

POLYSTYRENE MICROPLASTICS ELEVATE BLOOD GLUCOSE AND AGGRESSIVE BEHAVIOR IN SIAMESE FIGHTING FISH

Mikroplastik polistiren meningkatkan kadar glukosa darah dan perilaku agresif pada ikan cupang siam

Bagus Dwi Hari Setyono*, Lalu Wahyu Budi Utomo, Rangga Idris Affandi

Study Program of Aquaculture, Faculty of Agriculture, Universitas Mataram, Mataram, West Nusa Tenggara, Indonesia

Majapahit Sreet No. 62, Gomong, Selaparang District, Mataram, West Nusa Tenggara, 83115

*Corresponding Author: bagus.setyono@unram.ac.id

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ABSTRACT

This study evaluated the sublethal physiological and behavioral responses of Siamese fighting fish (*Betta splendens*) to waterborne polystyrene microplastic exposure during a 45-day rearing trial. A completely randomized design with four treatments and three replicates was used: 0, 50, 100, and 150 mg/L. Fish were reared individually, and the principal endpoints were blood glucose and aggression index. Blood glucose was measured at the end of the trial using a portable glucometer, whereas aggressive behavior was quantified as the number of aggressive acts per minute during a standardized 3-minute visual challenge. Data was analyzed using one-way ANOVA followed by Tukey's test, and the relationship between blood glucose and aggression was further examined using Pearson correlation based on individual observations. Blood glucose differed significantly among treatments ($F = 39.116$; $p < 0.001$) and increased from 87.3 ± 6.81 mg/dL in the control to 148.3 ± 10.41 mg/dL at 150 mg/L. Aggressive behavior also differed significantly ($F = 6.820$; $p = 0.014$), increasing from 6.0 ± 1.00 to 14.0 ± 1.00 events min^{-1} over the same concentration range. Individual-level re-analysis indicated a strong positive correlation between blood glucose and aggression ($r = 0.795$; $p = 0.002$). Taken together, these findings indicate that polystyrene microplastic exposure induced clear metabolic and behavioral responses in *B. splendens* and support the use of blood glucose and aggressive behavior as complementary sublethal biomarkers in ornamental-fish microplastic studies.

Keywords: Aggressive Behavior, Betta Fish, Blood Glucose, Ornamental Fish, Polystyrene Microplastics, Sublethal Biomarkers.

ABSTRAK

Studi ini mengevaluasi respons fisiologis dan perilaku sublethal ikan cupang (*Betta splendens*) terhadap paparan mikroplastik polistirena dalam air selama percobaan pemeliharaan 45 hari. Desain acak lengkap dengan empat perlakuan dan tiga ulangan digunakan: 0, 50, 100, dan 150 mg/L. Ikan dipelihara secara individual, dan parameter utama yang diukur adalah kadar glukosa darah dan indeks agresi. Kadar glukosa darah diukur pada akhir percobaan

menggunakan glukometer portabel, sedangkan perilaku agresif diukur sebagai jumlah tindakan agresif per menit selama tantangan visual standar 3 menit. Data dianalisis menggunakan ANOVA satu arah diikuti dengan uji Tukey, dan hubungan antara glukosa darah dan agresi selanjutnya diperiksa menggunakan korelasi Pearson berdasarkan pengamatan individual. Kadar glukosa darah berbeda secara signifikan antar perlakuan ($F = 39,116$; $p < 0,001$) dan meningkat dari $87,3 \pm 6,81$ mg/dL pada kelompok kontrol menjadi $148,3 \pm 10,41$ mg/dL pada konsentrasi 150 mg/L. Perilaku agresif juga berbeda secara signifikan ($F = 6,820$; $p = 0,014$), meningkat dari $6,0 \pm 1,00$ menjadi $14,0 \pm 1,00$ kejadian min-1 pada rentang konsentrasi yang sama. Analisis ulang tingkat individu menunjukkan korelasi positif yang kuat antara glukosa darah dan agresi ($r = 0,795$; $p = 0,002$). Secara keseluruhan, temuan ini menunjukkan bahwa paparan mikroplastik polistirena menginduksi respons metabolik dan perilaku yang jelas pada *B. splendens* dan mendukung penggunaan glukosa darah dan perilaku agresif sebagai biomarker subletal komplementer dalam studi mikroplastik pada ikan hias.

