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Biochemical and Histopathological Responses of the Freshwater Crab *Paratelphusa jacquemontii* (Rathbun) Exposed to Cypermethrin: A Chemical and Toxicological Assessment

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Abstract
Agricultural pesticides are being used more frequently in freshwater habitats, which could have negative effects on aquatic creatures that are not intended targets. In addition to being significant members of aquatic and semi-aquatic food webs, freshwater crabs may be helpful bioindicators of environmental pollution. The biochemical and histological reactions of the freshwater crab Paratelphusa jacquemontii (Rathbun) to the synthetic pyrethroid insecticide cypermethrin were examined in this work. Crabs were treated to cypermethrin for various experimental durations after being acclimated to controlled laboratory conditions. The Lowry method was used to estimate the protein content of certain tissues, while normal biochemical techniques were used to evaluate the glycogen and lipid content. After fixation, dehydration, paraffin embedding, sectioning, and hematoxylin-eosin staining, histological changes in gill tissue were assessed.
Keywords: *Paratelphusa jacquemontii, cypermethrin, freshwater crab, pesticide toxicity, protein, glycogen, lipid, gill histology, biochemical biomarkers, environmental toxicology.*

1. Introduction

Agricultural intensification has resulted in increased use of synthetic pesticides for the control of insect pests. Pesticide residues can enter aquatic habitats through surface runoff, drainage; spray drift, and unintentional discharge, even though they are intended to target agricultural pests. Therefore, pesticide doses that are relevant to the ecosystem may be present in aquatic creatures that live in agricultural environments. Freshwater crabs play a significant ecological role in both terrestrial and aquatic food webs. They may come into touch with a range of chemical hazards due to their intimate relationship with sediment, water, and agricultural settings. As a result, their physiological and biochemical reactions can reveal important details about pollution in the environment.

Numerous research examining pesticide and heavy-metal toxicity have made use of the freshwater crab known as Paratelphusa jacquemontii in toxicological literature. Previous studies on this crab revealed metabolic alterations after copper exposure, such as decreased amounts of protein, sugar, and lipids in the hemolymph. After long-term exposure to the organophosphate pesticide malathion, other studies have shown changes in enzyme activity. Acid phosphatase, alkaline phosphatase, GOT, GPT, and ATPase activities were shown to have changed. A common synthetic pyrethroid for controlling agricultural pests is cypermethrin. Because polluted water and sediment may expose non-target aquatic creatures, its presence in aquatic settings is concerning.

The gills, hepatopancreas, and muscle of P. jacquemontii have shown significant histological damage in earlier experiments with cypermethrin, especially when combined with chlorpyrifos. Gill lesions that were reported included haemorrhage, oedema, necrosis, fusion of secondary lamellae, and epithelial lifting.

The present investigation was undertaken to evaluate the biochemical and histopathological effects of cypermethrin exposure in *P. jacquemontii*. Particular emphasis was placed on changes in tissue protein content and gill architecture. The study also establishes a framework for integrating biochemical chemistry with histopathological biomarkers in freshwater pesticide toxicology.

2. Literature Review

The freshwater crab *Paratelphusa jacquemontii* has been used as a suitable biological model for investigating the toxic effects of environmental contaminants. Maharajan et al. (2015) investigated the histological effects of combined chlorpyrifos and cypermethrin (Nurocombi) in *P. jacquemontii* following 28 days of sublethal exposure. The study reported pronounced pathological changes in the gills, including epithelial lifting, edema, necrosis, fusion of secondary lamellae and hemorrhage. Alterations were also observed in the hepatopancreas and

muscle, demonstrating that pesticide exposure can produce tissue-specific damage in freshwater crabs.

Chronic exposure to insecticides has also been associated with serious pathological changes in other crab species. Shen et al. (2021) investigated long-term exposure of the Chinese mitten crab *Eriocheir sinensis* to low concentrations of β-cypermethrin and trichlorfon. The study demonstrated pathological damage to the hepatopancreas and other tissues and suggested a relationship between chronic insecticide exposure and hepatopancreatic necrosis. Changes in immune-related and detoxification enzymes further demonstrated that prolonged exposure to low concentrations of insecticides can produce physiological disturbances even when concentrations are below acute toxicity levels.

