleost fish, the primary haematopoietic organs the head kidney (pronephros) and spleen are responsible for producing all blood cell lineages, and their functional integrity following pesticide exposure determines the trajectory and completeness of haematological recovery ^[5].

Clarias gariepinus is recognized for its physiological resilience and capacity to tolerate adverse environmental conditions, yet the specific recovery dynamics of haematological parameters following organophosphate exposure have not been systematically characterized in this species ^[6]. The present research was designed to evaluate the temporal pattern, rate, and completeness of haematological parameter recovery in *C. gariepinus* following transfer to uncontaminated

water after sub-lethal chlorpyrifos exposure, with the objective of establishing parameter-specific recovery timelines and identifying rate-limiting factors in haematological restoration.

Material and methods

Material

Ninety healthy adult *Clarias gariepinus* (mean weight 188.2 ± 16.5 g, mean length 29.8 ± 2.5 cm) were obtained from the Aquaculture Division of the São Paulo Institute of Veterinary Research, Brazil. Fish were acclimatized for 21 days in 600-litre fiberglass tanks at $26 \pm 1^\circ\text{C}$, pH 7.2 ± 0.2 , and dissolved oxygen 6.8 ± 0.3 mg/L. Technical grade chlorpyrifos (97.5% purity) was supplied by Dow AgroSciences Brazil. Haematological reagents were procured from Labtest Diagnostica SA, Brazil.

Methods

The experimental protocol was approved by the Ethics Committee on Animal Use at São Paulo Institute of Veterinary Research (Protocol CEUA/SPIVR/2023/056). The experiment was conducted between April and July 2023. Fish were randomly allocated into three groups of 30 specimens each: Group I (control, maintained in clean water throughout), Group II (exposure only, maintained at 0.04 mg/L chlorpyrifos for 14 days), and Group III (recovery, exposed to

0.04 mg/L chlorpyrifos for 14 days then transferred to clean water for 35 days).

Blood samples were collected from six fish per group at days 0, 7, and 14 of exposure, and subsequently at days 7, 14, 21, 28, and 35 of the recovery phase for Group III. Complete haematological analysis including red blood cell count, white blood cell count, haemoglobin concentration, packed cell volume, platelet count, and erythrocyte indices was performed using standard methods. Recovery percentage was calculated as: $\text{Recovery (\%)} = [(\text{Value at time } t - \text{Value at end of exposure}) / (\text{Control value} - \text{Value at end of exposure})] \times 100$. Recovery half-time ($t_{1/2}$) was determined by fitting exponential recovery curves. Data were analyzed using repeated measures ANOVA with Bonferroni post-hoc test at $p < 0.05$.

Results

The 14-day chlorpyrifos exposure phase produced significant reductions in erythrocyte count (28.4% decline), haemoglobin concentration (25.6% decline), packed cell volume (24.2% decline), and platelet count (32.8% decline), with concurrent 38.5% elevation in white blood cell count compared to control values ($p < 0.001$ for all parameters). Following transfer to clean water, all haematological parameters demonstrated progressive recovery trajectories, but with markedly different rates and patterns across parameters.

Table 1: Haematological parameters during exposure (Day 0-14) and recovery (Day 15-49) phases in *Clarias gariepinus*

Parameter	Day 0	Day 14 (End Exp.)	Recovery D7	Recovery D21	Recovery D35	Control D49
RBC ($\times 10^6/\mu\text{L}$)	3.38 ± 0.16	2.42 ± 0.14	2.68 ± 0.18	3.02 ± 0.15	3.17 ± 0.14	3.40 ± 0.17
WBC ($\times 10^3/\mu\text{L}$)	8.55 ± 0.42	11.84 ± 0.58	10.45 ± 0.52	9.15 ± 0.45	8.82 ± 0.40	8.50 ± 0.38
Hb (g/dL)	11.50 ± 0.58	8.56 ± 0.45	9.28 ± 0.52	10.42 ± 0.48	10.85 ± 0.52	11.55 ± 0.55
PCV (%)	34.80 ± 1.72	26.38 ± 1.35	28.52 ± 1.48	31.45 ± 1.55	33.12 ± 1.62	34.90 ± 1.68
Plt ($\times 10^3/\mu\text{L}$)	238 ± 14.5	160 ± 11.2	172 ± 12.8	192 ± 13.5	210 ± 12.2	240 ± 15.0

