

## **WATERBORNE POLYSTYRENE MICROPLASTIC EXPOSURE DEPRESSES GROWTH PERFORMANCE OF SIAMESE FIGHTING FISH (*Betta splendens*)**

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### **ABSTRACT**

This study evaluated the effects of waterborne polystyrene microplastic exposure on the growth performance of Siamese fighting fish (*Betta splendens*) over a 45-day rearing period. A completely randomized design with four treatments and three replicates was applied: 0, 50, 100, and 150 mg/L. The evaluated growth variables comprised absolute length gain, specific length growth rate, absolute weight gain, specific growth rate, feed conversion ratio (FCR), and survival rate (SR). Data were analyzed using one-way ANOVA followed by Tukey's test at the 5% significance level. Polystyrene microplastic exposure significantly affected absolute length gain, specific length growth rate, absolute weight gain, and specific growth rate ( $p < 0.05$ ). Fish reared without microplastic exposure consistently showed the highest growth performance, whereas the 150 mg/L treatment produced the lowest values across the principal growth endpoints. By contrast, FCR tended to increase with increasing microplastic concentration, but the effect was not statistically significant ( $p > 0.05$ ). SR remained 100% in all treatments, indicating that the tested exposure levels produced sublethal rather than lethal effects under the present experimental conditions. Water quality remained within a range generally tolerated by betta fish, suggesting that the observed response was more closely associated with microplastic exposure than with overt deterioration of the rearing medium. Overall, waterborne polystyrene microplastic exposure reduced the growth performance of *B. splendens* and should be considered a relevant emerging stressor in ornamental fish culture.

**Key words:** *Betta splendens*, Growth Performance, Ornamental Fish, Polystyrene, Waterborne Microplastics.

### **INTRODUCTION**

Microplastics are widely recognized as emerging contaminants in aquatic ecosystems and are increasingly relevant to aquaculture because they may enter culture systems through source water, feed ingredients, and domestic or industrial waste streams. In fish, microplastic exposure has been associated with physiological disturbances, metabolic alteration, growth impairment, and other sublethal effects that

can reduce production performance even in the absence of mortality (Banaee et al., 2024; Ghosh, 2025; Iheanacho et al., 2023; Russell & Sidaoui-Haddad, 2021; Wang et al., 2024).

Within the aquaculture context of West Nusa Tenggara, microplastic contamination has already been documented in aquaculture-related environments, including the cultivation area of Ekas Bay and whiteleg shrimp farming systems in North Lombok. In addition, dietary exposure to PVC microplastics has been reported to reduce the growth and survival of Nile tilapia. Together, these findings indicate that microplastics are not merely a global concern but also a realistic issue in aquaculture systems located geographically close to the present study site (Putrajab et al., 2024; Setyono et al., 2024; Sumsanto et al., 2024).

Growth is a particularly informative endpoint because it integrates feed intake, digestive efficiency, nutrient assimilation, energy allocation, and overall physiological condition. Recent meta-analytical evidence indicates that microplastics generally depress growth performance across fish species, although effect size depends on particle size, polymer type, exposure route, exposure duration, and the species tested (Wang et al., 2024). Recent reviews have likewise emphasized that growth impairment frequently co-occurs with intestinal histopathology, oxidative stress, metabolic dysfunction, and disrupted energy homeostasis (Banaee et al., 2024; Ghosh et al., 2025; Hasan et al., 2024; Hodkovicova et al., 2022; Kim et al., 2021). Polystyrene is among the plastic types most used in microplastic toxicology studies because it is widely available and relatively easy to prepare. Experimental studies in fish have shown that exposure to polystyrene micro- and nanoplastics can reduce growth, alter metabolism, damage digestive tissues, and modulate molecular responses. Such patterns have been reported in medaka, tilapia, guppy, goldfish, channel catfish, zebrafish, large yellow croaker, rare minnow, and other freshwater fish, although effect severity varies among species and experimental protocols (Gonzalez-Doncel et al., 2022; Huang et al., 2020; Jiang et al., 2022; Lai et al., 2021; Mbugani et al., 2022; Medriano & Bae, 2022; Rashid et al., 2025; Romano et al., 2020; Umamaheswari et al., 2021; Wang et al., 2022; Zhang et al., 2022).

