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PVC MICROPLASTIC EXPOSURE IN BROODSTOCK WATER REDUCES FERTILIZATION, HATCHING, AND SURVIVAL OF ZEBRAFISH

Paparan mikroplastik PVC dalam air induk mengurangi pembuahan, penetasan, dan kelangsungan hidup ikan zebra

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ABSTRACT

This study evaluated the effect of polyvinyl chloride (PVC) microplastic exposure through broodstock rearing water on the fertilization rate (FR), hatching rate (HR), and survival rate (SR) of zebrafish (*Danio rerio*). Fish were exposed for 30 days in a completely randomized design with four PVC doses, namely 0, 5, 10, and 15 mg/L, each with three replicates. After exposure, broodstock were spawned to obtain FR and HR data, whereas SR was calculated during the rearing period. One-way ANOVA showed that PVC dose significantly affected FR ($F(3,8)=53.48$; $P<0.001$), HR ($F(3,8)=70.44$; $P<0.001$), and SR ($F(3,8)=9.33$; $P=0.005$). Duncan's multiple range test separated all FR and HR treatments into different homogeneous subsets, indicating a clear dose-related decline. FR decreased from 90.83% in the control to 68.43% at 15 mg/L, whereas HR declined from 94.77% to 75.17%. SR showed a more moderate pattern: the control, 5 mg/L, and 10 mg/L remained in the same homogeneous subset, while 15 mg/L formed a distinct subset with a mean of 81.67%. Water quality remained relatively stable during the experiment, with temperature at 29.1-29.3 °C, pH at 8.29-8.49, and dissolved oxygen at 7.0-7.5 mg/L. These findings indicate that, under the present experimental conditions, early reproductive endpoints were more sensitive to PVC microplastic exposure than survival.

Keywords: Fertilization Rate, Hatching Rate, Microplastic PVC, Survival Rate, Zebrafish

ABSTRAK

Penelitian ini mengevaluasi pengaruh paparan mikroplastik polivinil klorida (PVC) melalui air pemeliharaan induk terhadap tingkat pembuahan (FR), tingkat penetasan (HR), dan tingkat kelangsungan hidup (SR) ikan zebra (*Danio rerio*). Ikan dipaparkan selama 30 hari dalam desain acak lengkap dengan empat dosis PVC, yaitu 0, 5, 10, dan 15 mg/L, masing-masing dengan tiga ulangan. Setelah paparan, induk dipijahkan untuk mendapatkan data FR dan HR, sedangkan SR dihitung selama periode pemeliharaan. ANOVA satu arah menunjukkan bahwa dosis PVC secara signifikan mempengaruhi FR (F (3,8) = 53,48; P<0,001), HR (F (3,8) =70,44; P<0,001), dan SR (F (3,8) = 9,33; P = 0,005). Uji rentang berganda Duncan memisahkan semua perlakuan FR dan HR ke dalam subset homogen yang berbeda, menunjukkan penurunan yang jelas terkait dosis. FR menurun dari 90,83% pada kontrol menjadi 68,43% pada 15 mg/L, sedangkan HR menurun dari 94,77% menjadi 75,17%. SR menunjukkan pola yang lebih moderat: kontrol, 5 mg/L, dan 10 mg/L tetap berada dalam subset homogen yang sama, sementara 15 mg/L membentuk subset yang berbeda dengan rata-rata 81,67%. Kualitas air tetap relatif stabil selama percobaan, dengan suhu 29,1-29,3 °C, pH 8,29-8,49, dan oksigen terlarut 7,0-7,5 mg/L. Temuan ini menunjukkan bahwa, dalam kondisi percobaan saat ini, titik akhir reproduksi awal lebih sensitif terhadap paparan mikroplastik PVC daripada kelangsungan hidup.

Kata Kunci: Tingkat Pembuahan, Tingkat Penetasan, PVC Mikroplastik, Tingkat Kelangsungan Hidup, Ikan Zebra

INTRODUCTION

Microplastics are increasingly recognized as relevant contaminants in aquaculture and aquatic ecotoxicology because of their persistence, mobility, and capacity to enter fish through water and feed. In fish, microplastic exposure has been associated with oxidative stress, behavioral disturbance, tissue alteration, impaired growth, and reduced reproductive performance. Recent syntheses further show that the magnitude of these responses depends on polymer type, particle size, concentration, life stage, and exposure pathway (Bhat et al., 2024; Wang et al., 2024; Liu et al., 2024; Yi et al., 2024; Yön Ertuğ et al., 2024).