Recent studies have increasingly emphasized oxidative stress as an important mechanism of cypermethrin toxicity. Sharma et al. (2021), investigating chronic cypermethrin exposure in *Catla catla*, reported changes in superoxide dismutase, catalase, glutathione-S-transferase and reduced glutathione, together with persistent elevation of lipid peroxidation. Histological and ultrastructural examination revealed tissue degeneration, vacuolation, mitochondrial damage and other cellular abnormalities. The study demonstrated a relationship between oxidative stress and tissue damage and supported the use of multiple biomarkers for evaluating chronic cypermethrin toxicity.

A 2023 review of pesticide toxicity in aquatic organisms emphasized that biochemical and histopathological parameters provide complementary information about pesticide-induced stress. Biochemical changes may reflect alterations in metabolism, immunity and physiological regulation, whereas histopathological changes provide direct evidence of tissue injury. Consequently, combining biochemical and histological biomarkers can provide a more comprehensive assessment of aquatic pollution than relying on a single endpoint.

Recent experimental research has further strengthened the evidence for multiple-biomarker assessment of cypermethrin toxicity. A 2024 study on common carp (*Cyprinus carpio*) reported that chronic cypermethrin exposure produced oxidative stress, altered hematobiochemical parameters, immune dysfunction, genotoxicity and histopathological damage. The study highlighted the usefulness of integrating physiological, biochemical, genotoxic and histological endpoints for assessing the chronic effects of cypermethrin.

More recent work in aquatic invertebrates has provided additional evidence for the biochemical and molecular effects of cypermethrin. A 2025 study on the clam *Ruditapes philippinarum* found that cypermethrin exposure activated antioxidant and detoxification responses and increased oxidative damage markers such as malondialdehyde and 8-hydroxydeoxyguanosine. Transcriptomic analysis indicated involvement of detoxification metabolism, oxidative stress, immune responses and apoptosis-related pathways. These findings are relevant to freshwater crustaceans because they demonstrate that cypermethrin toxicity may involve interconnected biochemical, cellular and molecular responses rather than a single toxicological pathway.

A recent 2026 publication specifically addressing *Paratelphusa jacquemontii* proposed an integrated framework involving contaminant analysis, histopathology and biomarker assessment. The study emphasized analysis of tissues such as gill, hepatopancreas and muscle, together with antioxidant and acetylcholinesterase-related biomarkers, for evaluating environmental contamination and potential ecological and human-health implications.

2. Materials and Methods

Collection and acclimatization

For the study, freshwater crabs known as Paratelphusa jacquemontii (Rathbun) were chosen. In the study area of Digras, District Yavatmal, Maharashtra, India, live, healthy crabs of roughly similar size were gathered from freshwater habitat. The animals were kept in appropriate freshwater containers after being properly transferred to the lab. Before the experiment started, the crabs were acclimated. The water's temperature and pH were kept at roughly 25°C and 7.0, respectively, during acclimatisation. During acclimatisation, the animals were kept in a lab setting and fed the proper food. Throughout the investigation, the water quality and overall health of the experimental animals were observed. Because of its widespread usage in agriculture and possible effects on aquatic creatures that are not the intended target, cypermethrin was chosen as the experimental toxicant.

A suitable stock solution was prepared according to the concentration required for the experimental design. The experimental concentration was selected as a sublethal concentration so that biochemical and histopathological responses could be studied without producing immediate mortality.

Experimental design

The animals were split up into groups for experimentation and control. Without being exposed to cypermethrin, the control group was kept in the same lab conditions. The chosen sublethal concentration of cypermethrin was administered to experimental groups. After 7, 14, 21, and 28 days, sampling was done. Appropriate tissues were gathered for histological and biochemical investigation at each sampling interval.

3. Biochemical Analysis

Protein estimation

With the appropriate adjustment for tissue samples, the Lowry method was used to determine protein content. Following homogenisation in the proper buffer, tissue samples were centrifuged. For protein estimation, the resultant supernatant was utilised. Using an appropriate protein standard, a standard calibration curve was created to determine the protein concentration.

Glycogen estimation

The anthrone technique was used to estimate tissue glycogen. Before being reacted with anthrone reagent, tissue samples were homogenised and properly treated. Spectrophotometry was used to measure the resulting colour intensity. The standard curve was used to compute the glycogen content, which was then expressed per unit weight of tissue. During pesticide-induced stress, changes in glycogen concentration were regarded as a sign of modified energy utilisation and carbohydrate metabolism..

Lipid estimation

A suitable lipid extraction method based on organic-solvent extraction was used to assess the total lipid content. After homogenising the tissue, appropriate organic solvents were used for extraction. Gravimetric analysis or the chosen analytical techniques were used to quantify the isolated lipid fraction. Changes in energy metabolism and membrane-associated biochemical reactions were thought to be indicated by lipid accumulation or depletion.

