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INTESTINAL DAMAGE AND BIOCHEMICAL CHANGES IN HELMINTH-INFECTED FRESHWATER FISHES FROM KELZAR DAM, MAHARASHTRA, INDIA

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ABSTRACT
Helminth parasites are among the major pathogens affecting freshwater fishes and may produce severe pathological and metabolic disturbances. The present study investigated histopathological alterations and biochemical changes in helminth-infected freshwater fishes collected from Kelzar Dam, Maharashtra, India. A total of 468 fishes belonging to eight species were examined during June 2021 to May 2025. Histopathological examination of intestine and liver tissues was carried out using standard Haematoxylin and Eosin staining methods. Biochemical estimation of protein, lipid and glycogen was performed using standard laboratory methods. Infected fishes showed villi erosion, haemorrhage, inflammatory infiltration, vacuolization and granultoma formation. Severe tissue damage was mainly associated with Pallisentis ophiocephali and Camallanus sp. infections. Significant reduction in protein (20.1%), lipid (23.1%) and glycogen (27.7%) was observed in infected fishes compared to non-infected controls ($p < 0.05$). Carnivorous fishes showed greater biochemical depletion than herbivorous and omnivorous species. Increased parasite burden was positively associated with metabolic reduction. Such pathological and metabolic disturbances may reduce fish growth, market quality and reservoir fishery productivity. The findings indicate that helminth infection significantly affects fish health and reservoir fishery productivity.

KEYWORDS: *Helminth infection, Histopathology, Biochemical, Freshwater fishes, Glycogen depletion*

INTRODUCTION
Freshwater fishes contribute significantly to inland fisheries and nutritional security in India. However, parasitic infections remain one of the major biological factors affecting fish health, growth and productivity (Mishra *et al*., 2021). Helminth parasites including nematodes, cestodes, trematodes and acanthocephalans commonly infect freshwater fishes and produce tissue damage, physiological stress and metabolic disturbances (Kumar and Sharma, 2022).

Histopathological examination is considered an effective approach for evaluating tissue damage caused by fish parasites. Intestinal helminths commonly produce epithelial degeneration, villi erosion, haemorrhage and inflammatory infiltration in infected fishes (Deshmukh *et al*., 2023). Acanthocephalans are highly pathogenic because their proboscis penetrates deeply into intestinal tissues causing severe tissue disruption and necrosis (Borkar *et al*., 2023). Nematode larvae may also migrate into liver tissues and induce vacuolization, granuloma formation and hepatocellular degeneration (Patil *et al*., 2021). Biochemical constituents such as protein, lipid and glycogen are important indicators of physiological stress in fishes. Helminth infection increases energy demand due to tissue repair and immune response, resulting in depletion of biochemical reserves (Sharma *et al*., 2022). Glycogen depletion is generally more pronounced because carbohydrates are utilized as the primary source of energy during stress conditions (Jadhav and Kaur, 2023). Reduction in protein and lipid content has also been reported in parasitized freshwater fishes due to impaired nutrient absorption and metabolic imbalance (Suryawanshi *et al*., 2024). Recent studies have emphasized the importance of integrated histopathological and biochemical investigations for understanding the impact of parasitic infection on fish health and fisheries management (Pawar and Gaikwad, 2024). However, information regarding histopathological and biochemical alterations in helminth-infected fishes from Kelzar Dam, Maharashtra, is limited. Therefore, the present study was undertaken to evaluate tissue damage and biochemical changes associated with helminth infection in freshwater fishes from this reservoir ecosystem.

MATERIALS AND METHODS
Study Area and Fish Collection
Kelzar Dam (locally called Gopal Sagar) is situated in Baglan Taluka of Nashik district, Maharashtra, India (20°39′06″N, 73°58′24″E). This earthfill dam was constructed across the Aram River in 1981 and has a water spread area of approximately 4.5 km², experiencing a tropical monsoonal climate with summer, monsoon and winter seasons. Freshwater fishes were collected monthly from three sampling sites (inlet, middle, outlet) during June 2021 to May 2025 using cast nets and gill nets. A total of 468 fishes belonging to eight species (*Catla catla*, *Labeo rohita*, *Cirrhinus mrigala*, *Channa punctatus*, *Clarias batrachus*, *Mystus seenghala*, *Oreochromis niloticus*, *Puntius sophore*) were examined. Infected fishes showing visible helminth infection and non-infected control fishes were selected for histopathological and biochemical

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analysis. Tissues from three infected fishes per species and three non-infected controls were processed for further study.

