

Prevalence and intensity of ectoparasites in giant freshwater prawn: Implications for aquaculture

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Article information

Article history:

Received 07 February 2025

Accepted 02 July 2025

Published 20 August 2025

Keywords:

Low productivity

Mortality

Organic matter

Temnocephala sp.

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Abstract

The giant freshwater prawn (GFP) is highly susceptible to ectoparasite infestations, particularly under suboptimal management conditions. This study investigates the prevalence and intensity of ectoparasites on various prawn body parts, including the gills, body surface, swimming legs, and walking legs. A total of 180 prawns were sampled from the GFP culture ponds located in the Subang region of West Java, Indonesia. These samples were then analyzed microscopically. Identified ectoparasites included protozoans (*Epistylis* sp., *Zoothamnium* sp., *Vorticella* sp.) and *Temnocephala* sp. The prevalence and intensity levels were as follows: *Temnocephala* sp. (100%; 907 individuals/prawn), *Zoothamnium* sp. (27%; 35 individuals/prawn), *Vorticella* sp. (27%; 26 individuals/prawn), and *Epistylis* sp. (29%; 4 individuals/prawn). The gills exhibited the highest prevalence (100%) and intensity (550 individuals/prawn), severely impacting respiration and increasing mortality risk. These findings highlight the urgent need for effective parasite management strategies in GFP aquaculture to minimize economic losses and enhance sustainability.

DOI: [10.3389/ijvs.2025.157309.4116](https://doi.org/10.3389/ijvs.2025.157309.4116). ©Authors, 2025, College of Veterinary Medicine, University of Mosul.

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Introduction

Giant freshwater prawn (*Macrobrachium rosenbergii*) is an economically significant species in global aquaculture. However, its cultivation faces numerous challenges due to a combination of biotic and abiotic stressors, including disease outbreaks and ectoparasite infestations, which can lead to substantial economic losses (1). Ectoparasites pose a serious threat to prawn health by impairing feeding, hindering growth, and increasing mortality. Several ectoparasitic ciliates, including *Zoothamnium*, *Trichodina*, and *Vorticella*, have been reported to colonize the exoskeleton and appendages of prawns, disrupting their physiological functions and negatively impacting production yields (2). The association between ectoparasitic infections and secondary disease, including viral infections, highlights the necessity of implementing robust biosecurity measures and effective management strategies to mitigate their impact and sustain production (3,4). Ectoparasites of *M. rosenbergii*,

including protozoans and monogeneans, attach to the external surface, feeding on epidermal tissues or body fluids (5). These infestations can result in impaired feeding, slowed growth, increased susceptibility to disease, and in severe cases, mortality (6). The impact of ectoparasite infections is particularly pronounced in intensive aquaculture systems, where high stocking densities and poor water quality create favorable conditions for parasite proliferation (7). Crustaceans, both in cultured and natural environments, serve as hosts to a variety of protozoan and metazoan of parasites, some which exhibit considerable pathogenicity, adversely affecting growth and reproductive performance (8). Ectoparasite infestations in freshwater giant prawns, particularly *Macrobrachium* species, have been documented across various geographical regions, demonstrating high prevalence and intensity. In the Amazon, *Macrobrachium amazonicum* has been reported to harbor crustacean ectoparasites such as *Probopyrus pandalicola*, *P. floridensis* and *P. bithynis*, which reside in the gill cavities and

significantly affect host health and condition factors (9). Similarly, in India, *M. rosenbergii* is commonly infested with ciliates such as *Zoothamnium*, *Trichodina* and *Vorticella*. Studies conducted in West Bengal have identified ectoparasites with higher prevalence in larger prawns, suggesting that infestation rates are influenced by size and seasonal variations (10). In Asia, the viral, bacterial, and parasitic infections of *M. rosenbergii* have been recognized as major contributors to pond production losses (10). However, while studies on penaeid shrimp parasites have been extensively conducted in Indonesia, research on the ectoparasite burden in giant freshwater prawn farming remains limited. Understanding the presence and interactions of ecto- and endoparasites in GFP aquaculture is crucial for formulating effective management strategies to mitigate their negative effects (4).

This study aims to investigate the prevalence and intensity of ectoparasites in giant freshwater prawn (*M. rosenbergii*) in aquaculture ponds with different rearing times in Subang, West Java, Indonesia. By identifying the key factors influencing parasite infestation and their associated economic impact, this research will contribute to the development of sustainable disease management practices in giant freshwater prawn aquaculture.

Materials and methods

Ethical approval

This study was conducted with the approval of Research Ethic Committee, National Research and Innovation Agency, under protocol number Jakarta/4/2/25.