The Siamese fighting fish (*Betta splendens*) is a high-value freshwater ornamental species and a practical model organism for behavioral and physiological research. The species is relatively easy to maintain, shows considerable environmental tolerance, and is frequently used in small-scale laboratory studies. Nonetheless, information on the growth response of betta fish to microplastic exposure in rearing water remains limited compared with that available for other model species (Lichak et al., 2022; Norazmi-Lokman et al., 2020; Ramos et al., 2025). This gap is important because the ornamental fish industry depends heavily on stable growth performance, efficient feed utilization, and consistent husbandry quality.

Accordingly, the present study aimed to evaluate the effects of waterborne polystyrene microplastic exposure on the growth performance of Siamese fighting fish during a 45-day rearing period. The article specifically focuses on growth- and production-related responses, namely length gain, weight gain, feed conversion ratio, and survival rate. By adopting this narrower and more targeted scope, the study is intended to provide clearer experimental evidence on the relevance of microplastics as sublethal stressors in ornamental fish culture. Particle preparation followed a simple mechanical approach commonly used in preliminary studies, and the findings should therefore be interpreted while considering the absence of more detailed particle-size characterization (McColley et al., 2023).

## METHODS

The experiment was conducted for 45 days, from October to November 2025, in the Fish Production and Reproduction Laboratory, Aquaculture Study Program, Faculty of Agriculture, University of Mataram. The test animals were Siamese fighting fish approximately 2–3 months old, with an initial total length of 2.5 cm and an initial body weight of 1.5 g. Each experimental unit consisted of one fish maintained individually in an aquarium measuring 15 × 20 × 20 cm.

A completely randomized design was applied with four concentrations of polystyrene microplastics: 0 mg/L (control), 50 mg/L, 100 mg/L, and 150 mg/L, with three replicates per treatment. The microplastics were prepared from commercial polystyrene material by cutting, blending, drying, and sieving until an operational particle size of less than 5 mm was obtained. Accordingly, the material was treated as microplastic based on the target size range, although particle-size distribution and particle shape were not instrumentally characterized.

Fish were acclimated before stocking and then reared for 45 days under the assigned exposure conditions. Commercial pellet feed was provided twice daily, at 08 am and 4 pm, at 3% of biomass. The rearing medium was partially renewed once per week by siphoning 50% of the water volume and replacing it with fresh water mixed with microplastics at the corresponding treatment concentration so that the intended exposure level could be maintained throughout the trial.

The primary response variables were absolute length gain, specific length growth rate, absolute weight gain, specific growth rate, feed conversion ratio (FCR), and survival rate (SR). Water-quality parameters monitored during the trial included temperature, dissolved oxygen (DO), pH, and ammonia. Statistical analysis was performed using one-way ANOVA at the 95% confidence level, followed by Tukey's test when treatment effects were significant. All analyses were based on final measurements obtained from each experimental unit.

## RESULTS

Across treatments, the control descriptively produced the highest values for absolute length gain, specific length growth rate, absolute weight gain, and specific growth rate, whereas these variables tended to decline as microplastic concentration increased and reached their lowest values at 150 mg/L. One-way ANOVA showed significant treatment effects on absolute length gain ( $F = 14.700$ ;  $p = 0.001$ ), specific length growth rate ( $F = 14.295$ ;  $p = 0.001$ ), absolute weight gain ( $F = 11.417$ ;  $p = 0.003$ ), and specific growth rate ( $F = 12.555$ ;  $p = 0.002$ ), but not on FCR ( $F = 3.654$ ;  $p = 0.063$ ). Survival remained 100% in all treatments, indicating that the observed responses represent sublethal rather than lethal effects under the present conditions.

