

Research Article

Impact of high-density polyethylene (HDPE) microplastics exposure on *Penaeus vannamei* survival

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ABSTRACT. High-density polyethylene microplastics (HDPE-MPs) represent an emerging threat to aquatic ecosystems and aquaculture species, such as *Penaeus vannamei*. This study examined the impact of PE-MPs on the survival and growth of shrimp postlarvae and juveniles under controlled laboratory conditions. Bioassays were conducted using HDPE-MPs (34-50 μm) at concentrations ranging from 5 to 625 mg L^{-1} . Survival, specific growth rate (SGR), and LC_{50} values were assessed. Ingestion of MPs was confirmed in postlarvae using fluorescence microscopy with Nile red staining. Postlarvae showed 100% mortality at 625 mg L^{-1} , with an LC_{50} of 21.89 mg L^{-1} , while juveniles reached 70% mortality at 125 mg L^{-1} , with an LC_{50} of 30.6 mg L^{-1} . Both stages exhibited a significant dose-dependent reduction in survival with MPs ($P < 0.05$). SGR declined markedly at concentrations of $\geq 100 \text{ mg L}^{-1}$. Behavioral alterations, lethargy, and reduced feeding were observed, along with the accumulation of MPs in the tissues of dead shrimp, indicating physiological disruption. Postlarvae exhibited greater sensitivity to LC_{50} concentrations, although juveniles may have a relatively higher particle ingestion capacity when considering biomass and size proportions. This study provides the first LC_{50} values for *P. vannamei* postlarvae and juveniles exposed solely to HDPE-MPs, highlighting the role of organism size and developmental stage in susceptibility. These results underscore the urgent need to establish toxicity thresholds for MPs in aquaculture species and inform strategies to mitigate their impact. Understanding MP-related risks is essential for enhancing biosecurity and sustainability in shrimp farming systems.

Keywords: *Penaeus vannamei*; shrimp; microplastics; polyethylene; survival; LC_{50} ; aquaculture

INTRODUCTION

Plastics, essential materials in modern society, pose a serious environmental concern: approximately 10% of annual plastic waste reaches marine ecosystems (Ghosh 2025). One of the most widely used and frequently identified polymers in the aquatic environment is polyethylene (PE), with projections indicating a significant increase in its use by 2060, which is categorized into low-density polyethylene (LDPE) and high-density polyethylene (HDPE) (Oliveira et al. 2020, OECD 2022). According to Hsieh et al. (2021), the PE is the most common plastic found in marine environments due to its high global production, variable density (LDPE and HDPE), high flexibility, and strong resistance to degradation compared to other common pollutants like polypropylene (PP) and polystyrene (PS). These properties enhance its persistence and distribution within the water column, unlike other polymers, thereby underscoring the prevalence of HDPE in aquatic environments due to its high resistance to degradation (Oliveira et al. 2020). In aquatic environments, synthetic polymers are subject to various abiotic degradation processes, including photodegradation and hydrolytic reactions, which progressively weaken their molecular structure, leading to the fragmentation of these plastic materials (Barlucchi et al. 2024, Dudek et al. 2025). Consequently, most synthetic polymers, like PE-based, breaks down forming particles classified as macroplastics (>25 mm), mesoplastics (5-25 mm), microplastics (MPs) (<5 mm), and nanoplastics (NPs) (<1 µm), according to Ramsperger et al. (2023), and this breakdown makes it easier for aquatic organisms to ingest. Additionally, MPs can vary in shape and origin (primary or secondary) and are persistent pollutants that affect all levels of the aquatic food chain (Auta et al. 2017, Bernal et al. 2020, Li et al. 2021). Among the polymers that produce MPs, HDPE particles are considered one of the most strongly linked to various deficiencies, abnormalities, and harm in aquatic species (Hsieh et al. 2021, Musa et al. 2025).

Therefore, a wide variety of marine organisms ingest MPs due to their small size; they can enter trophic networks through primary producers such as phytoplankton and algae, facilitating their transfer across food webs (Bellás et al. 2016, Bordbar et al. 2018, Timaná-Morales et al. 2024). These particles eventually accumulate in marine sediments, where benthic organisms are particularly exposed (Vaid et al. 2021). Many of these contaminants can be ingested by humans through the consumption of seafood products (Vaid et al. 2021, Hariharan et al. 2022). Therefore,

MPs as HDPE-MPs compromise the health of cultured species by inducing toxicity, oxidative stress, inflammation, and reproductive disorders (Ory et al. 2017, Wang et al. 2021, Leela et al. 2025). Additionally, these pollutants can disrupt trophic interactions and lead to long-term ecological disturbances (Clark et al. 2016, Thacharodi et al. 2024).

In aquaculture, crustaceans such as shrimp are particularly vulnerable to MP contamination, making it essential to assess their ingestion capacity and the associated risks (Lusher et al. 2017, Kim et al. 2021, Páez-Osuna et al. 2024). Among them, the white shrimp *Penaeus vannamei*, considered a cornerstone species in global aquaculture production (FAO 2024), is especially at risk due to its benthic feeding habits and continuous exposure to contaminated sediments (Frank et al. 2020), which may be attributed to their multipart gastrointestinal tract (GIT) and voracious feeding habits, causing sublethal effects as: gastrointestinal blockages, reduce feeding efficiency, contribute to poor nutritional status, suppress growth, and damaging vital organs such as the hepatopancreas (Devriese et al. 2015, Courtene-Jones et al. 2017, Jamieson et al. 2019, Hossain et al. 2020, Daniel et al. 2020, Han et al. 2022, Valencia-Castañeda et al. 2022, Yu et al. 2023).