4. Histopathological Analysis

Tissue preparation

Gill tissues were carefully dissected from control and experimental crabs. The tissues were fixed in **Bouin's fixative** to preserve cellular and tissue architecture. Following fixation, tissues were dehydrated through a graded series of alcohol, cleared using a suitable clearing agent and embedded in paraffin wax. Sections of approximately **5 µm thickness** were prepared using a microtome.

5. Statistical Analysis

Depending on the lab design, biochemical data should be presented as mean ± standard error (SE) or mean ± standard deviation (SD). Depending on the experimental design, suitable statistical techniques like one-way or two-way ANOVA should be used to assess the impact of exposure duration and treatment. Tukey's multiple-comparison test can be used for post-hoc comparison when ANOVA shows significant differences. Statistical significance may be defined as a probability value of $p < 0.05$.

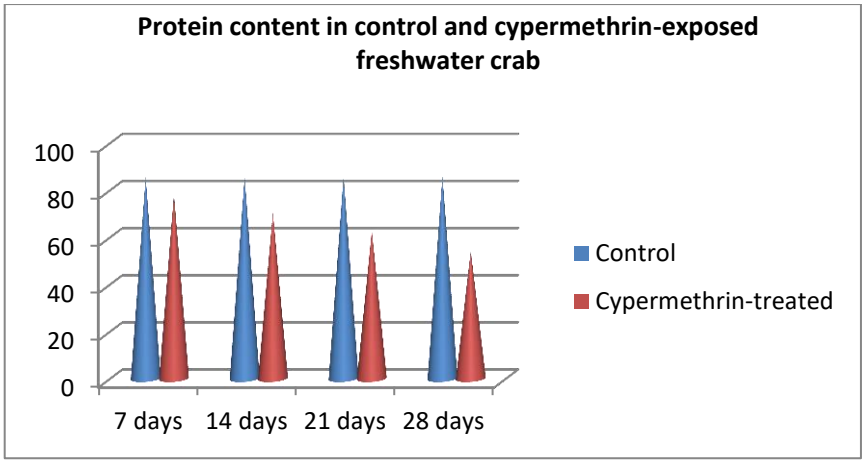
6. Results

Effect of cypermethrin on protein content

The biochemical observations demonstrated a progressive reduction in protein content in cypermethrin-exposed crabs during the experimental period.

Table 1. Protein content in control and cypermethrin-exposed freshwater crab

Exposure period	Control	Cypermethrin-treated
7 days	85.6	78.3
14 days	85.8	70.5
21 days	86.1	62.8
28 days	86.0	54.2

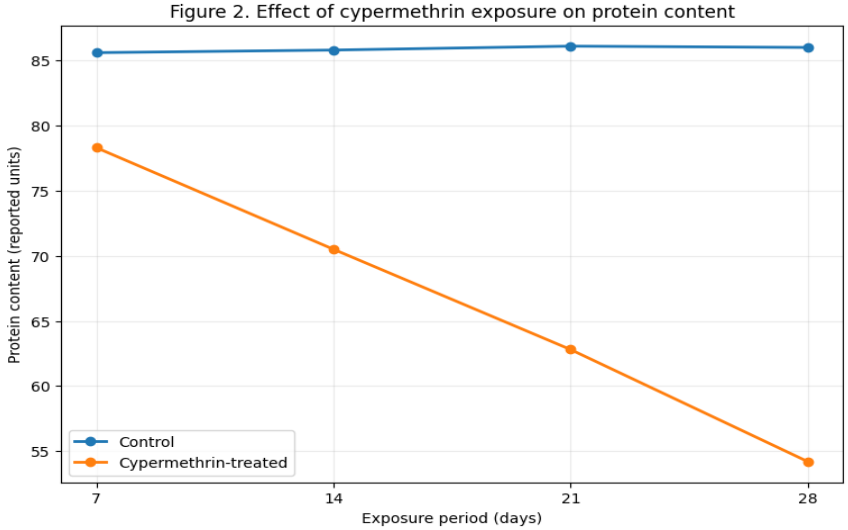


The control group showed relatively stable protein values throughout the experimental period, ranging from 85.6 to 86.1. In contrast, the cypermethrin-treated group exhibited a progressive decline from 78.3 at 7 days to 54.2 at 28 days.