Histopathological Analysis

Intestinal and liver tissues were fixed in Bouin's fixative for 18–24 hours. Tissues were dehydrated through ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. Sections of 5–6 µm thickness were prepared using a rotary microtome and stained with Haematoxylin and Eosin following standard histological procedures (Humason, 1979). Histological sections were examined under a compound microscope and photomicrographs were recorded.

Biochemical Analysis

Liver and muscle tissues were homogenized in chilled phosphate buffer and centrifuged at 10,000 rpm for 15 minutes at 4°C. Total protein was estimated using the Folin-phenol method (Lowry *et al*., 1951), while total lipid was estimated following the method of Folch *et al*. (1957). Glycogen estimation was carried out using Anthrone reagent method. All biochemical analyses were performed in triplicate.

Statistical Analysis

Biochemical values were expressed as Mean ± SD. Student's t-test was applied to compare infected and non-infected fishes. Statistical significance was considered at $p < 0.05$.

RESULTS

Histopathological Alterations

Histopathological examination revealed marked tissue damage in infected fishes. Intestinal tissues infected with *Pallisentis ophiocephali* showed deep penetration of the proboscis, severe haemorrhage and destruction of intestinal villi. Infection with *Camallanus* sp. produced moderate villi erosion and inflammatory infiltration, whereas *Genarchopsis* sp. caused mild epithelial degeneration. Liver tissues infected with *Contracaecum* larvae exhibited vacuolization, necrosis and granuloma formation. The severity of tissue damage varied according to parasite type and infection intensity. Carnivorous fishes showed comparatively greater pathological alterations than herbivorous fishes. Detailed histopathological observations are presented in **Table 1.**

Table 1. Histopathological alterations observed in helminth-infected freshwater fishes

Organ	Parasite	Major pathological alteration	Severity
Intestine	*Pallisentis ophiocephali*	Deep tissue penetration and haemorrhage	Severe
Intestine	*Camallanus* sp.	Villi erosion and inflammatory infiltration	Moderate
Intestine	*Genarchopsis* sp.	Mild epithelial degeneration	Mild
Stomach	*Procamallanus* sp.	Mucosal inflammation	Moderate
Liver	*Contracaecum* larvae	Vacuolization and necrosis	Moderate
Liver	*Contracaecum* larvae	Granuloma formation	Mild

