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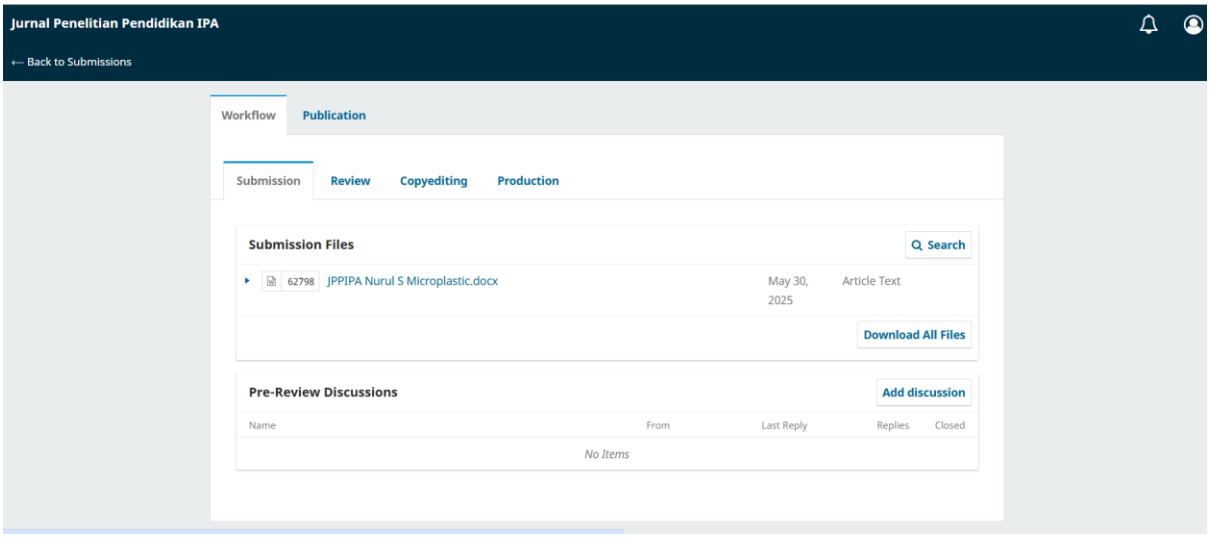

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The Effect of Polystyrene Microplastic Exposure in the Rearing Water on Muscle Morphology of Mutiara Catfish (*Clarias gariepinus* Burchell, 1822)

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Abstract: Polystyrene (PS) is one of the most abundance microplastics in freshwater that can lead to its accumulation in fish. This study aims to determine the effect of PS microplastic abundance in the rearing water on muscle tissue, diameter and area of myofibers in Mutiara catfish. A total of 120 fishes were exposed to PS microplastics in the rearing water for 28 days at concentrations of 0 mg/L, 1 mg/L, 10 mg/L, and 100 mg/L. Muscle samples from the right abdominal were collected and prepared using paraffin method with hematoxylin-eosin staining. Microplastics in muscle tissue was extracted with KOH 10%. Microplastics were observed using stereo microscope. Myofibers diameter was measured using Image-Raster. The data were analyzed using the Kruskal Wallis test. The results showed a significant difference between treatments ($P<0.05$) in all parameters. The lowest abundance of microplastics in the muscle found in control, while the highest was in T3. The smallest myofiber diameter and area was in T2, with the largest in control. Exposure of PS microplastics affects the abundance of microplastics in muscle, reduce myofiber diameter and area. This research emphasizes the need for further investigation into the broader effects of microplastics on aquatic organisms and human health.

Keywords: Microplastics; Myofiber; Polystyrene; Rearing water

Introduction

Microplastics are found in various sizes, shapes and concentrations in aquatic ecosystems (Smith et al., 2018). The primary sources of microplastics include plastic materials such as plastic bags, beverage bottles, beauty products, and cleaning agents (Nainggolan et al., 2022). The wide range of polymer types in microplastics, from high-density to low-density plastics, allows them to be carried by water currents, leading to their presence in both water and sediment. Numerous studies have reported the detection of microplastics in freshwater ecosystems, particularly in river areas. Research has identified microplastics in the Serang River, Yogyakarta, with abundances ranging from 1488 particles kg^{-1} to 384.58 particles kg-.[1] (Ismiyati et al., 2023). Microplastics in water pose significant negative impacts on the environment and aquatic organisms (Utami et al., 2021).

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The presence of microplastics in rivers can lead to their accumulation in aquaculture waters. When aquaculture water is contaminated with microplastics, it can degrade the water quality and adversely affect fish farming operations (Vieira Dantas Filho et al., 2023). Research indicates that the Pelayaran River in Sidoarjo, which supplies water for tilapia (*Oreochromis niloticus*) farming, is contaminated with microplastics, leading to the pollution of the farmed tilapia (Al-Fatih, 2022).

Polystyrene is one type of microplastic polymer frequently detected in aquatic environments (Victoria, 2017). It mainly originates from food packaging, craft materials and decorative items (Melyna, 2021). Research on the Mahakam River found that polystyrene accounted for 6.45% of the total plastic waste, with microplastics comprising 12.5% of the total abundance (Kurniawan et al., 2023). Polystyrene plastics degrade into microplastics through processes such as ultraviolet radiation exposure and weathering, which break the material into microparticles (Hwang et al., 2020).

Microplastics can be ingested by fish through their gills, digestive tract and muscles (Sawalman et al., 2021). Microplastics present in water can enter fish muscles via the bloodstream (Akhbarizadeh et al., 2018). When microplastics enter the digestive tract, they can be transported through the blood vessels and eventually accumulate in muscle tissue (Guanting et al., 2021). Previous research has shown that exposure to microplastics at a concentration of 100 mg/L in tilapia (*Oreochromis niloticus*) can cause muscle damage (Hamed et al., 2021). One of the commonly cultivated and consumed fish species is Mutiara catfish (*Clarias gariepinus* Burchell, 1822) (Iswanto et al., 2016). Therefore, the purpose of this study was to determine the effect of polystyrene microplastic exposure in the rearing water on muscle morphology of Mutiara catfish. This study holds significant implications for environmental health, aquaculture productivity and food safety.

Method

Research Time and Location

This research was conducted from January to November 2024. Maintenance and treatment took place in Dengok Kulon, Bugisan, Prambanan, Klaten, while data preparation, collection, and analysis were carried out at the Laboratory of Animal Structure and Physiology, Universitas Ahmad Dahlan.

Experimental Design

The study was an experimental design consisting of four treatments: a control group with 0 mg/L of polystyrene microplastic, T1 with 1 mg/L, T2 with 10 mg/L, and T3 with 100 mg/L of polystyrene microplastic. A completely randomized design (CRD) was employed, with the treatments administered over 28 days using a total of 120 Mutiara catfishes. Research parameter included the abundance of microplastics in muscle tissue, the diameter of myofibers and the myofiber area.

Microplastics Exposure

Microplastic preparation was carried out by pulverizing polystyrene plastic with a blender, followed by filtering through a 35-mesh sieve. The microplastics were weighed according to the treatment concentrations. Mutira catfish were acclimatized for ten days by being given food at 08.00 and 16.00 WIB, equivalent to 3% of their body weight (Tang et al., 2024). Microplastic exposure was conducted during water changes every two days for 28 days, based on the designated exposure concentrations.

Muscle Tissue Preparation

At the end of the treatment, the fish were fasted for 24 hours before being weighed and their organs harvested on day 29. Muscle tissues were weighed and fixed in a 10% formalin solution (Zulfadhli et al., 2016). The preparation process included dehydration with graded alcohol (70% to absolute), de-alcoholization with toluol solution infiltration, embedding in paraffin, sectioning with a microtome, fixation using Mayer's albumin, and staining with hematoxylin-eosin. The prepared tissue slides were examined under a light microscope at 40x and 100x magnification, with observations made in five fields of view per slide. Documentation was carried out using a Beta View camera system. Muscle parameters, including myofiber diameter and area, were measured using Image Raster.

Microplastic Extraction

Microplastic abundance analysis was carried out starting with muscle organs placed in 30 mL flask bottles containing a 10% potassium hydroxide (KOH) solution. The samples were left at room temperature for three days to allow digestion. After three days, the contents of the flask bottles were poured into empty bottles equipped with a funnel and filter paper. The filter paper was left to dry completely before distilled water was added to dissolve any microplastics adhering to the filter. The resulting solution was examined under a stereo microscope at 4x magnification, and the

microplastic abundance was quantified (Nurul Suwartiningsih, 2023).

Data Analysis

The raw data were initially processed using Microsoft Excel, followed by statistical analysis with IBM SPSS version 20. Differences between treatments were considered significant at $P < 0.05$ for all measured parameters and were evaluated using a one-way analysis of variance (ANOVA). When significant differences were detected, further analysis was conducted using Duncan's Multiple Range Test (DMRT).

Result and Discussion

Microplastic Abundance

The results showed that the abundance of microplastics in the muscles of Mutiara catfish was lowest in the control group (0.00 ± 0.00 particles/g) and highest in the T3 (2.63 ± 2.72 particles/g). Statistical analysis indicated that T3 was significantly different from the control and other treatment groups ($P < 0.05$). Regression analysis demonstrated that exposure to polystyrene microplastics in rearing water for 28 days significantly affected the abundance of microplastics in the muscles of Mutiara catfish ($P < 0.05$). This suggests a positive correlation, where higher concentrations of microplastics in the rearing water led to greater microplastic accumulation in the fish muscles, as shown in Table 1.

Table 1. Polystyrene microplastic abundance in Mutiara catfish muscle

Treatment	Microplastic abundance (particles/g)
Control	0,00 ± 0,00^{a}
T1	0,85 ± 1,48^{ab}
T2	0,74 ± 1,28^{ab}
T3	2,63 ± 2,72^{b}

Description: superscript different letters indicate there is a significant difference ($P < 0.05$) and vice versa

The research showed that microplastic exposure starting from 1 mg/L in the rearing media, the abundance of microplastics in the Mutiara catfish muscle was also high. Previous research found that exposure to nanoplastic polystyrene (NPPs) at a concentration of 1 mg/L for three days in zebrafish (*Danio rerio*) resulted in NPPs accumulation of 49 ± 17 mg/g of muscle tissue (Chen et al., 2017). The high abundance of microplastics in fish organs is influenced by the increasing concentration of microplastic particles in the water, allowing microplastics to enter the fish through food ingestion (Roch et al., 2020).

The detection of microplastics in the muscles of Mutiara catfish in this study indicates bioaccumulation, or the accumulation of contaminant particles in the body (Miller et al., 2020). When microplastics enter the gastrointestinal tract, larger particles may settle or be excreted in feces. However, as microplastics break down into smaller particles, they can travel through the bloodstream and accumulate in muscle tissue. Particles around 5 µm in size can infiltrate the gills, intestines, and liver, while microplastics approximately 20 µm in size can penetrate the gills and intestines (Guanting et al., 2021). Particles smaller than 100 µm are capable of reaching muscle tissue (Barboza et al., 2020).

Diameter and Area of Mutiara Catfish Myofiber

The results showed that the diameter of myofiber in Mutiara catfish muscle was lowest in T2 (83.58 ± 25.01 µm) and highest in control (122.11 ± 40.06 µm). The area of myofiber in Mutiara catfish muscle was lowest in T2 (5.96 ± 3.77x10^3 µm^2) and highest in control (12.94 ± 8.65 x10^3 µm^2) (Table 2). Statistical analysis revealed that both myofiber diameter and area were significantly different between control and the other treatment groups ($P < 0.05$). However, there were no significant differences in myofiber diameter and area among the P1, P2, and P3 groups. Regression analysis indicated that polystyrene microplastic exposure in the rearing water for 28 days significantly affected both the myofiber diameter and area in Mutiara catfish ($P < 0.05$).

Table 2. Diameter and area of Mutiara catfish myofiber

Treatment	Diameter of myofiber (µm)	Area of myofiber (x10^3µm^2)
Control	122,11 ± 40,06^{b}	12,49 ± 8,65^{b}
T1	87,80 ± 26,81^{a}	6,60 ± 4,87^{a}
T2	83,58 ± 25,01^{a}	5,96 ± 3,77^{a}
T3	86,32 ± 18,21^{a}	6,10 ± 2,52^{a}

Description: superscript different letters indicate there is a significant difference ($P < 0.05$) and vice versa

The results in Table 2 indicate that Mutiara catfish exposed to polystyrene microplastics for 28 days experienced a reduction in both myofiber diameter and area. The regulation of myofiber diameter is influenced by several factors including myogenesis, muscle atrophy and apoptosis (L. Zhang et al., 2023). Previous research demonstrated that exposure to polystyrene microplastics (PS-MPs) at a concentration of 1000 µg/L for 7 days in goldfish larvae (*Carassius auratus*) caused damage to mesenchymal muscle cells and led to muscle fiber atrophy (Yang et al., 2020). The changes in myofiber diameter can be observed in Figure 1.

