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Polyvinyl Chloride Microplastic Exposure Alters Body Colour and Reduces Condition Factor in Guppy (*Poecilia reticulata*)

Rifki Rijal Hapriawan, Bagus Dwi Hari Setyono*, Muhammad Sumsanto

Study Program of Aquaculture, Department of Fisheries and Marine Science,
Faculty of Agriculture, University of Mataram
Jl. Majapahit No. 62, Mataram, West Nusa Tenggara 83115, Indonesia

Correspondence:
bagus.setyono@unram.ac.id

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ABSTRACT

Polyvinyl chloride (PVC) microplastics may compromise fish health, but visible sublethal responses in ornamental fish remain poorly documented. This study evaluated whether nominal PVC microplastic exposure altered CIE L*a*b* body-colour indices, Fulton's condition factor, and survival of guppy (*Poecilia reticulata*). A completely randomized design included 0, 5, 15, and 20 mg/L PVC treatments, each with three aquaria stocked with 10 fish and maintained for 45 days. Treatment means were evaluated by one-way analysis of variance and Duncan's multiple range test. The 15 and 20 mg/L treatments produced higher lightness and lower redness and yellowness than the control and 5 mg/L treatments. At 20 mg/L, survival declined to 66.67 ± 15.28% and the condition factor declined to 0.96 ± 0.13. These results show a marked fading of red and yellow body-colour components at 15 to 20 mg/L, while reduced survival and condition factor were most evident at 20 mg/L. Digital CIE L*a*b* metrics may therefore provide a practical visual endpoint for screening sublethal responses of ornamental fish to nominal PVC microplastic exposure. The study did not test the physiological mechanisms that generated the colour response.

INTRODUCTION

Microplastics are persistent contaminants that occur in freshwater and marine systems, including water, sediments, feed, and cultured organisms. Their ingestion or contact may alter growth, survival, physiological regulation, oxidative balance, feeding behaviour, reproduction, and microbial communities in fish. The magnitude of these responses depends on polymer type, particle size, concentration, exposure route, and duration (Azevedo-Santos *et al*., 2019; Galafassi *et al*., 2021; Parker *et al*., 2021; Salerno *et al*., 2021; Wootton *et al*., 2021; Hasan *et al*., 2024; Wang *et al*., 2024; Banaee *et al*., 2025; Ghosh *et al*., 2025; Wu *et al*., 2025). Microplastic pollution also raises concerns for fisheries production and seafood security (Kibria, 2023).

Polyvinyl chloride is an important polymer of concern because of its wide use in pipes, packaging, and consumer products. When PVC fragments, it produces particles that can act as physical stressors and may also contain additives that can leach into water. The potential risk therefore includes particle effects and additive-related chemical exposure (Turner & Filella, 2021; Henkel *et al*., 2022; Haque & Fan, 2023). Experimental studies have linked PVC or other microplastics with reduced fish growth, oxidative disturbance, impaired health status, and lower survival under some exposure conditions (Xia *et al*., 2020; Masud & Cable, 2023; Kelly *et al*., 2024; Putrajab *et al*., 2024). Local studies also confirm that microplastic contamination occurs in aquaculture areas in West Nusa Tenggara, including Ekas Bay and whiteleg shrimp cultivation systems in North Lombok (Sumsanto *et al*., 2024; Setyono *et al*., 2024a).

In ornamental fish, body colour has biological and economic value. Red and yellow colouration depend largely on carotenoid availability, pigment metabolism, chromatophore function, and environmental conditions. Lightness and structural effects are also influenced by the organisation of pigment cells and reflective tissues (Nakano & Wiegertjes, 2020; Lau *et al*., 2023; Liu *et al*., 2024). Thus, a shift in colour may indicate early physiological disturbance before overt mortality occurs. Guppy (*Poecilia reticulata*) is suitable for this approach because it has prominent body colour, is readily cultured, and has economic importance in ornamental fish production (Setyono *et al*., 2025). Recent work also indicates that microplastics can impair guppy structural colour through oxidative pathways (Ren *et al*., 2025).

Evidence on PVC microplastic exposure and quantitative body-colour responses in guppy remains limited. This study therefore evaluated the effects of nominal PVC microplastic exposure on lightness (L*), redness (a*), yellowness (b*), Fulton's condition factor, and survival rate in guppy. The working premise was that body-colour indices could provide a practical visual endpoint for detecting sublethal disturbance in ornamental fish.

METHODS

Study Period and Location

The study was conducted from October to November 2025 at the Laboratory of Fish Production and Reproduction, Study Program of Aquaculture, Faculty of Agriculture, University of Mataram, Indonesia.

