JPPIPA 12 (7) (2026)

Jurnal Penelitian Pendidikan IPA

http://jppipa.unram.ac.id/index.php/jppipa/index

Polystyrene Microplastics in Aquatic Rearing Systems: Impacts on Growth Performance and Histomorphometry of the Giant Freshwater Prawn (*Macrobrachium rosenbergii*)

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Received: March 16, 2026
Revised: June 20, 2026
Accepted: July 25, 2026
Published: July 31, 2026

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DOI: 10.29303/jppipa.v12i7.14817

Abstract: Polystyrene (PS) microplastics are widely detected in aquatic environments, particularly in freshwater ecosystems, and may pose ecological risks to aquatic organisms. However, information on how PS microplastics affect the giant freshwater prawn, an economically important freshwater aquaculture species, remains limited, particularly at the tissue level. This study therefore investigated the effects of PS microplastics in rearing media on microplastic accumulation, growth performance, and histomorphometry of the giant freshwater prawn. Following a three-day acclimation period, prawns were exposed to four concentrations of PS microplastics (0, 1, 10, and 100 mg/L) for 14 days. Growth parameters (length and weight), microplastic abundance in organs (gills, intestine, and muscle), and histomorphometry of the gills, intestine, and muscle were evaluated using hematoxylin–eosin staining. Data were analyzed using analysis of variance or Kruskal–Wallis tests followed by Duncan's test and regression analysis. The results showed that microplastic abundance in the rearing media significantly increased microplastic accumulation in the gills and muscle ($p < 0.05$), but not in the intestine ($p > 0.05$). Exposure to PS microplastics did not significantly affect growth performance or gill lamella length ($p > 0.05$). However, significant alterations were observed on intestinal histological parameters and muscle myofiber structure ($p < 0.05$). These findings demonstrate that PS microplastics can induce tissue-level alterations in *M. rosenbergii* despite the absence of short-term growth effects.

Keywords: Ecotoxicology; Freshwater aquaculture; *Macrobrachium rosenbergii*; Organ histology; Tissue alteration

Introduction

The rapid increase in global plastic production since 1950s has made plastic materials an integral part of modern human activities. Their flexibility, durability, light weight, and low production cost have led to continuously increasing consumption. Most plastic waste undergoes degradation processes on land before eventually fragmenting into smaller particles known as microplastics (Hale et al., 2020). Microplastics are plastic particles from approximately 0.1 to 5,000 µm in size and may originate either from plastics manufactured in small sizes (primary microplastics) or from the breakdown of debris of larger plastic, through biological, chemical, and physical processes (EFSA, 2016; GESAMP, 2015).

Due to their resistance and persistence to degradation, they have become ubiquitous pollutants in terrestrial and aquatic ecosystems (Lusher et al., 2017). Recent global assessments indicate that the production

How to Cite:
Suwartiningsih, N., Yusuf, L. H., Swita, R., Marsela, R., Wijayanti, D. E., Aszhara, A. F., & Novianti, I. (2026). Polystyrene Microplastics in Aquatic Rearing Systems: Impacts on Growth Performance and Histomorphometry of the Giant Freshwater Prawn (Macrobrachium rosenbergii). *Jurnal Penelitian Pendidikan IPA*, *12*(7), 316–328. https://doi.org/10.29303/jppipa.v12i7.14817

of plastic annually, up to 400 million metric tons, while approximately 9–14 million metric tons every year, plastic waste enter aquatic ecosystems. Without effective mitigation, plastic waste enter aquatic environments is predicted to increase substantially over the coming decades, further elevating the risk of microplastic exposure to freshwater organisms (OECD, 2022; UNEP, 2023). It is estimated in 2030, that microplastics in marine could double, if current production and disposal trends continue (Hale et al., 2020). Microplastics in aquatic environments increases the exposure to aquatic organisms. Micoplastic particles may enter directly to the body through ingestion of water, or indirectly when organisms consume the contaminated prey (Lusher et al., 2017; Vendel et al., 2017; Yona et al., 2020).

Once inside the organism, microplastics can cause several biological effects. Physically, they may obstruct the digestive tract and induce false satiety, which can interfere with feeding behavior (Gall & Thompson, 2015). In addition, physiological impacts is from the additives of plastic or adsorbed contaminants such as bisphenol compounds and phthalates, which can cause endocrine-disrupting and potentially carcinogenic properties (Wright et al., 2013). Microplastic surfaces may also act as substrates for microbial biofilm formation, including pathogenic microorganisms, potentially affecting digestion, metabolism, reproduction, and behavior of aquatic organisms (Hale et al., 2020).

Among the various kinds of microplastics that detected in aquatic environments, polystyrene is one of the most frequently reported polymers in freshwater systems (Nugnes et al., 2022). Polystyrene is produced from styrene monomers polymerization, and is widely used in food packaging, household products, and various industrial applications (Nurhadi et al., 2017). Its high resistance to biodegradation contributes significantly to environmental plastic accumulation (Woo et al., 2020). Moreover, styrene monomers have been classified as potentially carcinogenic compounds that may pose health risks when entering biological systems (Kik et al., 2020). Environmental weathering processes, including ultraviolet radiation and physical degradation, can fragment polystyrene products into microplastic particles that subsequently disperse in aquatic environment (Hwang et al., 2020).