Zebrafish (Danio rerio) is a highly informative model for evaluating aquatic contaminants because it has a short life cycle, high fecundity, externally developing embryos, and sensitive early life stages. Its extensive use in toxicology also makes it possible to relate waterborne exposure to reproductive performance and developmental outcomes with relatively high resolution (Bhagat et al., 2020; Silva Brito et al., 2022; Zhao et al., 2024; Rojoni et al., 2024).

Studies on zebrafish have shown that microplastic exposure can impair gonadal condition, alter reproductive physiology, and reduce reproductive success. Most available evidence, however, has been generated using polystyrene particles or nanoplastics rather than PVC. This matters because PVC may carry additives and co-contaminants that modify its biological effects. PVC exposure has been associated with tissue alterations in zebrafish, and related work in other fish models suggests possible effects on the hypothalamic-pituitary-gonadal axis and reproductive tissues (Asani et al., 2023; Liu, Liang, Fan, Zhou, Chang, & Li, 2023).

From an aquaculture perspective, early reproductive endpoints such as fertilization rate, hatching rate, and survival rate are particularly relevant because they directly influence seed quality and hatchery output. Indonesian aquaculture systems have also begun to report microplastic contamination and biological responses in cultured species. Local studies have documented microplastic contamination in aquaculture areas and reported performance or hematological changes in tilapia exposed to PVC or polystyrene microplastics, underlining the practical relevance of this issue for culture systems (Saomadia et al., 2024; Putrajab et al., 2024; Sumsanto et al., 2024).

Based on this background, the present study evaluated the effects of PVC microplastic exposure through broodstock rearing water on the fertilization rate, hatching rate, and survival rate of zebrafish. The study was designed to produce a focused dataset on early reproductive success and to test the working hypothesis that increasing PVC dose would reduce these endpoints under controlled culture conditions.

METHODS

Experimental Design and PVC Exposure

The experiment was conducted from September to October 2025 at the Laboratory of Production and Reproduction, Faculty of Agriculture, Universitas Mataram. A completely randomized design was applied with four PVC microplastic doses in rearing water, namely 0 mg/L (control), 5 mg/L, 10 mg/L, and 15 mg/L, with three replicates per treatment, giving a total of 12 experimental units.

Zebrafish broodstock were acclimated for seven days before exposure. Experimental units consisted of 20-L freshwater containers equipped with continuous aeration. According to the experimental record used for the present manuscript, each unit was monitored from 20 fish at the start of rearing for survival assessment, and broodstock were standardized for spawning after the 30-day exposure period. Water exchange during rearing was followed by re-addition of PVC according to the assigned treatment dose.

PVC microplastics were prepared from PVC material that was cut into small pieces, blended, dried, and sieved before being dispersed into the rearing water. Because instrumental particle-size characterization was not performed, the present study interprets the findings as responses to laboratory-prepared PVC microplastics rather than to a narrowly defined particle fraction.

Spawning, Reproductive Endpoints, and Water Quality

After 30 days of exposure, broodstock from each experimental unit were spawned to obtain fertilization and hatching data. Fertilization rate (FR) was calculated as the percentage of fertilized eggs relative to total eggs released, whereas hatching rate (HR) was calculated as the percentage of hatched eggs relative to fertilized eggs. Survival rate (SR) was calculated as the percentage of fish surviving at the end of rearing relative to the number recorded at the start of the rearing period.

Water quality was monitored to support the interpretation of treatment effects. The observed parameters were temperature, pH, and dissolved oxygen (DO). These parameters were used as supporting variables to confirm that major differences among treatments were not driven by water-quality instability.

Statistical Analysis

FR, HR, and SR data were analyzed using one-way analysis of variance (ANOVA). When a significant treatment effect was detected, Duncan's multiple range test was used to compare treatment means at $P<0.05$. Results are presented as mean ± standard deviation.