Tukey's post hoc test indicated that, for absolute length gain, the control did not differ significantly from 50 mg/L ( $p > 0.05$ ), but it was significantly higher than 100 and 150 mg/L ( $p < 0.05$ ); the 50 mg/L treatment was also significantly higher than 150 mg/L ( $p < 0.05$ ), whereas the comparisons between 50 and 100 mg/L and between 100 and 150 mg/L were not significant ( $p > 0.05$ ). A similar pattern was observed for specific length growth rate: the control was comparable to 50 mg/L ( $p > 0.05$ ), 150 mg/L was significantly lower than both the control and 50 mg/L ( $p < 0.05$ ), and 100 mg/L occupied an intermediate position without differing significantly from either 50 or 150 mg/L ( $p >$

0.05). For absolute weight gain and specific growth rate, the control differed significantly from all exposed treatments ( $p < 0.05$ ), whereas the three exposure groups did not differ significantly from one another ( $p > 0.05$ ).

Mean  $\pm$  SD plots for each growth-related variable are presented in Figures 1–5 to illustrate the direction and magnitude of treatment responses. Figure 1 shows a clear decline in absolute length gain from the control toward the highest exposure level, with the lowest values recorded at 150 mg/L.

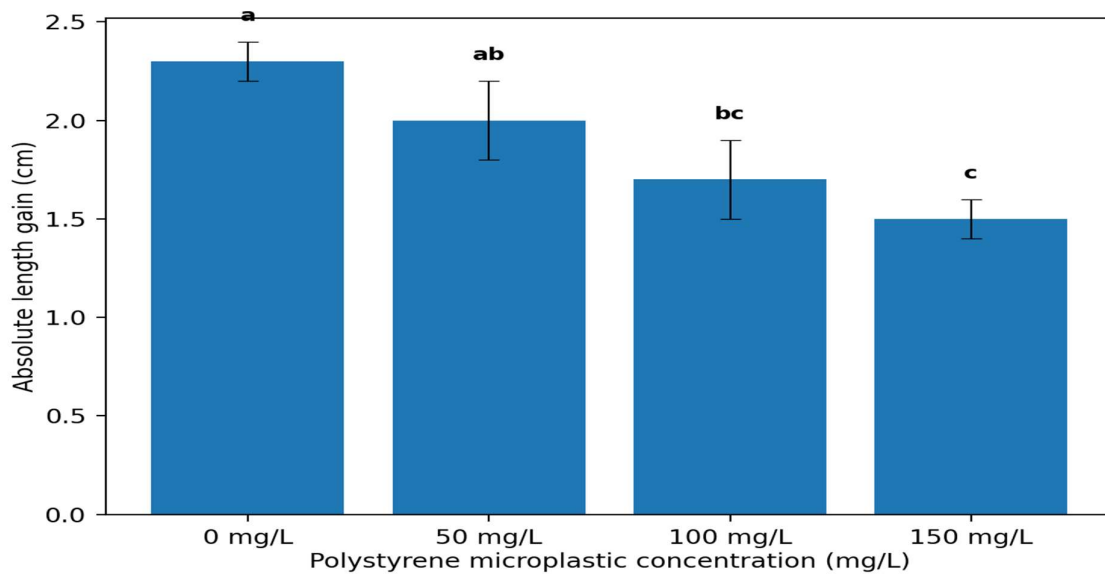


Figure 1. Mean  $\pm$  SD absolute length gain of Siamese fighting fish exposed to different concentrations of polystyrene microplastics during a 45-day rearing period. Different lowercase letters above bars indicate significant differences among treatments according to Tukey's test ( $p < 0.05$ ).

A similar downward trend was observed for specific length growth rate (Figure 2). Consistent with the post hoc analysis, the control and 50 mg/L treatments did not differ significantly ( $p > 0.05$ ), whereas 150 mg/L was significantly lower than the control and 50 mg/L ( $p < 0.05$ ).

