

Sublethal Exposure to Polystyrene Microplastics Alters Swimming Behaviour and Reduces Survival of Whiteleg Shrimp (*Litopenaeus vannamei*)

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ABSTRACT

Polystyrene microplastics (PS) in aquaculture systems may impair shrimp behaviour and survival, yet experimental evidence directly linking exposure intensity with easily observable behavioural indicators remains limited. This study evaluated the effects of sublethal PS exposure on swimming behaviour and survival of whiteleg shrimp during a 45-day rearing period. A completely randomized design was applied using four treatments: 0, 10, 20, and 30 mg/L PS, each with three replicates. PL-30 whiteleg shrimp were stocked at 20 individuals per 20-L container. Swimming behaviour was repeatedly assessed using three categories: BN (normal swimming), BL (weak swimming), and BL;DD (weak swimming with bottom-resting tendency). Survival data were analysed using ANOVA followed by Duncan's multiple range test at a 5% significance level, whereas behavioural observations were interpreted descriptively. Behavioural deterioration followed a consistent dose-related pattern. The control group remained normal throughout the observation period, while the 20–30 mg/L treatments shifted earlier toward BL and BL;DD. Survival was highest in the control ($73.33 \pm 5.77\%$) and declined to 31.67–36.67% in PS-exposed groups. ANOVA indicated a significant treatment effect on survival ($F(3,8) = 28.263$; $p < 0.001$), and Duncan's test separated the control from all exposed treatments, whereas the 10–30 mg/L treatments remained in the same homogeneous subset. Because water quality remained within an acceptable range, the behavioural shift and reduced survival were more plausibly linked to PS exposure than to deterioration in rearing conditions. These findings highlight swimming behaviour as a practical early-warning indicator in shrimp culture exposed to microplastics.

INTRODUCTION

Whiteleg shrimp is one of the most important aquaculture commodities and its production performance is strongly dependent on stable rearing conditions and efficient biological responses to environmental stress. In recent years, microplastics have no longer been viewed solely as an issue of marine ecology, but also as contaminants of direct relevance to aquaculture because they have been detected in water, sediments, feed, and cultured organisms. Their presence in shrimp culture systems raises concern regarding production performance, animal health, and food safety (Ali *et al.*, 2024; Páez-Osuna *et al.*, 2024; Pal *et al.*, 2025; Naidu *et al.*, 2025). Local evidence from West Nusa Tenggara likewise shows that microplastic contamination has already been identified in shrimp farming areas and along the production chain. Sumsanto *et al.* (2024) reported the abundance and diversity of microplastics in the cultivation area of Ekas Bay, while Setyono *et al.* (2024) documented microplastic contamination in water, feed, and shrimp organs in whiteleg shrimp culture systems in North Lombok. These findings reinforce the ecological and practical relevance of the present experimental study.

Among the various polymer types, polystyrene deserves particular attention because it is widely used in packaging, styrofoam, and other materials that can become important sources of secondary particles in aquatic environments. In addition to being persistent, polystyrene may release plastic additives and undergo surface changes after environmental exposure, thereby increasing its potential interaction with aquatic tissues (Siddiqui *et al.*, 2023; Gulizia *et al.*, 2023; Wang *et al.*, 2024; Kwon, 2026).

In shrimp and other crustaceans, microplastic exposure has been associated with microbiota disruption, intestinal and hepatopancreatic histopathology, oxidative stress, metabolic alteration, immune disturbance, and increased susceptibility to co-occurring contaminants or pathogens. In whiteleg shrimp, recent studies have shown that microplastics may compromise tissue integrity, modulate apoptosis and autophagy pathways, alter metabolite profiles, and aggravate the effects of combined exposure with nitrite, metals, herbicides, or pathogenic bacteria (Duan *et al.*, 2021; Sun *et al.*, 2022; Zhou *et al.*, 2023; Zeng *et al.*, 2023; Seta *et al.*, 2023; Xing *et al.*, 2024; Chen *et al.*, 2024; Li *et al.*, 2024; Lv *et al.*, 2024; Thammatorn *et al.*, 2024; Li *et al.*, 2025; Bonilla-Aguirre *et al.*, 2026).

Nevertheless, most previous studies have emphasized molecular biomarkers or histological changes, whereas behavioural indicators that can be easily observed in production units have rarely been assessed in a systematic way. In practice, however, changes in swimming activity and feeding behaviour are often among the earliest visible signs of declining shrimp fitness. Within aquaculture systems, such indicators are highly valuable because they can be monitored rapidly and at relatively low cost as part of routine management and decision-making (Bardera *et al.*, 2020; Bardera *et al.*, 2021; Darodes de Tailly *et al.*, 2021; Xiong *et al.*, 2023; Zink & Wood, 2024; Zhang *et al.*, 2025).