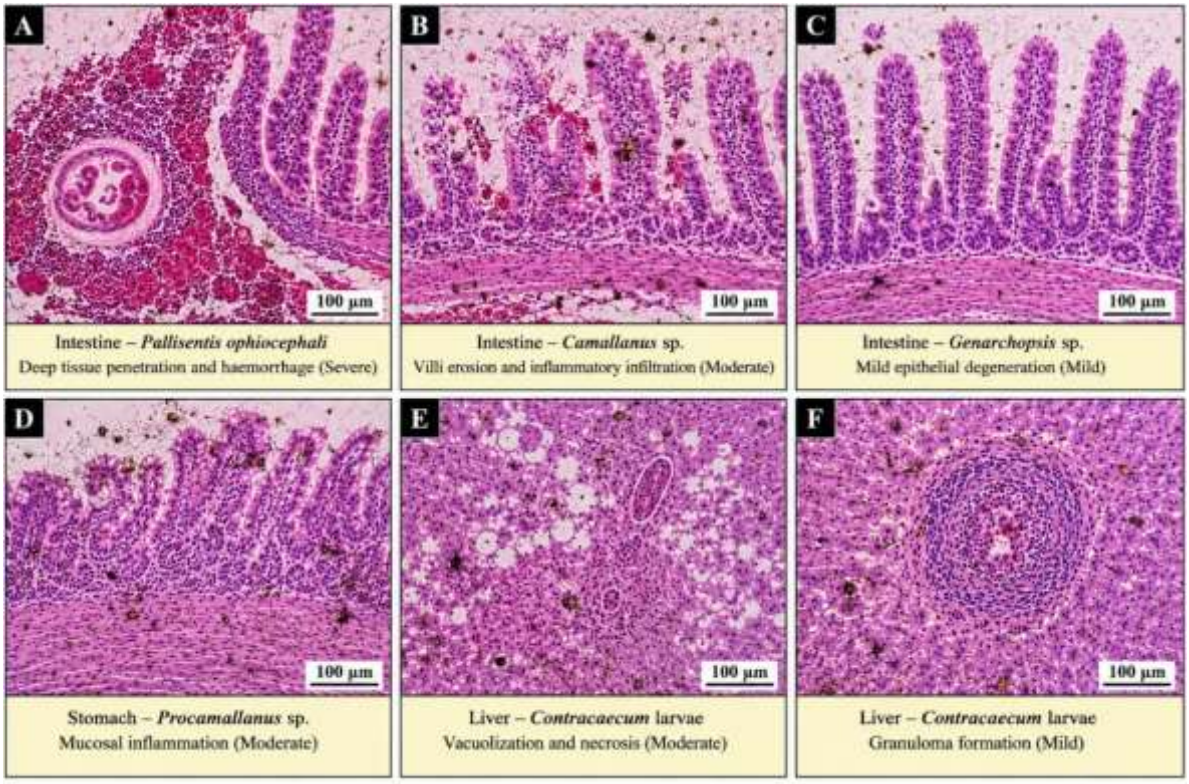

Figure 1. Histopathological alterations in helminth-infected freshwater fishes showing deep tissue penetration, villi erosion, epithelial degeneration, mucosal inflammation, vacuolization and granuloma formation in intestine, stomach and liver tissues.

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Biochemical Alterations in Helminth Infected Fishes

Biochemical analysis revealed significant reduction in protein, lipid and glycogen levels in infected fishes compared to non-infected controls ($p < 0.05$). Glycogen showed maximum depletion (27.7%) followed by lipid (23.1%) and protein (20.1%). Reduction in glycogen indicated increased energy utilization during parasitic stress. Lipid depletion suggested mobilization of stored energy reserves, while protein reduction reflected impaired nutrient absorption and tissue degeneration. Detailed biochemical values are presented in **Table 2.**

Table 2. Comparative biochemical parameters between infected and non-infected freshwater fishes

Parameter	Non-infected (Mean ± SD)	Infected (Mean ± SD)	Reduction (%)	*p*-value
Protein (mg/g)	82.4 ± 4.6	65.8 ± 3.9	20.1	$p < 0.05$
Lipid (mg/g)	38.6 ± 2.4	29.7 ± 2.1	23.1	$p < 0.05$
Glycogen (mg/g)	24.2 ± 1.8	17.5 ± 1.5	27.7	$p < 0.05$