Moreover, transcriptomic analyses of *P. vannamei* have demonstrated that exposure to MPs (1-5 µm amino formaldehyde polymer) can induce cardiac muscle dysfunction, oxidative stress, and alterations in immune responses (Han et al. 2021). Furthermore, studies have reported up to 38 MPs per individual in shrimp farms, providing evidence of their potential for biomagnification due to chronic exposure and intake of these contaminants (Hossain et al. 2020, Valencia-Castañeda et al. 2022).

MPs ingestion in shrimp is size-dependent: shrimp consume particles proportional to their body size. Individuals weighing approximately 0.002 g ingest particles smaller than 400 µm, whereas those exceeding 2.5 g consume particles up to 3.2 mm. In wild *P. vannamei*, MPs have been found ranging from 403 ± 296 µm in shrimp of 19.2 ± 4.6 g and from 8 µm to 4.22 mm in farmed shrimp during the first 120 days of culture (Van Wyk et al. 1999, Li et al. 2021, Valencia-Castañeda et al. 2022, Naidu et al. 2024, Morris et al. 2025). Similarly, Valencia-Castañeda et al. (2024) reported that PE-based MPs were the most abundant in farmed *P. vannamei* shrimp in Mexico, followed by nylon (NY) and polyethylene terephthalate (PET).

Additionally, MPs can interact with pathogens, such as white spot syndrome virus (WSSV), thereby increasing virulence and suppressing immune responses

in shrimp (Shan et al. 2023). However, limited knowledge exists regarding the LC₅₀ values in juvenile organisms, which are particularly susceptible due to their reduced size and accelerated metabolism (Trestrail et al. 2021).

As highlighted by Boterell et al. (2025), toxicological assessments of MPs indicate that LC₅₀ values are generally only observed under controlled high-exposure conditions, while environmentally relevant concentrations predominantly elicit sublethal responses. Furthermore, these sublethal effects, which differ between acute and chronic exposures, are particularly important for ecotoxicological risk assessment, as they can compromise essential processes such as physiology, reproduction, or behavior, thus affecting long-term population sustainability (Roy et al. 2022). Studies indicate that MPs and NPs in shrimp generally produce sublethal effects; however, concentrations that do not result in mortality may still contribute to physiological disturbances (Hsieh et al. 2021, Hariharan et al. 2022, Ríos et al. 2023, Thammatorn et al. 2024). Das (2025) specifically reported that chronic exposure to HDPE reduced growth, metabolism, and physiological health in *Penaeus monodon*.

In contrast, studies on crustaceans such as *Artemia salina* have shown LC₅₀ values ranging from 40.9 to 52.0 µg mL⁻¹, which have been linked to sublethal effects, including neural impairment and epithelial tissue damage (Jeyavani et al. 2022). Additionally, other challenges with shrimp of the species *P. monodon*, *Marsupenaeus japonicus*, and *P. vannamei* were evaluated to determine the lethal effects of PE-MPs (300 mg L⁻¹). A 50% mortality rate has been reported for *P. monodon* and *M. japonicus*, while for *P. vannamei*, a 20% mortality rate was observed (Wang et al. 2021). Similarly, previous experiments have established that the 96 h LC₅₀ calculated for *Macrobrachium nipponense* juveniles exposed to PS-NPs (71.18 nm) was 396.4 mg L⁻¹ (Li et al. 2021); however, LC₅₀ equivalent data for *P. vannamei* exposed to HDPE-MPs are still lacking.

This study aimed to determine the tolerance thresholds of *P. vannamei* to HDPE-MPs, with a particular focus on their lethal effects under simulated extreme contamination scenarios. Although these conditions are not typically found in natural systems, they were designed to generate critical knowledge that addresses gaps regarding the shrimp's ability to tolerate acute MP exposure and its implications for aquaculture and the environment.

MATERIALS AND METHODS

Ethical considerations

The shrimp used in this study were handled and euthanized in accordance with the Mexican Official Standard NOM-062-Z00-1999, which establishes technical specifications for the production, care, and use of laboratory animals.

PE-MPs preparation and characterization

HDPE-MPs were produced by mechanically crushing clean commercial bottles labeled with the recycling code "2" (HDPE) using an electric angle grinder (TRUPER® ESMA-4-1/2A9, 800 W). The ground material was sifted to obtain particles between 34 and 50 µm (density of 0.94 g cm⁻³), based on previous research related to MPs in shrimp (Hossain et al. 2020, Hariharan et al. 2022, Páez-Osuna et al. 2024, Valencia-Castañeda et al. 2024, Morris et al. 2025). Particle size and shape were checked under a light microscope (Primo Star 1 Carl Zeiss®) at 10x and 40x magnification, and an electronic camera (OPTIKA® PRO VIEW C-B1) with LITEView Software was used to measure the particle size.