Changes in muscle tissue can occur due to the introduction of contaminants in the muscle (Di Giacinto et al., 2023). Styrene is a benzene-derived compound that has high toxicity (Majid et al., 2018). Previous research revealed that exposure to contaminants at concentrations of 44 µg/kg and 234 µg/kg in Yellow catfish (*Pelteobagrus fulvidraco*) over eight weeks led to a reduction in muscle myofiber diameter (Z. Y. Zhang et al., 2021). Another study showed that exposure of fonofos contaminants at a concentration of 2 mg/L for 96 hours caused a decrease in myofiber diameter in Zebrafish (*Danio rerio*) (Arman & Üçüncü, 2021).

This study found a decrease in both myofiber diameter and myofiber area, thus disrupting the growth of muscle myofiber. Myofiber growth that is not optimal will have an impact on the elasticity and compactness of the meat (Z. Y. Zhang et al., 2021). Moreover, muscle injuries may serve as an indicator of exposure to environmental contaminants (Ciamarro et al., 2015). Among such contaminants, microplastics pose a significant toxic risk to all organisms (Stapleton & Hai, 2023).

Conclusion

Exposure of PS microplastics in rearing water for 28 days affects the abundance of microplastics in muscle, reduce myofiber diameter and area of Mutiara catfish. This research emphasizes the need for further investigation into the broader effects of microplastics on aquatic organisms and human health.

Acknowledgments

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Author Contributions

N. Suwartiningsih and A. Fayzha Aszhara collaboratively conceived and designed the study. Computational analyses were carried out by A. Fayzha Aszhara and D. Eka Wijayanti. All authors contributed to the manuscript's writing. Revisions to the manuscript were made with input from all authors, who have reviewed and approved the final version and significantly contributed to the study.

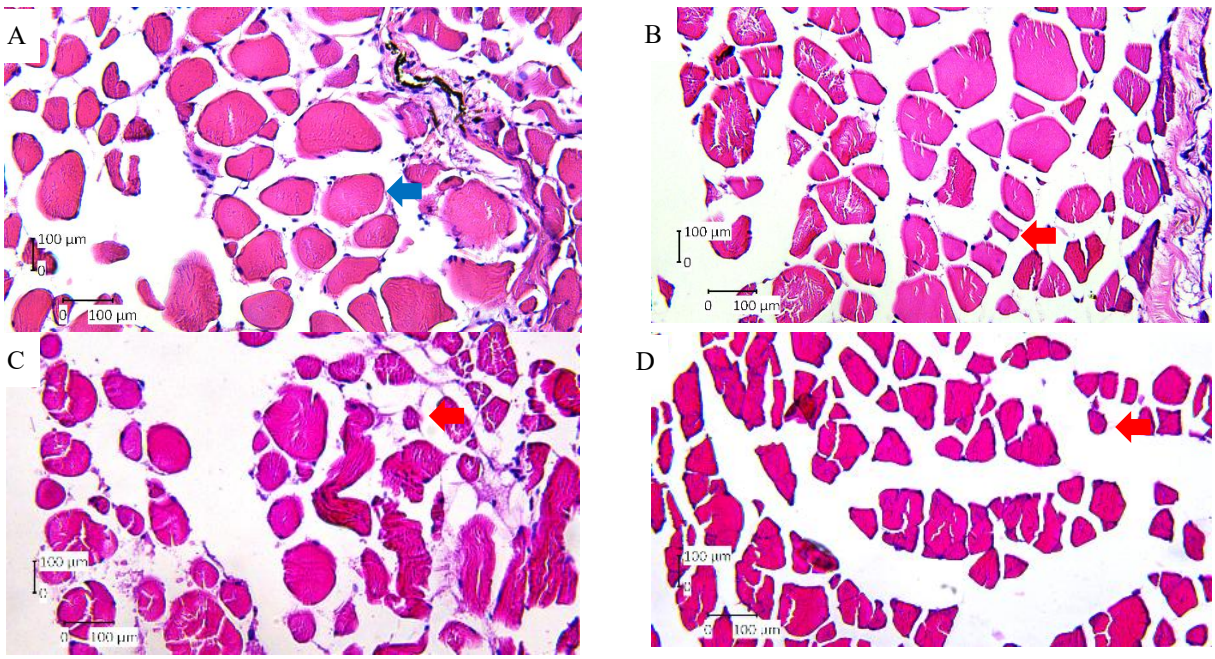

Figure 1. Diameter and area of Mutiara catfish myofiber after 28 days exposure to polysterene microplastics. A: control (0 mg/L); B: T1 (1 mg/L); C: T2 (10 mg/L); D: T3(100 mg/L). Blue arrows indicate normal myofiber diameter and red arrows indicate decreased myofiber diameter (hematoxylin-eosin staining; scale bar 100 µm)

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Conflicts of Interest
We hereby affirm that the preparation of this article was conducted without any conflict of interest that could compromise the objectivity or integrity of the findings.

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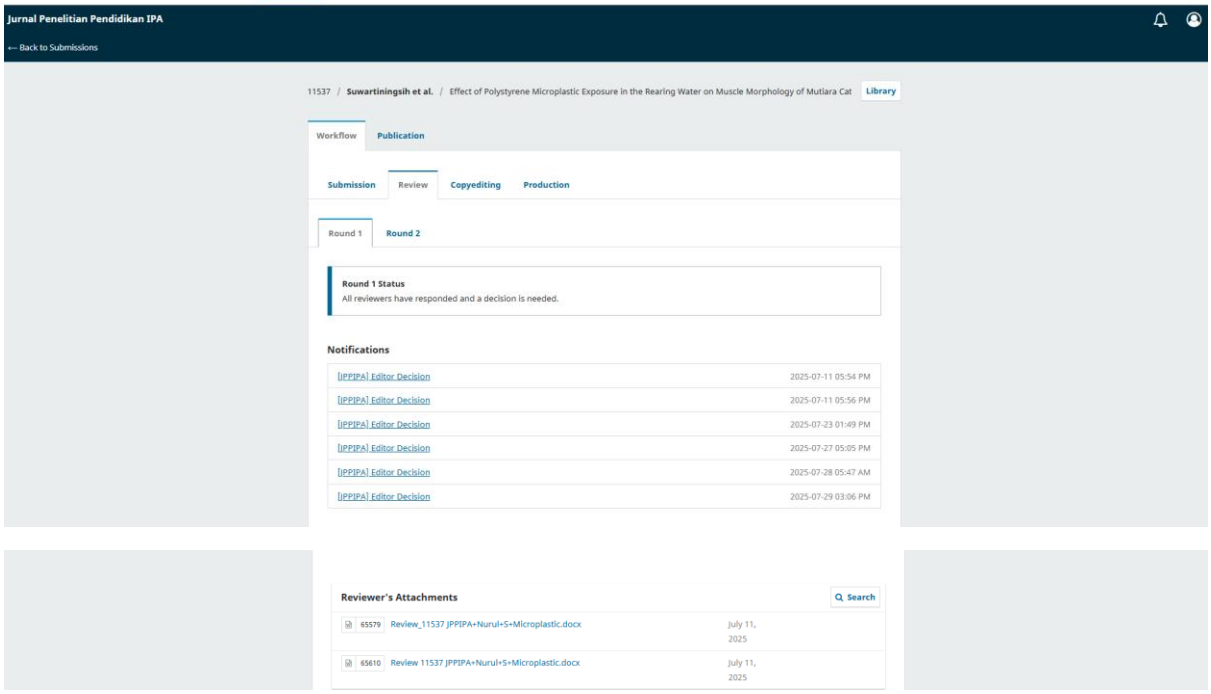

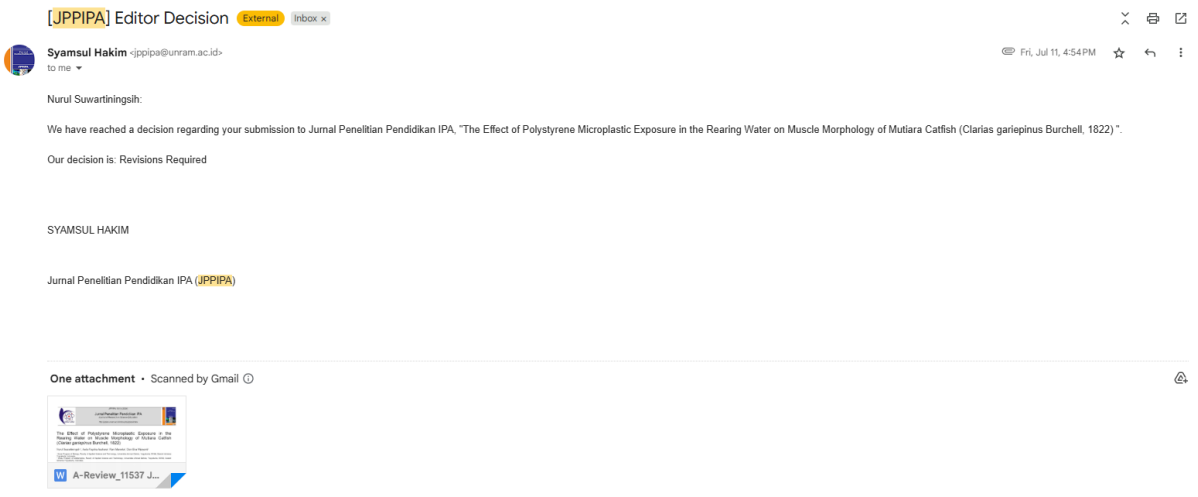

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Abstract: Polystyrene (PS) is one of the most abundance microplastics in freshwater that can lead to its accumulation in fish. This study aims to determine the effect of PS microplastic abundance in the rearing water on muscle tissue, diameter and area of myofibers in Mutiara catfish. A total of 120 fishes were exposed to PS microplastics in the rearing water for 28 days at concentrations of 0 mg/L, 1 mg/L, 10 mg/L, and 100 mg/L. Muscle samples from the right abdominal were collected and prepared using paraffin method with hematoxylin-eosin staining. Microplastics in muscle tissue was extracted with KOH 10%. Microplastics were observed using stereo microscope. Myofibers diameter was measured using Image-Raster. The data were analyzed using the Kruskal Wallis test. The results showed a significant difference between treatments ($P<0.05$) in all parameters. The lowest abundance of microplastics in the muscle found in control, while the highest was in T3. The smallest myofiber diameter and area was in T2, with the largest in control. Exposure of PS microplastics affects the abundance of microplastics in muscle, reduce myofiber diameter and area. This research emphasizes the need for further investigation into the broader effects of microplastics on aquatic organisms and human health.

Keywords: Microplastics; Myofiber; Polystyrene; Rearing water

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Introduction

Microplastics are found in various sizes, shapes and concentrations in aquatic ecosystems (Smith et al., 2018). The primary sources of microplastics include plastic materials such as plastic bags, beverage bottles, beauty products, and cleaning agents (Nainggolan et al., 2022). The wide range of polymer types in microplastics, from high-density to low-density plastics, allows them to be carried by water currents, leading to their presence in both water and sediment. Numerous studies have reported the detection of microplastics in freshwater ecosystems, particularly in river areas. Research has identified microplastics in the Serang River, Yogyakarta, with abundances ranging from 1488 particles kg^{-1} to 384.58 particles kg-.[1] (Ismiyati et al., 2023). Microplastics in water pose significant negative impacts on the environment and aquatic organisms (Utami et al., 2021).

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The presence of microplastics in rivers can lead to their accumulation in aquaculture waters. When aquaculture water is contaminated with microplastics, it can degrade the water quality and adversely affect fish farming operations (Vieira Dantas Filho et al., 2023). Research indicates that the Pelayaran River in Sidoarjo, which supplies water for tilapia (*Oreochromis niloticus*) farming, is contaminated with microplastics, leading to the pollution of the farmed tilapia (Al-Fatih, 2022).

Polystyrene is one type of microplastic polymer frequently detected in aquatic environments (Victoria, 2017). It mainly originates from food packaging, craft materials and decorative items (Melyna, 2021). Research on the Mahakam River found that polystyrene accounted for 6.45% of the total plastic waste, with microplastics comprising 12.5% of the total abundance (Kurniawan et al., 2023). Polystyrene plastics degrade into microplastics through processes such as ultraviolet radiation exposure and weathering, which break the material into microparticles (Hwang et al., 2020).