Against this background, the present study was designed to analyse the effects of sublethal exposure to polystyrene microplastics on swimming behaviour and survival of whiteleg shrimp during a 45-day rearing period. The manuscript deliberately focuses on behavioural response and survival in order to align the interpretation with the strength of the available dataset and to provide a more directly applicable perspective for aquaculture management.

METHODS

The experiment was conducted for 45 days, from June to July 2025, at the Aquatic Environment Laboratory and the Fish Production and Reproduction Laboratory, Faculty of Agriculture, University of Mataram. The study employed a completely randomized design with four polystyrene microplastic treatments: P1 = 0 mg/L (control), P2 = 10 mg/L, P3 = 20 mg/L, and P4 = 30 mg/L, each with three replicates, resulting in 12 experimental units. The selected exposure levels were adapted from previous crustacean studies using graded polystyrene microplastic/nanoplastic concentrations. Zeng *et al.* (2023) reported sublethal effects of polystyrene microplastics in *Litopenaeus vannamei* at 0.02-1 mg/L, whereas Li *et al.* (2021, 2023) employed graded polystyrene nanoplastic exposures of 0, 5, 10, 20, and 40 mg/L in *Macrobrachium nipponense*. Based on these studies, 10, 20, and 30 mg/L were selected in the present study to represent progressively increasing sublethal exposure levels.

PL-30 whiteleg shrimp (*Litopenaeus vannamei*) were used as the experimental animals. Each experimental container was filled with 20 L of seawater and stocked with 20 shrimp. Prior to treatment exposure, the shrimp were acclimated for more than 24 h. During the rearing period, shrimp were fed at 5% of total biomass per day, divided into three feeding times. Siphoning and water exchange were performed every 2 days.

Polystyrene microplastics were prepared from styrofoam by crushing, drying, sieving, and weighing the material according to the assigned treatment dose before being introduced into the culture medium. The main response variables evaluated in this study were swimming behaviour and survival rate (SR).

Swimming behaviour was assessed in four consecutive observation sessions during the experimental period. Behavioural responses were classified as BN (normal swimming), BL (weak swimming), and BL;DD (weak swimming accompanied by a tendency to remain on the bottom). Because the behavioural data were recorded as categorical observations in each session, they were analysed descriptively and compared across treatments to evaluate the consistency of response patterns.

Survival rate was determined at the end of the experiment using the following equation:

$$SR (\%) = (N_t / N_0) \times 100$$

where N_t is the number of shrimps surviving at the end of the experiment and N_0 is the initial number of shrimps stocked. The calculation of SR followed Effendie (1979).

Water quality was monitored throughout the experiment to ensure that the observed responses were not primarily attributable to deterioration of the culture medium. The measured parameters included temperature, dissolved oxygen (DO), salinity, and pH. Temperature was measured using a thermometer, DO using a DO meter, salinity using a hand refractometer, and pH using a digital pH meter. All parameters were recorded twice daily, in the morning and afternoon, throughout the rearing period.

Survival-rate data were analysed by one-way analysis of variance (ANOVA) at a 95% confidence level using SPSS version 16.0. When significant differences among treatments were detected, Duncan's multiple range test was applied for post hoc comparison.

RESULTS

Swimming Behaviour Response

Behavioural observations revealed a consistent pattern of change with increasing PS dose. During the first observation session, all treatments still displayed normal swimming

(BN). Differences among doses became apparent in subsequent sessions. The control remained in the BN category throughout all observations, whereas the 10 mg/L treatment shifted to weak swimming (BL) during the third and fourth sessions. At 20 mg/L, the shift toward BL appeared earlier and by the fourth session the shrimp had reached the combined BL;DD category. The most severe pattern occurred at 30 mg/L, where BL was already observed in the second session and BL;DD persisted during the third and fourth sessions. To facilitate interpretation of this pattern, Figure 1 presents a visual summary redrawn in English from the same observation matrix.