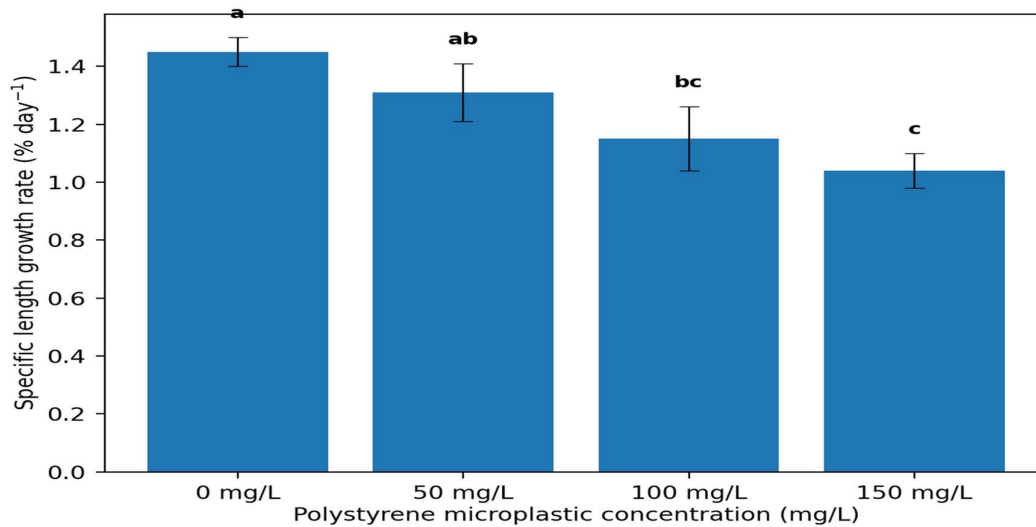


Figure 2. Mean  $\pm$  SD specific length growth rate of Siamese fighting fish exposed to different concentrations of polystyrene microplastics during a 45-day rearing period. Different lowercase letters above bars indicate significant differences among treatments according to Tukey's test ( $p < 0.05$ ).

The absolute weight-gain profile likewise showed a marked reduction after microplastic exposure, with the control significantly higher than all exposed treatments ( $p < 0.05$ ), whereas the exposed groups did not differ significantly from one another ( $p > 0.05$ ) (Figure 3).