Microplastics can be ingested by fish through their gills, digestive tract and muscles (Sawalman et al., 2021). Microplastics present in water can enter fish muscles via the bloodstream (Akhbarizadeh et al., 2018). When microplastics enter the digestive tract, they can be transported through the blood vessels and eventually accumulate in muscle tissue (Guanting et al., 2021). Previous research has shown that exposure to microplastics at a concentration of 100 mg/L in tilapia (*Oreochromis niloticus*) can cause muscle damage (Hamed et al., 2021). One of the commonly cultivated and consumed fish species is Mutiara catfish (*Clarias gariepinus* Burchell, 1822) (Iswanto et al., 2016). Therefore, the purpose of this study was to determine the effect of polystyrene microplastic exposure in the rearing water on muscle morphology of Mutiara catfish. This study holds significant implications for environmental health, aquaculture productivity and food safety.

Method

Research Time and Location

This research was conducted from January to November 2024. Maintenance and treatment took place in Dengok Kulon, Bugisan, Prambanan, Klaten, while data preparation, collection, and analysis were carried out at the Laboratory of Animal Structure and Physiology, Universitas Ahmad Dahlan.

Experimental Design

The study was an experimental design consisting of four treatments: a control group with 0 mg/L of polystyrene microplastic, T1 with 1 mg/L, T2 with 10 mg/L, and T3 with 100 mg/L of polystyrene microplastic. A completely randomized design (CRD) was employed, with the treatments administered over 28 days using a total of 120 Mutiara catfishes. Research parameter included the abundance of microplastics in muscle tissue, the diameter of myofibers and the myofiber area.

Microplastics Exposure

Microplastic preparation was carried out by pulverizing polystyrene plastic with a blender, followed by filtering through a 35-mesh sieve. The microplastics were weighed according to the treatment concentrations. Mutira catfish were acclimatized for ten days by being given food at 08.00 and 16.00 WIB, equivalent to 3% of their body weight (Tang et al., 2024). Microplastic exposure was conducted during water changes every two days for 28 days, based on the designated exposure concentrations.

Muscle Tissue Preparation

At the end of the treatment, the fish were fasted for 24 hours before being weighed and their organs harvested on day 29. Muscle tissues were weighed and fixed in a 10% formalin solution (Zulfadhli et al., 2016). The preparation process included dehydration with graded alcohol (70% to absolute), de-alcoholization with toluol solution infiltration, embedding in paraffin, sectioning with a microtome, fixation using Mayer's albumin, and staining with hematoxylin-eosin. The prepared tissue slides were examined under a light microscope at 40x and 100x magnification, with observations made in five fields of view per slide. Documentation was carried out using a Beta View camera system. Muscle parameters, including myofiber diameter and area, were measured using Image Raster.

Microplastic Extraction

Microplastic abundance analysis was carried out starting with muscle organs placed in 30 mL flask bottles containing a 10% potassium hydroxide (KOH) solution. The samples were left at room temperature for three days to allow digestion. After three days, the contents of the flask bottles were poured into empty bottles equipped with a funnel and filter paper. The filter paper was left to dry completely before distilled water was added to dissolve any microplastics adhering to the filter. The resulting solution was examined under a stereo microscope at 4x magnification, and the

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microplastic abundance was quantified (Nurul Suwartiningsih, 2023).

Data Analysis

The raw data were initially processed using Microsoft Excel, followed by statistical analysis with IBM SPSS version 20. Differences between treatments were considered significant at P < 0.05 for all measured parameters and were evaluated using a one-way analysis of variance (ANOVA). When significant differences were detected, further analysis was conducted using Duncan's Multiple Range Test (DMRT).

Result and Discussion

Microplastic Abundance

The results showed that the abundance of microplastics in the muscles of Mutiara catfish was lowest in the control group (0.00 ± 0.00 particles/g) and highest in the T3 (2.63 ± 2.72 particles/g). Statistical analysis indicated that T3 was significantly different from the control and other treatment groups ($P < 0.05$). Regression analysis demonstrated that exposure to polystyrene microplastics in rearing water for 28 days significantly affected the abundance of microplastics in the muscles of Mutiara catfish ($P < 0.05$). This suggests a positive correlation, where higher concentrations of microplastics in the rearing water led to greater microplastic accumulation in the fish muscles, as shown in Table 1.

Table 1. Polystyrene microplastic abundance in Mutiara catfish muscle

Treatment	Microplastic abundance (particles/g)
Control	0,00 ± 0,00[a]
T1	0,85 ± 1,48[ab]
T2	0,74 ± 1,28[ab]
T3	2,63 ± 2,72[b]

Description: superscript different letters indicate there is a significant difference ($P < 0.05$) and vice versa

The research showed that microplastic exposure starting from 1 mg/L in the rearing media, the abundance of microplastics in the Mutiara catfish muscle was also high. Previous research found that exposure to nanoplastic polystyrene (NPPs) at a concentration of 1 mg/L for three days in zebrafish (*Danio rerio*) resulted in NPPs accumulation of 49 ± 17 mg/g of muscle tissue (Chen et al., 2017). The high abundance of microplastics in fish organs is influenced by the increasing concentration of microplastic particles in the water, allowing microplastics to enter the fish through food ingestion (Roch et al., 2020).

The detection of microplastics in the muscles of Mutiara catfish in this study indicates bioaccumulation, or the accumulation of contaminant particles in the body (Miller et al., 2020). When microplastics enter the gastrointestinal tract, larger particles may settle or be excreted in feces. However, as microplastics break down into smaller particles, they can travel through the bloodstream and accumulate in muscle tissue. Particles around 5 µm in size can infiltrate the gills, intestines, and liver, while microplastics approximately 20 µm in size can penetrate the gills and intestines (Guanting et al., 2021). Particles smaller than 100 µm are capable of reaching muscle tissue (Barboza et al., 2020).

Diameter and Area of Mutiara Catfish Myofiber

The results showed that the diameter of myofiber in Mutiara catfish muscle was lowest in T2 (83.58 ± 25.01 µm) and highest in control (122.11 ± 40.06 µm). The area of myofiber in Mutiara catfish muscle was lowest in T2 (5.96 ± 3.77x10^3 µm^2) and highest in control (12.94 ± 8.65 x10^3 µm^2) (Table 2). Statistical analysis revealed that both myofiber diameter and area were significantly different between control and the other treatment groups ($P < 0.05$). However, there were no significant differences in myofiber diameter and area among the P1, P2, and P3 groups. Regression analysis indicated that polystyrene microplastic exposure in the rearing water for 28 days significantly affected both the myofiber diameter and area in Mutiara catfish ($P < 0.05$).

Table 2. Diameter and area of Mutiara catfish myofiber

Treatment	Diameter of myofiber (µm)	Area of myofiber (x10^3µm^2)
Control	122,11 ± 40,06[b]	12,49 ± 8,65[b]
T1	87,80 ± 26,81[a]	6,60 ± 4,87[a]
T2	83,58 ± 25,01[a]	5,96 ± 3,77[a]
T3	86,32 ± 18,21[a]	6,10 ± 2,52[a]

Description: superscript different letters indicate there is a significant difference ($P < 0.05$) and vice versa

The results in Table 2 indicate that Mutiara catfish exposed to polystyrene microplastics for 28 days experienced a reduction in both myofiber diameter and area. The regulation of myofiber diameter is influenced by several factors including myogenesis, muscle atrophy and apoptosis (L. Zhang et al., 2023). Previous research demonstrated that exposure to polystyrene microplastics (PS-MPs) at a concentration of 1000 µg/L for 7 days in goldfish larvae (*Carassius auratus*) caused damage to mesenchymal muscle cells and led to muscle fiber atrophy (Yang et al., 2020). The changes in myofiber diameter can be observed in Figure 1.

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Changes in muscle tissue can occur due to the introduction of contaminants in the muscle (Di Giacinto et al., 2023). Styrene is a benzene-derived compound that has high toxicity (Majid et al., 2018). Previous research revealed that exposure to contaminants at concentrations of 44 µg/kg and 234 µg/kg in Yellow catfish (*Pelteobagrus fulvidraco*) over eight weeks led to a reduction in muscle myofiber diameter (Z. Y. Zhang et al., 2021). Another study showed that exposure of fonofos contaminants at a concentration of 2 mg/L for 96 hours caused a decrease in myofiber diameter in Zebrafish (*Danio rerio*) (Arman & Üçüncü, 2021).

This study found a decrease in both myofiber diameter and myofiber area, thus disrupting the growth of muscle myofiber. Myofiber growth that is not optimal will have an impact on the elasticity and compactness of the meat (Z. Y. Zhang et al., 2021). Moreover, muscle injuries may serve as an indicator of exposure to environmental contaminants (Ciamarro et al., 2015). Among such contaminants, microplastics pose a significant toxic risk to all organisms (Stapleton & Hai, 2023).

Conclusion

Exposure of PS microplastics in rearing water for 28 days affects the abundance of microplastics in muscle, reduce myofiber diameter and area of Mutiara catfish. This research emphasizes the need for further investigation into the broader effects of microplastics on aquatic organisms and human health.

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Author Contributions
N. Suwartiningsih and A. Fayzha Aszhara collaboratively conceived and designed the study. Computational analyses were carried out by A. Fayzha Aszhara and D. Eka Wijayanti. All authors contributed to the manuscript's writing. Revisions to the manuscript were made with input from all authors, who have reviewed and approved the final version and significantly contributed to the study.

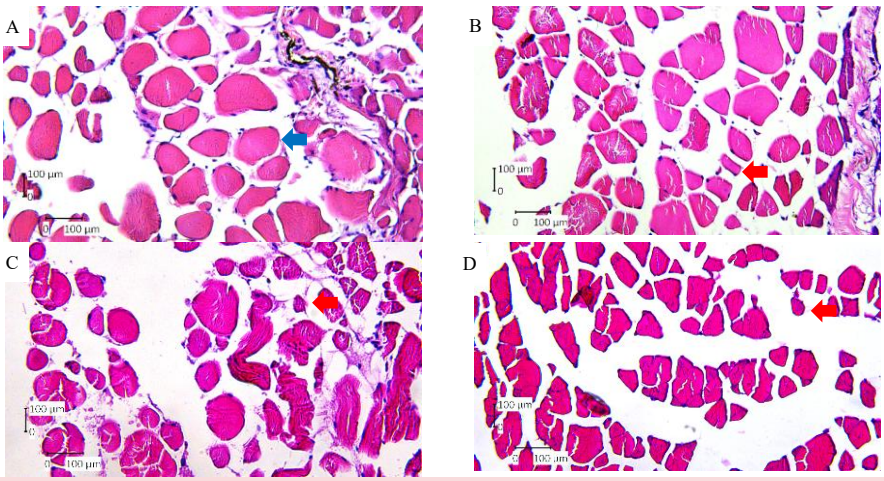

Figure 1. Diameter and area of Mutiara catfish myofiber after 28 days exposure to polysterene microplastics. A: control (0 mg/L); B: T1 (1 mg/L); C: T2 (10 mg/L); D: T3(100 mg/L). Blue arrows indicate normal myofiber diameter and red arrows indicate decreased myofiber diameter (hematoxylin-eosin staining; scale bar 100 µm)

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Conclusions should be more detailed and specific.

Lack of Generalization:
Try to generalize your research findings.

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Explain the practical implications of your research findings.

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Conceptualization, C. H. S. A, P. M. Z, T. R, R. A. E, M. N. S.; methodology, C. H. S. A.; validation, P. M. Z. and T. R.; formal analysis, R. A. E.; investigation, M. N. S., and C. H. S. A.; resources, P. M. Z. and T. R.; data curation, R. A. E.: writing—original draft preparation, M. N. S and C. H. S. A.; writing—review and editing, P. M. Z.: visualization, and T. R. and R. A. E. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest
We hereby affirm that the preparation of this article was conducted without any conflict of interest that could compromise the objectivity or integrity of the findings.