RESULTS

Early Reproductive Performance

PVC microplastic exposure reduced all three reproductive performance variables measured in this study, although the magnitude of decline differed among endpoints. Mean values, standard deviations, and Duncan grouping results are presented in Table 1.

Table 1. Mean ± SD of fertilization rate, hatching rate, and survival rate of zebrafish after PVC microplastic exposure. Different superscript letters within the same column indicate significant differences according to Duncan's multiple range test (P<0.05).

Treatment	Fertilization rate (%)	Hatching rate (%)	Survival rate (%)
0 mg/L	90.83 ± 2.13a	94.77 ± 1.56a	96.67 ± 2.89a
5 mg/L	85.03 ± 2.82b	87.47 ± 0.91b	91.67 ± 2.89a
10 mg/L	79.20 ± 2.65c	83.73 ± 1.40c	90.00 ± 5.00a

Treatment	Fertilization rate (%)	Hatching rate (%)	Survival rate (%)
15 mg/L	68.43 ± 1.00d	75.17 ± 2.50d	81.67 ± 2.89b

One-way ANOVA showed that PVC dose significantly affected FR ($F(3,8)=53.48$; $P<0.001$), HR ($F(3,8)=70.44$; $P<0.001$), and SR ($F(3,8)=9.33$; $P=0.005$). Duncan's multiple range test separated all FR and HR treatments into different homogeneous subsets, indicating a stepwise decline with increasing dose. By contrast, SR showed a more moderate response: the control, 5 mg/L, and 10 mg/L remained in the same homogeneous subset, whereas 15 mg/L formed a distinct subset.

To facilitate visual interpretation of the dose-response pattern, the three principal reproductive endpoints are summarized in Figure 1.

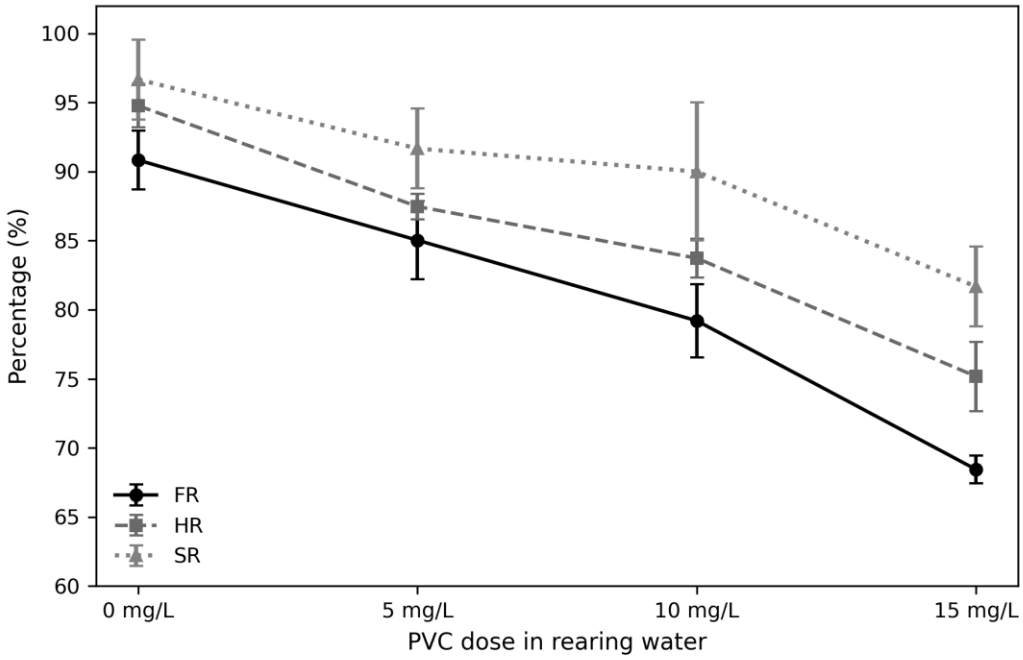

Figure 1. Combined response of fertilization rate (FR), hatching rate (HR), and survival rate (SR) of zebrafish after PVC microplastic exposure through broodstock rearing water. Error bars represent standard deviation.

