Water Air Soil Pollut (2025) 236:168
https://doi.org/10.1007/s11270-025-07800-x

A Comprehensive Investigation of Microplastic Contamination and Polymer Toxicity in Farmed Shrimps; *L. vannamei* and *P. monodon*

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Received: 7 June 2024 / Accepted: 8 February 2025 / Published online: 20 February 2025

Abstract Microplastic (MP) pollution poses a significant threat to marine ecosystems, seafood safety, and human health. This study investigates the accumulation of microplastics in two commercially important shrimp species, *Litopenaeus vannamei* (*L. vannamei*) and *Penaeus monodon* (*P. monodon*), sourced from cluster farming sites in Puttalam, Sri Lanka. Shrimp exoskeletons and edible soft tissues underwent rigorous microplastic analysis, including density separation, alkali digestion, stereo microscopy, and Raman spectroscopy. The results revealed high microplastic contamination, with *L. vannamei* containing an average of 4.99 ± 1.81 MP particles/g and *P. monodon* containing 1.87 ± 0.55 MP particles/g. Microplastic sizes varied, with *L. vannamei* predominantly contaminated with 100–250 µm particles and *P. monodon* with 500 µm—1000 µm particles. Fiber morphotypes were prevalent in *L. vannamei*, while blue-colored microplastics were dominant in *P. monodon*. These comprised polystyrene (PS), nylon 6,6, and polyethylene (PE) which were identified by Raman spectroscopy. Additionally, the study investigated the acute toxicity effects of microplastic polymer combinations using a zebrafish embryo model (FET236 assay). Zebrafish embryos exposed to polyethylene-nylon 6,6 combinations exhibited significant adverse effects on hatching, survival, and heart function at lower concentrations, while polyethylene terephthalate-polystyrene

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11270-025-07800-x.

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combinations showed no considerable effects. These findings underscore the urgent need for monitoring and managing microplastic contamination in shrimp farming areas. Future research should focus on elucidating the ecological impacts and human health risks associated with microplastic exposure.

Keywords Microplastics · Farmed shrimps · Polystyrene · Polymer toxicity · Zebrafish embryo model

1 Introduction

Plastics have permeated nearly every aspect of modern day–to–day life with their unparalleled functional properties, becoming one of the most versatile and widely used materials in the world. Global plastic production has increased during the last decade and reached up to 390.7 million tons in 2021 (Pourebrahimi & Pirooz, 2023). Plastics have a wide range of applications in automobiles, construction, packaging, textiles, personal care products, electrical and electronics, agriculture, and many more (Shamsuyeva & Endres, 2021). Microplastics (MPs) are tiny plastic particles less than 5 mm in diameter (Ashrafy et al., 2023). Based on the origin, microplastics are classified into primary microplastics and secondary microplastics (Ziani et al., 2023). Primary microplastics (< 5 mm in diameter) are originally made in dimensions such as microbeads, microfibers, and plastic pellets, while secondary microplastics are produced via the breakdown of larger plastic materials (Yee et al., 2021).

The prevalence of microplastics in marine habitats has made them bioavailable to a plethora of marine organisms. These microplastics are ingested by marine organisms by being confused as food or by ingestion of water contaminated with microplastics (Vital et al., 2021). Factors such as color, size, shape, aggregation, and abundance of microplastics affect their bioavailability in marine organisms (Wang et al., 2021; Paul et al., 2024; Öztekin et al., 2024;). As microplastics can easily travel through the trophic levels of food webs, they can cause health problems in a range of organisms. According to studies, microplastics have caused several ecotoxicological effects on a broad variety of fish species, via physical, chemical, and biological processes (Wang et al., 2020). In previous studies, microplastics have been detected in commercial fish species such as common mackerel (*Trachurus trachurus*), Atlantic hake (*Merluccius merluccius*), seabass (*Dicentrarchus labrax*), striped red mullets (*Mulus surmuletus*) and Nile tilapia (*Oreochromis niloticus*) as well as in bivalves such as mussels (*Mytilus* spp.) and oysters (*Crassostrea virginica* and *Ostrea edulis*) that are used for human consumption (Vital et al., 2021; Ditlhakanyane et al., 2023).

The most popular crustacean seafood in the world is considered to be shrimp, due to its unique flavor, odor, high dietary value, delicate and tender texture and high protein content (Das & Mishra, 2023) which is sourced through capture from natural habitats (ocean, freshwater, brackish water) or harvesting from aquaculture. However, the non-selective feeding behavior and size similarity between sediment and planktonic prey items with that of microplastics, there is a high chance for the accumulation of microplastics in shrimps (Thammatorn & Palić, 2022). This emphasizes the trophic transfer and accumulation of microplastics in seafood, posing a significant threat to food security and human health, particularly given the popularity of shrimp in the human diet (Curren et al., 2020; Timilsina et al., 2023; Vitheepradit & Prommi, 2023). Sri Lanka is fortunate with a broad coastal area with abundant natural resources containing lagoons and estuaries. Therefore, seafood farming and consumption is a popular activity in Sri Lanka, as it provides a major source of daily protein intake with fresh shrimps containing approximately 19.4 g of protein per 100 g, and contributing to 87% of the total energy needs (Dayal et al., 2013). For years, the primary shrimp species cultivated in Asia was *Penaeus monodon* (*P. monodon*). However, due to its ability to resist certain diseases, *Litopenaeus vannamei* was introduced to replace *P. monodon* as the dominant cultivated species (De Silva et al., 2021). Mundal Lake and Dutch Canal brackish water systems which are connected with Puttalam lagoon serve as the major water source for *P. monodon* and *L. vannamei* shrimp farming (IUCN, 2012). In the specific case of the Puttalam Lagoon in Sri Lanka, densely populated urban settlements, intense fishing activities, substantial riverine discharge during intense monsoon seasons, and restricted water exchange with the open ocean has contributed to the entrapment of