Experimental Design and Fish Husbandry

The experiment used a completely randomized design with four nominal PVC microplastic treatments and three replicate aquaria per treatment. The treatments were P1, 0 mg/L; P2, 5 mg/L; P3, 15 mg/L; and P4, 20 mg/L. Each aquarium was stocked with 10 guppy measuring 3 to 7 cm in total length, resulting in 12 experimental units. The exposure period was 45 days. The aquarium was treated as the experimental unit for statistical analysis.

The treatment concentrations were selected by adopting the waterborne PVC microplastic exposure range previously applied to Nile tilapia (*Oreochromis niloticus*), namely 5, 15, and 20 mg/L (Setyono *et al*., 2024b). That study used the same graded nominal concentrations and reported significant effects on survival and growth-related performance. Accordingly, the present experiment used 5 mg/L as the lower treatment level and 15 and 20 mg/L as higher experimental challenge levels to examine dose-related sublethal responses in guppy. These concentrations were selected for laboratory hazard characterization and should not be interpreted as direct estimates of microplastic concentrations in natural waters.

Guppy were fed twice daily at 08:00 and 17:00 h at 3% of biomass per day. Siphoning was carried out weekly, followed by 50% water replacement. PVC treatment was expressed as a nominal concentration. Actual particle concentrations were not measured over the exposure period.

PVC Microplastic Preparation

PVC particles were prepared from flexible PVC pipe material. The pipe was ground, dried, and sieved through a 60-mesh screen with a 0.40 mm aperture. Material passing the sieve was weighed according to treatment dose and introduced into the culture medium. The protocol established a maximum sieve-defined particle size but did not quantify particle-size distribution, particle number, morphology, or additive composition.

Body-colour Analysis, Condition Factor, and Survival

Fish were photographed under standardised lighting against a neutral background. Digital images were analysed in Adobe Photoshop 2020 after conversion to Lab colour mode. Lightness was expressed on the L* axis, redness on the a* axis, and yellowness on the b* axis. The imaging protocol provided comparative colour values across treatments. The study did not measure chromatophore density, carotenoid concentration, or oxidative biomarkers.

Fulton's condition factor was calculated at the end of the experiment as $K = 100W/L^3$, where W is body weight in g and L is total length in cm. Survival rate was calculated as SR (%) = $(N_t/N_0) \times 100$, where N_t is the number of fish surviving at the end of the experiment and N_0 is the number stocked at the beginning of the experiment.

Statistical Analysis

Treatment data were analysed by one-way analysis of variance at a 95% confidence level. Duncan's multiple range test was used to separate treatment means when the analysis of variance was significant. Results are presented as mean ± standard deviation from three replicate aquaria.

RESULTS

All response variables differed among treatments (Table 1). Survival rate, the three CIE L*a*b* indices, and condition factor were therefore retained as the principal endpoints for interpretation.

Table 1. Survival Rate, CIE L*a*b* Body-colour Indices, and Condition Factor of Guppy After 45 Days of Nominal PVC Microplastic Exposure

Treatment	SR (%)	L*	a*	b*	K
P1 (0 mg/L)	100.00 ± 0.00^a	40.33 ± 7.64^a	52.33 ± 5.13^a	46.67 ± 7.51^a	1.44 ± 0.17^a
P2 (5 mg/L)	100.00 ± 0.00^a	45.33 ± 0.58^a	55.67 ± 6.03^a	48.67 ± 6.43^a	1.45 ± 0.17^a
P3 (15 mg/L)	100.00 ± 0.00^a	77.00 ± 5.57^b	1.33 ± 2.52^b	5.67 ± 4.51^b	1.20 ± 0.04^{ab}
P4 (20 mg/L)	66.67 ± 15.28^b	65.00 ± 0.00^b	-3.00 ± 1.00^b	8.67 ± 2.52^b	0.96 ± 0.13^b

Note: Values are mean ± SD (n = 3 aquaria). Different superscript letters within the same column indicate significant differences according to Duncan's multiple range test ($p < 0.05$).

CIE L*a*b* Body-colour Responses

Nominal PVC treatment was associated with a pronounced change in all three body-colour components. Lightness differed among treatments ($p < 0.001$). L* was higher in P3 and P4 than in P1 and P2. The highest mean L* occurred in P3 at 77.00 ± 5.57, compared with 40.33 ± 7.64 and 45.33 ± 0.58 in P1 and P2, respectively. P3 and P4 did not differ from one another.