Water Quality

Water-quality variables remained relatively stable during the experiment. Temperature ranged from 29.1 to 29.3 °C, pH from 8.29 to 8.49, and DO from 7.0 to 7.5 mg/L. The summary is shown in Table 2.

Table 2. Water-quality range and mean values during the experiment.

Parameter	Range	Mean
Temperature (°C)	29.1-29.3	29.21
pH	8.29-8.49	8.40
DO (mg/L)	7.0-7.5	7.16

DISCUSSION

The present study shows that PVC microplastic exposure through broodstock rearing water reduced early reproductive performance in zebrafish. The strongest and most consistent responses were observed in FR and HR, both of which declined progressively and were separated into four distinct Duncan groups. This pattern indicates that reproductive impairment was already evident before or during fertilization and became more pronounced during embryonic development. In aquaculture terms, such a response is important because losses at the fertilization and hatching stages directly reduce larval output and hatchery efficiency.

The decline in FR suggests that waterborne PVC exposure before spawning likely affected gamete quality or broodstock reproductive condition. This interpretation is consistent with previous zebrafish studies showing that microplastics can disrupt gonadal condition, reproductive physiology, endocrine balance, or maternal metabolic homeostasis (Qiang & Cheng, 2021; Gupta et al., 2023; Adhikari et al., 2024; Adhikari et al., 2025; Zheng et al., 2024). Although the present study did not measure hormones, oxidative biomarkers, or gonadal gene expression, the observed stepwise reduction in FR supports the view that reproductive endpoints are sensitive indicators of microplastic stress.

HR followed the same dose-related decline as FR, which indicates that the effect of broodstock exposure extended beyond fertilization to embryo viability and hatching success. Previous research on zebrafish embryos and early life stages has shown that microplastics can delay hatching, reduce embryo quality, and interfere with early development, either alone or in combination with other contaminants (Santos et al., 2020; De Marco et al., 2022; Lu et al., 2022; Mansuri et al., 2024; Sun et al., 2024; Zhou et al., 2024). Under hatchery conditions, this means that contamination risk may be expressed first through reduced seed quality rather than through immediate mortality of broodstock.

SR also decreased, but the statistical pattern was weaker than for FR and HR because only the highest dose differed clearly from the other treatments. This suggests that, within the 30-day exposure period used here, survival was less sensitive than early reproductive endpoints. The same general interpretation is supported by broader syntheses indicating that microplastic effects on fish survival can be present but are highly dependent on polymer type, particle characteristics, concentration, and life stage (Wang et al., 2024; Liu et al., 2024; Yi et al., 2024). In other words, the present data support the practical conclusion that FR and HR may function as earlier warning endpoints than SR in broodstock-based assessments.

The relatively narrow ranges of temperature, pH, and dissolved oxygen among treatments strengthen the inference that the observed differences primarily reflected PVC exposure rather than major water-quality instability. This is especially relevant to aquaculture-oriented interpretation. Local studies in Indonesian culture systems have already documented microplastic contamination in farming areas and biological effects in cultured fish exposed to PVC or polystyrene microplastics (Saomadia et al., 2024; Putrajab et al., 2024; Sumsanto et al., 2024). Therefore, the present zebrafish findings should not be viewed only as a model-system response, but also as evidence that microplastic contamination has potential implications for broodstock management and hatchery performance in aquaculture settings.

This study has several limitations. The PVC particles were not instrumentally characterized for size distribution or morphology, and mechanistic biomarkers were not measured. In addition, the study focused on three practical endpoints rather than a full physiological profile of broodstock and larvae. Even so, the strength of the present manuscript lies in its clear and statistically coherent response pattern across reproductive endpoints that are directly relevant to seed production.

CONCLUSION

PVC microplastic exposure through broodstock rearing water significantly reduced the fertilization rate, hatching rate, and survival rate of zebrafish. The most sensitive responses were observed in fertilization and hatching, both of which declined stepwise with increasing PVC dose and formed distinct Duncan groups across all treatments. Survival also declined, but a clear separation occurred only at 15 mg/L. Under the present experimental conditions, these results indicate that early reproductive endpoints are more sensitive than survival for detecting the impact of PVC microplastics on broodstock performance.

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