Study Site

The study was conducted at a government-operated fish breeding facility in Subang, West Java, Indonesia, a site for its diverse aquaculture practices and multiple stages of giant freshwater prawn (GFP) rearing. The prawns were cultured in earthen ponds using a semi-intensive system, with a stocking density of 10 individuals per square meter.

Sample collection

A total of 180 prawns were randomly collected from three earthen ponds, each representing a distinct rearing period (2, 4, and 6 months). A random sample of 60 prawns was obtained from each pond using a cast net, thus ensuring a representative sample of the prawn population. The collected Specimens were immediately placed in sterile containers and transported to the Fish Health Laboratory at the Fish Breeding Research Centre in Subang for further examination.

Dissection and parasite identification

To assess ectoparasite infestation, the specimens underwent meticulous dissection, which was performed under a stereomicroscope for precise observation. Key

anatomical regions targeted for examination included the gills, body surface, swimming legs, and walking legs-areas frequently associated with ectoparasite colonization. The swimmerets (pleopods) and walking legs were carefully dissected using sterile surgical scissors, mounted onto glass slides for microscopic analysis. To enhance visibility and facilitate analysis, a drop of physiological NaCl solution was applied to each slide before being covered with a cover glass. The prepared slides were then examined under a microscope at magnifications of 40x and 100x, allowing for detailed observation and identification of ectoparasites. Mucus samples from the carapace and gills were collected by gently scraping the surface with a sterilized toothpick, spread onto glass slides, and analyzed under the same microscopic conditions. The morphological identification of ectoparasites was based on established taxonomic keys, focusing on body shape, size, and distinguishing anatomical features (11-13).

Quantification of parasite infestation

Following identification, ectoparasite quantification was conducted by systematically counting individual parasites per prawn. The recorded data were organized in a structured spreadsheet for statistical analysis, facilitating the evaluation of infestation intensity across different rearing periods. This approach enabled the determination of the relationship between prawn growth stage and ectoparasite prevalence.

Data analysis

The prevalence (P) and mean intensity (MI) of the ectoparasites infestations were calculated using standard epidemiological formulas (14): prevalence (%) = $(\sum \text{ectoparasite infected prawns} / \sum \text{examined prawn}) / 100$; Intensity = $\sum \text{observed ectoparasite} / \sum \text{infected prawn}$. The severity of infections was categorized according to established classification criteria, as presented in tables 1 and 2 (15,16).

Table 1: Categories of ectoparasite prevalence levels

Rules %	Category	Information
100-99	Always	Very severe infection
98-90	Almost always	Severe infection
89-70	Usually	Moderate infection
69-50	Very often	Widespread infection
49-30	Generally	Common infection
29-10	Often	Frequent infection
9-1	Sometimes	Infection sometimes
< 1-0.01	Rarely	Rare infection
<0.1-0.01	Very rarely	Very rare infection
<0.01	Almost never	Infection never

Water quality monitoring

To evaluate environmental influences on ectoparasites prevalence, water quality parameters in the giant freshwater ponds were systematically monitored over 60 days. Weekly

in-situ measurements included temperature, pH, and dissolved oxygen using a multi-parameter water quality checker. Additionally, ammonia (NH₃) and nitrite (NO₂) levels were analysed in a laboratory using a spectrophotometer, employing the phenate and sulfanilamide methods respectively.

Table 2: The intensity level of ectoparasitic infection

Intensity (parasite/prawn)	Category
Very low	1
Low	1-5
Medium	6-55
Severe	51-100
Awfully	>100
Superinfection	>1000

Results

The presence of two types of ectoparasites infecting giant freshwater prawns was observed: metazoa (*Temnocephala* sp.) and protozoa (*Zoothamnium* sp., *Vorticella* sp., and *Epistylis* sp.) (Figure 1). The prevalence and intensity of the ectoparasites were documented in Table 3. The gills of giant freshwater prawns are highly susceptible to parasitic infections, as evidenced by a 100% prevalence rate and an intensity of 550 parasites per infected prawn (Table 4). This vulnerability is likely due to the gills' exposed and delicate nature, making them a prime target for parasites. The *Temnocephala* population on the giant freshwater prawn gills is shown in Figure 2. The body carapace and swimming legs also show signs of infection, although to a lesser extent, with prevalence rates of 28.3 and 26.7%, respectively. All cultured giant freshwater prawns, regardless of age 2, 4, or 6 months, were infected with ectoparasites. The intensity of ectoparasite infections increased with increasing prawn size (Table 5). In line with the data in Table 3, the intensity of ectoparasite infection also increased in larger prawns (Table 6). The ectoparasite population is significantly influenced by pond conditions, particularly the concentration of dissolved organic matter. Pond ammonia and nitrite levels serve as indicators of the level of organic matter in the pond. Water quality parameters over the 60-day observation period are presented in table 7.

