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Research Article

A case of *Piscicola* sp. infestation in polyculture systems in Meghalaya: Prevalence, physiological impacts, and species-specific responses

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Abstract

This study reports the first occurrence of *Piscicola* sp. leech infestation in aquaculture systems in Meghalaya, India. The research examined the prevalence of leech infestation across six fish species in polyculture ponds and assessed the physiological impacts on infested fish. Among 300 fish specimens examined, common carp showed the highest prevalence (44%), followed by mrigal (30%) and rohu (16%), while catla remained uninfested. Infested fish, particularly common carp and mrigal, exhibited significant physiological changes, including reduced hematocrit, hemoglobin, and red blood cell counts, indicating anemia. Elevated white blood cell counts, plasma glucose, and cortisol levels in infested fish suggested active immune and stress responses. The study also observed lower condition factors in infested common carp and mrigal, potentially impacting growth rates and meat quality. The species-specific susceptibility and physiological responses highlight the complex nature of host-parasite interactions in polyculture systems. These findings emphasize the need for tailored management strategies and further research into resistance mechanisms observed in less susceptible species to mitigate the impact of leech infestations on aquaculture productivity in the region.

Keywords: Carp polyculture, Fish physiology, Hill aquaculture, Leech infestation, *Piscicola*

Highlights

- First report of *Piscicola* sp. leech infestation in Meghalaya's aquaculture systems.
- Infested fish, notably common carp and mrigal, showed significant anemia and stress.
- Leech infestation is linked to reduced condition factors in susceptible species, affecting yield.
- Findings stress need for tailored management in polyculture systems and further research.

INTRODUCTION

Aquaculture in the hill state of Meghalaya, a northeastern state of India, is characterized by polyculture systems that include various carp species such as common carp (*Cyprinus carpio*), mrigal (*Cirrhinus mrigala*), rohu (*Labeo rohita*), catla (*Catla catla*), gonius (*Labeo gonius*), and Java barb (*Barbonymus gonionotus*). While these systems offer economic benefits, they also present unique challenges in parasite management. Leeches of the genus *Piscicola* are known fish ectoparasites that can significantly impact fish health and aquaculture productivity (Bielecki *et al.*, 2011). Despite the potential threat posed by these parasites, there is limited information on leech infestations in fish from India in general and Northeast India in particular. This information is crucial for understanding the potential impact of leech infestations on fish health and for developing appropriate management strategies in aquaculture systems (Sahoo *et al.*, 2013). This study reports the first occurrence of leech infection in the aquaculture system in Meghalaya, India. We describe the prevalence of leech infestation across different fish species and present data on the physiological parameters of infested fish.

MATERIALS AND METHODS

The study was conducted at Ri Bhoi district of Meghalaya (25.93° N, 92.06° E) in June 2023. A total of 300 fish specimens (50 each of common carp, mrigal, rohu, catla, gonius, and Java barb) were randomly collected from different ponds at the Fisheries Farm, ICAR, Meghalaya, using cast nets and drag nets. Each fish was carefully examined for the presence of leeches, with particular attention to the skin, fins, and gills. Leeches were carefully removed using forceps and preserved in 70% ethanol for morphological identification (Kaygorodova and Matveenko, 2023). The prevalence of infestation was calculated for each fish species.

To assess the impact of leech infestation on fish health, we estimated several physiological parameters in both infested and non-infested fish of each species.

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Blood samples were collected from the caudal vein of 10 infested and 10 non-infested fish for each species using heparinized syringes. Hematocrit (Hct) values were determined using microhematocrit centrifugation method (Witeska *et al*., 2022). Hemoglobin (Hb) was measured using the cyanmethemoglobin method (Dacie and Lewis, 1995). Red blood cell (RBC) count and white blood cell (WBC) count were performed using a Neubauer hemocytometer following standard dilution protocols (Blaxhall and Daisley, 1973). Plasma glucose was estimated using a commercial glucose oxidase activity assay kit (Sigma-Aldrich) following the manufacturer's instructions. Plasma cortisol levels were determined using a commercial ELISA kit (Cortisol ELISA, Sigma-Aldrich) according to the manufacturer's protocol. Additionally, we recorded the total length (cm) and body weight (g) of all sampled fish. The condition factor (K) was calculated using the formula: $K = (W \times 100)/L^3$, where W is the weight in grams, and L is the total length in centimeters.

Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test to compare physiological parameters among different fish species and between infested and non-infested groups. Statistical significance was set at $p \leq 0.05$.