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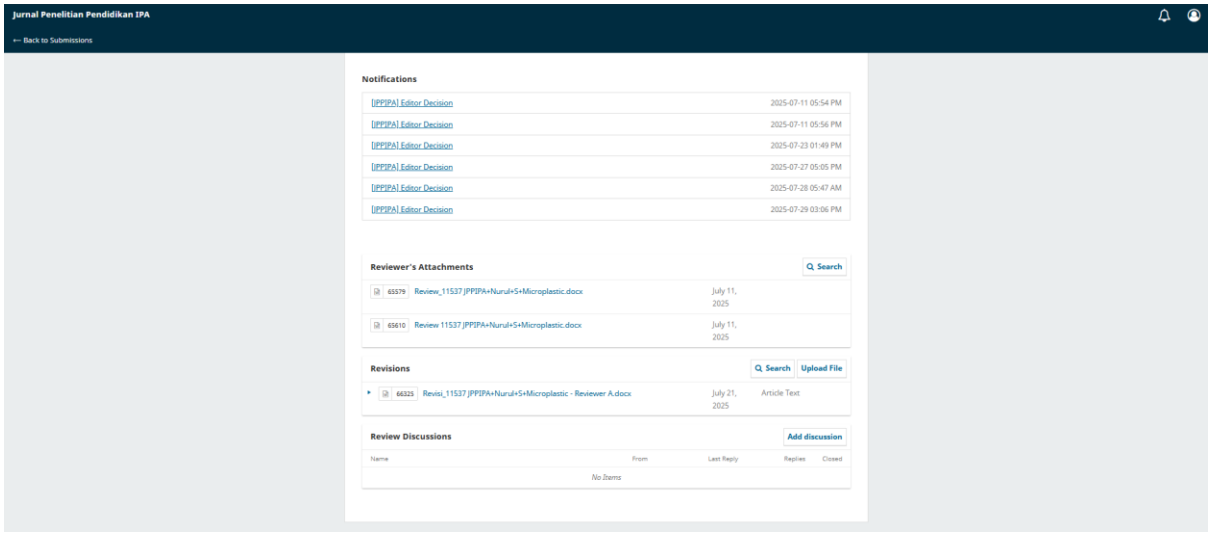

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Dear Editor,

Thank you for your decision and the opportunity to revise our manuscript entitled *"The Effect of Polystyrene Microplastic Exposure in the Rearing Water on Muscle Morphology of Mutiara Catfish (Clarias gariepinus Burchell, 1822)"*.

We appreciate the valuable feedback provided. We have carefully revised the manuscript based on the comments and suggestions from the reviewers to improve its overall quality. The revised manuscript, along with the response to reviewers' comments, is hereby submitted for your kind consideration.

Thank you once again for your consideration.

Sincerely,
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The Effect of Polystyrene Microplastic Exposure in the Rearing Water on Muscle Morphology of Mutiara Catfish (*Clarias gariepinus* Burchell, 1822)

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Abstract: Polystyrene (PS) is one of the most abundant microplastics in freshwater and can accumulate in fish tissues. This study investigated the effect of PS microplastic exposure in rearing water on the abundance of microplastics in muscle, and the diameter and area of myofibers in Mutiara catfish (*Clarias gariepinus*). A total of 120 fish were exposed to PS microplastics at concentrations of 0, 1, 10, and 100 mg/L for 28 days. Muscle samples from the right abdomen were processed using the paraffin method and stained with hematoxylin-eosin. Microplastics were extracted using 10% KOH and observed under a stereo microscope. Myofiber diameter and area were measured with Image Raster software. Data were analyzed using the Kruskal-Wallis test. Results showed significant differences among treatments ($P<0.05$) for all parameters. The control group had the lowest microplastic abundance and the largest myofiber size, while the highest accumulation occurred at 100 mg/L, and the smallest myofiber diameter and area were found at 10 mg/L. Exposure to PS microplastics leads to accumulation in muscle tissue and a reduction in myofiber dimensions. These findings highlight the potential adverse impacts of microplastics on fish health and underline the importance of further studies regarding ecological and human health implications.

Keywords: Microplastic exposure; Muscle tissue; Myofiber area; Myofiber diameter; Polystyrene toxicity

Introduction

Microplastics are increasingly recognized as emerging pollutants in aquatic ecosystems, appearing in various polymers, shapes, and concentrations (Smith et al., 2018). These particles originate primarily from consumer products such as plastic bags, beverage bottles, personal care items, and cleaning agents (Nainggolan et al., 2022). Due to their diverse polymer composition, ranging from high to low density plastics, microplastics are easily transported by water currents and accumulate in both water columns and sediments (Owowenu et al., 2023). Numerous studies have reported the detection of microplastics in freshwater ecosystems, particularly in river areas (Dhea et al., 2023; Lofty et al., 2023). Research has identified microplastics

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Example: Susilawati, S., Doyan, A., Muliyadi, L., & Hakim, S. (2019). Growth of tin oxide thin film by aluminum and fluorine doping using spin coating Sol-Gel techniques. *Jurnal Penelitian Pendidikan IPA, 1*(1), 1-4. https://doi.org/10.29303/jppipa.v1i1.264

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in the Pekalongan River estuary, with abundances ranging from 45.2 to 99.1 particles L^{-1} in surface water, dominated by fragment and film morphologies (Ismanto et al., 2023). Microplastics pose ecological risks by negatively impacting aquatic organisms and degrading environmental quality (Li et al., 2023; Yuranda et al., 2024).

Rivers contaminated with microplastics may transfer these particles into aquaculture systems (Santikanuri et al., 2025), thereby affecting farmed fish. Microplastic contamination in aquaculture environments can deteriorate water quality, compromise fish health, and ultimately reduce aquaculture productivity (Filho et al., 2023). Research findings indicate that tilapia (*Oreochromis niloticus*) reared in the Pelayaran River, Sidoarjo, contain microplastic residues, attributed to pollution in the surrounding aquatic environment (Al-Fatih, 2022).

Among the various types of microplastics, polystyrene is commonly detected in aquatic habitats (Jones et al., 2020) originating from food and beverage packaging as well as the automotive industry (Nurhadi et al., 2017). Research on the Mahakam River found that polystyrene accounted for 6.45% of the total plastic waste, with microplastics comprising 12.5% of the total abundance (Kurniawan et al., 2023). Environmental exposure, especially ultraviolet radiation and weathering, accelerates the breakdown of polystyrene into microplastic fragments (Hwang et al., 2020).

Microplastics can be ingested by fish through their gills, digestive tract and muscles (Sawalman et al., 2021). Microplastics present in water can enter fish muscles via the bloodstream (Akhbarizadeh et al., 2018). When microplastics enter the digestive tract, they can be transported through the blood vessels and eventually accumulate in muscle tissue (Liu et al., 2022). Previous research has shown that exposure to microplastics at a concentration of 100 mg/L in tilapia (*Oreochromis niloticus*) can cause muscle damage (Hamed et al., 2021). One of the commonly cultivated and consumed fish species is Mutiara catfish (*Clarias gariepinus* Burchell, 1822) (Iswanto et al., 2016). Despite growing concern over microplastic contamination, the histopathological effects of polystyrene microplastics on muscle morphology in Mutiara catfish remain understudied. Most research has focused on gastrointestinal tissues, with limited attention to muscle damage in economically important aquaculture species under controlled exposure

The novelty of this research lies in evaluating the sub-lethal and tissue-specific effects of polystyrene microplastic exposure on Mutiara catfish muscle morphology. This study provides critical insight into how microplastics may compromise fish meat quality, a concern for both environmental health and food safety. Therefore, this research is important to understand potential histological damage caused by microplastic contamination in aquaculture systems. The findings may inform sustainable fish farming practices, guide risk assessments of microplastic exposure in aquatic food chains, and support the development of environmental policies to mitigate plastic pollution impacts on food-producing aquatic ecosystems.

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Give logical reasons why this research is important to do

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Method

Research Time and Location

This research was conducted from January to November 2024. Maintenance and treatment took place in Dengok Kulon, Bugisan, Prambanan, Klaten, while data preparation, collection, and analysis were carried out at the Laboratory of Animal Structure and Physiology, Universitas Ahmad Dahlan.

Experimental Design

This study employed an experimental approach using a completely randomized design (CRD) with four treatment groups: a control group (0 mg/L polystyrene microplastic), T1 (1 mg/L), T2 (10 mg/L), and T3 (100 mg/L). Each treatment was replicated six times using separate rearing tanks, with five Mutiara catfish (*Clarias gariepinus*) allocated per tank, resulting in a total of 120 fish. The exposure period lasted for 28 days. The parameters observed in this study included the abundance of microplastics in muscle tissue, the diameter of myofibers, and the area of myofibers.

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Commented [NS9R8]: Thank you for the valuable feedback. We appreciate the suggestion to include a flow or chart to improve clarity. However, we have chosen not to include a flowchart at this stage, as the narrative has been revised to provide a clearer and more structured explanation of the process. We believe the improved narrative sufficiently conveys the intended information in a coherent and comprehensible manner. Nonetheless, we remain open to revisiting this should further clarification be required.

Microplastics Exposure

Microplastic preparation was carried out by pulverizing polystyrene plastic with a blender, followed by filtering through a 35-mesh sieve. The microplastics were weighed according to the treatment concentrations. Mutira catfish were acclimatized for ten days by being given food at 08.00 and 16.00 WIB, equivalent to 3% of their body weight (Tang et al., 2024). Microplastic exposure was conducted during water changes every two days for 28 days, based on the designated exposure concentrations.

Muscle Tissue Preparation

At the end of the treatment, the fish were fasted for 24 hours before being weighed and their organs harvested on day 29. Muscle tissues were weighed and fixed in a 10% formalin solution (Zulfadhli et al., 2016).

The preparation process included dehydration with graded alcohol (70% to absolute), de-alcoholization with toluol solution infiltration, embedding in paraffin, sectioning with a microtome, fixation using Mayer's albumin, and staining with hematoxylin-eosin. The prepared tissue slides were examined under a light microscope at 40x and 100x magnification, with observations made in five fields of view per slide. Documentation was carried out using a Beta View camera system. Muscle parameters, including myofiber diameter and area, were measured using Image Raster.

Microplastic Extraction

Microplastic abundance analysis was carried out starting with muscle organs placed in 30 mL flask bottles containing a 10% potassium hydroxide (KOH) solution. The samples were left at room temperature for three days to allow digestion. After three days, the contents of the flask bottles were poured into empty bottles equipped with a funnel and filter paper. The filter paper was left to dry completely before distilled water was added to dissolve any microplastics adhering to the filter. The resulting solution was examined under a stereo microscope at 4x magnification, and the microplastic abundance was quantified (Nurul Suwartiningsih, 2023).

Data Analysis

The raw data were initially processed using Microsoft Excel, followed by statistical analysis with IBM SPSS version 20. Differences between treatments were considered significant at $P < 0.05$ for all measured parameters and were evaluated using a one-way analysis of variance (ANOVA). When significant differences were detected, further analysis was conducted using Duncan's Multiple Range Test (DMRT).

Result and Discussion

Microplastic Abundance

The results showed that the abundance of microplastics in the muscles of Mutiara catfish was lowest in the control group (0.00 ± 0.00 particles/g) and highest in the T3 (2.63 ± 2.72 particles/g). Statistical analysis indicated that T3 was significantly different from the control and other treatment groups ($P < 0.05$). Regression analysis demonstrated that exposure to polystyrene microplastics in rearing water for 28 days significantly affected the abundance of microplastics in the muscles of Mutiara catfish ($P < 0.05$). This suggests a positive correlation, where higher concentrations of microplastics in the rearing water led to greater microplastic accumulation in the fish muscles, as shown in Table 1.

Table 1. Polystyrene microplastic abundance in Mutiara catfish muscle

Treatment	Microplastic abundance (particles/g)
Control	0,00 ± 0,00[a]
T1	0,85 ± 1,48[ab]
T2	0,74 ± 1,28[ab]
T3	2,63 ± 2,72[b]

Description: superscript different letters indicate there is a significant difference ($P < 0.05$) and vice versa

The research showed that microplastic exposure starting from 1 mg/L in the rearing media, the abundance of microplastics in the Mutiara catfish muscle was also high. Previous research found that exposure to nanoplastic polystyrene (NPPs) at a concentration of 1 mg/L for three days in zebrafish (*Danio rerio*) resulted in NPPs accumulation of 49 ± 17 mg/g of muscle tissue (Chen et al., 2017). The high abundance of microplastics in fish organs is influenced by the increasing concentration of microplastic particles in the water (Popi et al., 2025; Rofiq & Sari, 2022; Wahid & Joesidawati, 2024), and vice verca (Fitriyani et al., 2025) allowing microplastics to enter the fish through food ingestion (Roch et al., 2020; Yona et al., 2022).

The detection of microplastics in the muscles of Mutiara catfish in this study indicates bioaccumulation, or the accumulation of contaminant particles in the body (Miller et al., 2020; Utomo & Muzaki, 2022). When microplastics enter the gastrointestinal tract, larger particles may settle or be excreted (Purnama et al., 2021) in feces. However, as microplastics break down into smaller particles, they can travel through the bloodstream (Nurwahyunani et al., 2022) and accumulate in muscle tissue (Yona et al., 2021). Particles around 5 µm in size can infiltrate the gills, intestines, and liver, while microplastics approximately 20 µm in size can penetrate the gills and intestines (Guanting et al., 2021). Particles smaller than 100 µm are capable of reaching muscle tissue (Barboza et al., 2020).