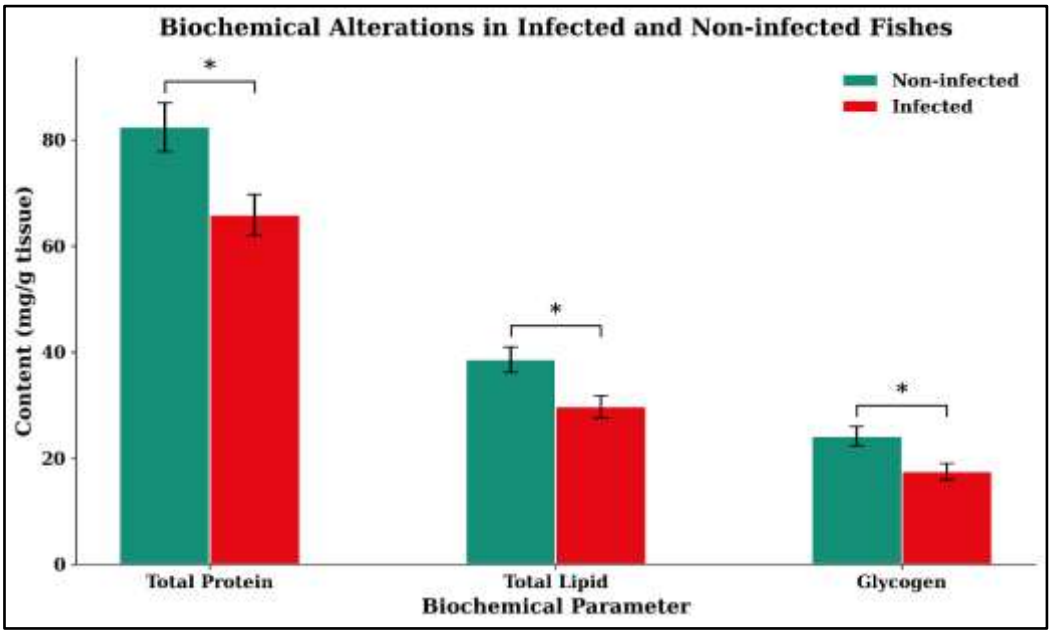

Figure 2. Comparative biochemical parameters in helminth-infected and non-infected freshwater fishes from Kelzar Dam. Protein, lipid and glycogen contents showed significant reduction in infected fishes compared to non-infected controls ($p < 0.05$).

Species-wise Biochemical Alterations in Helminth Infected Fishes

Variation in biochemical depletion was observed among different fish species. Carnivorous fishes such as *Channa punctatus*, *Clarias batrachus* and *Mystus seenghala* showed comparatively higher depletion of protein, lipid and glycogen than herbivorous and omnivorous fishes. *Channa punctatus* exhibited maximum glycogen reduction (31.2%) indicating severe metabolic stress. Herbivorous fishes showed comparatively lower biochemical depletion. These findings suggest that feeding behaviour and parasite burden influence the severity of metabolic disturbance in fishes. Species-wise biochemical reduction is presented in **Table 3.**

Table 3. Species-wise biochemical reduction in infected freshwater fishes

Fish species	Feeding habit	Protein reduction (%)	Lipid reduction (%)	Glycogen reduction (%)
Channa punctatus	Carnivorous	28.4	24.8	31.2
Clarias batrachus	Carnivorous	24.1	21.6	27.4
Mystus seenghala	Carnivorous	22.3	19.8	25.6
Labeo rohita	Herbivorous	13.4	10.6	14.1
Catla catla	Herbivorous	14.8	11.2	15.3
Oreochromis niloticus	Omnivorous	10.5	8.2	11.3

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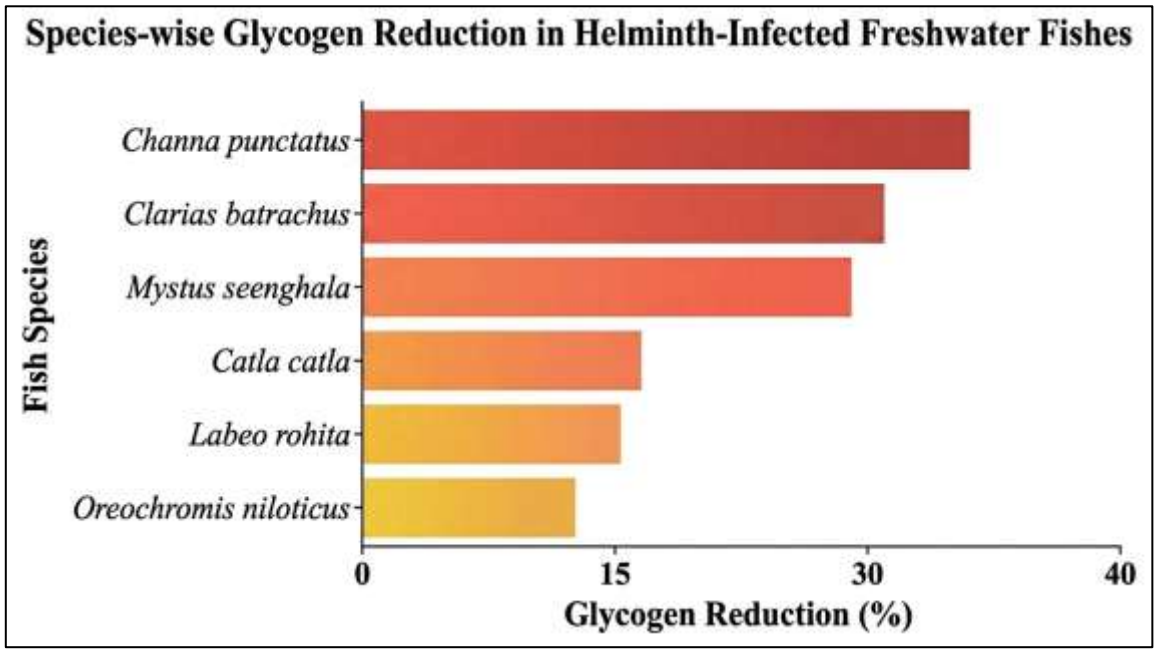