MPs were separated from other particles using a saturated ZnCl₂ solution (1.7 g cm⁻³), following the method of Crutchett & Bornt (2024) for isolating medium and high-density MPs. The recovered MPs were washed by sonication for 20 min, placing 300 µg in microtubes (1.5 mL) containing 500 µL of sterile wash solution (triple-distilled water with 0.01% Tween® 20), then centrifuged for 20 min at 5,000 g and 15°C in a benchtop microcentrifuge (Frontier™ Series 5000, model FC5515R, OHAUS®, USA).

Floating particles were collected with a micropipette, transferred to sterile 1.5 mL microtubes, and dried at 35°C for 48 h. To identify the polymer type of the MPs, a sample (5-10 mg, analyzed in triplicate) was examined using an Alpha II (Bruker®) Fourier-transformed infrared spectrometer with attenuated total reflectance (FTIR-ATR) following the specifications for PE (Bredács et al. 2021) and HDPE (Karlsson et al. 2020). The final particles were stored in sterile microtubes (1.5 mL) for use in bioassays.

Experimental animals

Postlarvae (10 ± 5 mg) and juvenile shrimp (6 ± 0.73 g) were obtained from Acuacultura Integral S.A. de C.V. in Bahía Matanchén, Nayarit, Mexico. The shrimp were acclimated to laboratory conditions for 15 days, after which they were transferred to the experimental units under identical conditions and maintained for an

additional 3 days before the beginning of the experiment; in filtered seawater (20 µm, 30‰ salinity) previously disinfected with 0.05% sodium hypochlorite for 48 h under continuous aeration. Tween 20 (0.01%) was added as a surfactant to facilitate the dispersion of HDPE-MPs. Shrimps were maintained under constant aeration and fed twice daily (8:00 and 17:00 h) with commercial feed (Purina®; 36% protein, 5% fat, 3% fiber). Postlarvae were fed at 25% of their biomass per day, whereas juveniles received 10% of their biomass per day. Physicochemical parameters were monitored daily in each bioassay to ensure optimal conditions for shrimp culture, as reported by Brock & Main (1994): dissolved oxygen (DO) levels of 4.0-10.0 mg L⁻¹, pH levels of 8.1-9.0, and temperatures of 23-30°C, with salinity ranging from 15 to 35.

Concentration and quantification of HDPE-MPs

Treatments with increasing concentrations of HDPE-MPs were evaluated for each bioassay, simulating extreme scenarios to determine lethality levels. The concentrations of HDPE-MPs used in the bioassays were quantified under a Primo Star 1 Carl Zeiss® microscope using a Neubauer chamber to determine particle shape, size, and the corresponding particle concentration in MPs mL⁻¹.

Bioassay 1: impact of HDPE-MPs on postlarvae

The 14-day bioassay was conducted using postlarvae (10 ± 5 mg) in 3 L glass tanks (20 ind per tank, in triplicate) with seawater (30‰ salinity). Five treatments were evaluated with increasing concentrations of HDPE-MPs: I) 0 mg L⁻¹, II) 50 mg L⁻¹, III) 100 mg L⁻¹, IV) 250 mg L⁻¹, and V) 500 mg L⁻¹. Behavioral changes were recorded, including hyperactivity, erratic swimming, lethargy, and inappetence, and were compared against the control group. Water was completely replaced every five days. Mortality was recorded twice daily, and survival rate (SR) and specific growth rate (SGR) were calculated at the end of the experiment using the formula:

$$SR (\%) = N_f / N_i \times 100$$

where N_f is the number of shrimp alive at the end of the experiment, and N_i is the initial number of shrimp at the start of the experiment (Escobar-Gil et al. 2017).

$$SGR (\% d^{-1}) = 100 \times [\ln(W_f) - \ln(W_i)] / t$$

where W_f is the mean final weight, W_i is the mean initial weight, and t is the duration in days (Ricker 1979).

Bioassay 2: LC₅₀ determination in postlarvae

A five day bioassay was conducted using postlarvae (10 ± 5 mg) in 3 L glass tanks (20 ind per tank, in triplicate) filled with seawater (30‰ salinity). Five treatments were tested: I) 0 mg L⁻¹, II) 5 mg L⁻¹, III) 25 mg L⁻¹, IV) 125 mg L⁻¹, and V) 625 mg L⁻¹. Behavioral parameters and other signs of stress were monitored. The SR was calculated as previously described, and LC₅₀ was determined using the Probit method. For HDPE-MPs ingestion analysis, dead shrimp were placed in 1.5 mL microtubes containing 0.5 mL of sterile triple-distilled water and washed five times for 1 min using a vortex. The survivor shrimp were euthanized by immersion in an ice-water slurry (0-4°C) according to the NOM-062-ZOO-1999. Samples were then stored at -20°C.

HDPE-MPs intake analysis in postlarvae

The MPs intake in shrimps collected during bioassay 2 was compared among all treatments. The samples (10 ind per tank, with five dead and five alive) were digested in 10% KOH for 48 h at 40°C, following the method described by Wang et al. (2023). They were then filtered using Whatman® cellulose filters (15 mm diameter, 2 µm pore size). PE-MPs were identified by fluorescence microscopy using protocols established by Dowarah et al. (2020) and González et al. (2024), with modifications. The samples were stained with 0.1 mL of Nile Red (Sigma Aldrich®, 0.1 M in acetone) for 30 min in the dark. The stained particles were visualized under a UV microscope (Carl Zeiss®) equipped with an Axio-Lab® camera at 430 nm.