Diameter and Area of Mutiara Catfish Myofiber

The results showed that the diameter of myofiber in Mutiara catfish muscle was lowest in T2 (83.58 ± 25.01 µm) and highest in control (122.11 ± 40.06 µm). The area of myofiber in Mutiara catfish muscle was lowest in T2 (5.96 ± 3.77x10^3 µm^2) and highest in control (12.94 ± 8.65 x10^3 µm^2) (Table 2). Statistical analysis revealed that both myofiber diameter and area were significantly different between control and the other treatment groups ($P < 0.05$). However, there were no significant differences in

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Delete the number column, replace Indonesian with English.
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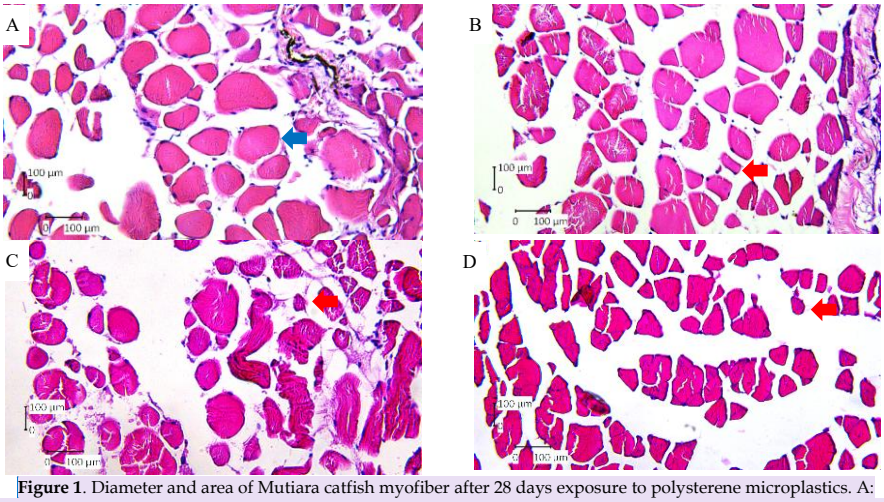

Figure 1. Diameter and area of Mutiara catfish myofiber after 28 days exposure to polysterene microplastics. A: control (0 mg/L); B: T1 (1 mg/L); C: T2 (10 mg/L); D: T3(100 mg/L). Blue arrows indicate normal myofiber diameter and red arrows indicate decreased myofiber diameter (hematoxylin-eosin staining; scale bar 100 µm)

myofiber diameter and area among the P1, P2, and P3 groups. Regression analysis indicated that polystyrene microplastic exposure in the rearing water for 28 days significantly affected both the myofiber diameter and area in Mutiara catfish ($P < 0.05$).

Table 2. Diameter and area of Mutiara catfish myofiber

Treatment	Diameter of myofiber (µm)	Area of myofiber ($x10^3 µm^2$)
Control	122,11 ± 40,06[b]	12,49 ± 8,65[b]
T1	87,80 ± 26,81[a]	6,60 ± 4,87[a]
T2	83,58 ± 25,01[a]	5,96 ± 3,77[a]
T3	86,32 ± 18,21[a]	6,10 ± 2,52[a]

Description: superscript different letters indicate there is a significant difference ($P < 0.05$) and vice versa

The results in Table 2 indicate that Mutiara catfish exposed to polystyrene microplastics for 28 days experienced a reduction in both myofiber diameter and area. The regulation of myofiber diameter is influenced by several factors including myogenesis, muscle atrophy and apoptosis (L. Zhang et al., 2023). Previous research demonstrated that exposure to polystyrene microplastics (PS-MPs) at a concentration of 1000 µg/L for 7 days in goldfish larvae (*Carassius auratus*) caused damage to mesenchymal muscle cells and led to muscle fiber atrophy (Yang et al., 2020). The changes in myofiber diameter can be observed in Figure 1.

Changes in muscle tissue can occur due to the introduction of contaminants in the muscle (Di Giacinto et al., 2023; Setyono et al., 2024). Styrene is a benzene-derived compound that has high toxicity (Aldi, 2024; Majid et al., 2018). Previous research revealed that exposure to contaminants at concentrations of 44 µg/kg and 234 µg/kg in Yellow catfish (*Pelteobagrus fulvidraco*) over eight weeks led to a reduction in muscle myofiber diameter (Z. Y. Zhang et al., 2021). Another study showed that exposure of fonofos contaminants at a concentration of 2 mg/L for 96 hours caused a decrease in myofiber diameter in Zebrafish (*Danio rerio*) (Arman & Üçüncü, 2021).

This study found a decrease in both myofiber diameter and myofiber area, thus disrupting the growth of muscle myofiber (Moenek & Toelle, 2021). Myofiber growth that is not optimal will have an impact on the elasticity and compactness of the meat (Suwiti et al., 2015; Z. Y. Zhang et al., 2021). Moreover, muscle injuries may serve as an indicator of exposure to environmental contaminants (Ciamarro et al., 2015). Among such contaminants, microplastics pose a significant toxic risk to all organisms (Aulia et al., 2023; Stapleton & Hai, 2023).

Conclusion

This study demonstrated that exposure to polystyrene (PS) microplastics in rearing water for 28 days significantly increased microplastic accumulation in muscle tissue and reduced both myofiber diameter and area in Mutiara catfish (*Clarias gariepinus*). These histological alterations indicate muscle atrophy,

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suggesting that microplastic contamination can compromise fish health and meat quality. The findings emphasize the potential ecological risks of microplastics and their implications for food safety, particularly in aquaculture systems. This highlights the urgent need for improved environmental monitoring and regulatory policies to reduce microplastic pollution and safeguard both aquatic ecosystems and public health.

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Author Contributions

Conceptualization, N.S. and A.F.A.; methodology, N.S. and A.F.A.; validation, N.S.; formal analysis, A.F.A.; investigation, A.F.A. and D.E.W.; resources, N.S.; data curation, A.F.A.; writing—original draft preparation, A.F.A.; writing—review and editing, N.S., A.F.A., and D.E.W.; visualization, A.F.A.; supervision, N.S. All authors have read and approved the final version of the manuscript.

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Lack of Detail:
Conclusions should be more detailed and specific.

Lack of Generalization:
Try to generalize your research findings.

Practical Implications:
Explain the practical implications of your research findings.

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Conceptualization, C. H. S. A, P. M. Z, T. R, R. A. E, M. N. S.; methodology, C. H. S. A.; validation, P. M. Z. and T. R.; formal analysis, R. A. E.; investigation, M. N. S., and C. H. S. A.; resources, P. M. Z. and T. R.; data curation, R. A. E.: writing—original draft preparation, M. N. S and C. H. S. A.; writing—review and editing, P. M. Z.: visualization, and T. R. and R. A. E. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest
We hereby affirm that the preparation of this article was conducted without any conflict of interest that could compromise the objectivity or integrity of the findings.

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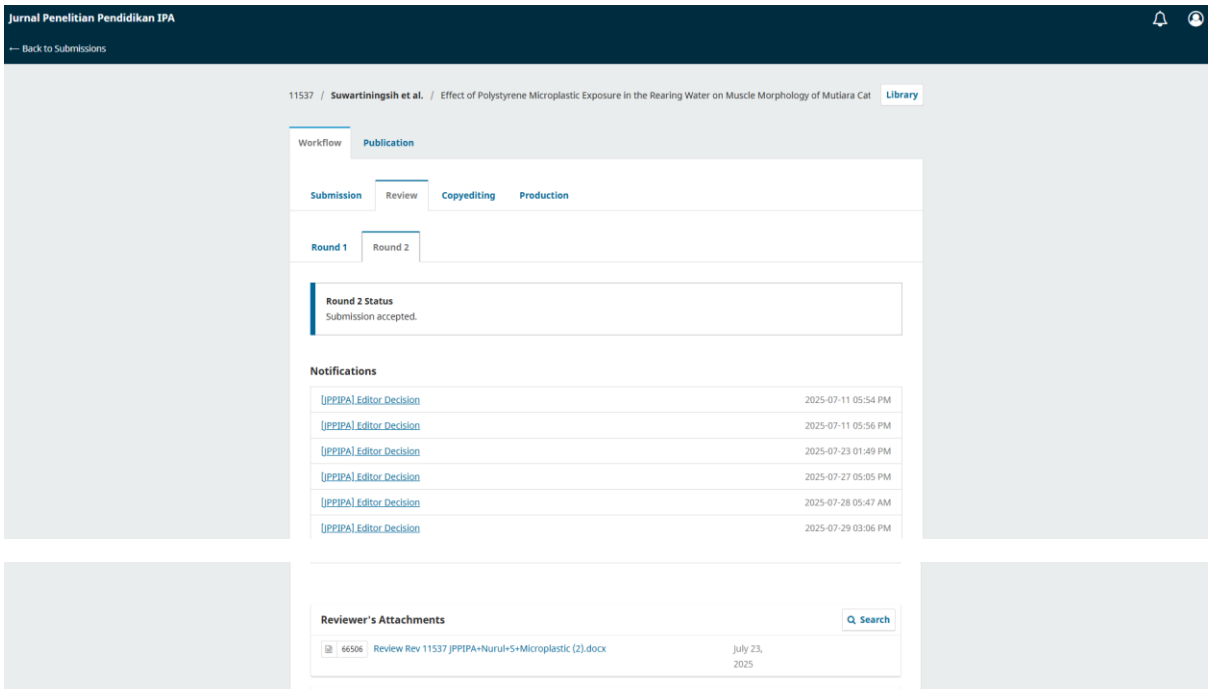

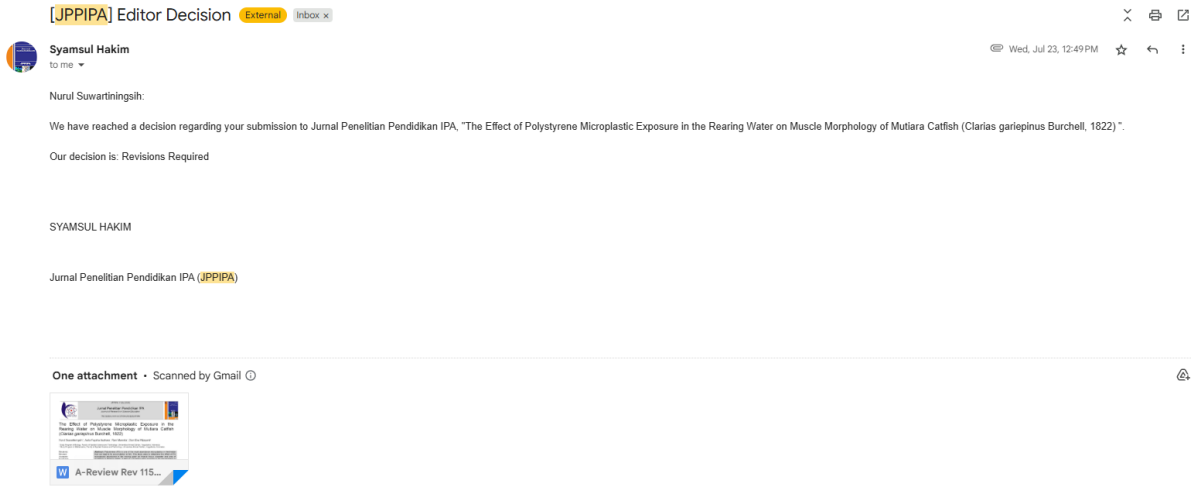

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The Effect of Polystyrene Microplastic Exposure in the Rearing Water on Muscle Morphology of Mutiara Catfish (*Clarias gariepinus* Burchell, 1822)

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Abstract: Polystyrene (PS) is one of the most abundant microplastics in freshwater and can accumulate in fish tissues. This study investigated the effect of PS microplastic exposure in rearing water on the abundance of microplastics in muscle, and the diameter and area of myofibers in Mutiara catfish (*Clarias gariepinus*). A total of 120 fish were exposed to PS microplastics at concentrations of 0, 1, 10, and 100 mg/L for 28 days. Muscle samples from the right abdomen were processed using the paraffin method and stained with hematoxylin-eosin. Microplastics were extracted using 10% KOH and observed under a stereo microscope. Myofiber diameter and area were measured with Image Raster software. Data were analyzed using the Kruskal-Wallis test. Results showed significant differences among treatments ($P<0.05$) for all parameters. The control group had the lowest microplastic abundance and the largest myofiber size, while the highest accumulation occurred at 100 mg/L, and the smallest myofiber diameter and area were found at 10 mg/L. Exposure to PS microplastics leads to accumulation in muscle tissue and a reduction in myofiber dimensions. These findings highlight the potential adverse impacts of microplastics on fish health and underline the importance of further studies regarding ecological and human health implications.