Research on the effects of microplastics on aquatic biota has expanded considerably. In crustaceans, most studies have focused on commercially important species such as *Litopenaeus vannamei*. Previous studies shows that microplastic exposure may alter nutritional composition and gut microbiota in shrimp, potentially affecting long-term growth performance (Chae et al., 2019). Another studies also show the accumulation of microplastics in various shrimp organs, including the head, digestive tract, and body tissues, indicating that digestive organs are the major sites of accumulation of microplastic (Yoon et al., 2022). Similarly, debris and microplastics have been detected in the stomach and intestine of *Macrobrachium* species, suggesting widespread environmental exposure in freshwater prawns (Tee-hor et al., 2023). Recent studies explore the histological and toxicological consequences of microplastic exposure. In *L. vannamei*, microplastic have been detected in the digestive tract and in the other tissues such as the exoskeleton, muscle, and hepatopancreas (Vitheepradit & Prommi, 2023). Furthermore, exposure of microplastics and chemical pollutants, such as glyphosate can induce oxidative stress, histological damage, and immune disturbances in shrimp digestive tissues (Thammatorn et al., 2024). In Indonesia, reports on microplastic contamination in cultured shrimp have also emerged; most studies remain limited to identifying microplastic types and organ accumulation without evaluating broader biological impacts (Setyono et al., 2024). Overall, previous studies have primarily emphasized the occurrence and accumulation of microplastics, whereas quantitative evaluations linking tissue accumulation with growth performance and histomorphometric alterations remain limited. Consequently, the physiological consequences of microplastic accumulation in economically important freshwater crustaceans are still not fully understood.

Biologically, ingested or absorbed microplastics can induce oxidative stress by the increase production of reactive oxygen species (ROS), resulting in lipid peroxidation, inflammation, cellular damage, and disruption of homeostasis. These processes can impair intestinal nutrient absorption, alter gill function, and inhibit muscle fiber development, ultimately affecting organism growth and overall health (Hamed et al., 2021; Stapleton & Hai, 2023).

Giant freshwater prawn considered one of the most economically important freshwater aquaculture commodities, with increasing demand at both national and international markets (Khasani et al., 2023; Pillai & Panda, 2024). The species is broadly cultured due to its large body size, rapid growth rate, and relatively low disease prevalence, making it economically attractive for aquaculture development (Khairunnisa et al., 2023). Despite its high economic value, the effects of microplastics on this species have received limited research attention. Previous research has largely concentrated on the detection of microplastics in aquatic ecosystems and their accumulation in the tissues of aquatic organisms (Gopal et al., 2022; Jitkaew et al., 2023), with limited evaluation of potential impacts on growth performance or organ histology. Furthermore, no previous study has comprehensively evaluated the relationships among environmental polystyrene microplastic concentration, tissue-specific microplastic accumulation, growth performance, and histomorphometric responses of multiple organs (gills, intestine, and muscle) under controlled laboratory exposure. This represents an important knowledge gap because tissue-level alterations may occur before measurable changes in growth become apparent.

Based on this knowledge gap, we hypothesized that increasing concentrations of PS microplastics in the rearing medium would increase tissue-specific microplastic accumulation and induce histomorphometric alterations in the gills, intestine, and muscle, which may subsequently affect growth performance. Therefore, this study seeks to evaluate the effects of polystyrene microplastic exposure in the rearing medium on organ-specific microplastic abundance, growth performance, and gill, intestine, and muscle histomorphometric characteristics of giant freshwater prawn.

Method

Experimental Animals, Sampling, and Experimental Design

The study involved 72 healthy giant freshwater prawns aged four months. Prior to the experiment, all prawns were acclimatized under laboratory conditions for three days. Following acclimatization, the experiment employed a completely randomized design (CRD) with four treatment groups based on polystyrene microplastic concentrations in the rearing medium: K (0 mg/L), P1 (1 mg/L), P2 (10 mg/L), and P3 (100 mg/L) (Ding et al., 2018).

Each treatment consisted of six replicate aquaria, with three prawns maintained in each aquarium. One aquarium containing three prawns represented one experimental unit, resulting in a total of 24 experimental units (72 prawns). The treatment allocation was performed randomly using a lottery method to minimize allocation bias.

Growth performance was evaluated using all experimental animals. Upon completion of the exposure period, three prawns were randomly selected from each treatment group for subsequent analysis using a stratified random sampling technique for microplastic abundance and histomorphometric analyses. This sampling approach ensured representative sampling of each treatment while minimizing sampling bias.

The overall experimental workflow consisted of the following sequential stages: (1) acclimatization of prawns; (2) preparation of rearing media containing different concentrations of PS microplastics; (3) random allocation of prawns into treatment groups; (4) exposure for 14 days; (5) measurement of growth performance; (6) organ sampling; (7) microplastic extraction and quantification; and (8) histological and histomorphometric analyses.

Microplastic and Rearing Media Preparation

Microplastic particles were prepared from polystyrene plastic. The material was ground using a blender for five minutes (Uy & Johnson, 2022). The resulting particles were then sieved using a 35-mesh sieve. Microplastics with particle sizes ≤250 µm were used for the experimental treatments.

The experimental units consisted of plastic containers holding 20 L, each of which was supplied with 10 L of water. PS microplastics were added according to the designated treatment concentrations. Continuous aeration was provided throughout the experiment to maintain homogeneous suspension of the microplastic particles in the rearing media.

Acclimatization and Exposure

Prior to treatment, all prawns were acclimatized for three days under laboratory conditions. Throughout the acclimation period, the prawns received commercial pellets twice a day at 08:00 and 16:00 WIB, at a feeding rate equivalent to 3% of their total biomass. Following acclimatization, the experimental treatments were applied continuously for 14 consecutive days. The feeding protocol was continued throughout the exposure period. Water quality was assessed on a weekly basis by monitoring dissolved oxygen (DO), temperature, and pH to maintain optimal conditions for prawn rearing.