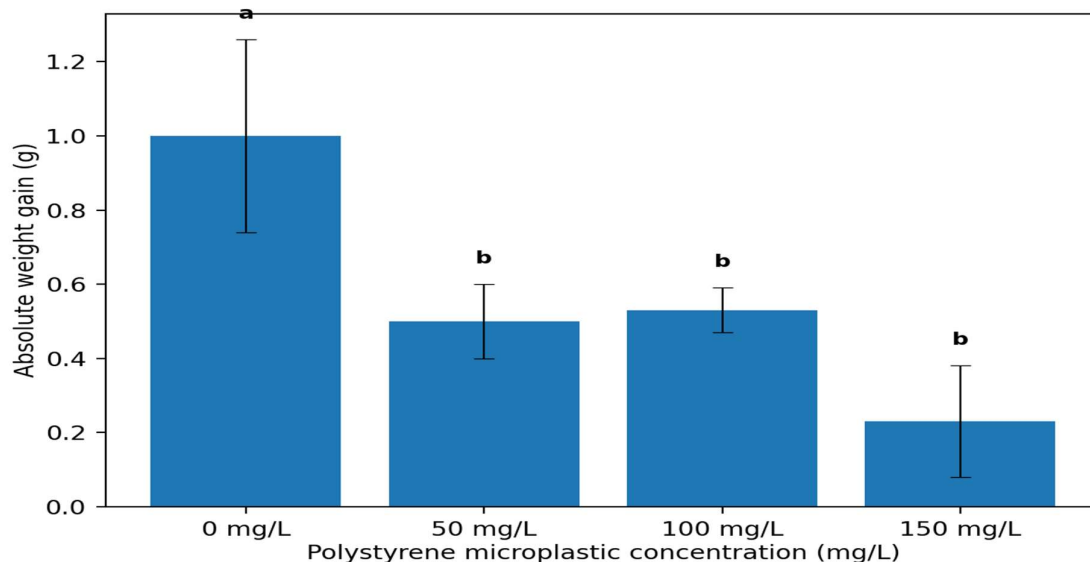


Figure 3. Mean  $\pm$  SD absolute weight gain of Siamese fighting fish exposed to different concentrations of polystyrene microplastics during a 45-day rearing period. Different lowercase letters above bars indicate significant differences among treatments according to Tukey's test ( $p < 0.05$ ).

The same overall tendency was evident for specific growth rate, with the control significantly higher than all exposed treatments ( $p < 0.05$ ), whereas the three exposed groups did not differ significantly from one another ( $p > 0.05$ ) (Figure 4).

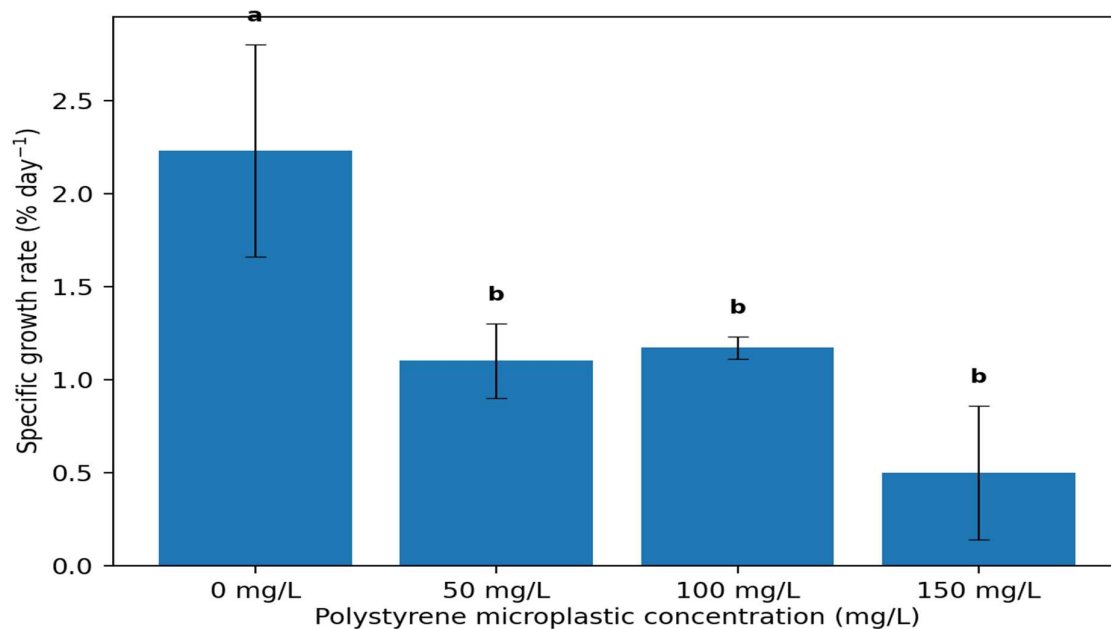


Figure 4. Mean  $\pm$  SD specific growth rate of Siamese fighting fish exposed to different concentrations of polystyrene microplastics during a 45-day rearing period. Different lowercase letters above bars indicate significant differences among treatments according to Tukey's test ( $p < 0.05$ ).

For FCR, the largest mean value occurred at 150 mg/L, but the overall treatment effect was not statistically significant ( $p > 0.05$ ), and this treatment also showed the widest spread of observations (Figure 5).