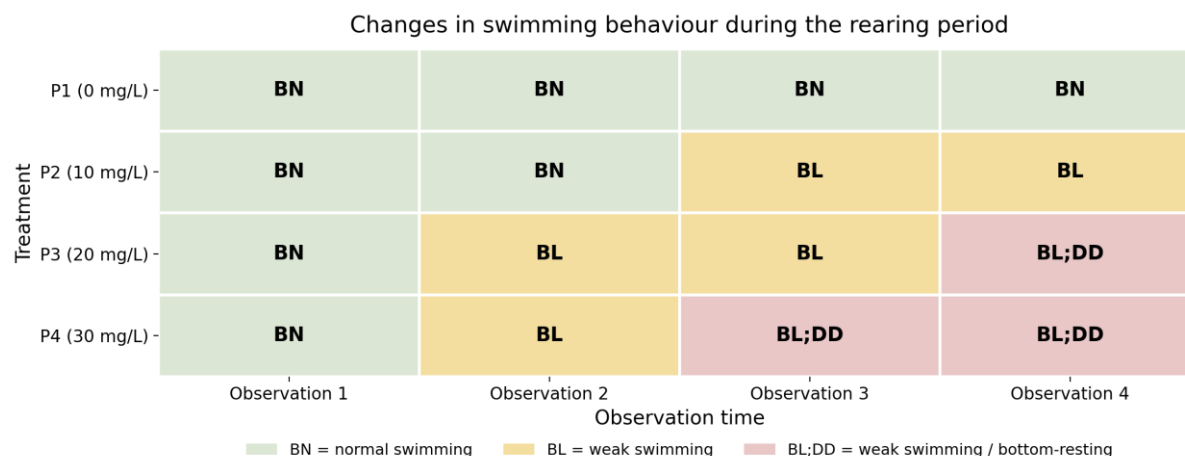


Figure 1. Visual Summary of Changes in the Swimming-Behaviour Response of Whiteleg Shrimp During the Rearing Period

Survival

Survival declined in a clear treatment-related pattern. The highest mean SR was observed in control at $73.33 \pm 5.77\%$, whereas the PS-exposed treatments yielded $36.67 \pm 7.63\%$ (P2), $36.67 \pm 2.88\%$ (P3), and $31.67 \pm 7.63\%$ (P4). The detailed post-hoc grouping is presented in Table 1.

Table 1. Survival of Whiteleg Shrimp under Different Polystyrene Microplastic Treatments

Treatment	SR (%)	Duncan Group	Interpretation
P1 (0 mg/L)	73.33 ± 5.77	b	Significantly different from P2–P4
P2 (10 mg/L)	36.67 ± 7.63	a	Same subset as P3–P4
P3 (20 mg/L)	36.67 ± 2.88	a	Same subset as P2–P4
P4 (30 mg/L)	31.67 ± 7.63	a	Same subset as P2–P3

According to the original ANOVA output, treatment exerted a significant effect on SR ($F(3,8) = 28.263$; $p < 0.001$). Duncan’s test separated P1 from all PS-exposed treatments, whereas P2, P3, and P4 remained in the same homogeneous subset. In other words, PS exposure generally reduced survival relative to the control, but within the 10–30 mg/L range the exposed groups were not further separated statistically.

To preserve traceability to the source manuscript while improving publication readiness, Figure 2 presents the SR graph redrawn in English and synchronized with the original SPSS output.

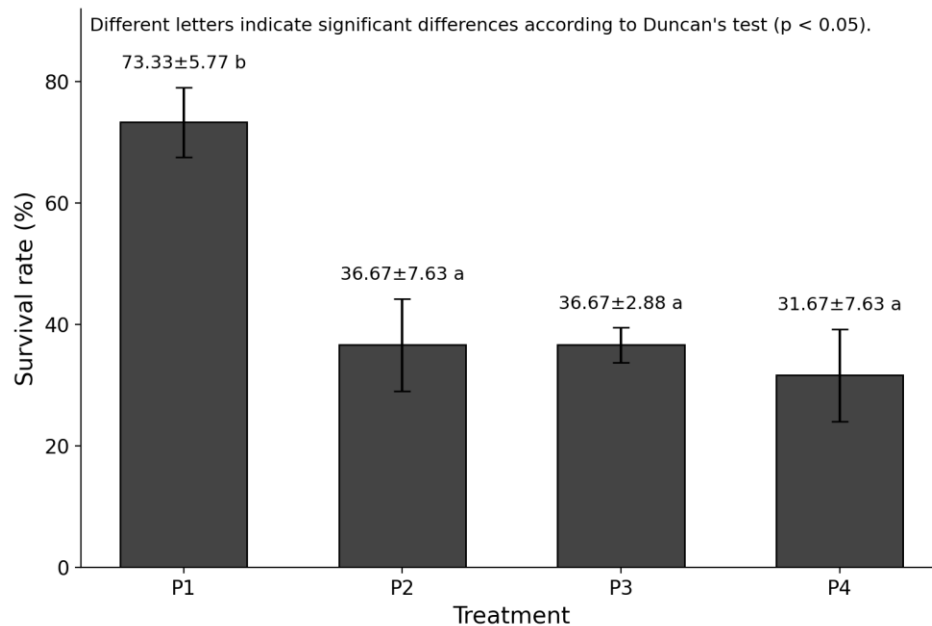


Figure 2. Survival Rate of Whiteleg Shrimp under Different Concentrations of Polystyrene Microplastics