Growth Measurement

Body length and body weight were measured immediately before the initiation of microplastic exposure and again following the 14-day exposure period. Total body length was measured using a digital caliper extending from the rostral tip to the posterior extremity of the tail. Body weight was determined using an analytical balance.

Organ Sampling

Upon completion of the exposure period, prawns were fasted for 24 hours before organ sampling. Three individuals from each treatment group were randomly selected for organ analyses. The gills, intestine, and approximately 1 cm of right abdominal muscle were dissected, weighed, and processed for microplastic extraction and histological analyses.

Microplastic Extraction

Organ samples designated for microplastic analysis were placed in flask bottles containing 10% potassium hydroxide (KOH) solution and left at room temperature until complete tissue digestion. After digestion, the solution was filtered using filter paper and allowed to dry for three days. The filter paper was subsequently placed in a flask and rinsed thoroughly with distilled water. The resulting liquid was transferred to a Petri dish for microscopic examination. Microplastic abundance was determined by counting the particles detected in each organ.

Histological Observation

Organ samples designated for histological analysis were fixed in Davidson's AFA fixative solution. Histological preparation included washing with 70% alcohol, graded dehydration (70% to absolute alcohol), clearing with toluol, paraffin infiltration and embedding, sectioning using a microtome, mounting with Mayer's albumin, and hematoxylin–eosin staining. The prepared specimens were subsequently examined using a light microscope.

For gill tissues, the observed parameter was lamella length. In intestinal tissues, villus height was determined as the distance between the villus apex and the villus–crypt junction, whereas crypt depth was measured from crypt base to the corresponding junction. The surface area of the intestinal villi was subsequently calculated using the following formula:

Villus surface area = 2 × 3.14 × (villus diameter ÷ 2) × villus height

The ratio between villus height and crypt depth was subsequently calculated. For muscle tissues, myofiber diameter and myofiber area were measured.

Data Analysis

Data on microplastic abundance in organs, growth parameters (body length and weight), and histological characteristics of gills, intestine, and muscle were analyzed using inferential statistical methods. Differences among treatments were tested Statistical comparisons were performed using ANOVA, whereas Kruskal–Wallis test was applied when the data did not satisfy the assumptions of homogeneity of variance and normality (Field, 2018). Significant differences were further examined using DMRT Test or appropriate pairwise comparisons as post-hoc procedures. Regression analysis was additionally employed to assess the relationship between increasing concentrations of PS microplastics in the rearing medium and the measured parameters. Statistical analyses were conducted using IBM SPSS 27.0 for Windows, with results considered statistically significant at $p < 0.05$.

Result and Discussion

Microplastic Abundance

This study investigated the effects of exposure of polystyrene microplastic through the rearing medium on the growth performance and organ histology in giant freshwater prawns. The lowest microplastic abundance in the gills was recorded in the control group (1.67 ± 1.53 particles/sample), whereas the highest microplastic abundance was observed in T3 (5.67 ± 2.08 particles/sample) (Table 1), with a significant difference detected among the treatment groups ($p < 0.05$). In the digestive tract, microplastic abundance was lowest in both the control group (0.67 ± 1.15 particles/sample) and T1 (0.67 ± 0.58 particles/sample), whereas T3 exhibited the greatest abundance (7.67 ± 10.69 particles/sample). Despite these differences, microplastic abundance in the digestive tract did not differ significantly among treatments ($p > 0.05$). In muscle tissue, no microplastic particles were detected in the control group (0.00 ± 0.00 particles/g), whereas the highest abundance was recorded in T3 (2.33 ± 1.53 particles/g). Statistical analysis revealed a significant difference between the control and exposed groups ($p < 0.05$), whereas the differences among T1, T2, and T3 were not statistically significant ($p > 0.05$).

Regression analysis demonstrated a significant association between PS microplastic levels in the rearing medium and their abundance in the gill and muscle tissues ($p < 0.05$). However, the concentration of PS microplastics in the rearing medium was not significantly associated with their abundance in the digestive tract ($p > 0.05$).

Table 1. Abundance of PS Microplastics in the Organs of Giant Freshwater Prawn (*Macrobrachium rosenbergii*) after 14 days of PS microplastic exposure

Treatment	Gills (particles/ sample)	Digestive Tract (particles/ sample)	Muscle (particles/g
Control (0 mg/L)	1.67 ± 1.53^{a}	0.67 ± 1.15^{a}	0.00 ± 0.00^{a}
T1 (1 mg/L)	2.00 ± 1.00^{a}	0.67 ± 0.58^{a}	0.67 ± 0.58^{ab}
T2 (10 mg/L)	3.67 ± 1.53^{b}	4.00 ± 5.00^{a}	2.00 ± 1.00^{b}
T3 (100 mg/L)	5.67 ± 2.08^{b}	7.67 ± 10.69^{a}	2.33 ± 1.53^{b}

Note: Different superscript letters indicate significant differences ($p < 0.05$).

Table 1 shows a clear tendency that increasing microplastic concentrations in the rearing medium corresponded with increasing numbers of particles detected in prawn organs. This pattern indicates that higher environmental exposure to microplastics increases the probability of particle uptake and accumulation in aquatic organisms, consistent with previous studies (Güven et al., 2017; Roch et al., 2020; Wright et al., 2013).

One of the organs where microplastics were detected was the gill, which serves as the primary respiratory organ and is directly exposed to medium. Microplastics accumulation in gills is associated with the respiratory ventilation process, during which organisms continuously pump water across the gill surface to obtain oxygen. Suspended microplastic particles may therefore be transported through the water flow and adhere to the gill tissues (Aryani et al., 2023; Pradit et al., 2023; Putra et al., 2025).