Micrographs were captured at 10x and 40x magnification. Sterile filters were used as blanks throughout the process for every sample to detect background contamination. Contaminant particles in blank filters were quantified and subtracted from sample data to correct for background interference.

Bioassay 3: LC₅₀ in juvenile shrimp

This 14-day bioassay was performed using juvenile shrimp (6 ± 0.73 g) in 18 L glass tanks (20 ind per tank, in triplicate) with seawater (30‰ salinity). Four treatments were tested with increased concentrations of HDPE-MPs: I) 0 mg L⁻¹, II) 5 mg L⁻¹, III) 25 mg L⁻¹, and IV) 125 mg L⁻¹. Behavioral changes and physiological signs were recorded. At the end of the experiment, SR and SGR were calculated, and the LC₅₀ was estimated using the Probit method.

Statistical analysis

To evaluate differences among treatments, a normality test and Bartlett's test for homogeneity of variances were performed ($P > 0.05$). A one-way ANOVA followed by Tukey's HSD test ($P < 0.05$) was used to compare SR and SGR. Survival data were arcsine-transformed before analysis (Daniel 1997). All statistical analyses were conducted using Statistica 7 software.

RESULTS

HDPE-MPs particle characterization and count

The FTIR-ATR analysis detected characteristic PE peak readings within the ranges of 3,000-2,800, 1,500-1,300, and 720 cm^{-1} (Fig. 1), and the corresponding peaks for HDPE at 1,471 and 1,377 cm^{-1} (Fig. 2), thereby confirming the polymer identity of the samples. Particle analysis revealed that the HDPE-MPs appeared as semi-spherical and irregular fragments with no visible fibers. Table 1 presents the HDPE-MPs concentrations (mg L^{-1}) and the corresponding particle count per mL (MPs mL^{-1}).

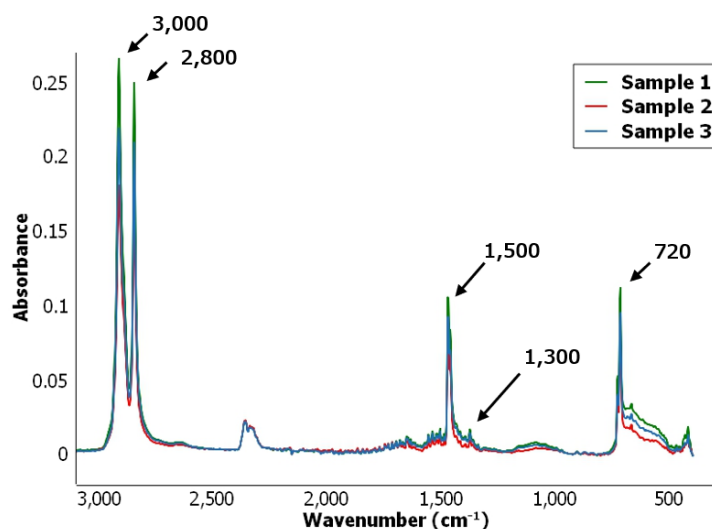


Figure 1. Characteristic polyethylene (PE) peaks of Fourier-transformed infrared spectrometer with attenuated total reflectance (FTIR-ATR) spectra at 3,000-2,800, 1,500-1,300, and 720 cm^{-1} of the microplastics sample.

Bioassay 2: LC₅₀ determination in postlarvae

Physicochemical parameters remained within optimal culture ranges: DO $4.1 \pm 0.2 \text{ mg L}^{-1}$, pH 8.2 ± 0.2 , temperature $26.2 \pm 1.3^\circ\text{C}$, and salinity 31 ± 1 (Brock & Main 1994). Shrimp in treatments IV and V showed signs of lethargic swimming and inappetence. Survival

Bioassay 1: Effects of HDPE-MP exposure on postlarvae

The physicochemical parameters remained within optimal ranges for shrimp culture: DO $4.0 \pm 0.3 \text{ mg L}^{-1}$, pH 8.2 ± 0.2 , temperature $26.8 \pm 0.9^\circ\text{C}$, and salinity 30 ± 2 (Brock & Main 1994).

Shrimp in treatments III, IV, and V exhibited altered behavior, including inappetence, erratic swimming, and lethargy, compared to other groups. Postlarvae survival was 98.3% in the control group (I), decreasing to 80% (II, 50 mg L^{-1}), 86% (III, 100 mg L^{-1}), 68% (IV, 250 mg L^{-1}), and 66% (V, 500 mg L^{-1}) (Fig. 3). Statistical analysis revealed significant differences ($P < 0.05$) between the control and all treatments, indicating that HDPE-MPs exerted an adverse, dose-dependent effect on survival, particularly at higher concentrations (IV and V). The SGR results showed significant differences ($P < 0.05$) between the control group (I) and treatments III, IV, and V. The most significant reduction in growth was observed at 100 mg L^{-1} of HDPE-MPs (Fig. 4).

rates were 100% (I), 60% (II, 5 mg L^{-1}), 70% (III, 25 mg L^{-1}), 23% (IV, 125 mg L^{-1}), and 0% (V, 625 mg L^{-1}) (Fig. 5). Significant differences ($P < 0.05$) were found between the control and all other treatments, with a dose-dependent increase in mortality. Probit analysis estimated an LC₅₀ of 21.89 mg L^{-1} , corresponding to 2.47 MPs mL^{-1} for this study.