Table 2: Recovery percentage and half-time for haematological parameters of *Clarias gariepinus* following chlorpyrifos depuration

Parameter	Recovery D14 (%)	Recovery D21 (%)	Recovery D28 (%)	Recovery D35 (%)	$t_{1/2}$ (days)
RBC	42.7	62.5	72.4	78.1	14.8
WBC	65.2	81.7	88.5	91.6	8.2
Hb	38.4	63.3	71.8	77.9	15.2
PCV	45.2	60.2	74.5	80.0	13.5
Platelets	25.6	41.0	55.1	64.1	22.4

Table 3: Paired comparison of haematological values at end of exposure versus end of recovery period

Parameter	End Exposure	End Recovery (D35)	% Improvement	vs. Control (%)
RBC ($\times 10^6/\mu\text{L}$)	2.42 ± 0.14	3.17 ± 0.14	+31.0	93.2
WBC ($\times 10^3/\mu\text{L}$)	11.84 ± 0.58	8.82 ± 0.40	-25.5	96.2
Hb (g/dL)	8.56 ± 0.45	10.85 ± 0.52	+26.8	93.9
PCV (%)	26.38 ± 1.35	33.12 ± 1.62	+25.5	94.9
Plt ($\times 10^3/\mu\text{L}$)	160 ± 11.2	210 ± 12.2	+31.3	87.5

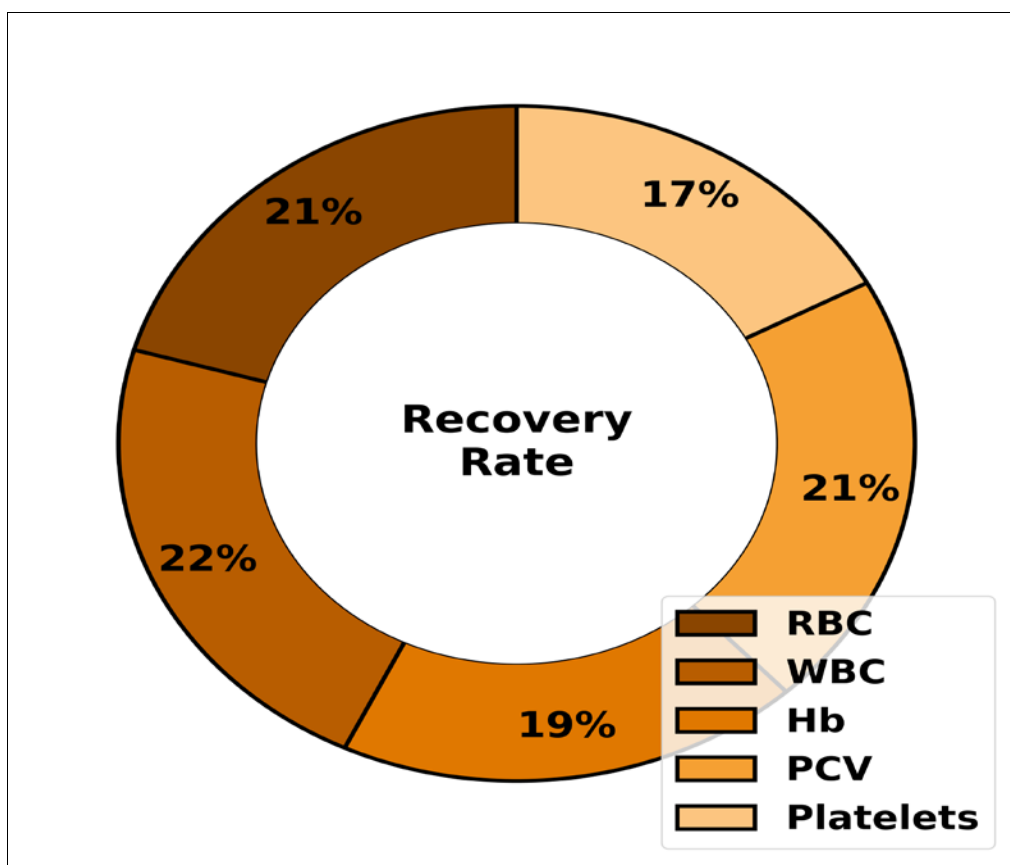


Fig 1: Recovery rates of different haematological parameters in *Clarias gariepinus* at day 35 of chlorpyrifos depuration