Microplastics were also detected in digestive tract, where they may enter through several pathways, including ingestion with food, inadvertent uptake through contaminated water or ingestion of prey containing microplastic particles (Roch et al., 2020). In this study, microplastics likely entered the digestive tract during feeding activity. However, the results showed that microplastic abundance in the rearing medium did not significantly affect the abundance detected in the digestive tract. This may occur because microplastics accumulated in the digestive tract can be excreted through feces or translocated to other tissues (Gall & Thompson, 2015).

The size of microplastic particles is another important factor influencing their accumulation. Larger microplastic particles tend to be retained in the digestive tract and eventually excreted, whereas particles of smaller dimensions can cross the intestinal epithelial barrier and subsequently reach other tissues (Aryani et al., 2024). After entering the digestive tract, some microplastics may translocate through the circulatory system and accumulate in other tissues, including muscle. The present results indicate that microplastic abundance gradually decreased from the digestive tract to muscle tissue, suggesting that only a fraction of ingested particles can penetrate the epithelial barrier and reach systemic circulation.

Previous research reported that in brown shrimp (*Crangon crangon*), only very small microplastics (0.1 μm) were capable of being absorbed from the intestine and transported to other tissues (Korez et al., 2020). Similar findings were reported in whiteleg shrimp (*Penaeus vannamei*), where microplastics were detected not only in the intestine but also in muscle tissue (Vitheepradit & Prommi, 2023), with fewer particles detected in muscle tissue than in the digestive tract (Suwartiningsih et al., 2023). These findings indicate that microplastic particle size is a critical factor determining the ability of particles to translocate between tissues in aquatic organisms.

Growth Performance

The smallest increase in body length was recorded for T3 (0.55 ± 0.42 cm), whereas the control group exhibited the greatest increase (0.75 ± 0.69 cm) (Table 2). Nevertheless, the differences among the treatment groups were not statistically significant ($p > 0.05$). A similar pattern was observed for weight gain, with T3 showing the lowest value (1.91 ± 1.33 g) and the control group showing the highest (3.39 ± 1.61 g); however, these differences were also not significant ($p > 0.05$). Furthermore, regression analysis showed that exposure to different PS microplastic concentrations in the rearing medium for 14 days had no significant effect on either body length increment or weight gain in the prawns ($p > 0.05$).

Table 2. Growth Performance of Giant Freshwater Prawn (*Macrobrachium rosenbergii*) after 14 Days of PS Microplastic Exposure

Treatment	Length increment (cm/day)	Weight gain (g/day)
Control (0 mg/L)	0.75 ± 0.69[a]	3.55 ± 1.50[a]
T1 (1 mg/L)	0.68 ± 0.45[a]	2.39 ± 1.48[a]
T2 (10 mg/L)	0.60 ± 0.43[a]	1.96 ± 1.41[a]
T3 (100 mg/L)	0.55 ± 0.42[a]	1.91 ± 1.33[a]

Note: Different superscript letters indicate significant differences ($p < 0.05$).

The findings indicated that PS microplastic concentrations in the rearing medium did not significantly influence body length gain in giant freshwater prawns. The absence of significant differences is likely because the concentration of microplastics applied had not reached a level capable of disrupting physiological processes associated with growth, such as cell division in skeletal tissues. Consequently, the body elongation process of the prawns was able to proceed normally.

In addition, the present study also demonstrated that microplastic exposure did not significantly affect weight gain. This condition may be attributed to the relatively low concentration of microplastics used, which did not alter the physical quality of the rearing medium, such as water turbidity or dissolved oxygen availability. As a result, the organisms were still able to maintain metabolic processes, nutrient utilization, and physiological activities required for growth.

Although no statistically significant effects were detected, the data pattern indicated a declining trend in length increment and weight gain with increasing concentrations of PS microplastics in the rearing medium. A similar pattern was reported in a study using juvenile giant freshwater prawns, where body length decreased by 15.6% and body weight decreased by 29.6% following exposure to 1 mg/L PS microplastics in the rearing medium for 42 days (Zhou et al., 2026). Exposure to PS microplastics is suspected to alter feeding behavior and disrupt food absorption in juvenile giant freshwater prawns, which may consequently reduce growth performance (Jaikumar et al., 2021).

Histomorphometry

Gills

Gill lamellae were shortest in the control group (93.27 ± 41.62 μm), while the greatest mean length was recorded in T2 (108.52 ± 64.38 μm) (Table 3). Despite this variation, lamella length did not differ significantly between treatments ($p > 0.05$). Regression analysis likewise revealed no significant effect of PS microplastic exposure through the rearing medium on gill lamella length ($p > 0.05$). The histological appearance of the gill lamellae following 14 days of PS microplastic exposure is shown in Figure 1.

Exposure to PS microplastics through the rearing medium did not significantly alter gill lamella length in giant freshwater prawns. Nevertheless, the data pattern indicated a tendency for lamella length to increase with increasing microplastic concentration in the medium. This tendency suggests the presence of the organism's physiological response to the introduction of foreign particles into its aquatic surroundings. When microplastics accumulate on gill tissues, these particles may potentially obstruct water flow and interfere with oxygen absorption occurring at the lamellar surface (Yudiati et al., 2009). Such disturbances may trigger structural changes in gill tissues as a response to environmental stress.