RESULTS

Morphological characteristics of leeches: The leeches identified in this study exhibited typical characteristics of the genus *Piscicola*. They possessed a cylindrical, elongated body with distinct anterior and posterior suckers. Clear segmentation was observed, with 14 body segments between the suckers. The anterior sucker featured two pairs of eyes arranged in a trapezoidal pattern. The leeches displayed a pale greenish-brown coloration with darker pigmentation patterns on the dorsal surface. Adult specimens ranged from 10-45 mm in length. The posterior sucker was noticeably larger than the anterior one. Upon closer examination, lateral crop caeca were visible through the semi-transparent body wall, a distinguishing feature of the genus *Piscicola* (Fig. 1). While these characteristics strongly indicated the genus, species-level identification would require more detailed microscopic examination and potentially genetic analysis. The specimens were preserved for future studies to determine the exact species.

Prevalence of leech infestation: The prevalence of leech infestation varied significantly among the six fish species examined in the polyculture ponds. Table 1 presents the detailed prevalence data for each species. Common carp showed the highest prevalence at 44%, with 22 out of 50 examined fish being infested. Mrigal followed with a 30% prevalence rate, where 15 out of 50 fish were infested. Rohu exhibited a lower prevalence of 16%, with 8 infested fish out of 50 examined. Gonius and Java barb showed relatively low prevalence rates of 6% and 4%, respectively, with only 3 and 2 infested fish

Table 1. Prevalence of *Piscicola* sp. infestation in different fish species

Fish species	Number examined	Number infested	Prevalence (%)
Common carp	50	22	44.0[a]
Mrigal	50	15	30.0[b]
Rohu	50	8	16.0[c]
Gonius	50	3	6.0[d]
Java barb	50	2	4.0[d]
Catla	50	0	0.0[e]

Different superscript letters indicate significant differences ($p<0.05$) between species; CD ($p=0.05$) = 3.2%

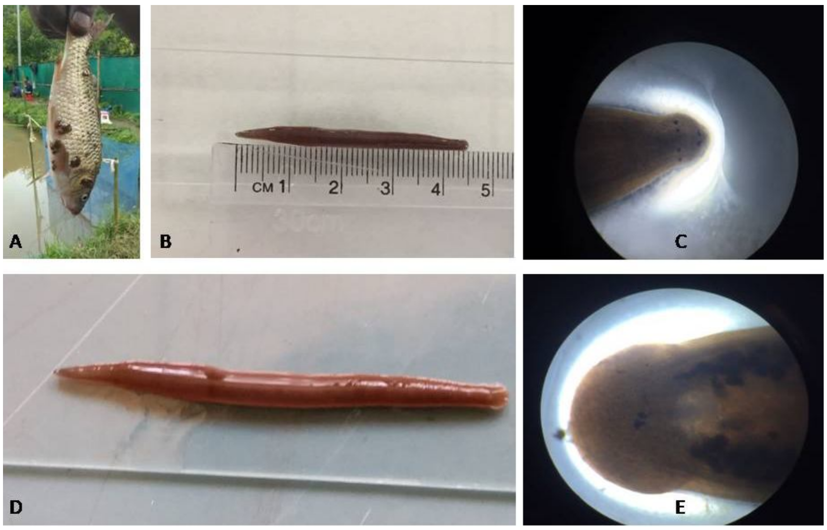

Fig. 1. A: Infested fish caught from polyculture pond showing leech attachments; B: Leech specimen measured against centimeter scale, showing length of approximately 4-5 cm; C: Microscopic view of leech anterior sucker; D: Preserved leech specimen displaying elongated body shape; E: Microscopic view of leech posterior sucker

out of 50 for each species. Notably, catla remained completely uninfested, with no leeches found on any of the 50 examined specimens.

Physiological parameters: The study assessed various physiological parameters in both infested and non-infested fish to evaluate the impact of leech infestation on fish health, with detailed results presented in Table 2. Key findings highlight the pronounced effects of infestation on different fish species. Hematocrit (Hct) levels were significantly lower in infested fish, with common carp showing the most drastic reduction, averaging 27.1% compared to 35.2% in non-infested fish. Mrigal also displayed decreased Hct levels, averaging 29.3% in infested fish versus 33.8% in non-infested fish, while rohu exhibited a significant but less pronounced difference.

Hemoglobin (Hb) levels were notably reduced in infested fish, with common carp experiencing the most substantial drop, showing a mean Hb of 7.2 g/dL compared to 9.8 g/dL in non-infested fish. Mrigal and rohu also showed significant reductions, though less severe than those observed in common carp. The red blood cell (RBC) count was significantly lower in infested fish, especially in common carp and mrigal. Infested common carp had an average RBC count of $1.6\times10^6/\mu L$ compared to $2.1\times10^6/\mu L$ in non-infested fish, with a similar trend in mrigal, while the difference in rohu was less pronounced.