Keywords: Microplastic exposure; Muscle tissue; Myofiber area; Myofiber diameter; Polystyrene toxicity

Introduction

Microplastics are increasingly recognized as emerging pollutants in aquatic ecosystems, appearing in various polymers, shapes, and concentrations (Smith et al., 2018). These particles originate primarily from consumer products such as plastic bags, beverage bottles, personal care items, and cleaning agents (Nainggolan et al., 2022). Due to their diverse polymer composition, ranging from high to low density plastics, microplastics are easily transported by water currents and accumulate in both water columns and sediments (Owowenu et al., 2023). Numerous studies have reported the detection of microplastics in freshwater ecosystems, particularly in river areas (Dhea et al., 2023; Lofty et al., 2023). Research has identified microplastics

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in the Pekalongan River estuary, with abundances ranging from 45.2 to 99.1 particles L^{-1} in surface water, dominated by fragment and film morphologies (Ismanto et al., 2023). Microplastics pose ecological risks by negatively impacting aquatic organisms and degrading environmental quality (Li et al., 2023; Yuranda et al., 2024).

Rivers contaminated with microplastics may transfer these particles into aquaculture systems (Santikanuri et al., 2025), thereby affecting farmed fish. Microplastic contamination in aquaculture environments can deteriorate water quality, compromise fish health, and ultimately reduce aquaculture productivity (Filho et al., 2023). Research findings indicate that tilapia (*Oreochromis niloticus*) reared in the Pelayaran River, Sidoarjo, contain microplastic residues, attributed to pollution in the surrounding aquatic environment (Al-Fatih, 2022).

Among the various types of microplastics, polystyrene is commonly detected in aquatic habitats (Jones et al., 2020) originating from food and beverage packaging as well as the automotive industry (Nurhadi et al., 2017). Research on the Mahakam River found that polystyrene accounted for 6.45% of the total plastic waste, with microplastics comprising 12.5% of the total abundance (Kurniawan et al., 2023). Environmental exposure, especially ultraviolet radiation and weathering, accelerates the breakdown of polystyrene into microplastic fragments (Hwang et al., 2020).

Microplastics can be ingested by fish through their gills, digestive tract and muscles (Sawalman et al., 2021). Microplastics present in water can enter fish muscles via the bloodstream (Akhbarizadeh et al., 2018). When microplastics enter the digestive tract, they can be transported through the blood vessels and eventually accumulate in muscle tissue (Liu et al., 2022). Previous research has shown that exposure to microplastics at a concentration of 100 mg/L in tilapia (*Oreochromis niloticus*) can cause muscle damage (Hamed et al., 2021). One of the commonly cultivated and consumed fish species is Mutiara catfish (*Clarias gariepinus* Burchell, 1822) (Iswanto et al., 2016). Despite growing concern over microplastic contamination, the histopathological effects of polystyrene microplastics on muscle morphology in Mutiara catfish remain understudied. Most research has focused on gastrointestinal tissues, with limited attention to muscle damage in economically important aquaculture species under controlled exposure

The novelty of this research lies in evaluating the sub-lethal and tissue-specific effects of polystyrene microplastic exposure on Mutiara catfish muscle morphology. This study provides critical insight into how microplastics may compromise fish meat quality, a concern for both environmental health and food safety. Therefore, this research is important to understand potential histological damage caused by microplastic contamination in aquaculture systems. The findings may inform sustainable fish farming practices, guide risk assessments of microplastic exposure in aquatic food chains, and support the development of environmental policies to mitigate plastic pollution impacts on food-producing aquatic ecosystems.

Method

Research Time and Location

This research was conducted from January to November 2024. Maintenance and treatment took place in Dengok Kulon, Bugisan, Prambanan, Klaten, while data preparation, collection, and analysis were carried out at the Laboratory of Animal Structure and Physiology, Universitas Ahmad Dahlan.

Experimental Design

This study employed an experimental approach using a completely randomized design (CRD) with four treatment groups: a control group (0 mg/L polystyrene microplastic), T1 (1 mg/L), T2 (10 mg/L), and T3 (100 mg/L). Each treatment was replicated six times using separate rearing tanks, with five Mutiara catfish (*Clarias gariepinus*) allocated per tank, resulting in a total of 120 fish. The exposure period lasted for 28 days. The parameters observed in this study included the abundance of microplastics in muscle tissue, the diameter of myofibers, and the area of myofibers.

Microplastics Exposure

Microplastic preparation was carried out by pulverizing polystyrene plastic with a blender, followed by filtering through a 35-mesh sieve. The microplastics were weighed according to the treatment concentrations. Mutira catfish were acclimatized for ten days by being given food at 08.00 and 16.00 WIB, equivalent to 3% of their body weight (Tang et al., 2024). Microplastic exposure was conducted during water changes every two days for 28 days, based on the designated exposure concentrations.

Muscle Tissue Preparation

At the end of the treatment, the fish were fasted for 24 hours before being weighed and their organs harvested on day 29. Muscle tissues were weighed and fixed in a 10% formalin solution (Zulfadhli et al., 2016).

The preparation process included dehydration with graded alcohol (70% to absolute), de-alcoholization with toluol solution infiltration, embedding in paraffin, sectioning with a microtome, fixation using Mayer's albumin, and staining with hematoxylin-eosin. The prepared tissue slides were examined under a light microscope at 40x and 100x magnification, with observations made in five fields of view per slide. Documentation was carried out using a Beta View camera system. Muscle parameters, including myofiber diameter and area, were measured using Image Raster.

Microplastic Extraction

Microplastic abundance analysis was carried out starting with muscle organs placed in 30 mL flask bottles containing a 10% potassium hydroxide (KOH) solution. The samples were left at room temperature for three days to allow digestion. After three days, the contents of the flask bottles were poured into empty bottles equipped with a funnel and filter paper. The filter paper was left to dry completely before distilled water was added to dissolve any microplastics adhering to the filter. The resulting solution was examined under a stereo microscope at 4x magnification, and the microplastic abundance was quantified (Suwartiningsih & Nafi'a, 2023).

Data Analysis

The raw data were initially processed using Microsoft Excel, followed by statistical analysis with IBM SPSS version 20. Differences between treatments were considered significant at $P < 0.05$ for all measured parameters and were evaluated using a one-way analysis of variance (ANOVA). When significant differences were detected, further analysis was conducted using Duncan's Multiple Range Test (DMRT).

Result and Discussion

Microplastic Abundance

The results showed that the abundance of microplastics in the muscles of Mutiara catfish was lowest in the control group (0.00 ± 0.00 particles/g) and highest in the T3 (2.63 ± 2.72 particles/g). Statistical analysis indicated that T3 was significantly different from the control and other treatment groups ($P < 0.05$). Regression analysis demonstrated that exposure to polystyrene microplastics in rearing water for 28 days significantly affected the abundance of microplastics in the muscles of Mutiara catfish ($P < 0.05$). This suggests a positive correlation, where higher concentrations of microplastics in the rearing water led to greater microplastic accumulation in the fish muscles, as shown in Table 1.

Table 1. Polystyrene microplastic abundance in Mutiara catfish muscle

Treatment	Microplastic abundance (particles/g)
Control	0,00 ± 0,00[a]
T1	0,85 ± 1,48[ab]
T2	0,74 ± 1,28[ab]
T3	2,63 ± 2,72[b]

Description: superscript different letters indicate there is a significant difference ($P < 0.05$) and vice versa

The research showed that microplastic exposure starting from 1 mg/L in the rearing media, the abundance of microplastics in the Mutiara catfish muscle was also high. Previous research found that exposure to nano plastic polystyrene (NPPs) at a concentration of 1 mg/L for three days in zebrafish (*Danio rerio*) resulted in NPPs accumulation of 49 ± 17 mg/g of muscle tissue (Chen et al., 2017). The high abundance of microplastics in fish organs is influenced by the increasing concentration of microplastic particles in the water (Popi et al., 2025; Rofiq & Sari, 2022; Wahid & Joesidawati, 2024), and vice versa (Fitriyani et al., 2025) allowing microplastics to enter the fish through food ingestion (Roch et al., 2020; Yona et al., 2022).

The detection of microplastics in the muscles of Mutiara catfish in this study indicates bioaccumulation, or the accumulation of contaminant particles in the body (Miller et al., 2020; Utomo & Muzaki, 2022). When microplastics enter the gastrointestinal tract, larger particles may settle or be excreted (Purnama et al., 2021) in feces. However, as microplastics break down into smaller particles, they can travel through the bloodstream (Nurwahyunani et al., 2022) and accumulate in muscle tissue (Yona et al., 2021). Particles around 5 µm in size can infiltrate the gills, intestines, and liver, while microplastics approximately 20 µm in size can penetrate the gills and intestines (Guanting et al., 2021). Particles smaller than 100 µm are capable of reaching muscle tissue (Barboza et al., 2020).

Diameter and Area of Mutiara Catfish Myofiber

The results showed that the diameter of myofiber in Mutiara catfish muscle was lowest in T2 (83.58 ± 25.01 µm) and highest in control (122.11 ± 40.06 µm). The area of myofiber in Mutiara catfish muscle was lowest in T2 (5.96 ± 3.77x10^3 µm^2) and highest in control (12.94 ± 8.65 x10^3 µm^2) (Table 2). Statistical analysis revealed that both myofiber diameter and area were significantly different between control and the other treatment groups ($P < 0.05$). However, there were no significant differences in

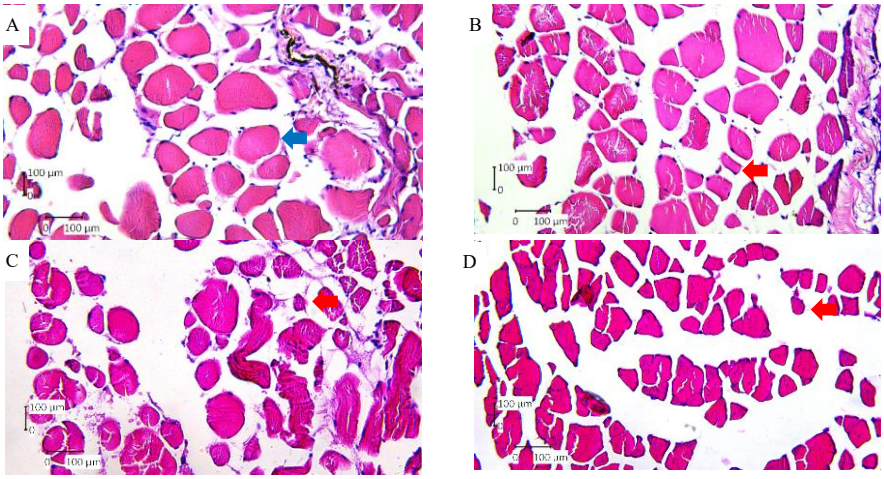

Figure 1. Diameter and area of Mutiara catfish myofiber after 28 days exposure to polysterene microplastics. A: control (0 mg/L); B: T1 (1 mg/L); C: T2 (10 mg/L); D: T3(100 mg/L). Blue arrows indicate normal myofiber diameter and red arrows indicate decreased myofiber diameter (hematoxylin-eosin staining; scale bar 100 µm)

myofiber diameter and area among the P1, P2, and P3 groups. Regression analysis indicated that polystyrene microplastic exposure in the rearing water for 28 days significantly affected both the myofiber diameter and area in Mutiara catfish ($P < 0.05$).

Table 2. Diameter and area of Mutiara catfish myofiber

Treatment	Diameter of myofiber (µm)	Area of myofiber (x10³µm²)
Control	122,11 ± 40,06^{b}	12,49 ± 8,65^{b}
T1	87,80 ± 26,81^{a}	6,60 ± 4,87^{a}
T2	83,58 ± 25,01^{a}	5,96 ± 3,77^{a}
T3	86,32 ± 18,21^{a}	6,10 ± 2,52^{a}

Description: superscript different letters indicate there is a significant difference ($P < 0.05$) and vice versa

The results in Table 2 indicate that Mutiara catfish exposed to polystyrene microplastics for 28 days experienced a reduction in both myofiber diameter and area. The regulation of myofiber diameter is influenced by several factors including myogenesis, muscle atrophy and apoptosis (Zhang et al., 2023). Previous research demonstrated that exposure to polystyrene microplastics (PS-MPs) at a concentration of 1000 µg/L for 7 days in goldfish larvae (*Carassius auratus*) caused damage to mesenchymal muscle cells and led to muscle fiber atrophy (Yang et al., 2020). The changes in myofiber diameter can be observed in Figure 1.