Redness and yellowness also differed among treatments (both $p < 0.001$). The a* and b* values in P1 and P2 remained positive and high, whereas both indices declined sharply in P3 and P4. At 20 mg/L, a* reached -3.00 ± 1.00 and b* reached 8.67 ± 2.52. The response pattern shows that 15 to 20 mg/L nominal PVC exposure was associated with a lighter appearance and a loss of red and yellow colour components (Figure 1).

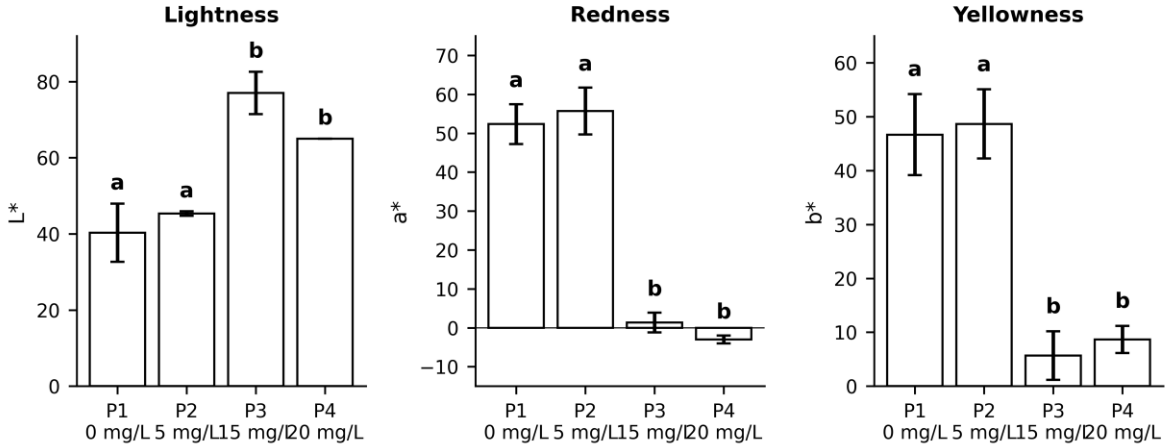

Figure 1. CIE L*a*b* Body-Colour Responses of Guppy After 45 Days of Nominal PVC Microplastic Exposure. Values are Mean ± SD (n = 3). Different Letters Indicate Significant Differences Among Treatments According to Duncan's Multiple Range Test ($p < 0.05$).

Survival Rate and Condition Factor

Survival rate differed among treatments ($p = 0.001$). P1, P2, and P3 maintained 100% survival, whereas P4 declined to 66.67 ± 15.28%. P4 was significantly lower than the other treatments. The condition factor also differed among treatments ($p = 0.008$). P4 had the lowest K value at 0.96 ± 0.13 and was lower than P1 and P2. P3 had an intermediate K value of 1.20 ± 0.04 and did not differ from the higher or lower group (Figure 2).

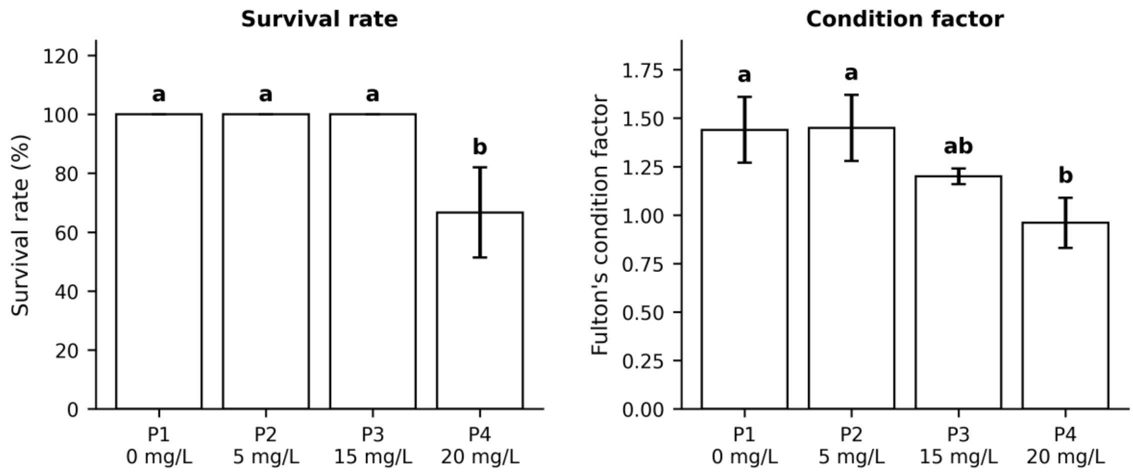

Figure 2. Survival Rate and Fulton's Condition Factor of Guppy After 45 Days of Nominal PVC Microplastic Exposure. Values are Mean ± SD (n = 3). Different Letters Indicate Significant Differences Among Treatments According to Duncan's Multiple Range Test ($p < 0.05$).