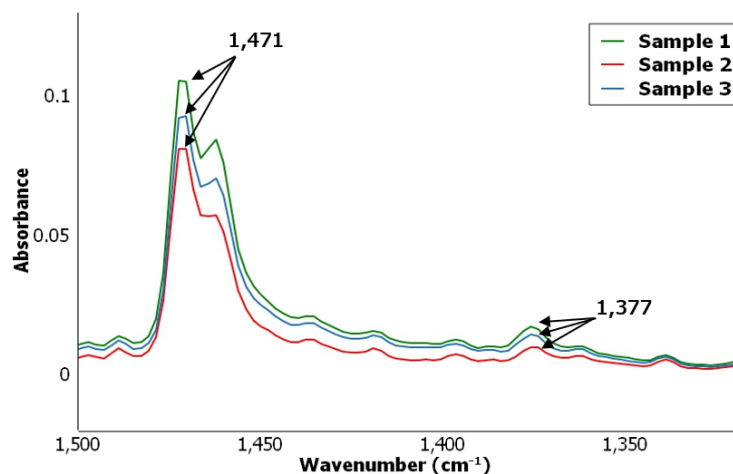


Figure 2. Characteristic high-density polyethylene (HDPE) peaks of Fourier-transformed infrared spectrometer with attenuated total reflectance (FTIR-ATR) spectra at 1,471 and 1,377 cm^{-1} of the MPs sample.

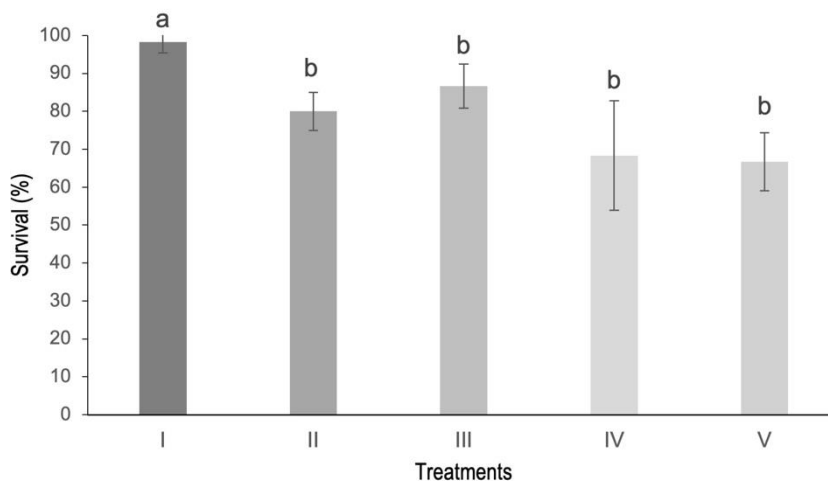


Figure 3. Survival of *P. vannamei* postlarvae exposed to high-density polyethylene microplastics (HDPE-MPs). Treatments: I) 0 mg L^{-1} , II) 50 mg L^{-1} , III) 100 mg L^{-1} , IV) 250 mg L^{-1} , V) 500 mg L^{-1} . Data are shown as mean \pm standard deviation. Different letters indicate significant differences ($P < 0.05$).

Table 1. Concentration of polyethylene microplastics (PE-MP) particles (mg L^{-1}) used in the survival bioassays of *P. vannamei*. Values are presented as mean \pm standard deviation.

Concentrations (mg L^{-1})	Particle quantities (MPs mL^{-1})
5	0.58 ± 0.625
25	3.09 ± 0.133
30	3.71 ± 0.313
50	9.50 ± 0.164
100	17.64 ± 0.142
125	22.38 ± 0.137
250	38.27 ± 0.185
500	67.59 ± 0.104
625	81.26 ± 0.238

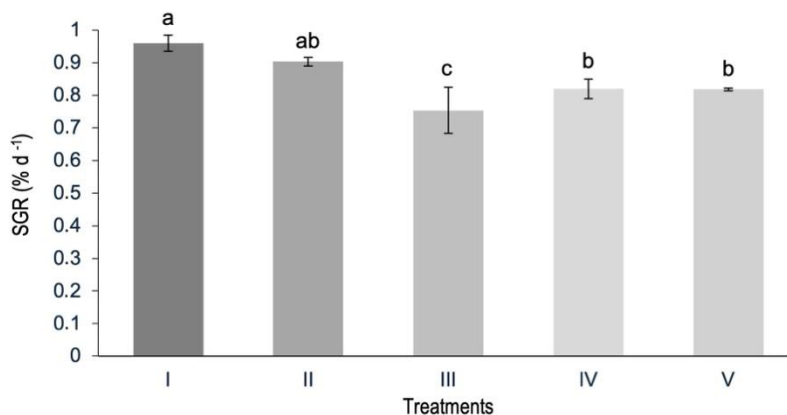


Figure 4. Specific growth rate (SGR) of *P. vannamei* postlarvae exposed to high-density polyethylene microplastics (HDPE-MPs). Treatments: I) 0 mg L⁻¹, II) 50 mg L⁻¹, III) 100 mg L⁻¹, IV) 250 mg L⁻¹, V) 500 mg L⁻¹. Data are shown as mean \pm standard deviation. Different letters indicate significant differences ($P < 0.05$).