Kata kunci: Perilaku Agresif, Ikan Betta, Glukosa Darah, Ikan Hias, Mikroplastik Polistirena, Biomarker Subletal

INTRODUCTION

Microplastics are now recognized as pervasive contaminants in aquatic ecosystems and have become increasingly relevant to aquaculture because exposure may occur through culture water, feed ingredients, and waste-derived particles entering production systems. In fish, microplastic exposure has been associated with oxidative stress, metabolic disturbance, altered gene expression, neurobehavioral disruption, and a wide range of other sublethal responses. At the same time, the magnitude and even the direction of these responses remain strongly context dependent, varying with polymer type, particle size, exposure route, duration, and species-specific sensitivity. Claims regarding mechanism therefore need to be framed cautiously and in proportion to the strength of the data (Banaee *et al.*, 2024; Ghosh, 2025; Hasan *et al.*, 2024; Wang *et al.*, 2024).

Within Indonesian aquaculture, this issue is also relevant at the regional scale. Microplastic contamination has already been documented in aquaculture areas of Ekas Bay and in whiteleg shrimp farming systems in North Lombok, indicating that exposure risk is not merely hypothetical but increasingly plausible in managed aquatic environments close to the present study area (Sumsanto *et al.*, 2024; Setyono *et al.*, 2024). Although those studies did not examine blood glucose or aggressive behavior, they strengthen the practical relevance of evaluating sublethal endpoints in cultured ornamental fish.

Blood glucose is among the most widely used physiological indicators of stress in fish. Activation of the hypothalamus–pituitary–interrenal axis promotes catecholamine and cortisol release, which in turn stimulates glycogenolysis and gluconeogenesis to provide rapidly available energy. Elevated blood glucose is therefore commonly interpreted as a signal of disturbed energy homeostasis or increased physiological load. Recent studies further suggest that microplastic exposure may interfere with glucose metabolism, hepatic function, and broader energy-regulation pathways, making blood glucose a particularly informative endpoint for sublethal toxicological screening (Brun *et al.*, 2019; Wang *et al.*, 2022; Zhang *et al.*, 2025).

Behavioral endpoints are equally important because they may respond earlier than growth depression or mortality. In fish, microplastic exposure has been linked to altered swimming activity, sociality, anxiety-related responses, and changes in neurotransmitter-regulated behavior. Aggressive behavior is especially relevant in *Betta splendens* because territorial defense and threat display are central features of the species' behavioral repertoire. Even modest disturbance in neuroendocrine balance may therefore be expressed as altered

flaring, chasing, and attack intensity (Al Jabri *et al.*, 2024; Li *et al.*, 2024; Rojoni *et al.*, 2024; Zanona *et al.*, 2025).

Betta splendens is a useful model for this purpose because it is easy to maintain individually, responds clearly to social stimuli, and represents a high-value freshwater ornamental species. Compared with zebrafish and medaka, however, information on microplastic-induced physiological and behavioral responses in betta fish remains limited. This gap matters not only from a toxicological perspective but also from a husbandry perspective, because ornamental-fish culture depends on maintaining both physiological stability and predictable behavioral quality (Lichak *et al.*, 2022; Ramos *et al.*, 2025).

Against this background, the present study aimed to evaluate the effects of waterborne polystyrene microplastic exposure on blood glucose and aggressive behavior in Siamese fighting fish during a 45-day rearing period. The study further tested whether both endpoints changed in the same directional pattern across treatments and whether individual variation in blood glucose was positively associated with individual variation in aggression. By positioning these variables as complementary sublethal biomarkers, this article seeks to move beyond generic statements that microplastics merely “increase stress” and instead provide a more precise biological interpretation for ornamental-fish research.

METHODS

The experiment was conducted for 45 days, from 15 October to 28 November 2025, in the Fish Production and Reproduction Laboratory, Aquaculture Study Program, Faculty of Agriculture, University of Mataram. Healthy Siamese fighting fish approximately 2–3 months old were selected as test animals. Initial body size was relatively uniform, with an initial length of about 2.5 cm and an initial body weight of about 1.5 g. Fish were reared individually, with one fish assigned to each aquarium measuring 15 × 20 × 20 cm. Individual housing was selected because *Betta splendens* is routinely maintained singly in experimental settings to minimize social interference and injury risk during behavioral observation (Lichak *et al.*, 2022).