Table 3. Gill Histomorphometry of Giant Freshwater Prawn (*Macrobrachium rosenbergii*) after 14 days of PS Microplastic Exposure

Treatment	Lamella length (μm)
Control (0 mg/L)	93.27 ± 41.62^a
T1 (1 mg/L)	95.50 ± 44.75^a
T2 (10 mg/L)	108.52 ± 64.38^a
T3 (100 mg/L)	104.82 ± 34.94^a

Note: Different superscript letters indicate significant differences ($p < 0.05$).

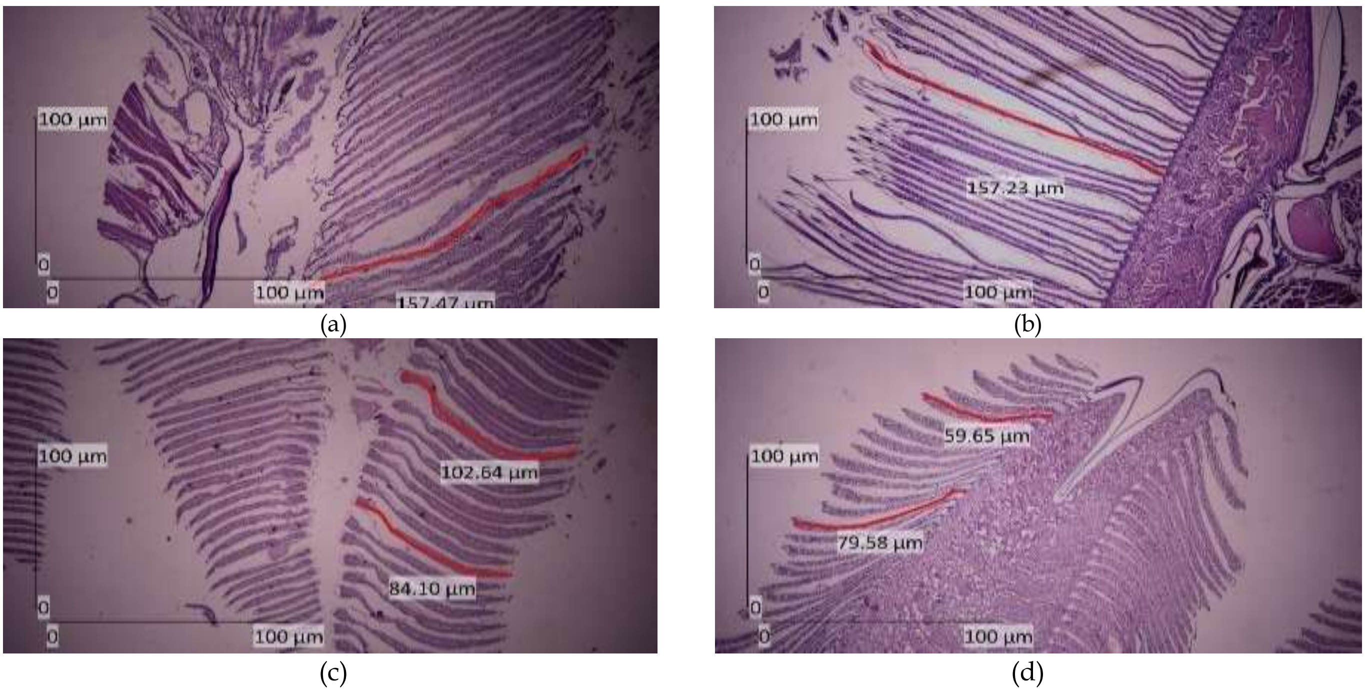


Figure 1. Gills of giant freshwater prawn (*Macrobrachium rosenbergii*) after 14 days of PS microplastic exposure: (a) Control (0 mg/L); (b) T1 (1 mg/L); (c) T2 (10 mg/L); and (d) T3 (100 mg/L)

Accumulation of microplastics in gills has also been reported to cause physical damage to gill filaments, leading to histopathological changes (Barboza et al., 2018), such as lamellar hypertrophy (Silveyra et al., 2023). Under such conditions, aquatic organisms may perform physiological adjustments to maintain efficient gas exchange. One possible adaptive response is the elongation of lamellae, which increases the respiratory surface area so that oxygen demand for metabolism can still be fulfilled.

A comparable adaptive response has been documented in the shore crab (*Carcinus maenas*) following exposure to PS microplastics for 24 hours did not reduce oxygen consumption because the organism was able to maintain gas exchange through enhanced lamellar function or changes in water flow within the branchial chamber (Watts et al., 2016). A comparable response was also observed in Mutiara catfish (*Clarias gariepinus*) (Suwartiningsih et al., 2023). Therefore, the tendency toward lamella elongation observed in this study is likely an adaptive response of giant freshwater prawns to microplastic exposure in the rearing environment.