In contrast, the white blood cell (WBC) count was elevated in infested fish, most notably in common carp, which had a mean WBC count of $28.7\times10^3/\mu L$ compared to $18.5\times10^3/\mu L$ in non-infested fish. Mrigal also exhibited a significant increase, though the change in rohu was less pronounced. Plasma glucose levels were elevated in infested fish, indicating a stress response, with common carp showing the highest increase, averaging 92.6 mg/dL compared to 65.3 mg/dL in non-infested fish. Mrigal also showed a significant increase, while the change in rohu was less marked. Similarly, plasma cortisol levels, another stress indicator, were significantly higher in infested fish, with common carp showing a dramatic increase to 72.9 ng/mL compared to 38.2 ng/mL in non-infested fish. Mrigal and rohu also exhibited significant increases, though less severe than those seen in common carp. Finally, the condition factor, which reflects the overall health and nutritional status of the fish, was lower in infested fish, with common carp showing the most pronounced reduction, averaging 1.43 compared to 1.68 in non-infested fish. Mrigal also showed a significant reduction, while the change in rohu was less pronounced.

Species-specific responses: The physiological responses to leech infestation demonstrated a clear correlation with species-specific susceptibility patterns. Common carp, which showed the highest infestation prevalence (44%), exhibited the most severe physiological alterations: significant reductions in hematocrit (27.1% vs 35.2%),

Table 2. Physiological parameters of infested and non-infested fish (mean ± SD)

Parameter	Status	Common carp	Mrigal	Rohu	Gonius	Java barb
Hematocrit (%)	Non-infested	35.2±2.8	33.8±2.5	32.5±2.3	32.7±2.1	32.9±2.2
	Infested	27.1±3.1***	29.3±2.8**	29.8±2.5*	30.1±2.2	31.3±2.0
Hemoglobin (g/dL)	Non-infested	9.8±0.7	9.2±0.6	8.9±0.5	8.8±0.5	8.9±0.4
	Infested	7.2±0.8***	8.1±0.7**	8.2±0.6*	8.3±0.4	8.5±0.3
RBC ($\times10^6/\mu L$)	Non-infested	2.1±0.2	2.0±0.2	1.9±0.2	1.9±0.1	2.0±0.1
	Infested	1.6±0.2***	1.7±0.2**	1.8±0.2	1.8±0.1	1.9±0.1
WBC ($\times10^3/\mu L$)	Non-infested	18.5±2.1	17.8±1.9	16.9±1.8	17.2±1.7	17.0±1.6
	Infested	28.7±3.3***	24.5±2.7***	19.2±2.1	18.5±1.5	17.8±1.3
Glucose (mg/dL)	Non-infested	65.3±5.7	62.7±5.1	60.5±4.8	61.8±4.5	61.2±4.2
	Infested	92.6±8.1***	78.4±6.9**	67.3±5.5	65.2±4.3	63.7±3.9
Cortisol (ng/mL)	Non-infested	38.2±4.5	35.6±4.1	33.7±3.8	34.5±3.6	34.1±3.4
	Infested	72.9±7.8***	58.2±6.5***	45.1±5.2**	41.3±3.5	39.8±3.2
Condition factor	Non-infested	1.68±0.12	1.72±0.11	1.70±0.10	1.71±0.09	1.70±0.08
	Infested	1.43±0.15***	1.55±0.13**	1.62±0.12	1.65 ± 0.08	1.67±0.07

Significance levels: * $p<0.05$, ** $p<0.01$, *** $p<0.001$ (compared to non-infested fish of the same species). For gonius and Java barb, statistical comparisons were not performed due to the low number of infested individuals. Catla is not included in the table as no infested individuals were found.

hemoglobin (7.2 vs 9.8 g/dL), and RBC counts (1.6 vs 2.1×106/µL), along with markedly elevated WBC counts (28.7 vs 18.5×10^3/µL), plasma glucose (92.6 vs 65.3 mg/dL), and cortisol levels (72.9 vs 38.2 ng/mL). These changes were accompanied by the lowest condition factor (1.43 vs 1.68) among all species. Mrigal, with the second-highest prevalence (30%), displayed moderate but significant alterations across all parameters, particularly in stress indicators (glucose: 78.4 vs 62.7 mg/dL; cortisol: 58.2 vs 35.6 ng/mL). Rohu, showing lower susceptibility (16% prevalence), exhibited significant changes primarily in hematological parameters (hematocrit: 29.8% vs 32.5%) and stress indicators (cortisol: 45.1 vs 33.7 ng/mL), while other parameters showed minimal alterations. Gonius and Java barb, with very low prevalence rates (6% and 4%, respectively), showed negligible physiological changes, though statistical comparisons were limited by the small number of infested individuals. Catla's complete resistance to infestation (0% prevalence) precluded any comparison of physiological parameters. This clear gradient of physiological impacts corresponding to species susceptibility suggests potential differences in host-parasite interactions and resistance mechanisms among these species, with common carp and mrigal being most vulnerable while catla appears to possess effective resistance mechanisms.