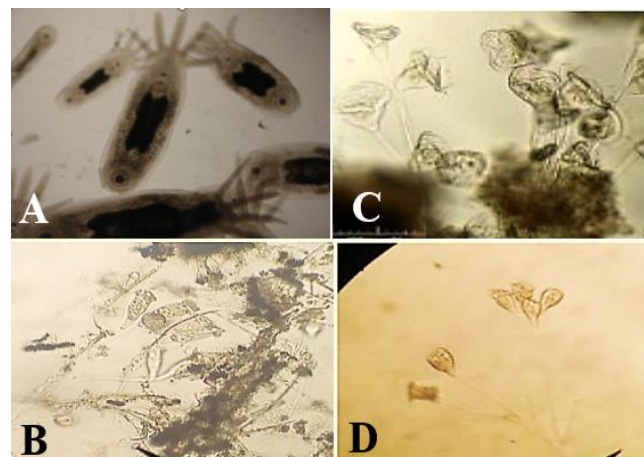


Figure 1: The ectoparasites collected from the giant freshwater prawn: (a) *Temnocephala* sp. (10x); (b) *Epistylis* sp. (40x); (c) *Vorticella* sp. (40x); (d) *Zoothamnium* sp (40x).

Table 3: Ectoparasite in giant freshwater prawn

Ectoparasite	Prevalence (%)	Intensity
<i>Temnocephala</i> spp.	100	907
<i>Zoothamnium</i> sp.	27	35
<i>Vorticella</i> sp.	27	26
<i>Epistylis</i> sp.	29	4



Figure 2: The *Temnocephala* population on the giant freshwater prawn gills.

Table 4: Prevalence and intensity of ectoparasite in the different organs of giant freshwater prawn

Observed organ	Parasite number	Infected prawn number	Prevalence (%)	Intensity
Swimming legs	400	16	26,7	25
Walking legs	0	0	0	0
Body carapace	663	17	28,3	39
Gills	33,000	60	100	550

Table 5: Prevalence and intensity of ectoparasite in giant freshwater prawn in different rearing periods

Rearing period	Observed prawn number	Infected prawn number	Parasite number	Prevalence (%)	Intensity
4 months	60	60	6,767	100	338
6 months	60	60	11,659	100	583
8 months	60	60	41,616	100	2,081

Table 6: Prevalence and intensity of ectoparasite in giant freshwater prawn based on prawn total body length

Interval Class	Observed prawn number	Infected prawn number	Parasite number	Prevalence (%)	Intensity
9.1 – 11.5 cm	10	10	2954	100	295
11.6 – 13.0 cm	15	15	7193	100	476
13.1 – 14.0 cm	13	13	8321	100	640
14.1 – 17.0 cm	22	22	35726	100	1624

Table 7: Water quality parameters of the giant freshwater pond during 60 days observation

Parameters	Rearing duration (months)			Optimum level (17)
	4	6	8	
Temperature (°C)	31.6 - 34.0	31.6 - 34.6	31.3-34.7	26 - 30
pH	7.70 - 8.53	7.75 - 8.53	7.79-8.82	7.0 - 8.5
Dissolve oxygen (mg L ⁻¹)	3.82 - 7.70	3.8 - 7.70	3.4-5.92	3.0 - 7.0
NH ₃ (mg L ⁻¹)	0.0- 0.0137	0.0 - 0.014	0.0-0.015	< 0.3
NO ₂ (mg L ⁻¹)	0.003-0.013	0.003-0.013	0.003-0.015	< 0.1

Discussion

The body shape of *Temnocephala* species generally has a flattened body shape like a leaf (17,18). The cirrus (reproductive structure) is often conical with different surfaces, one flat and the other concave (12). The distal area of the introvert (body part) is characterized by spines, including a strong, oblique, sclerotized (hardened) ring and two rows of long spines. The intestine has no partition (12). Many *Temnocephala* species are commensal ectosymbionts, meaning they live on other organisms' surfaces without causing damage. For example, they are often found on freshwater crabs and mollusks' gills or body surfaces (12). These flatworms are commonly found in regions such as South America and Australia's freshwater environments. In their study, (19). found that *Temnocephalida* parasites on cultured *Cherax quadricarinatus* were more than the number in the wild. Crayfish reared at high density will be affected by environmental stress and water quality issues, leading to an increased prevalence of parasites of temnocephalid (20). An increased abundance of unutilized nutrients in closed culture ecosystems contributes to low water quality and, therefore, may lead to an increased prevalence of *Temnocephalid* (21).