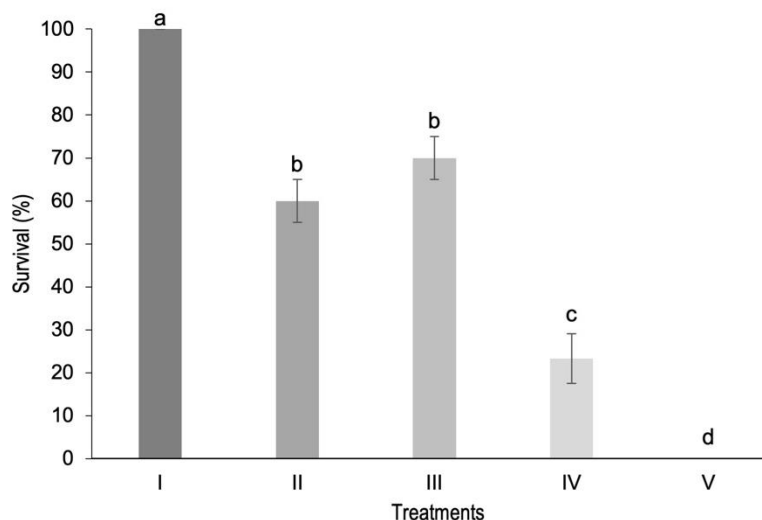


Figure 5. Survival of *P. vannamei* postlarvae exposed to high-density polyethylene microplastics (HDPE-MPs). Treatments: I) 0 mg L⁻¹, II) 5 mg L⁻¹, III) 25 mg L⁻¹, IV) 125 mg L⁻¹, V) 625 mg L⁻¹. Data are shown as mean \pm standard deviation. Different letters indicate significant differences ($P < 0.05$).

Ingestion analysis of HDPE-MPs in bioassay 2

Fluorescence microscopy confirmed the presence of HDPE-MPs in all treatments during bioassay 2. In the blank sample (Fig. 6a), larger MPs (>100 μ m) likely originating from environmental contamination were observed (3.75 ± 1.48 MPs per filter). In treatment I (Fig. 6b), no detectable MPs were found inside the exoskeleton of surviving shrimp. In treatment II (Fig. 6c), HDPE-MPs were detected in the caudal exoskeleton; in treatment III (Fig. 6d), HDPE-MPs were visible within the intestinal tract; in treatment IV (Fig. 6e), HDPE-MPs were observed both internally and in the caudal region; and in treatment V (Fig. 6f), high internal accumulation in the caudal region was evident.

Bioassay 3: LC₅₀ in juvenile shrimp

Physicochemical conditions were within optimal shrimp culture limits: DO 4.0 ± 0.2 mg L⁻¹, pH 8.3 ± 0.1 , temperature 28.0 ± 1.2 °C, and salinity 3.0 ± 0.1 (Brock & Main 1994). Survival rates were 100% (I), 86.7% (II), 40% (III), and 30% (IV), respectively (Fig. 7). All HDPE-MP treatments exhibited significantly lower survival rates compared to the control ($P < 0.05$). Treatment II differed significantly ($P < 0.05$) from III and IV, indicating more severe effects at higher HDPE-MP concentrations. The estimated LC₅₀ from Probit analysis was 30.6 mg L⁻¹, equivalent to 3.712 MPs mL⁻¹ in this study. Behavioral abnormalities in treatments III and IV included aggression, hyperactivity, inappetence,