Digestive Tract

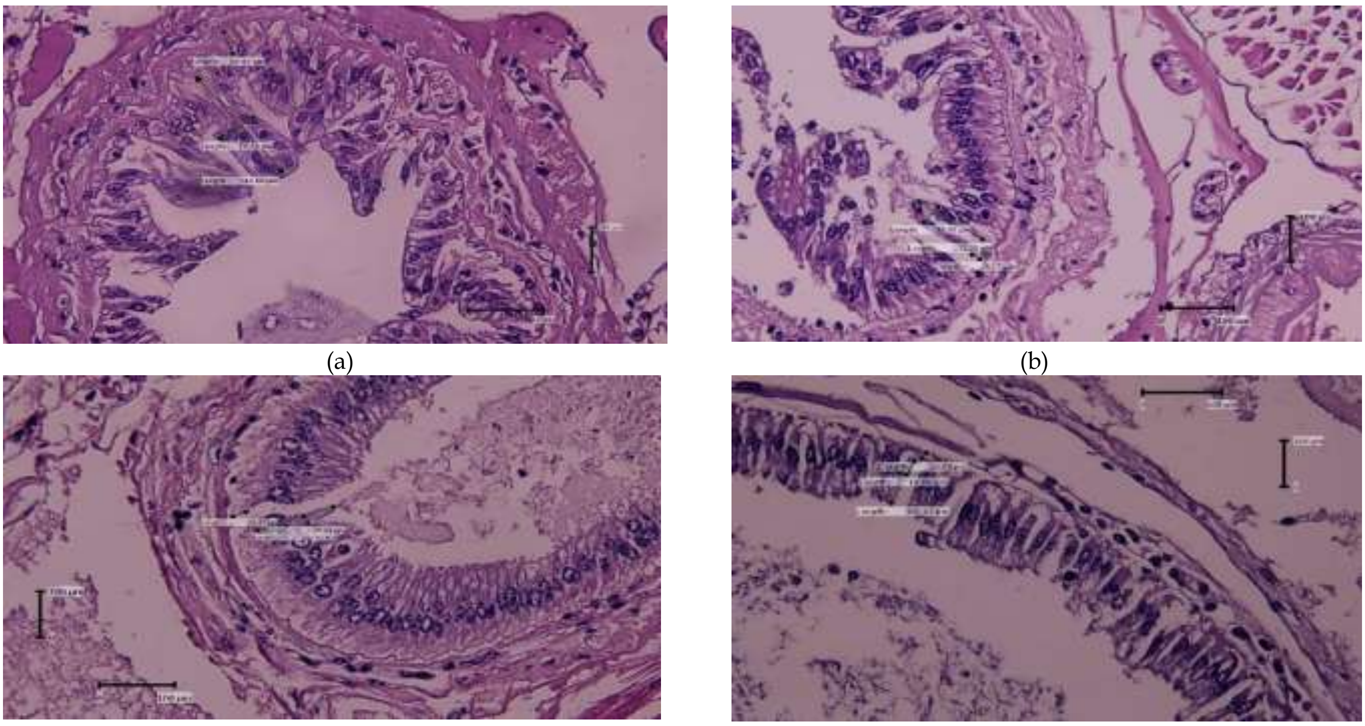
Villus height decreased to its lowest value in T3 (72.83 ± 15.22 μm), whereas the control group exhibited the greatest height (152.12 ± 51.05 μm) (Table 4), with significant differences detected among treatments ($p < 0.05$). Crypt depth followed a similar pattern, showing the smallest value in T3 (21.32 ± 1.91 μm) and the largest in the control group (34.32 ± 11.36 μm), with significant variation among treatments ($p < 0.05$). Likewise, the villus-to-crypt ratio reached its lowest value in T3 (3.43 ± 0.73), while the highest ratio occurred in the control group (4.87 ± 2.16). The lowest villus surface area was observed in T3 ((29.99 ± 11.83) × 10^2 μm²), whereas the highest value was found in the control group ((154.12 ± 124.46) × 10^2 μm²).

Regression analysis revealed that microplastic abundance in the rearing medium significantly affected the height of intestinal villi, depth of the crypts, villus-to-crypt ratio, and villus surface area ($p < 0.05$).

Table 4. Intestinal Histomorphometry of Giant Freshwater Prawn (*Macrobrachium rosenbergii*) after 14 days of PS Microplastic Exposure

Treatment	Villus height (μm)	Crypt depth (μm)	Villus–crypt ratio	Villus area (×10^2 μm²)
Control (0 mg/L)	152.12 ± 51.05^c	34.32 ± 11.36^c	4.87 ± 2.16^c	154.12 ± 124.46^c
T1 (1 mg/L)	112.94 ± 31.00^b	25.79 ± 4.88^b	4.54 ± 1.54b^c	95.27 ± 53.85^b
T2 (10 mg/L)	86.70 ± 17.19^a	22.65 ± 4.02^{ab}	3.93 ± 1.01^{ab}	42.71 ± 16.40^a
T3 (100 mg/L)	72.83 ± 15.22^a	21.32 ± 1.91^a	3.43 ± 0.73^a	29 .99 ± 11.83^a

Note: Different superscript letters indicate significant differences ($p < 0.05$).



(a) (b) (c) (d)

Figure 2. Intestinal histology of giant freshwater prawn (*Macrobrachium rosenbergii*) after 14 days of PS microplastic exposure; (a) Control (0 mg/L); (b) T1 (1 mg/L); (c) T2 (10 mg/L); and (d) T3 (100 mg/L)

The findings demonstrated that PS microplastic levels in the rearing water significantly altered intestinal morphology, as reflected by changes in villus dimensions, crypt depth, the villus-to-crypt ratio, and intestinal surface area. Increasing microplastic concentrations in the medium were accompanied by increasingly evident morphological changes, including shortening of the villi, shallower crypts, a decrease in the villus–crypt ratio, and a reduction in villus surface area. This pattern indicates that the presence of microplastics may disrupt the structural integrity of the intestinal mucosa.

This tissue damage is presumed to occur due to continuous abrasion and irritation of the intestinal epithelium while microplastic particles remain in the digestive tract, resulting in repeated tissue repair processes that ultimately trigger morphological alterations (Mbugani et al., 2022). Similar effects have also been reported in zebrafish (*Danio rerio*), where microplastic exposure caused damage to the intestinal villus structure (Lei et al., 2018).