In addition to parasites of the temnocephalid genus, parasites of the protozoan class (*Zoothamnium* sp., *Vorticella* sp., and *Epistelis* sp.) were also found to infect giant freshwater prawns, but with lower prevalence. According to these three parasites can infect the prawns by attacking the

gills and other external organs (22,23). This can cause respiratory and other health problems. These protozoa belong to a broader group of ectoparasitic protozoa that have been shown to affect various fish species, with potential economic and health impacts in aquaculture (24,25). Despite the presence of these protozoa, the prevalence of specific species in giant freshwater prawn species remains relatively low compared to other parasites. This low prevalence can be attributed to various ecological and biological factors influencing the distribution and intensity of protozoan infections in aquatic environments (25).

Protozoan parasites, including ciliates such as *Zoothamnium* sp., *Vorticella* sp., and *Epistelis* sp., have been identified as parasites on a wide variety of aquatic animals, causing diseases that can impact fish health and aquaculture productivity. These parasites have been found attached to the skin, gills, or external surfaces of fish and shrimp in freshwater environments, causing irritation and potential secondary infections. The low prevalence of these protozoa in giant freshwater prawn species may be due to specific environmental conditions or host immune responses that limit their spread and impact (24). These parasites cause irritation and damage to the host's skin and gills, leading to secondary infections and increased mortality (25). A comprehensive understanding of the prevalence and impact of protozoan parasites is essential for developing effective management and control strategies in aquaculture (24). Despite their low prevalence, monitoring these parasites is important to prevent potential outbreaks and ensure the

health and productivity of aquaculture systems (25). Although the occurrence of *Zoothamnium* sp., *Vorticella* sp., and *Epistylis* sp. in giant freshwater species is relatively low, it is still essential to consider broader ecological and environmental factors that may influence their presence.

Carapace had a moderate prevalence rate of 28.3%. Infection intensity was 39 parasites per infected shrimp, indicating a significant but less severe infestation than gills. The prevalence rate for swimming legs was 26.7%, with an intensity of 25 parasites per infected shrimp, indicating a less critical site for parasite attachment. No parasites were found on walking legs, indicating that this organ is not a common site for parasite attachment in this study. The data suggests that gills are the predominantly targeted by the parasite in the prawns, characterized by high prevalence and intensity. In addition, moderate infections observed in the body carapace and swim legs indicate that these areas also require attention, albeit to a lesser extent. The prevalence of *temnocephalids* in both farmed and natural *Cherax* sp. was most prevalent in the gills (26).

The gills are the primary site of infection, with a prevalence rate of 100% and a high intensity of 550 parasites per shrimp, indicating extreme susceptibility (27). Previous studies have shown that parasitic infections in gills in histopathological studies show that gill infections can cause severe tissue damage, including necrosis and inflammation, impairing respiratory function (28). Factors such as exposure to heavy metals like lead can exacerbate gill damage, leading to further susceptibility to infection (29-31).

The gills were the most affected organ, with a prevalence rate of 100% among sampled prawns. The high prevalence of gill parasites suggests the need for control strategies that focus on gill health. This could include the use of mechanical filters to remove free parasite stages from the water, as has been shown to be effective for other parasites such as *Ichthyophthirius* (32). Consider using an integrated control approach, which has been shown to be effective in aquaculture. For example, a combination of mechanical filtration, the use of safe biochemicals, and the selection of *M. rosenbergii* strains that are more resistant to parasite infection (32). Infection intensity was very high, with 550 parasites per infected shrimp. This high intensity suggests that the gills are highly susceptible to parasite infection, most likely due to their open and fragile nature. The prevalence and intensity of gill infections in our study were significantly higher compared to recent findings of other parasitic infections in *M. rosenbergii*, such as *Enterocytozoon hepatopenaei* (EHP), which has been reported to have prevalence rates ranging from 4.9% to 18.2% (31). This stark difference highlights the potential severity of *Temnocephala* spp. infections and underscores the need for targeted control measures to protect this vital organ and ensure prawn health and productivity.