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, each with three replicates. 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. Thus, the material was treated as microplastic based on the target size range, although the particle-size distribution and particle shape were not instrumentally characterized. The operational size criterion followed the conventional use of the <5 mm threshold in fish microplastic studies, although the lack of finer characterization remains an important limitation for interpretation (Banaee *et al.*, 2024; Ghosh, 2025).

Fish were acclimated before stocking and reared for 45 days. Commercial pellet feed was administered twice daily, at 8 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 appropriate treatment concentration to maintain the intended exposure.

The principal response variables were blood glucose and aggressive behavior. Blood glucose was measured at the end of the trial after a fasting period of approximately 12–24 h to reduce the immediate effect of feeding on glucose values. Blood samples were collected carefully from the caudal region and immediately applied to a portable glucometer strip. This endpoint was selected because blood glucose is widely used as a rapid secondary stress indicator in fish and has been linked to polystyrene-associated metabolic disturbance in previous studies (Brun *et al.*, 2019; Wang *et al.*, 2022; Zhang *et al.*, 2025). Aggressive behavior was assessed using a standardized visual challenge with a conspecific stimulus fish. The recorded behaviors included flaring, chasing, and attacking during a 3-min observation period.

The aggression index was calculated as the number of aggressive acts per minute. Use of a standardized social challenge was considered appropriate because *Betta splendens* is an established model for territorial and aggression-related assays (Lichak *et al.*, 2022). Survival rate was recorded as a supporting variable.

Statistical analysis was performed using one-way ANOVA at the 95% confidence level, followed by Tukey's test when treatment effects were significant. In addition, Pearson correlation analysis was performed on individual observations to assess whether blood glucose and aggression changed in a coordinated manner across fish.

RESULTS

Polystyrene microplastic exposure induced clear physiological and behavioral responses in Siamese fighting fish (Table 1). The lowest mean blood glucose was observed in the control group, whereas the highest value occurred at 150 mg/L. A similar concentration-associated increase was observed for aggression index. One-way ANOVA indicated a highly significant treatment effect on blood glucose ($F = 39.116$; $p < 0.001$) and a significant treatment effect on aggression index ($F = 6.820$; $p = 0.014$).

Table 1. Polystyrene Microplastic Exposure Induced Clear Physiological and Behavioral Responses

Treatment	Blood glucose (mg/dL)	Aggression index (events min ⁻¹)
0 mg/L	87.3 ± 6.81 ^a	6.0 ± 1.00 ^a
50 mg/L	105.0 ± 5.00 ^{ab}	10.7 ± 1.53 ^{ab}
100 mg/L	120.0 ± 5.00 ^b	12.0 ± 4.00 ^b
150 mg/L	148.3 ± 10.41 ^c	14.0 ± 1.00 ^b

Mean ± SD blood glucose and aggression index of Siamese fighting fish after 45 days of polystyrene microplastic exposure. Different superscript letters within a column indicate significant differences according to Tukey's test ($p < 0.05$).

One-way ANOVA showed a highly significant treatment effect on blood glucose ($F = 39.116$; $p < 0.001$). Tukey's test indicated that the control did not differ significantly from 50 mg/L ($p = 0.064$) but differed significantly from 100 mg/L ($p = 0.002$) and 150 mg/L ($p < 0.001$). The 50 mg/L treatment also did not differ significantly from 100 mg/L ($p = 0.122$), whereas 150 mg/L remained significantly higher than both intermediate treatments. Overall, blood glucose showed the clearest concentration-associated pattern among the endpoints evaluated.

Aggressive behavior was also significantly affected by treatment ($F = 6.820$; $p = 0.014$). The aggression index at 50 mg/L was descriptively higher than that of the control, although the difference was not statistically significant ($p = 0.128$). By contrast, the control differed significantly from both 100 mg/L ($p = 0.046$) and 150 mg/L ($p = 0.011$). Differences among exposed groups were not significant, indicating that the behavioral endpoint was responsive to exposure, but statistically less resolved than blood glucose.