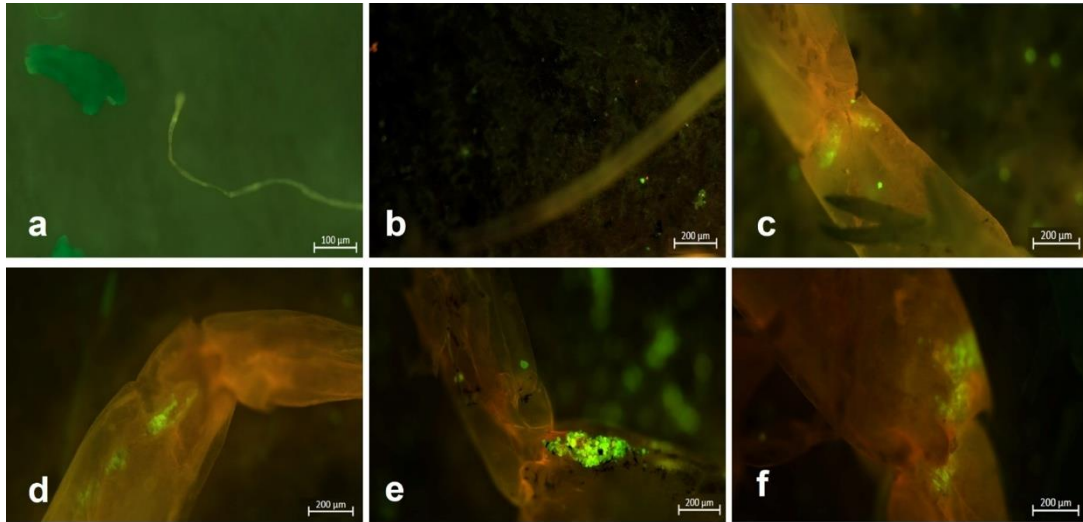


Figure 6. Fluorescent micrographs (430 nm) of *P. vannamei* postlarvae from bioassay 2 (LC_{50}). Images at 10x magnification. a) Blank (environmental contamination), b) treatment I (control, 0 mg L^{-1}), c) treatment II (5 mg L^{-1}), d) treatment III (25 mg L^{-1}), e) treatment IV (125 mg L^{-1}), f) treatment V (625 mg L^{-1}). Light intensity was adjusted to highlight high-density polyethylene microplastics (HDPE-MPs).

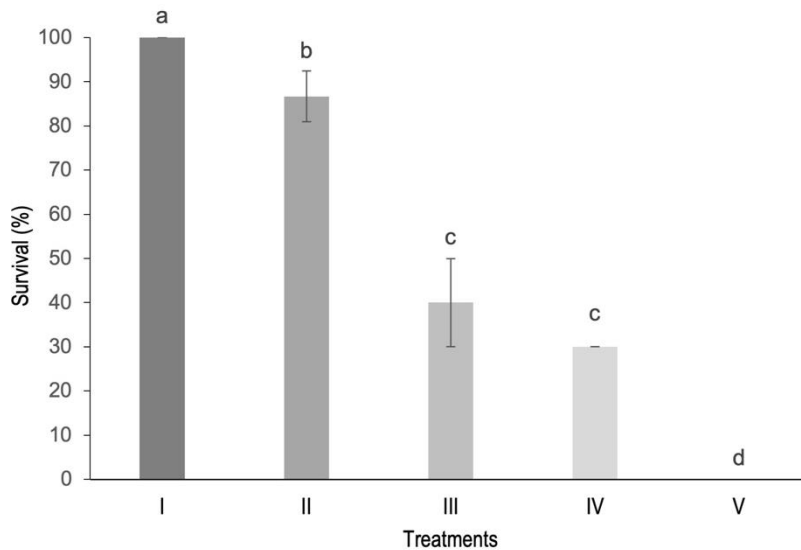


Figure 7. Survival of juvenile *P. vannamei* exposed to high-density polyethylene microplastics (HDPE-MPs). Treatments: I) 0 mg L^{-1} , II) 5 mg L^{-1} , III) 25 mg L^{-1} , IV) 125 mg L^{-1} . Data are shown as mean \pm standard error. Different letters indicate significant differences ($P < 0.05$).

and bluish pigmentation. The SGR in juveniles was 1.17% (I), compared to significantly lower values in treatments II (0.42%), III (0.50%), and IV (0.42%) (Fig. 8). These differences were statistically significant ($P < 0.05$), confirming the negative impact of HDPE-MPs on growth performance.

DISCUSSION

The accumulation and impacts of PE-MPs in aquatic species depend on various factors, including the physicochemical properties of the polymers and the physiological, behavioral, and trophic characteristics of the organisms exposed to them (Timilsina et al. 2023). Feeding habits, in particular, can increase susceptibility

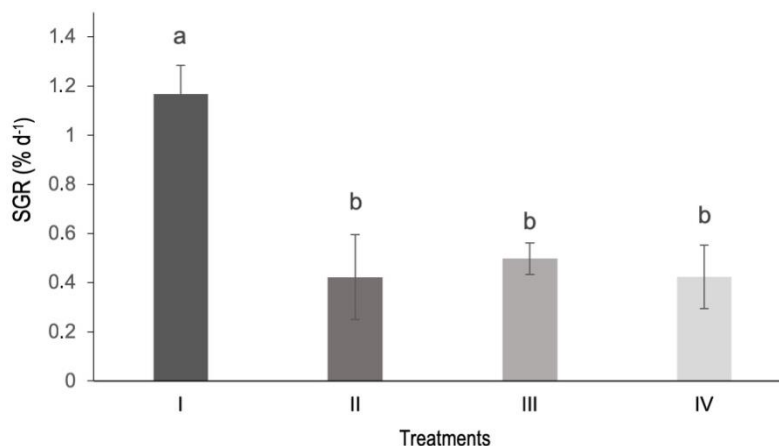


Figure 8. Specific growth rate (SGR) of juvenile *P. vannamei* exposed to high-density polyethylene microplastics (HDPE-MPs). Treatments: I) 0 mg L⁻¹, II) 5 mg L⁻¹, III) 25 mg L⁻¹, IV) 125 mg L⁻¹. Data are shown as mean \pm standard deviation. Different letters indicate significant differences ($P < 0.05$).

to these contaminants (Karlsson et al. 2017, Courtene-Jones et al. 2019, Seta et al. 2023, Timaná-Morales et al. 2024).