Changes in muscle tissue can occur due to the introduction of contaminants in the muscle (Di Giacinto et al., 2023; Setyono et al., 2024). Styrene is a benzene-derived compound that has high toxicity (Aldi, 2024; Majid et al., 2018). Previous research revealed that exposure to contaminants at concentrations of 44 µg/kg and 234 µg/kg in Yellow catfish (*Pelteobagrus fulvidraco*) over eight weeks led to a reduction in muscle myofiber diameter (Zhang et al., 2021). Another study showed that exposure of fonofos contaminants at a concentration of 2 mg/L for 96 hours caused a decrease in myofiber diameter in Zebrafish (*Danio rerio*) (Arman & Üçüncü, 2021).

This study found a decrease in both myofiber diameter and myofiber area, thus disrupting the growth of muscle myofiber (Moenek & Toelle, 2021). Myofiber growth that is not optimal will have an impact on the elasticity and compactness of the meat (Suwiti et al., 2015; Zhang et al., 2021). Moreover, muscle injuries may serve as an indicator of exposure to environmental contaminants (Ciamarro et al., 2015). Among such contaminants, microplastics pose a significant toxic risk to all organisms (Aulia et al., 2023; Stapleton & Hai, 2023).

Conclusion

This study demonstrated that exposure to polystyrene (PS) microplastics in rearing water for 28 days significantly increased microplastic accumulation in muscle tissue and reduced both myofiber diameter and area in Mutiara catfish (*Clarias gariepinus*). These histological alterations indicate muscle atrophy,

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suggesting that microplastic contamination can compromise fish health and meat quality. The findings emphasize the potential ecological risks of microplastics and their implications for food safety, particularly in aquaculture systems. This highlights the urgent need for improved environmental monitoring and regulatory policies to reduce microplastic pollution and safeguard both aquatic ecosystems and public health.

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Author Contributions

Conceptualization, N.S. and A.F.A.; methodology, N.S. and A.F.A.; validation, N.S.; formal analysis, A.F.A.; investigation, A.F.A. and D.E.W.; resources, N.S.; data curation, A.F.A.; writing—original draft preparation, A.F.A.; writing—review and editing, N.S., A.F.A., and D.E.W.; visualization, A.F.A.; supervision, N.S. All authors have read and approved the final version of the manuscript..

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Conflicts of Interest
We hereby affirm that the preparation of this article was conducted without any conflict of interest that could compromise the objectivity or integrity of the findings.

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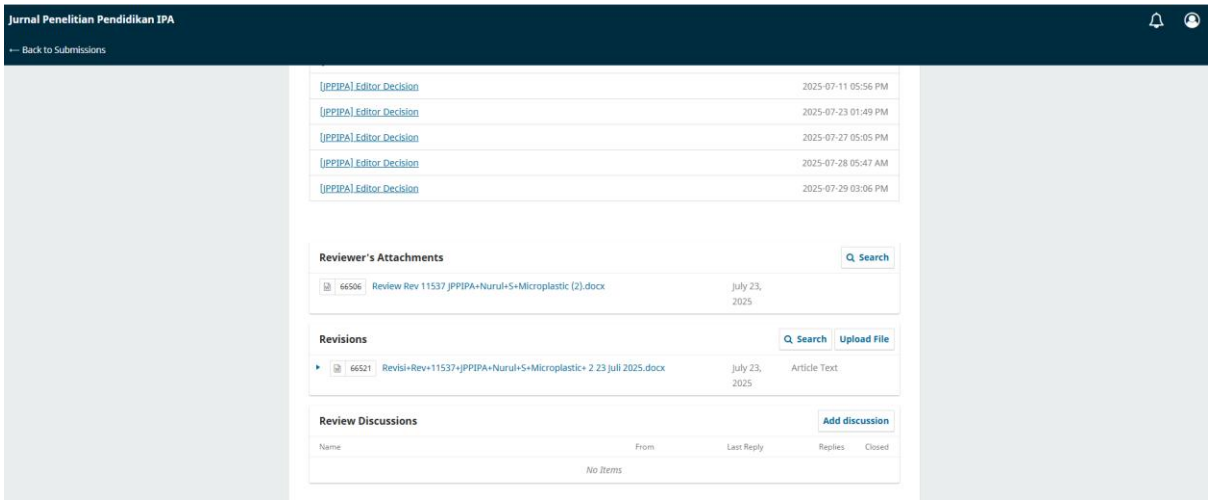
Sumbit Revisi Tahap 2 (23 Juli 2025)



Nurul Suwartiningsih <nurul.suwartiningsih@bio.uad.ac.id>
to Syamsul

Wed, Jul 23, 5:21 PM

Dear Editor Syamsul Hakim,

Thank you for your email and for the opportunity to revise our manuscript entitled *"The Effect of Polystyrene Microplastic Exposure in the Rearing Water on Muscle Morphology of Mutiara Catfish (Clarias gariepinus Burchell, 1822)"*.

We truly appreciate the constructive comments and suggestions provided by the reviewers. In response, we have carefully revised the manuscript in accordance with all recommendations. The necessary improvements have been made to enhance the clarity, depth, and scientific quality of the paper. A detailed point-by-point response to the reviewers' comments has also been included.

We hope the revised version meets the journal's standards and look forward to your favorable consideration.

Sincerely,
Nurul Suwartiningsih

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The Effect of Polystyrene Microplastic Exposure in the Rearing Water on Muscle Morphology of Mutiara Catfish (*Clarias gariepinus* Burchell, 1822)

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Abstract: Polystyrene (PS) is one of the most abundant microplastics in freshwater and can accumulate in fish tissues. This study investigated the effect of PS microplastic exposure in rearing water on the abundance of microplastics in muscle, and the diameter and area of myofibers in Mutiara catfish (*Clarias gariepinus*). A total of 120 fish were exposed to PS microplastics at concentrations of 0, 1, 10, and 100 mg/L for 28 days. Muscle samples from the right abdomen were processed using the paraffin method and stained with hematoxylin-eosin. Microplastics were extracted using 10% KOH and observed under a stereo microscope. Myofiber diameter and area were measured with Image Raster software. Data were analyzed using the Kruskal-Wallis test. Results showed significant differences among treatments ($P<0.05$) for all parameters. The control group had the lowest microplastic abundance and the largest myofiber size, while the highest accumulation occurred at 100 mg/L, and the smallest myofiber diameter and area were found at 10 mg/L. Exposure to PS microplastics leads to accumulation in muscle tissue and a reduction in myofiber dimensions. These findings highlight the potential adverse impacts of microplastics on fish health and underline the importance of further studies regarding ecological and human health implications.

Keywords: Microplastic exposure; Muscle tissue; Myofiber area; Myofiber diameter; Polystyrene toxicity

Introduction

Microplastics are increasingly recognized as emerging pollutants in aquatic ecosystems, appearing in various polymers, shapes, and concentrations (Smith et al., 2018). These particles originate primarily from consumer products such as plastic bags, beverage bottles, personal care items, and cleaning agents (Nainggolan et al., 2022). Due to their diverse polymer composition, ranging from high to low density plastics, microplastics are easily transported by water currents and accumulate in both water columns and sediments (Owowenu et al., 2023). Numerous studies have reported the detection of microplastics in freshwater ecosystems, particularly in river areas (Dhea et al., 2023; Lofty et al., 2023). Research has identified microplastics

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Example: Susilawati, S., Doyan, A., Muliyadi, L., & Hakim, S. (2019). Growth of tin oxide thin film by aluminum and fluorine doping using spin coating Sol-Gel techniques. *Jurnal Penelitian Pendidikan IPA, 1*(1), 1-4. https://doi.org/10.29303/jppipa.v1i1.264

in the Pekalongan River estuary, with abundances ranging from 45.2 to 99.1 particles L^{-1} in surface water, dominated by fragment and film morphologies (Ismanto et al., 2023). Microplastics pose ecological risks by negatively impacting aquatic organisms and degrading environmental quality (Li et al., 2023; Yuranda et al., 2024).

Rivers contaminated with microplastics may transfer these particles into aquaculture systems (Santikanuri et al., 2025), thereby affecting farmed fish. Microplastic contamination in aquaculture environments can deteriorate water quality, compromise fish health, and ultimately reduce aquaculture productivity (Filho et al., 2023). Research findings indicate that tilapia (*Oreochromis niloticus*) reared in the Pelayaran River, Sidoarjo, contain microplastic residues, attributed to pollution in the surrounding aquatic environment (Al-Fatih, 2022).

Among the various types of microplastics, polystyrene is commonly detected in aquatic habitats (Jones et al., 2020) originating from food and beverage packaging as well as the automotive industry (Nurhadi et al., 2017). Research on the Mahakam River found that polystyrene accounted for 6.45% of the total plastic waste, with microplastics comprising 12.5% of the total abundance (Kurniawan et al., 2023). Environmental exposure, especially ultraviolet radiation and weathering, accelerates the breakdown of polystyrene into microplastic fragments (Hwang et al., 2020).

Microplastics can be ingested by fish through their gills, digestive tract and muscles (Sawalman et al., 2021). Microplastics present in water can enter fish muscles via the bloodstream (Akhbarizadeh et al., 2018). When microplastics enter the digestive tract, they can be transported through the blood vessels and eventually accumulate in muscle tissue (Liu et al., 2022). Previous research has shown that exposure to microplastics at a concentration of 100 mg/L in tilapia (*Oreochromis niloticus*) can cause muscle damage (Hamed et al., 2021). One of the commonly cultivated and consumed fish species is Mutiara catfish (*Clarias gariepinus* Burchell, 1822) (Iswanto et al., 2016). Despite growing concern over microplastic contamination, the histopathological effects of polystyrene microplastics on muscle morphology in Mutiara catfish remain understudied. Most research has focused on gastrointestinal tissues, with limited attention to muscle damage in economically important aquaculture species under controlled exposure

The novelty of this research lies in evaluating the sub-lethal and tissue-specific effects of polystyrene microplastic exposure on Mutiara catfish muscle morphology. This study provides critical insight into how microplastics may compromise fish meat quality, a concern for both environmental health and food safety. Therefore, this research is important to understand potential histological damage caused by microplastic contamination in aquaculture systems. The findings may inform sustainable fish farming practices, guide risk assessments of microplastic exposure in aquatic food chains, and support the development of environmental policies to mitigate plastic pollution impacts on food-producing aquatic ecosystems.

Method

Research Time and Location

This research was conducted from January to November 2024. Maintenance and treatment took place in Dengok Kulon, Bugisan, Prambanan, Klaten, while data preparation, collection, and analysis were carried out at the Laboratory of Animal Structure and Physiology, Universitas Ahmad Dahlan.

Experimental Design

This study employed an experimental approach using a completely randomized design (CRD) with four treatment groups: a control group (0 mg/L polystyrene microplastic), T1 (1 mg/L), T2 (10 mg/L), and T3 (100 mg/L). Each treatment was replicated six times using separate rearing tanks, with five Mutiara catfish (*Clarias gariepinus*) allocated per tank, resulting in a total of 120 fish. The exposure period lasted for 28 days. The parameters observed in this study included the abundance of microplastics in muscle tissue, the diameter of myofibers, and the area of myofibers.

Microplastics Exposure

Microplastic preparation was carried out by pulverizing polystyrene plastic with a blender, followed by filtering through a 35-mesh sieve. The microplastics were weighed according to the treatment concentrations. Mutira catfish were acclimatized for ten days by being given food at 08.00 and 16.00 WIB, equivalent to 3% of their body weight (Tang et al., 2024). Microplastic exposure was conducted during water changes every two days for 28 days, based on the designated exposure concentrations.

Muscle Tissue Preparation

At the end of the treatment, the fish were fasted for 24 hours before being weighed and their organs harvested on day 29. Muscle tissues were weighed and fixed in a 10% formalin solution (Zulfadhli et al., 2016). The preparation process included dehydration with graded alcohol (70% to absolute), de-alcoholization with toluol solution infiltration, embedding in paraffin,

sectioning with a microtome, fixation using Mayer's albumin, and staining with hematoxylin-eosin. The prepared tissue slides were examined under a light microscope at 40x and 100x magnification, with observations made in five fields of view per slide. Documentation was carried out using a Beta View camera system. Muscle parameters, including myofiber diameter and area, were measured using Image Raster.