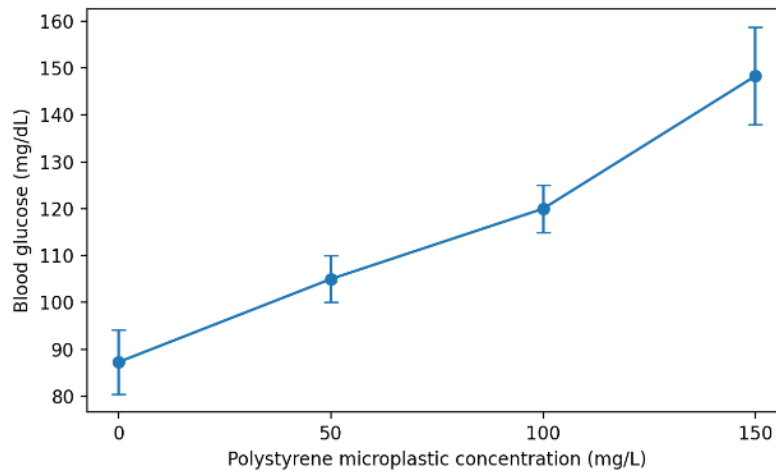


Figure 1. Blood Glucose of Siamese Fighting Fish Exposed to Different Concentrations of Polystyrene Microplastics During the 45-day Rearing Period. Error Bars Indicate Standard Deviation.

Pearson correlation analysis based on individual fish data showed a strong positive relationship between blood glucose and aggression ($r = 0.795$; $p = 0.002$). Across individual observations, fish with higher blood glucose tended to show higher aggression indices. Although correlation does not establish causality, the result supports the view that both variables shifted in a coherent biological direction under polystyrene microplastic exposure.

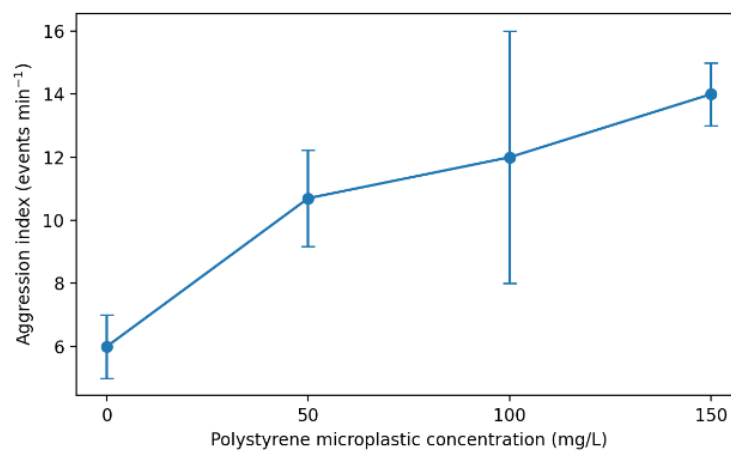


Figure 2. Aggression Index of Siamese Fighting Fish Exposed to Different Concentrations of Polystyrene Microplastics During the 45-day Rearing Period. Error Bars Indicate Standard Deviation.

Table 2. Summary of Principal Inferential Statistics Used in the Study

Analysis	Statistic	p-value	Interpretation
Blood glucose	ANOVA $F = 39.116$	< 0.001	Significant treatment effect
Aggression index	ANOVA $F = 6.820$	0.014	Significant treatment effect
Glucose–aggression relationship	Pearson $r = 0.795$	0.002	Strong positive correlation

DISCUSSION

The present results indicate that waterborne exposure to polystyrene microplastics induced coordinated physiological and behavioral responses in *Betta splendens*. The steady increase in blood glucose across treatments, together with the upward shift in aggression relative to the control, is consistent with broader evidence that microplastics can alter both stress physiology and behavior in fish, even when mortality does not occur. Recent reviews and meta-analytical syntheses indicate that such responses vary substantially with polymer type, particle size, dose, exposure route, and species, but they are nevertheless recurrent enough to support the interpretation of microplastics as biologically relevant sublethal stressors in fish (Hasan *et al.*, 2024; Rojoni *et al.*, 2024; Ghosh, 2025; Zanona *et al.*, 2025).