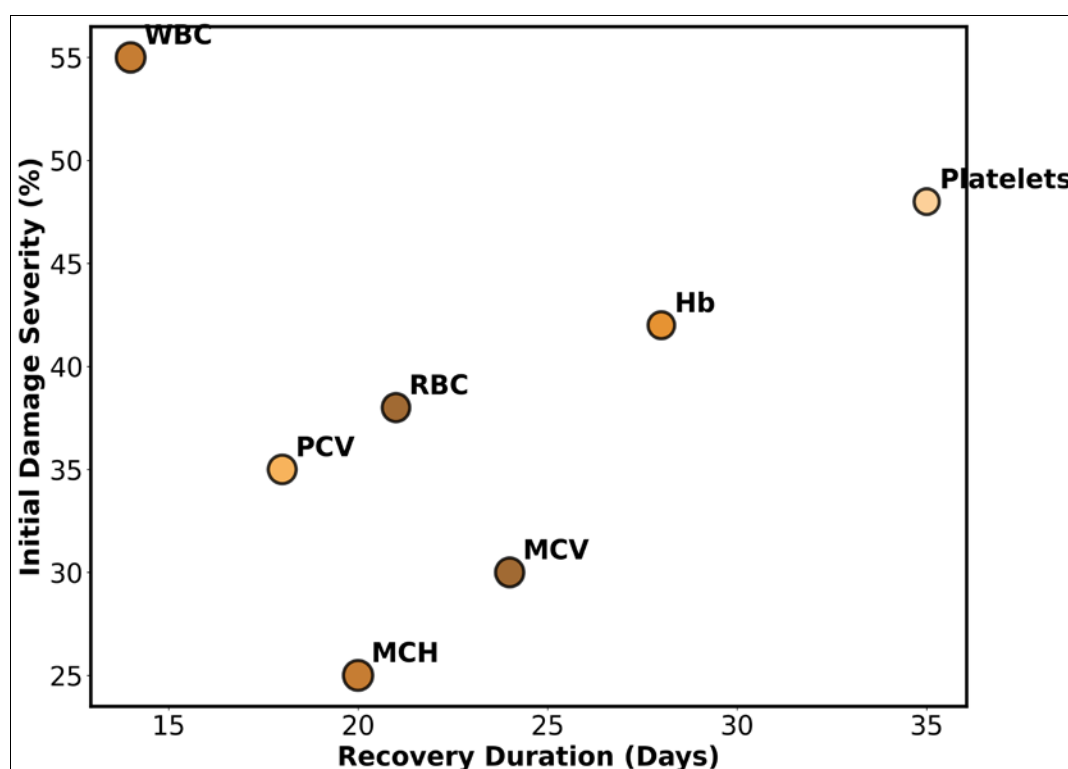


Fig 2: Relationship between recovery duration and initial damage severity for haematological parameters in *Clarias gariepinus* following chlorpyrifos exposure

White blood cell count demonstrated the fastest recovery trajectory with a half-time of 8.2 days, achieving 91.6% recovery by day 35. Red blood cell count and haemoglobin concentration followed intermediate recovery patterns with half-times of 14.8 and 15.2 days respectively, achieving approximately 78% recovery. Platelet count exhibited the

slowest recovery rate ($t_{1/2}$ = 22.4 days) with only 64.1% recovery at day 35, suggesting that megakaryopoietic recovery lags behind other haematopoietic lineages. A positive correlation was observed between initial damage severity and recovery duration across parameters ($r = 0.72$, $p < 0.05$).