Water Quality and Interpretation of the Treatment Effect

Interpretation of behavioural change and survival decline must be considered in the context of rearing-water quality. Throughout the study, temperature, DO, salinity, and pH remained within ranges considered suitable for whiteleg shrimp culture, as summarized in Table 2.

Table 2. Summary of Water Quality During the Rearing Period of Whiteleg Shrimp

Parameter	Measured Range	Interpretation
Temperature (°C)	27.5–29.5	Suitable/optimal
DO (mg/L)	6.5–7.1	Optimal
Salinity (ppt)	31–32	Optimal
pH	7.8–8.0	Optimal

DISCUSSION

Swimming Behaviour Response

Descriptively, Figure 1 shows that increasing PS concentration was followed by a stepwise behavioural shift from BN to BL and subsequently to BL;DD. Because the behavioural records in this study were categorical and were not subjected to inferential testing, interpretation is intentionally limited to pattern consistency across treatments rather than claims of statistical significance. Even so, the pattern remains biologically meaningful because swimming behaviour is a sensitive functional indicator of declining fitness, irritation of the respiratory or digestive system, and reduced general physiological capacity (Bardera *et al.*, 2020; Bardera *et al.*, 2021; Darodes de Tilly *et al.*, 2021).

The present findings are also consistent with previous studies indicating that microplastics may alter nervous-system function, ionoregulatory homeostasis, and microbiota–host balance, all of which may ultimately be expressed as behavioural disruption or reduced activity. Meta-analytic and review evidence in aquatic animals and invertebrates

likewise supports a link between microplastic exposure and neurotoxic or host-health effects (Fu *et al.*, 2022; Wang *et al.*, 2023; Xiong *et al.*, 2023; Jeong *et al.*, 2026). Accordingly, in the context of this study, the appearance of BL and BL;DD is more appropriately interpreted as an indicator of functional impairment associated with PS exposure than as direct proof of any single physiological mechanism.

Survival

From a practical perspective, the results indicate that even the lowest PS dose used in this study was associated with a pronounced reduction in survival relative to the control. Recent literature on whiteleg shrimp suggests that microplastics may damage the digestive tract and hepatopancreas, disrupt metabolism, intensify oxidative stress, and increase vulnerability to additional stressors, such that the cumulative sublethal burden may eventually be reflected in lower survival (Duan *et al.*, 2021; Zhou *et al.*, 2023; Zeng *et al.*, 2023; Seta *et al.*, 2023; Chen *et al.*, 2024; Xing *et al.*, 2024; Li *et al.*, 2024; Thammatorn *et al.*, 2024; Li *et al.*, 2025; Bonilla-Aguirre *et al.*, 2026). In line with this interpretation, Setyono *et al.* (2025) reported that sublethal PVC microplastic exposure also reduced growth performance and survival of whiteleg shrimp, further supporting the view that plastic-particle exposure at sublethal levels can materially impair cultured shrimp performance.

Water Quality and Interpretation of the Treatment Effect

Because these water-quality variables remained within acceptable limits, the deterioration in swimming response and survival under PS exposure is more reasonably attributed to the particle treatment than to generalized decline in the culture medium. This point is important because it indicates that the observed differences were unlikely to be mere artefacts of uncontrolled temperature, dissolved oxygen, salinity, or pH. Taken together, the results support the conclusion that PS exposure contributed directly to the decline in biological performance under rearing conditions that were otherwise suitable for shrimp culture.

CONCLUSION

Sublethal exposure to polystyrene microplastics altered the swimming-behaviour response and reduced the survival of whiteleg shrimp. Descriptively, increasing PS concentration was associated with a shift from normal swimming toward weak swimming and subsequent bottom-resting behaviour. Statistically, PS exposure significantly reduced survival relative to the control, as indicated by ANOVA ($F(3,8) = 28.263$; $p < 0.001$) and Duncan's test, which separated the control from all exposed treatments while leaving the 10–30 mg/L treatments within the same homogeneous subset. Because water quality remained within suitable rearing limits throughout the experiment, the behavioural shift and reduced survival are more appropriately interpreted as early biological responses to microplastic exposure.

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