Figure 3. Species-wise glycogen reduction (%) in helminth-infected freshwater fishes from Kelzar Dam, Maharashtra. Carnivorous fishes showed comparatively higher glycogen depletion than herbivorous and omnivorous fishes, indicating greater metabolic stress due to parasitic infection.

DISCUSSION

The present investigation demonstrated that helminth parasites produce severe histopathological alterations in infected freshwater fishes. Intestinal villi erosion, haemorrhage and inflammatory infiltration observed in infected fishes indicate direct mechanical damage caused by parasite attachment and feeding activity. Similar pathological alterations have been reported in helminth-infected fishes by Deshmukh *et al*. (2023) and Borkar *et al*. (2023). Deep penetration of the proboscis by *Pallisentis ophiocephali* may be responsible for extensive tissue destruction and haemorrhage observed in the present study. Moderate vacuolization and granuloma formation observed in liver tissues infected with *Contracaecum* larvae indicate chronic inflammatory response and hepatocellular degeneration. Similar hepatic alterations have been documented in freshwater fishes by Patil *et al*. (2021). Granuloma formation may act as a protective response to isolate parasitic larvae from surrounding tissues and reduce further tissue injury. Pawar and Gaikwad (2024) also reported comparable liver pathology in nematode-infected fishes from Maharashtra. Biochemical analysis revealed significant depletion of glycogen, lipid and protein content in infected fishes. Maximum glycogen depletion (27.7%) observed in the present study suggests rapid utilization of carbohydrate reserves during parasitic stress. Similar reduction in glycogen levels has been reported in infected fishes by Sharma *et al*. (2022) and Jadhav and Kaur (2023). Lipid depletion (23.1%) may result from mobilization of stored energy reserves, while protein reduction (20.1%) may occur due to impaired nutrient absorption and increased metabolic demand (Suryawanshi *et al*., 2024). Carnivorous fishes showed greater biochemical depletion compared to herbivorous and omnivorous fishes. This may be associated with higher parasite burden and feeding behaviour of predatory fishes. *Channa punctatus* showed maximum glycogen reduction (31.2%) because this species harboured the highest parasite load. Increased biochemical reduction with increasing parasite load indicates that heavy infection produces greater physiological stress and metabolic disturbance. Similar observations have also been reported in recent fish parasitological investigations by Deshmukh *et al*. (2023) and Pawar and Gaikwad (2024). Rao *et al*. (2025) further confirmed that carnivorous fishes accumulate more helminth parasites through infected prey consumption, leading to greater metabolic depletion. Such pathological and metabolic disturbances may reduce fish growth, market quality and reservoir fishery productivity. These findings highlight the need for effective parasite control strategies to minimize economic losses in reservoir fisheries.

CONCLUSION

The present study confirmed that helminth infection causes significant histopathological and biochemical alterations in freshwater fishes from Kelzar Dam. Severe villi erosion, haemorrhage, inflammatory infiltration, vacuolization and granuloma formation were observed in infected fishes. Significant depletion of glycogen (27.7%), lipid (23.1%) and protein (20.1%) confirmed metabolic stress associated with parasitic infection. Carnivorous fishes showed comparatively greater biochemical disturbance than herbivorous and omnivorous species. Increased parasite burden resulted in higher biochemical depletion indicating dose-dependent physiological stress. These findings suggest that helminth infection may adversely affect fish growth, survival and reservoir fishery productivity.

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