Changes in villus structure have important implications for the physiological function of the intestine. Villi are the primary structures involved in nutrient absorption, as their surface increases the contact area between the intestinal lumen and epithelial tissue. The larger the villus surface area, the greater the capacity for nutrient absorption (Muhammadar et al., 2023). Therefore, the shortening and narrowing of villi caused by microplastic exposure may reduce the efficiency of nutrient absorption. This condition may lead to a decrease in the amount of nutrients that can be absorbed by the organism, which may ultimately affect growth performance.

Muscle

The results showed that the lowest myofiber diameter was observed in T3 (30.60 ± 7.67 µm), while the highest value occurred in the control group (51.18 ± 14.04 µm) (Table 5), with significant differences among treatments ($p < 0.05$). Nevertheless, the comparison between T2 and T3 did not reveal a statistically significant difference ($p > 0.05$). Similarly, the lowest myofiber area was observed in T3 (24.03 ± 6.02 × 10^3 µm^2), whereas the highest value occurred in the control group (40.19 ± 11.03 × 10^3 µm^2). A significant differ among treatments was detected ($p < 0.05$), althoug B and T3 did not differ significantly. Regression analysis indicated that microplastic abundance in the rearing medium significantly affected myofiber diameter and area ($p < 0.05$).

Table 5. Muscle histomorphometry of giant freshwater prawn (*Macrobrachium rosenbergii*) after 14 days of PS microplastic exposure

Treatment	Myofiber diameter (µm)	Myofiber area (×10^3 µm^2)
Control (0 mg/L)	51.18 ± 14.04^c	40.19 ± 11.03^c
T1 (1 mg/L)	41.17 ± 8.27^b	32.33 ± 6.50^b
T2 (10 mg/L)	33.48 ± 7.75^a	26.29 ± 6.09^a
T3 (100 mg/L)	30.60 ± 7.67^a	24.03 ± 6.02^a

Note: Different superscript letters indicate significant differences ($p < 0.05$).

The present findings are in agreement with earlier research, which demonstrated that a seven-day exposure to PS microplastics at 1000 µg/L in goldfish larvae (*Carassius auratus*) caused damage to mesenchymal muscle cells, leading to muscle fiber atrophy (Yang et al., 2020). Morphological changes in muscle tissue are thought to occur due to the entry of environmental contaminants into muscle cells and tissues, thereby disrupting the structural stability of the tissue (Di Giacinto et al., 2023; Setyono et al., 2024). One compound that may contribute to these toxic effects is styrene, a benzene derivative known to possess high toxicity (Aldi, 2024; Majid et al., 2018).

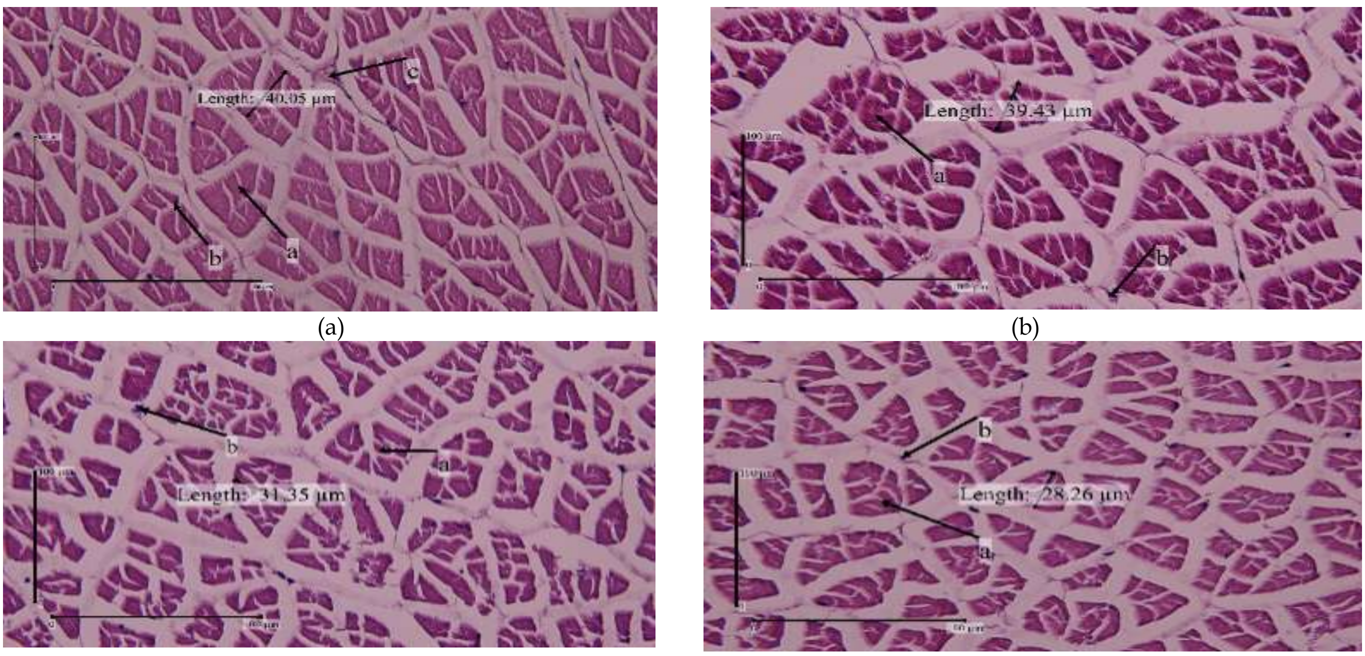


Figure 3. Muscle histology of giant freshwater prawn (*Macrobrachium rosenbergii*) after 14 days of PS microplastic exposure; (a) Control (0 mg/L); (b) T1 (1 mg/L); (c) T2 (10 mg/L); and (d) T3 (100 mg/L)

Several other studies have also reported similar patterns of muscle tissue damage resulting from exposure to various contaminants. Research on yellow catfish (*Pelteobagrus fulvidraco*) showed that contaminant exposure at exposure levels of 44 and 234 µg/kg administered over an eight-week period resulted in a reduction in myofiber diameter in muscle tissue (Zhang et al., 2021). In addition, exposure to the insecticide fonofos at 2 mg/L for 96 hours in zebrafish (*Danio rerio*) was also reported to cause a decrease in muscle fiber diameter (Arman & Üçüncü, 2021).