Relative to the corresponding control values, the approximate reductions were:

- **7 days:** 8.5%
- **14 days:** 17.8%
- **21 days:** 27.0%
- **28 days:** 37.0%

Figure 2. Effect of cypermethrin exposure on protein content



The greatest reduction was observed after 28 days of exposure.

Figure 1. Effect of cypermethrin exposure on protein content of *Paratelphusa jacquemontii* at different exposure periods.

Histopathological Results

The arrangement of the gill epithelium and lamellar structures was relatively typical in the control gills. On the other hand, as exposure duration increased, the gills exposed to cypermethrin showed growing structural abnormalities. Gill tissue degradation, gill lumen enlargement, cellular necrosis, vacuolation, lamellae fusion, epithelial lifting, oedema, hemorrhagic changes, and distortion of the normal lamellar structure were the main histological changes seen. These lesions show significant gill tissue deterioration after extended pesticide exposure. The gills' regular physiological processes, such as respiration, ion control, and internal homeostasis maintenance, may be hampered by the observed structural changes. Similar histopathological changes have been reported in freshwater crabs exposed to combined chlorpyrifos and cypermethrin, including epithelial lifting, edema, necrosis, secondary lamellar fusion, and hemorrhage, supporting the interpretation that gill tissue represents an important target of pesticide-induced toxic stress.

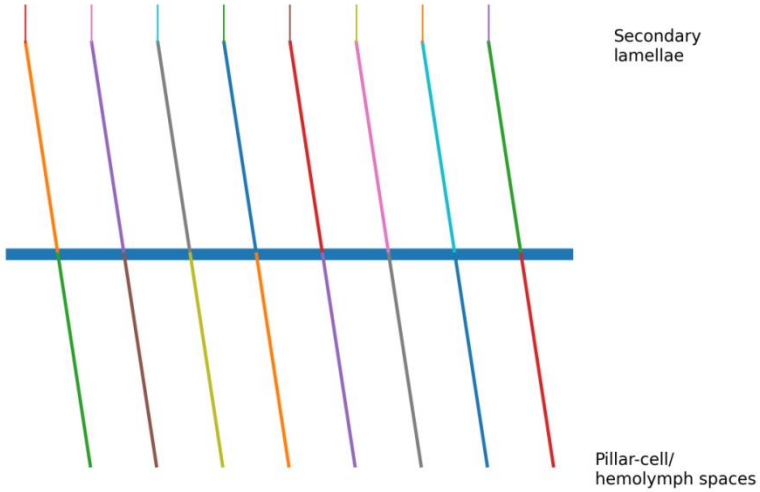


Figure 2: Normal Gill Structure

8. Discussion

The current study shows that exposure to cypermethrin significantly alters the freshwater crab Paratelphusa jacquemontii's biochemistry and histology. The treatment group's tissue protein content gradually decreased from 78.3 at 7 days to 54.2 at 28 days, but the control group's tissue protein content stayed mostly unchanged. Under pesticide-induced stress, this decrease implies higher protein breakdown, poorer synthesis, and increased use of proteins for energy. Protein and enzyme modifications are helpful markers of chemical stress, according to similar biochemical changes seen in crabs exposed to various pollutants and pesticides.

Histopathological examination further revealed degeneration, necrosis, vacuolation, lamellar fusion, epithelial lifting, edema, and hemorrhage in the gills. Since gills are immediately exposed to aquatic pollutants, these structural changes may affect respiration and osmoregulatory processes. Overall, cypermethrin-induced toxic stress in P. jacquemontii is strongly supported by the combined biochemical and histopathological responses, indicating that a multibiomarker approach is more helpful for evaluating pesticide toxicity than either biochemical or histological analysis alone.

Conclusion

The current study shows that the freshwater crab Paratelphusa jacquemontii exhibits quantifiable biochemical and histological changes as a result of extended exposure to cypermethrin.When compared to comparatively constant results in the control group, the exposed group's protein content gradually decreased from 78.3 at 7 days to 54.2 at 28 days, indicating significant metabolic disruption over extended pesticide exposure. Degeneration, vacuolation, necrosis, lamellar fusion, epithelial lifting, oedema, and haemorrhage are among the related gill lesions that offer structural proof of pesticide-induced harm.

An effective method for assessing environmental pesticide stress is the combined use of biochemical and histopathological indicators. The results also show that freshwater crabs may be useful as biological markers of aquatic pollution. A more thorough understanding of the molecular pathways underpinning cypermethrin toxicity would result from additional research including glycogen, lipid, antioxidant enzymes, oxidative-stress indicators, and pesticide residue studies.

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