DISCUSSION

The present study provides the first comprehensive report of *Piscicola* sp. leech infestation in aquaculture systems in Meghalaya, India, offering valuable insights into the prevalence, physiological impacts, and species-specific responses to this parasitic infection. The findings reveal a complex interplay between host species susceptibility and the physiological consequences of infestation, with significant implications for aquaculture management in the region.

Prevalence and species susceptibility: The observed pattern of leech infestation prevalence, with common carp being the most affected, followed by mrigal and rohu, while catla remained uninfested, suggests strong species-specific factors influencing host-parasite interactions. This differential susceptibility to leech infestation among fish species in polyculture systems aligns with observations reported in other studies (Woo, 2006; Nagasawa and Tanaka, 2011).

The susceptibility of common carp to leech infestation compared to other species, such as catla, may be influenced by several factors. One significant factor is the chemical composition of fish mucus, which plays a crucial role in protecting against parasites and pathogens. Species-specific differences in mucus composition can affect parasite susceptibility, with common carp mucus potentially being more attractive or less repellent to leeches (Reverter *et al.*, 2018). Additionally, the feeding behavior of common carp, which involves bottom-feeding and rooting in sediments, may increase their exposure to leech-infested areas of the pond (Sibbing, 1988).

Immune response differences also play a role, as common carp may have a less effective immune response against specific leech species. Variations in immune system components and responses among fish species have been documented (Alvarez-Pellitero, 2008). Furthermore, the habitat preferences of common carp may overlap more closely with the preferred microhabitats of leeches, as *Piscicola* species have specific microhabitat preferences within aquatic ecosystems (Bielecki *et al.*, 2011). Finally, differences in skin characteristics, such as skin thickness and scale patterns, could also influence the susceptibility of different fish species to leech attachment.

Physiological impacts: The physiological changes observed in infested fish, particularly in common carp and mrigal, provide critical insights into the impact of *Piscicola* sp. on fish health. The significant reduction in hematocrit, hemoglobin, and RBC counts in infested fish indicates anemia, a common consequence of blood-feeding parasites (Woo, 2006). This anemia can lead to reduced oxygen-carrying capacity of the blood, potentially affecting the fish's growth, reproduction, and overall fitness (Noga, 2010).

The severity of anemia observed in common carp (27.1% hematocrit in infested vs. 35.2% in non-infested) is comparable to findings in other studies on leech-infested fish (Santos *et al.*, 2020). This level of anemia can significantly impair physiological functions, potentially leading to reduced growth rates, decreased disease resistance, and increased mortality under stressful conditions.

The elevated WBC count in infested common carp and mrigal suggests an active immune response against the parasites. While this immune activation is a normal defensive mechanism, it comes at an energetic cost. This increased immune activity may divert energy resources from growth and other physiological processes, contributing to the observed lower condition factor in these species (Tavares-Dias and Moraes, 2007).

The higher plasma glucose levels in infested common carp and mrigal indicate a stress response. This is further supported by the elevated cortisol levels in these species.

Chronic stress can have far-reaching consequences, including suppressed immune function, making fish more susceptible to secondary infections and further compromising their health status (Barton, 2002). The long-term elevation of stress hormones can also negatively impact growth, reproduction, and overall fitness.

Species-specific responses: The differential physiological responses observed among fish species offer valuable insights into host-parasite interactions and potential resistance mechanisms. Common carp were the most severely affected, displaying pronounced changes across all physiological parameters. This severe response suggests that common carp may be particularly vulnerable to *Piscicola* sp. infestation, potentially due to a combination of higher susceptibility and less effective physiological coping mechanisms.

Mrigal also experienced significant physiological changes, though slightly less severe than those observed in common carp. This suggests that while mrigal is highly susceptible, it may possess a somewhat better ability to cope with the physiological stress of infestation. Rohu exhibited significant changes mainly in hematocrit, hemoglobin, and cortisol levels, with other parameters being less affected. This indicates a potentially higher tolerance to leech infestation compared to common carp and mrigal, possibly due to more effective physiological adaptation mechanisms or immune responses (Alvarez-Pellitero, 2008).