The decrease in myofiber diameter and area observed in this study indicates that muscle fiber growth was impaired. Suboptimal myofiber growth may reduce muscle tissue quality, particularly in terms of elasticity and structural compactness of the flesh (Moenek & Toelle, 2021; Suwiti et al., 2015; Zhang et al., 2021). Moreover, muscle tissue damage is often used as a biological indicator of environmental pollutant exposure (Ciamarro et al., 2015). Microplastics themselves have been widely reported to exhibit high toxicity potential toward various aquatic organisms (Aulia et al., 2023; Stapleton & Hai, 2023).

Mechanistically, PS microplastic accumulation within muscle tissue may facilitate the release of potentially harmful substances, including phthalates and bisphenol A. The presence of these compounds can promote reactive oxygen species (ROS) generation, consequently triggering oxidative stress. This oxidative imbalance may subsequently cause damage to cellular membranes and structural proteins responsible for maintaining muscle fiber integrity. Such damage may ultimately inhibit myogenesis and interfere with myofiber regeneration (Hamed et al., 2021; Novianti & Suwartiningsih, 2026).

The results obtained in the present study are in accordance with previous findings reported by Suwartiningsih et al. (2025), who found that exposure to 100 mg/L PS microplastics in catfish was associated with reductions in both myofiber diameter and cross-sectional area. These changes indicate histological alterations in muscle tissue as a response to microplastic exposure. Therefore, the reduction in myofiber size observed in this study further supports the hypothesis that PS microplastics can affect the structure and function of muscle tissue in aquatic organisms through toxicity mechanisms associated with oxidative stress.

Conclusion

This study demonstrated that 14-day exposure to PS microplastics (0–100 mg/L) resulted in accumulation and histomorphological alterations in the giant freshwater prawn. Microplastic abundance increased with exposure concentration, reaching 5.67 ± 2.08 particles/sample in gills and 2.33 ± 1.53 particles/g in muscle at 100 mg/L, compared with minimal or no particles in the control. Growth performance was not significantly affected; however, a decreasing trend was observed, with the lowest body length increment recorded in T3 (0.55 ± 0.42 cm) and the lowest weight gain (1.91 ± 1.33 g) at higher concentrations. Significant changes were observed in intestinal morphology, including reduced the depth of intestinal crypts, the ratio between villi and crypts, and the surface area of the intestinal villi, as well as decreased myofiber diameter and area in muscle tissue. These results indicate that PS microplastics can accumulate in tissues and induce structural damage in vital organs of the giant freshwater prawn. This study extends previous research by providing a comprehensive evaluation of the relationships among environmental polystyrene microplastic concentration, tissue-specific microplastic accumulation, growth performance, and histomorphometric responses in the gills, intestine, and muscle of *Macrobrachium rosenbergii* under standardized experimental conditions. These results enhance our understanding of the ecotoxicological impacts of PS microplastics on freshwater crustaceans and offer foundational information for microplastic pollution monitoring and sustainable freshwater shrimp aquaculture management. Furthermore, the observed tissue-level alterations suggest that histomorphometric parameters may serve as sensitive biomarkers for the early detection of microplastic-induced toxicity before measurable effects on growth become evident. Future studies should investigate the impacts of prolonged microplastic exposure and assess the toxicity associated with various polymer types, particle sizes, and shapes, and explore the underlying molecular mechanisms, including oxidative stress, inflammatory responses, and gene expression to gain deeper insight into the long-term consequences of microplastic contamination for freshwater aquaculture organisms.

Acknowledgments
Acknowledgment is given to Lembaga Penelitian dan Pengabdian kepada Masyarakat Universitas Ahmad Dahlan (LPPM UAD), for providing financial support that enabled the completion of this research.

Author Contributions
Conceptualization was led by N.S. Methodology was developed by N.S., L.H.Y., R.S., and R.M. Validation of the research procedures was conducted by N.S. and L.H.Y., while formal analysis was carried out by R.S. and R.M. The investigation was performed by N.S., L.H.Y., R.S., R.M., I.N., and D.E.W. Research resources were provided by N.S. Data curation was managed by L.H.Y., R.S. and R.M. The original draft of the manuscript was prepared by L.H.Y., R.S. and R.M. with contributions from A.F.A. and I.N. Manuscript review

and editing were completed by N.S. and D.E.W. Visualization was prepared by R.S., and R.M. Overall supervision of the research was carried out by N.S. All authors have read and approved the final version of the manuscript.

Funding
This research was funded by Lembaga Penelitian dan Pengabdian kepada Masyarakat Universitas Ahmad Dahlan (LPPM UAD) under grant number PD-279/SP3/LPPM-UAD/XII/2025 dated December 2, 2025.

Conflicts of Interest
The authors declare that this article was prepared in the absence of any conflicts of interest that might influence the impartiality or credibility of the reported results.

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