Microplastic Extraction

Microplastic abundance analysis was carried out starting with muscle organs placed in 30 mL flask bottles containing a 10% potassium hydroxide (KOH) solution. The samples were left at room temperature for three days to allow digestion. After three days, the contents of the flask bottles were poured into empty bottles equipped with a funnel and filter paper. The filter paper was left to dry completely before distilled water was added to dissolve any microplastics adhering to the filter. The resulting solution was examined under a stereo microscope at 4x magnification, and the microplastic abundance was quantified (Suwartiningsih & Nafi'a, 2023).

Data Analysis

The raw data were initially processed using Microsoft Excel, followed by statistical analysis with IBM SPSS version 20. Differences between treatments were considered significant at $P < 0.05$ for all measured parameters and were evaluated using a one-way analysis of variance (ANOVA). When significant differences were detected, further analysis was conducted using Duncan's Multiple Range Test (DMRT).

Result and Discussion

Microplastic Abundance

The results showed that the abundance of microplastics in the muscles of Mutiara catfish was lowest in the control group (0.00 ± 0.00 particles/g) and highest in the T3 (2.63 ± 2.72 particles/g). Statistical analysis indicated that T3 was significantly different from the control and other treatment groups ($P < 0.05$). Regression analysis demonstrated that exposure to polystyrene microplastics in rearing water for 28 days significantly affected the abundance of microplastics in the muscles of Mutiara catfish ($P < 0.05$). This suggests a positive correlation, where higher concentrations of microplastics in the rearing water led to greater microplastic accumulation in the fish muscles, as shown in Table 1.

Table 1. Polystyrene microplastic abundance in Mutiara catfish muscle

Treatment	Microplastic abundance (particles/g)
Control	0,00 ± 0,00^{a}
T1	0,85 ± 1,48^{ab}
T2	0,74 ± 1,28^{ab}
T3	2,63 ± 2,72^{b}

Description: superscript different letters indicate there is a significant difference ($P < 0.05$) and vice versa

The research showed that microplastic exposure starting from 1 mg/L in the rearing media, the abundance of microplastics in the Mutiara catfish muscle was also high. Previous research found that exposure to nano plastic polystyrene (NPPs) at a concentration of 1 mg/L for three days in zebrafish (*Danio rerio*) resulted in NPPs accumulation of 49 ± 17 mg/g of muscle tissue (Chen et al., 2017). The high abundance of microplastics in fish organs is influenced by the increasing concentration of microplastic particles in the water (Popi et al., 2025; Rofiq & Sari, 2022; Wahid & Joesidawati, 2024), and vice versa (Fitriyani et al., 2025) allowing microplastics to enter the fish through food ingestion (Roch et al., 2020; Yona et al., 2022).

The detection of microplastics in the muscles of Mutiara catfish in this study indicates bioaccumulation, or the accumulation of contaminant particles in the body (Miller et al., 2020; Utomo & Muzaki, 2022). When microplastics enter the gastrointestinal tract, larger particles may settle or be excreted (Purnama et al., 2021) in feces. However, as microplastics break down into smaller particles, they can travel through the bloodstream (Nurwahyunani et al., 2022) and accumulate in muscle tissue (Yona et al., 2021). Particles around 5 µm in size can infiltrate the gills, intestines, and liver, while microplastics approximately 20 µm in size can penetrate the gills and intestines (Guanting et al., 2021). Particles smaller than 100 µm are capable of reaching muscle tissue (Barboza et al., 2020).

Diameter and Area of Mutiara Catfish Myofiber

The results showed that the diameter of myofiber in Mutiara catfish muscle was lowest in T2 (83.58 ± 25.01 µm) and highest in control (122.11 ± 40.06 µm). The area of myofiber in Mutiara catfish muscle was lowest in T2 (5.96 ± 3.77x10^3 µm^2) and highest in control (12.94 ± 8.65 x10^3 µm^2) (Table 2). Statistical analysis revealed that both myofiber diameter and area were significantly different between control and the other treatment groups ($P < 0.05$). However, there were no significant differences in myofiber diameter and area among the P1, P2, and P3 groups. Regression analysis indicated that polystyrene microplastic exposure in the rearing water for 28 days

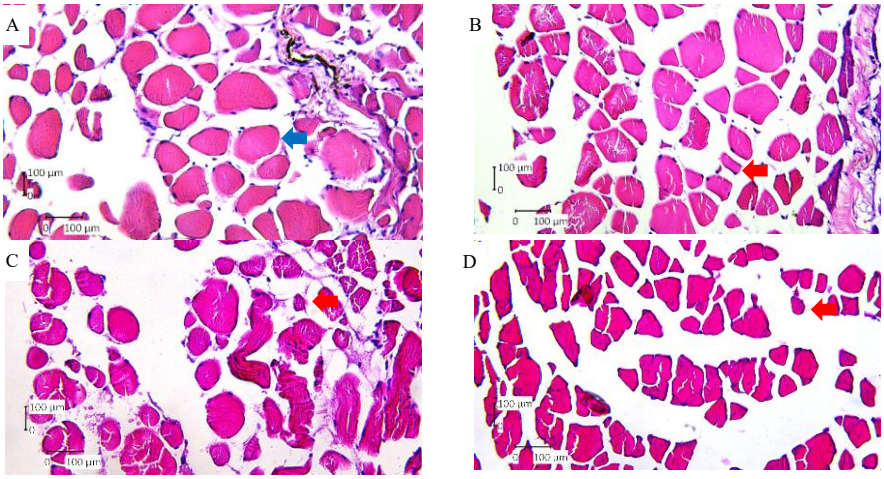

Figure 1. Diameter and area of Mutiara catfish myofiber after 28 days exposure to polysterene microplastics. A: control (0 mg/L); B: T1 (1 mg/L); C: T2 (10 mg/L); D: T3(100 mg/L). Blue arrows indicate normal myofiber diameter and red arrows indicate decreased myofiber diameter (hematoxylin-eosin staining; scale bar 100 µm)

significantly affected both the myofiber diameter and area in Mutiara catfish ($P < 0.05$). These findings are consistent with existing theories which suggest that exposure to toxic substances such as polystyrene microplastics can impair myogenesis and induce muscle atrophy, leading to a significant reduction in myofiber diameter and area.

Table 2. Diameter and area of Mutiara catfish myofiber

Treatment	Diameter of myofiber (µm)	Area of myofiber (x10³µm²)
Control	122,11 ± 40,06[b]	12,49 ± 8,65[b]
T1	87,80 ± 26,81[a]	6,60 ± 4,87[a]
T2	83,58 ± 25,01[a]	5,96 ± 3,77[a]
T3	86,32 ± 18,21[a]	6,10 ± 2,52[a]

Description: superscript different letters indicate there is a significant difference ($P < 0.05$) and vice versa

The results in Table 2 indicate that Mutiara catfish exposed to polystyrene microplastics for 28 days experienced a reduction in both myofiber diameter and area. The regulation of myofiber diameter is influenced by several factors including myogenesis, muscle atrophy and apoptosis (Zhang et al., 2023). Previous research demonstrated that exposure to polystyrene microplastics (PS-MPs) at a concentration of 1000 µg/L for 7 days in goldfish larvae (*Carassius auratus*) caused damage to mesenchymal muscle cells and led to muscle fiber atrophy (Yang et al., 2020). The changes in myofiber diameter can be observed in Figure 1.

Changes in muscle tissue can occur due to the introduction of contaminants in the muscle (Di Giacinto et al., 2023; Setyono et al., 2024). Styrene is a benzene-derived compound that has high toxicity (Aldi, 2024; Majid et al., 2018). Previous research revealed that exposure to contaminants at concentrations of 44 µg/kg and 234 µg/kg in Yellow catfish (*Pelteobagrus fulvidraco*) over eight weeks led to a reduction in muscle myofiber diameter (Zhang et al., 2021). Another study showed that exposure of fonofos contaminants at a concentration of 2 mg/L for 96 hours caused a decrease in myofiber diameter in Zebrafish (*Danio rerio*) (Arman & Üçüncü, 2021).

This study found a decrease in both myofiber diameter and myofiber area, thus disrupting the growth of muscle myofiber (Moenek & Toelle, 2021). Myofiber growth that is not optimal will have an impact on the elasticity and compactness of the meat (Suwiti et al., 2015; Zhang et al., 2021). Moreover, muscle injuries may serve as an indicator of exposure to environmental contaminants (Ciamarro et al., 2015). Among such contaminants, microplastics pose a significant toxic risk to all organisms (Aulia et al., 2023; Stapleton & Hai, 2023).

Conclusion

This study demonstrated that exposure to polystyrene (PS) microplastics in rearing water for 28 days significantly increased microplastic accumulation

Commented [Ac1]: add description correlation between data result and theories

Commented [NS2R1]: Thank you for the valuable suggestion. We have added a description that explains the correlation between our findings and relevant theoretical frameworks. Specifically, we included a sentence stating that the observed reduction in myofiber diameter and area in Mutiara catfish following polystyrene microplastic exposure is consistent with existing theories and previous studies, which indicate that toxic substances such as microplastics can impair myogenesis and induce muscle atrophy.

in muscle tissue and reduced both myofiber diameter and area in Mutiara catfish (*Clarias gariepinus*). The highest accumulation was observed at a concentration of 100 mg/L, reaching 2.63 particles/g, indicating that higher concentrations of microplastics in water lead to greater accumulation in muscle. This trend reflects patterns observed in other aquatic species, where microplastic presence in the environment directly influenced the level of contamination in fish organs.

Histological analysis also showed that fish in the treatment groups experienced significant reductions in myofiber diameter and area compared to the control. The control group exhibited average myofiber diameters of 122.11 µm and areas of 12.49 x10^3 µm², while exposed groups showed reduced diameters as low as 83.58 µm and areas down to 5.96 x10^3 µm². Similar muscle degradation patterns have been documented in other fish exposed to plastic contaminants and toxic substances, such as atrophied muscle fibers, disorganized tissue structures, and inhibited muscle growth, all of which impact meat quality.

These results confirm that even short-term exposure to PS microplastics at environmentally relevant concentrations can lead to bioaccumulation and muscle deterioration. The findings emphasize the potential ecological risks of microplastics and their implications for food safety, particularly in aquaculture systems. This highlights the urgent need for improved environmental monitoring and regulatory policies to reduce microplastic pollution and safeguard both aquatic ecosystems and public health.

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Author Contributions

Conceptualization, N.S. and A.F.A.; methodology, N.S. and A.F.A.; validation, N.S.; formal analysis, A.F.A.; investigation, A.F.A. and D.E.W.; resources, N.S.; data curation, A.F.A.; writing—original draft preparation, A.F.A.; writing—review and editing, N.S., A.F.A., and D.E.W.; visualization, A.F.A.; supervision, N.S. All authors have read and approved the final version of the manuscript..

Commented [Ac3]: Describe in detail the findings in the research and correlate them with relevant research results.

Commented [NS4R3]: Thank you for the insightful suggestion. We have revised the conclusion to include more detailed descriptions of our findings, including specific data on microplastic accumulation and myofiber alterations. We also added correlations with previous studies that reported similar patterns of muscle damage in fish exposed to plastic and toxic contaminants.

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Conflicts of Interest
We hereby affirm that the preparation of this article was conducted without any conflict of interest that could compromise the objectivity or integrity of the findings.

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Pemberitahuan Penerimaan Artikel untuk Dipublikasikan

(27 Juli 2025)

[JPPIPA] Editor Decision External Inbox ×

Syamsul Hakim Sun, Jul 27, 4:05 PM

to me

Nurul Suwartiningsih:

We have reached a decision regarding your submission to Jurnal Penelitian Pendidikan IPA, "The Effect of Polystyrene Microplastic Exposure in the Rearing Water on Muscle Morphology of Mutiara Catfish (Clarias gariepinus Burchell, 1822) ".

Our decision is to: Accept Submission

SYAMSUL HAKIM

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Nurul Suwartiningsih, Aulia Fayzha Aszhara, Rani Marsela, Dian Eka Wijayanti:

The editing of your submission, "The Effect of Polystyrene Microplastic Exposure in the Rearing Water on Muscle Morphology of Mutiara Catfish (Clarias gariepinus Burchell, 1822)," is complete. We are now sending it to production.

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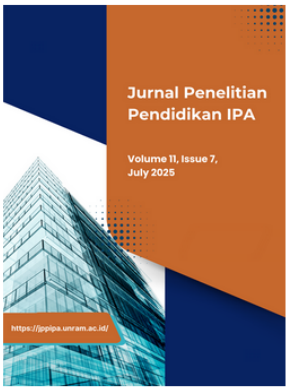

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