Descriptively, FCR increased with increasing microplastic concentration, with mean values of  $2.14 \pm 0.65$  in control,  $4.16 \pm 0.85$  at 50 mg/L,  $3.83 \pm 0.22$  at 100 mg/L, and  $11.81 \pm 7.73$  at 150 mg/L. However, variance in the highest treatment was also large, and ANOVA therefore did not detect a statistically significant difference. Accordingly, the FCR result is better interpreted as a tendency toward reduced feed-use efficiency rather than as conclusive comparative evidence among treatments.

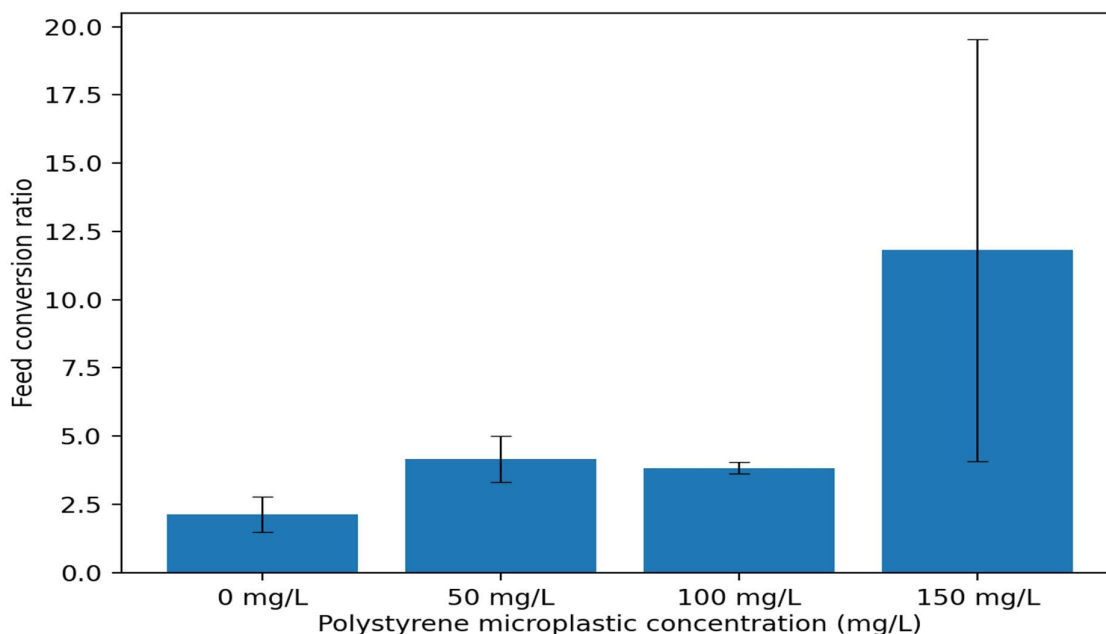


Figure 5. Mean  $\pm$  SD feed conversion ratio of Siamese fighting fish exposed to different concentrations of polystyrene microplastics during a 45-day rearing period. No significant treatment effect was detected for FCR (ANOVA,  $p > 0.05$ ).

## DISCUSSION

The principal finding of this study is that waterborne exposure to polystyrene microplastics depressed several growth endpoints in Siamese fighting fish while leaving survival unaffected under the tested conditions. This pattern is consistent with a sublethal stress response in which fish remain alive but show reduced performance in traits directly relevant to production and husbandry.

The direction of this response agrees with the broader literature on fish exposed to microplastics. Recent syntheses indicate that growth depression commonly occurs when microplastics disrupt digestive processes, alter metabolic balance, or increase the energetic cost of physiological maintenance (Banaee et al., 2024; Ghosh, 2025; Wang et al., 2024). Comparable outcomes have also been reported experimentally in tilapia, guppy, goldfish, and *Labeo rohita*, where microplastic exposure reduced body growth, worsened intestinal histology, and lowered nutrient-use efficiency (Huang et al., 2020; Mbugani et al., 2022; Rashid et al., 2025; Romano et al., 2020). The present findings therefore fall within a biologically plausible and well-supported response pattern.