DISCUSSION

PVC Exposure and Body-colour Fading

The results show a consistent body-colour response at 15 and 20 mg/L nominal PVC exposure. Higher L* values indicate a lighter body appearance, whereas the near-zero or negative a* values and low b* values indicate a marked loss of red and yellow colour components. In practical terms, the fish became paler and less saturated. The colour response was already evident at 15 mg/L, even though survival remained at 100% and condition factor was statistically intermediate. This pattern supports the use of visible colour change as an early sublethal endpoint in ornamental fish.

The present study did not measure the physiological pathway responsible for the colour change. Several mechanisms remain plausible. Microplastic exposure can disturb oxidative balance, metabolism, ion regulation, and nutrient allocation. These changes can reduce the availability or utilisation of pigments needed for visible colour expression (Kelly *et al*., 2024; Wang *et al*., 2024; Zink & Wood, 2024; Banaee *et al*., 2025; Ghosh *et al*., 2025). PVC also requires specific attention because polymer-associated additives can leach from PVC materials and may increase chemical stress beyond particle contact alone (Turner & Filella, 2021; Henkel *et al*., 2022).

Red and yellow colour in fish are strongly linked to carotenoid deposition and pigment-cell activity. Lightness and iridescence are also influenced by structural cells such as iridophores. Disturbance of these processes may alter colour appearance before a strong reduction in growth is visible (Nakano & Wiegertjes, 2020; Lau *et al*., 2023; Liu *et al*., 2024). Studies in fish have linked oxidative disturbance or toxic exposure with altered pigment metabolism and reduced colour quality (Shastak & Pelletier, 2023; Sokołowska & Kulczykowska, 2024; Zhang *et al*., 2024). More directly, Ren *et al*. (2025) reported structural-colour deterioration in guppy exposed to microplastics through oxidative pathways. The present colour pattern is consistent with those reports, but it does not prove an oxidative mechanism because oxidative biomarkers were not measured.

Condition Factor, Survival, and Aquaculture Relevance

The lower condition factor and survival rate at 20 mg/L indicate that the most severe treatment was associated with a broader decline in fish performance. Condition factor integrates weight relative to length and can decline when fish experience impaired feeding, energy reallocation, or chronic physiological stress. The present response is consistent with evidence that microplastic exposure can impair growth-related performance and survival in fish (Xia *et al*., 2020; Masud & Cable, 2023; Putrajab *et al*., 2024). The result should nonetheless be interpreted cautiously because feed intake, digestive condition, oxidative status, and tissue damage were not measured in this experiment.

For ornamental aquaculture, the economic consequence may occur before acute mortality. A fish that remains alive but loses red and yellow colour may have lower market appeal. This issue is relevant in West Nusa Tenggara because microplastic contamination has been documented in aquaculture environments and cultured organisms (Sumsanto *et al*., 2024; Setyono *et al*., 2024a). Routine visual assessment, supported by fixed lighting and digital colour analysis, may therefore help producers detect a decline in fish quality earlier than survival records alone.

Study Boundaries and Future Work

Several limits define the interpretation of this study. PVC exposure was represented by nominal dose rather than measured particle concentration over time. The water-replacement procedure did not document whether particles were re-dosed, and no particle-size distribution, particle count, or additive profile was reported. Water-quality conditions were not reported for this experiment. In addition, the digital imaging method did not include reported camera-calibration or colour-card procedures. These limitations do not negate the observed treatment pattern, but they limit precise attribution to a single particle or chemical mechanism.

Future research should quantify actual particle concentrations before and after water replacement, characterise particle size and morphology, record water-quality profiles, standardise image acquisition with a colour reference card, and measure carotenoid content, chromatophore status, oxidative biomarkers, feed intake, and growth. These additions would clarify whether CIE L*a*b* indices can serve as a robust routine biomarker of microplastic stress in ornamental fish.

CONCLUSION

Nominal PVC microplastic exposure for 45 days altered the body-colour profile of guppy. At 15 and 20 mg/L, lightness increased while redness and yellowness declined, indicating body-colour fading. Survival rate and Fulton's condition factor were lowest at 20 mg/L. The results indicate that CIE L*a*b* body-colour indices can provide a practical visual endpoint for detecting sublethal response in ornamental fish. Mechanistic attribution requires further work because the present study did not measure exposure concentration over time, water quality, pigment status, or oxidative biomarkers.

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