Diseases caused by parasitic infections are one of the main problems farmers face and a significant challenge in

aquaculture systems. In this study, the dominant parasites found to infect *M. rosenbergii* were *Temnocephala* spp., species with a prevalence rate of 100% derived from 180 prawn samples examined at three rearing periods (4, 6, and 8 months of rearing). The prevalence of *Temnocephala* spp. that you found (100%) is higher than the prevalence of *Enterocytozoon hepatopenaei* (EHP) in *M. rosenbergii* which has been reported to range from 4.9% to 18.2% (31). This suggests that *Temnocephala* spp. may be a more serious threat to *M. rosenbergii* cultivation. Furthermore, the high prevalence of *Temnocephala* spp. (100%) in this study also indicates a more severe infection rate compared to some other parasites reported in *M. rosenbergii*. For example, a recent study by Hooper *et al.* (33) reported that Covert Mortality Nodavirus (CMNV) infection in *M. rosenbergii* caused clinical symptoms such as pale hepatopancreas, soft shell, and muscle whitening and necrosis. Although CMNV had a significant impact, its prevalence was not as high as *Temnocephala* spp. in our study. It is important to note that research on parasites in *M. rosenbergii* in Indonesia is still limited. A study by Zakariah *et al.* (34) on gregarine parasites in *M. rosenbergii* in Malaysia showed a prevalence of 42.4%, which is much lower than our findings. Although this study was conducted in a neighboring country, it shows geographical variation in parasite prevalence in the same species.

Swim legs had a prevalence rate of 26.7% and an intensity of 25 parasites, suggesting that swim legs are a less important site for parasite attachment. Gill infections' high prevalence and intensity require targeted control measures to protect this vital organ to ensuring prawn health and productivity (30). Moderate-level infections on the carapace and swimming legs still require attention to prevent potential spread and their impact on shrimp health (29). Although the gills are the most affected organ, the absence of parasites on the walking legs indicates that not all body parts are equally susceptible. The complexity of parasite infections in shrimp and the need for comprehensive management strategies in aquaculture are influenced by the variability of infection levels across different body parts. Even though the parasite you found does not directly infect humans, high levels of infection can affect the quality and safety of *M. rosenbergii* food. This emphasizes the importance of good handling and processing practices in the aquaculture supply chain (33).

Conclusion

The giant freshwater prawns were infected by *Epistylis* sp., *Zoothamnium* sp., *Vorticella* sp., and *Temnocephala* sp. The gills were the most susceptible organ, and *Temnocephala* sp. was the most dominant parasite. The increasing intensity of infection with prawns size and culture period highlight the need for effective control measures, especially in older and larger prawns.

Acknowledgment

We are deeply grateful to the Research Institute for Fish Breeding, Ministry of Marine Affairs and Fisheries Indonesia for their sincere support and encouragement of this inquiry.

Conflict of interest

The authors announce that there are no conflicts of interest concerning the publication of this manuscript.

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انتشار وكثافة الطفيليات الخارجية في جمبري المياه العذبة العملاق: الآثار المترتبة على تربية الأحياء المائية

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الخلاصة

يعتبر جمبري المياه العذبة العملاق عرضة للإصابة بالطفيليات الخارجية، لا سيما في ظل ظروف الإدارة دون المستوى الأمثل. تبحث هذه الدراسة في انتشار وشدة الطفيليات الخارجية على أجزاء مختلفة من جسم الجمبري، بما في ذلك الخياشيم وسطح الجسم وأرجل السباحة وأرجل المشي. تم أخذ عينات من ما مجموعه ١٨٠ جمبريا من أحواض تربية القريدس الموجودة في منطقة سوبانج في جاوة الغربية بإندونيسيا. ثم تم تحليل هذه العينات مجهريا. وشملت الطفيليات الخارجية التي تم تحديدها الأوليات (*Epistylis* sp., *Zoothamnium* sp., *Vorticella* sp. و *Temnocephala* sp. كانت مستويات الانتشار والشدة على النحو التالي: *Temnocephala* sp. (١٠٠٪ ؛ ٩٠٧ أفراد/جمبري)، *Zoothamnium* sp. (٢٧٪ ؛ ٣٥ أفراد/جمبري)، *Vorticella* sp. (٢٧٪ ؛ ٢٦ أفراد/جمبري)، *Epistylis* sp. (٢٩٪ ؛ ٤ أفراد / جمبري). أظهرت الخياشيم أعلى معدل انتشار (١٠٠٪) وكثافة (٥٥٠ فردا/جمبري)، مما أثر بشدة على التنفس وزيادة خطر النفوق. تسلط هذه النتائج الضوء على الحاجة الملحة لاستراتيجيات فعالة لإدارة الطفيليات في تربية الأحياء المائية من أجل تقليل الخسائر الاقتصادية وتعزيز الاستدامة.