Results showed that exposure to HDPE-MPs under controlled conditions had a negative impact on the survival of *P. vannamei*. A clear dose-dependent effect was observed, where increased concentrations of HDPE-MPs significantly reduced survival, with juveniles being less susceptible than postlarvae. Notably, postlarvae survival decreased from 98.3% (control, 0 mg L⁻¹) to 66% at 500 mg L⁻¹ in bioassay 1 (Fig. 3). Furthermore, LC₅₀ values of 21.89 mg L⁻¹ for postlarvae and 30.6 mg L⁻¹ for juveniles were calculated in bioassays 2 and 3, respectively. Mortality rates of 30% (postlarvae) and 60% (juveniles) at 25 mg L⁻¹ (Figs. 3 and 7, respectively) indicate that both organism size and developmental stage are key determinants of sensitivity to HDPE-MPs. Despite the higher mortality observed in juvenile organisms at this concentration, the LC₅₀ estimate indicates increased tolerance to HDPE-MPs exposure in juveniles compared to postlarvae. Precision in MPs quantification is particularly critical for such comparisons at low concentrations where analytical variability increases (Wagner et al. 2022). These results align with prior studies reporting higher vulnerability during early shrimp stages, attributed to their higher relative feeding rates; according to van Wyk et al. (1999), juvenile *P. vannamei* (>2.5 g) ingest particles ranging from <400 μ m to 3.2 mm and can consume up to 10% of their body weight daily, a proportionally higher amount than

postlarvae (Pontes et al. 2008). Wang et al. (2021) reported 47, 53, and 20% mortality in *P. monodon*, *M. japonicus*, and *P. vannamei* juveniles, respectively, following 48 h exposure to 300 mg L⁻¹ PE-MPs (5 μ m). In contrast, Hariharan et al. (2022) found no significant mortality in *P. vannamei* postlarvae (4 mg) exposed for 25 days to 5 μ g L⁻¹ PE-MPs (32-43 μ m), although oxidative stress and progressive tissue accumulation were observed, suggesting sublethal physiological effects. Such factors, along with environmental conditions, may significantly impact shrimp survival in both aquaculture and natural settings (Valencia-Castañeda et al. 2022, Reunura & Prommi 2022).

Fluorescence microscopy (Nile red) revealed HDPE-MPs presence in the caudal exoskeleton and GI tract (treatments II-V), in contrast to their absence in surviving control organisms (treatment I). While some authors propose that MPs may pass through the digestive tracts of marine organisms without causing harm (Graham & Thompson 2009, Van Cauwenberghe et al. 2015), numerous studies have documented their bioaccumulation in critical tissues, such as the GI tract, stomach, gills, muscle, liver, hepatopancreas, and exoskeleton, along with immunosuppression, oxidative stress, and severe physiological disruption, particularly in benthic species like *P. vannamei* (Carreras-Colom et al. 2018, Kane et al. 2020, Duan et al. 2021, Hamilton et al. 2021). Additionally, behavioral effects were observed throughout all bioassays, most notably starvation, which was accompanied by either hyperactivity or lethargy. Similarly, previous studies

have reported that exposure to MPs can induce behavioral alterations such as inappetence, lethargy, and changes in movement speed (Hariharan et al. 2022, Timilsina et al. 2023).

Growth performance (SGR) was significantly impacted at ≥ 100 mg L⁻¹ in both life stages. In bioassay 1, postlarvae exposed to 100-500 mg L⁻¹ showed a marked reduction in growth (Fig. 4). In bioassay 3, control juveniles reached an SGR of 1.18%, whereas treated groups recorded 0.40-0.48% ($P < 0.05$), confirming that HDPE-MPs impair growth (Fig. 8). These outcomes are consistent with those of Han et al. (2021), who reported the downregulation of digestive and immune genes after MPs exposure, likely due to impaired nutrient assimilation, enzymatic inhibition, and disrupted intestinal homeostasis.

CONCLUSIONS

This study demonstrates that contamination by HDPE-MPs under extreme concentration scenarios significantly affects the survival and growth of *P. vannamei*, with postlarvae being more vulnerable to lethal effects and juveniles to cumulative ingestion. Moreover, acute exposure to HDPE-MPs induces behavioral changes, which should be appropriately assessed in future studies to determine the role of behavior in the evaluated variables. It is essential to implement mitigation measures (such as proper plastic waste management, bioremediation, and filtration systems) to reduce MPs risks and strengthen the biosecurity and sustainability of shrimp aquaculture systems.

Credit author contribution

J.V. Trejo-Flores: original draft writing, methodology, data curation, formal analysis, review, and editing; V. Peraza-Gómez: original draft writing, conceptualization, validation, supervision, project administration, formal analysis, review, and editing; E. Ortiz Espinoza: review and editing; J.A. Fierro-Coronado: methodology, formal analysis, review, and editing; M. Robles-Ravelero: formal analysis, review, and editing; E.D. Moreno-Medrano: microplastic analysis and characterization; D.R. Osuna-Laveaga: microplastic analysis and characterization; A. Luna-González: supervision and editing. All authors have read and accepted the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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