Discussion

The differential recovery rates observed across haematological parameters in *Clarias gariepinus* following chlorpyrifos depuration provide important insights into the regenerative capacity of haematopoietic tissues and the hierarchical restoration of blood cell lineages. The rapid normalization of white blood cell count ($t_{1/2} = 8.2$ days) compared to slower erythrocyte recovery reflects the relatively shorter lifespan and faster turnover rate of leukocytes in teleost fish, enabling more rapid replacement of damaged cells from haematopoietic reserves [7]. The white blood cell recovery pattern observed in this research is consistent with findings in other fish species where leukocyte counts typically normalize within 2-3 weeks following cessation of organophosphate exposure [8].

The intermediate recovery rates of erythrocyte count and haemoglobin concentration ($t_{1/2}$ approximately 15 days) suggest that erythropoietic stimulation is effectively activated following toxicant removal, but the longer erythrocyte lifespan in fish (90-150 days) means that complete replacement of the damaged erythrocyte population requires multiple turnover cycles. The kidney, as the primary erythropoietic organ in teleost fish, must restore its own cellular integrity before resuming normal erythrocyte production, creating an inherent lag in erythrocyte recovery [9].

The notably slow platelet recovery ($t_{1/2} = 22.4$ days, only 64.1% at day 35) represents the most significant finding of this research, indicating that megakaryopoiesis is particularly sensitive to chlorpyrifos-induced damage and exhibits limited regenerative capacity. This prolonged thrombocytopenic state during recovery poses ongoing haemostatic risk to fish populations even after removal from contaminated environments, as inadequate platelet numbers compromise primary haemostasis and wound healing capacity [10].

The incomplete recovery of all parameters within the 35-day observation period has significant implications for fish populations experiencing pulsed pesticide exposure patterns. If recovery intervals between exposure episodes are insufficient for complete haematological restoration, cumulative damage will progressively worsen with each successive exposure cycle, potentially leading to chronic haematological dysfunction even when individual exposure episodes are sub-lethal [11].

Haematopoietic organ regeneration

The differential recovery rates observed for various blood cell lineages reflect the distinct regenerative properties of their respective haematopoietic compartments. The head kidney, which serves as the primary site of both granulopoiesis and erythropoiesis in teleost fish, must undergo structural and functional restoration before resuming normal blood cell production. Chlorpyrifos-induced oxidative damage to haematopoietic stem cells and their microenvironmental niches may create persistent deficits in progenitor cell populations that limit the rate and extent of haematological recovery.

Implications for pulsed exposure scenarios

In natural aquatic environments, fish populations typically experience intermittent rather than continuous pesticide exposure, with concentration peaks coinciding with agricultural application events and subsequent washout following rainfall. The recovery timelines established in this research indicate that haematological restoration requires a

minimum of 5 weeks of depuration for substantial recovery, suggesting that exposure intervals shorter than this threshold will result in cumulative haematological deterioration across successive exposure cycles.

Conclusion

The primary contribution of this research is the establishment of parameter-specific recovery timelines for haematological restoration in *Clarias gariepinus* following chlorpyrifos depuration. The investigation demonstrates that chlorpyrifos-induced haematological damage is partially reversible upon removal of the toxicant, but recovery rates vary substantially across blood cell lineages.

The supporting evidence reveals a recovery hierarchy with white blood cell count normalizing fastest ($t_{1/2} = 8.2$ days, 91.6% recovery at day 35), followed by packed cell volume ($t_{1/2} = 13.5$ days, 80%), red blood cell count ($t_{1/2} = 14.8$ days, 78.1%), haemoglobin ($t_{1/2} = 15.2$ days, 77.9%), and platelet count demonstrating the slowest recovery ($t_{1/2} = 22.4$ days, 64.1%).

The field significance of these findings lies in their applicability to environmental management scenarios where fish populations experience intermittent chlorpyrifos exposure. The 35-day incomplete recovery documented here suggests that exposure-free intervals must exceed 5-6 weeks for near-complete haematological restoration, a timeline that may not be achievable during intensive agricultural seasons with repeated pesticide applications.

Open questions remaining for future investigation include the influence of exposure duration on recovery kinetics, the role of nutritional supplementation in accelerating haematological restoration, the effect of concurrent environmental stressors on recovery capacity, and the long-term consequences of repeated exposure-recovery cycles on haematopoietic tissue integrity.

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