Several mechanisms may plausibly underlie this pattern, although they were not measured directly in the present experiment. Microplastics that are ingested, or that remain in close contact with the digestive tract, may reduce nutrient-absorption efficiency and disturb energy homeostasis. At a broader level, polystyrene exposure has been linked to hepatic metabolomic alteration, gut dysbiosis, oxidative stress, and changes in the expression of genes related to growth and metabolism (Jiang et al., 2022; Kim et al., 2021; Medriano & Bae, 2022; Umamaheswari et al., 2021; Wang et

al., 2022; Zhang et al., 2022). In other fish, polystyrene exposure has also been reported to reduce muscle nutritional quality and alter lipid metabolism, suggesting that the associated stress may extend beyond the digestive tract and operate at the systemic level (Lai et al., 2021).

The nonsignificant FCR result warrants cautious interpretation. Numerically, FCR increased as microplastic concentration rose, especially at 150 mg/L, which is directionally consistent with impaired feed-use efficiency. However, the absence of statistical significance indicates that this pattern should not be overinterpreted as firm comparative evidence among treatments. The large variance observed in the highest-dose group suggests that the FCR response may be more variable than the direct growth endpoints measured here, or that the current experimental scale was insufficient to resolve the effect with greater precision.

Table 1. Water-quality ranges recorded during the rearing of Siamese fighting fish

Treatment	Temperature (°C)	DO (mg/L)	pH	Ammonia (mg/L)
0 mg/L	27.0–32.2	4.0–5.7	7.5–8.11	0–0.50
50 mg/L	28.7–32.1	4.2–5.4	7.7–8.90	0–0.15
100 mg/L	27.7–32.3	4.1–5.2	7.4–8.16	0–0.25
150 mg/L	27.5–32.0	4.0–5.5	7.8–8.13	0–0.25

Note: water-quality ranges are presented descriptively based on monitoring data collected during the rearing period.

Water-quality measurements presented in Table 1 provide additional context for interpreting the growth response. Temperature, dissolved oxygen, pH, and ammonia remained within ranges that still allowed Siamese fighting fish to survive throughout the rearing period. Because survival remained 100% in all treatments, the water-quality data support the interpretation that the rearing medium did not undergo severe deterioration that could, by itself, account for the observed reduction in growth. The recorded effects were therefore more plausibly associated with microplastic exposure and its accompanying sublethal physiological burden than with acute degradation of water quality alone.

From an aquaculture perspective, these findings are relevant because sublethal effects can still reduce production efficiency even when mortality does not occur. In ornamental-fish culture, slower growth and poorer feed-use efficiency may prolong rearing time, reduce uniformity, and lower production value. In that sense, the present results should be viewed as an early warning that microplastic contamination may compromise husbandry quality even before more overt outcomes, such as mortality, become evident (Ghosh et al., 2025; Hasan et al., 2024; Hodkovicova et al., 2022). These findings also align with studies showing that microplastics may affect performance attributes beyond growth, including physiology, histopathology, body coloration, and behavior (Hutton et al., 2024; Ren et al., 2025; Soliman et al., 2023;

Subaramaniyam et al., 2023; Zhang et al., 2025). Accordingly, the present study provides a useful basis for more mechanistic follow-up work in Siamese fighting fish, for example through integrated measurements of oxidative stress, intestinal histology, behavior, or metabolism.

## CONCLUSION

Waterborne exposure to polystyrene microplastics for 45 days reduced the growth performance of Siamese fighting fish, as indicated by declines in absolute length gain, specific length growth rate, absolute weight gain, and specific growth rate. FCR tended to increase at higher concentrations, although the differences were not statistically significant, while SR remained 100% in all treatments. Under the conditions of this study, polystyrene microplastics are therefore more appropriately viewed as sublethal stressors that suppress growth performance rather than as direct causes of mortality. Further studies incorporating finer particle characterization, larger sample sizes, and integrated physiological endpoints would strengthen the biological interpretation of these findings.

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