In contrast, gonius and Java barb displayed low infestation rates and minimal physiological changes, suggesting potential resistance mechanisms that warrant further investigation. The complete absence of infestation in catla is particularly noteworthy, pointing to highly effective resistance mechanisms. Understanding these mechanisms could provide valuable insights for developing selective breeding programs aimed at enhancing leech resistance in more susceptible species (Robinson *et al*., 2017).

Implications for aquaculture: The lower condition factor observed in infested common carp (1.43 vs. 1.68 in non-infested) and mrigal (1.55 vs. 1.72 in non-infested) is particularly concerning from an aquaculture perspective. Reduced condition factor is often associated with decreased growth rates and lower meat quality, which could have significant economic implications for fish farmers in the region (Sahoo *et al*., 2013). The combination of reduced growth, potential increased mortality, and the cost of parasite management could substantially impact the profitability of aquaculture operations.

The species-specific differences in susceptibility and physiological responses highlight the complex nature of host-parasite interactions in polyculture systems. These findings emphasize the need for tailored management strategies that consider the varying susceptibilities and responses of different fish species. For example, adjusting the species composition in polyculture systems to reduce the proportion of highly susceptible species like common carp might help mitigate the overall impact of leech infestations.

Ecological considerations: The impact of *Piscicola* sp. on wild fish populations in Meghalaya also warrants investigation. If these parasites are present in natural water bodies, they could pose a threat to native fish species, potentially affecting local biodiversity and ecosystem health (Hoffman and Williams, 1999). Understanding the distribution and impact of these parasites in natural ecosystems is crucial for both conservation efforts and sustainable aquaculture practices.

Future research directions: Several key areas for future research have emerged from this study.

First, a detailed species identification effort is needed to accurately determine the *Piscicola* species responsible for these infestations, involving both morphological and genetic analysis. Understanding the specific species involved will help tailor management strategies and control measures.

Second, investigating the resistance mechanisms in catla, as well as the low susceptibility observed in gonius and Java barb, is crucial. This research should explore the physiological, immunological, and behavioral factors that contribute to their resistance and lower susceptibility to leech infestations.

Third, assessing the long-term impacts of chronic leech infestation on fish growth, reproduction, and survival in aquaculture settings is important. This will provide insights into how prolonged exposure to leeches affects overall fish health and productivity.

Fourth, ecological studies should examine the prevalence and impact of *Piscicola* sp. in natural water bodies in Meghalaya. Understanding the distribution and ecological effects of these parasites in natural environments will inform more comprehensive management strategies.

Lastly, the development and testing of targeted management strategies are needed. This could include potential biological control methods or selective breeding programs aimed at increasing resistance to leech infestations. Effective management strategies will be essential for mitigating the impact of leeches on aquaculture and natural fish populations.

Management recommendations: Based on the findings of this study, we recommend several management strategies to effectively address leech infestations in polyculture systems. First, implementing regular health monitoring is crucial to detect leech infestations early. Routine health checks will enable prompt intervention and help mitigate the impact of infestations before they escalate. Additionally, maintaining optimal water quality is essential to reduce stress on fish, which can potentially lower their susceptibility to parasites. Good water management practices will support overall fish health and resilience against infestations.

Another important measure is the establishment of strict quarantine protocols for newly introduced fish. This will help prevent the introduction of leeches into uninfested ponds and limit the spread of infestations. Adjusting species composition in polyculture systems may also be beneficial, particularly by limiting the proportion of highly susceptible species such as common carp. Moreover, developing species-specific treatment protocols that consider the varying susceptibilities and physiological responses of different fish species can enhance the effectiveness of control measures. Finally, initiating selective breeding programs focused on leech resistance, particularly leveraging the apparent resistance observed in catla, could contribute to long-term management by enhancing resistance traits in future generations.

In conclusion, this study provides crucial insights into the complex dynamics of *Piscicola* sp. infestation in polyculture systems in Meghalaya. The species-specific susceptibility and physiological responses observed underscore the need for tailored management approaches in aquaculture. Furthermore, the findings open up new avenues for research into resistance mechanisms and the ecological implications of these parasites in both managed and natural aquatic ecosystems. As aquaculture continues to expand in the region, understanding and effectively managing such parasitic infestations will be crucial for ensuring sustainable and productive fish farming practices.

Conflict of interest: The author declares no conflict of interest in this study.

Author's contribution: CD: Involved in investigation, data generation, data curation, statistical analyses, methodology, preparing original draft and final editing.

Data availability statement: All the data supporting the research findings have been presented in this paper. The corresponding author is willing to provide the raw data upon reasonable request.

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