The blood-glucose response is biologically plausible within the framework of fish stress physiology. Hyperglycemia commonly reflects activation of the hypothalamus-pituitary-interrenal axis and the downstream mobilization of energy reserves through catecholamine- and cortisol-mediated processes. Similar metabolic disturbance has been reported in fish exposed to microplastics and particularly to polystyrene particles. In larval zebrafish, polystyrene nanoplastics disrupted both glucose metabolism and cortisol balance (Brun *et al.*, 2019), whereas in rare minnow and grass carp, polystyrene exposure was associated with hepatic metabolic shifts and glucose-metabolism disorders (Wang *et al.*, 2022; Zhang *et al.*, 2025). More recently, behavioral toxicity in zebrafish larvae was accompanied by disrupted mitochondrial energy metabolism and glucose-metabolism impairment under polypropylene exposure, reinforcing the view that altered glycemia may reflect broader bioenergetic dysregulation rather than an isolated endpoint (Hua *et al.*, 2024). Hematological and oxidative-stress disturbances reported in other exposed fish further support this interpretation (Jo *et al.*, 2025).

The behavioral results complement this interpretation, although their statistical structure was less sharply resolved than that of blood glucose. Aggression increased descriptively from the lowest to the highest concentration, but the post hoc pattern indicates that the clearest separation occurred between the control and the two upper exposure groups. This more conservative pattern is not unusual for behavioral endpoints, which often show greater between-individual variability than physiological biomarkers. Recent work in zebrafish has shown that microplastics can modify boldness, anxiety, sociability, locomotor activity, and exploratory behavior, with response magnitude partly influenced by prior personality state and exposure context (Chen *et al.*, 2022; Al Jabri *et al.*, 2024; Yu *et al.*, 2022). Reviews focused on fish neurobehavior similarly conclude that behavioral disruption is a recurrent but heterogeneous outcome of microplastic exposure (Hasan *et al.*, 2024; Rojoni *et al.*, 2024).

The strong positive correlation between blood glucose and aggression provides an additional layer of biological coherence. Although correlation does not establish causation, it indicates that individuals with higher glucose concentrations also tend to show stronger aggressive responses under the present test conditions. This interpretation is consistent with studies showing that microplastics can affect neurotransmission, dopaminergic signaling, and neurodevelopmental pathways that are relevant to behavioral regulation in fish. For example, water- and foodborne exposure in zebrafish altered dopamine and 5-HT concentrations and produced hyperactive responses (Yu *et al.*, 2022), photoaged polystyrene microplastics induced neurotransmission-related neurotoxicity in larvae (Li *et al.*, 2024), and recent studies have linked microplastic exposure with ADHD-like or depression-like behavioral phenotypes in zebrafish (Cai *et al.*, 2025; Yang *et al.*, 2025). At the same time, the latest systematic review and meta-analysis emphasized that neurobehavioral effects across fish studies remain highly heterogeneous, so the present correlation should be interpreted as supportive rather than definitive mechanistic evidence (Zanona *et al.*, 2025).

These findings should nevertheless be interpreted with caution. The study used a relatively small number of experimental units, one fish per aquarium, and microplastic particles that were not characterized in detail with respect to size distribution, shape, or surface properties. Accordingly, the present data are better suited to demonstrating an association between polystyrene exposure and sublethal physiological-behavioral disturbance than to establishing a specific mechanistic pathway. This restraint is important because recent reviews consistently note that comparability across fish studies is limited by differences in polymer identity, environmental aging, exposure concentration, duration, and endpoint selection (Hasan *et al.*, 2024; Rojoni *et al.*, 2024; Zanona *et al.*, 2025).

From an applied perspective, the findings are relevant to ornamental-fish culture because fish may remain alive while still experiencing marked physiological and behavioral disturbance. In the present study, all fish survived, yet both glucose and aggression increased substantially with exposure. For ornamental species, such sublethal changes matter because they may influence handling tolerance, welfare, display quality, and husbandry stability even before mortality or overt growth

CONCLUSION

Waterborne exposure to polystyrene microplastics for 45 days elevated blood glucose and increased aggressive behavior in Siamese fighting fish. Blood glucose showed the clearest concentration-associated pattern and was more statistically resolved than the aggression index, whereas correlation analysis indicated that both endpoints shifted in the same biological direction. Under the conditions of this study, blood glucose and aggressive behavior can therefore be regarded as complementary sublethal biomarkers for evaluating polystyrene microplastic exposure in *Betta splendens*.

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