microplastics within its boundaries (Bhathiya et al., 2024). In addition, continuous waste discharges from maritime and industrial activities have also contributed to the contamination of the lagoon water with microplastics (Bandara et al., 2023).

There are limited studies about the effect of microplastics on human health, while most of the studies use model organisms to analyze and extrapolate them to infer the impacts of microplastics on human health. Zebrafish (*Danio rerio*) is a popular model organism in a wide range of developmental toxicity studies (Malafaia et al., 2020). Previous toxicity analyses of microplastics using the zebrafish model have predominantly focused on polystyrene (PS) polymers rather than other polymer types. Different polymer types of microplastics have been frequently detected in marine ecosystems and aquatic organisms (Daniel et al., 2021; Hossain et al., 2020) Even so, the potentially harmful effects of different microplastic polymer types and at concentrations relevant to human exposure levels remain largely unknown highlighting the necessity for novel studies to better comprehend implications of microplastics on human health (Bhuyan, 2022; Jayavel et al., 2024; Li et al., 2023). Thus, for the first time, the current study focuses on the objectives that include, (i) conducting a comprehensive analysis of the microplastic accumulation in farmed *L. vannamei* and *P. monodon* shrimp species in Sri Lanka, (ii) evaluating the microplastic accumulation in the edible—soft portion and the external part of shrimps, and (iii) analyzing the synergistic acute toxicity of microplastics, assessing two polymer combinations using the zebrafish embryo model.

2 Materials and Methods

2.1 Sample Collection

Two shrimp species; White leg shrimp (*Litopenaeus vannamei*) and Black tiger shrimp (*Penaeus monodon*) were collected directly from two shrimp farms located at Udappuwa and Arachchikattuwa in the Puttalam district in July 2023 (Fig. 1). The freshly harvested four–month–old black tiger shrimps from the Udappuwa area and two–month–old white leg shrimps from Arachchikattuwa were selected based on habitat characteristics and body size, respectively. All shrimp specimens were carefully placed on clean stainless steel trays using metal forceps followed by immediate wrapping in aluminum foil, as a prevention of potential plastic contamination. To determine microplastics in the shrimps, a total of 20 *P. monodon* samples and 20 samples of *L. vannamei* were collected (Korez et al., 2020). The collected shrimps were preserved in the icebox and transferred to the laboratory of the University of Sri Jayewardenepura and stored at −40 °C in glass containers until being processed.

2.2 Sample Process to Observe Microplastics on the Outer Shell of the Shrimps

The preserved shrimp samples were defrosted in a metal tray where morphometric measurements were taken. Each shrimp specimen was carefully unwrapped from the aluminum foil, and its length was measured using a standard ruler while weight was recorded using an analytical balance. For microplastic extraction from the exoskeleton, individual shrimps were placed in separate glass beakers. A saturated NaCl solution (density 1.2 gcm^{-3}) was added to each beaker, and the mixture was stirred continuously for 40 min to facilitate the density separation of microplastics from the shrimp's exoskeleton (Hossain et al., 2020; Sevwandi Dharmadasa et al., 2021). The same specimens were retained for subsequent microplastic extraction from their edible portions. Density-separated microplastics were digested using 30% (v/v) hydrogen peroxide (H_2O_2) and iron (II) sulfate heptahydrate ($FeSO_4$) in the ice bath to remove organic materials (Hurley et al., 2018). Subsequently, saturated NaCl solution (density 1.2 gcm^{-3}) was added to the beaker, and density separation was performed to separate microplastics from the digested solution, and the suspension was filtered through the 11 µm filter papers (Whatman grade 1) using a vacuum filter. The filter papers were placed in a half-closed glass Petri dish and oven-dried at 50 ºC for 24 h.

2.3 Sample Process to Observe Microplastics in the Edible Soft Portion

Edible portions of each sampled individual were used to investigate microplastic contamination following the method described by Wakkaf et al., (2020a, 2020b) with modifications. The specimens were dissected separately using metal dissecting

utensils, and each edible soft portion (with GI tract = gastrointestinal tract) was collected into labeled glass containers and weights recorded separately. Subsequently, edible soft parts were digested by adding 10% KOH solution, with a rate of 20 mL per gram of soft tissues, and oven-dried at 60 °C for 24 h to remove all organic matter. Density separation was performed using saturated NaCl solution (density 1.2 gcm^{-3}) and kept covered overnight with aluminum foil at room temperature to enable the separation of microplastics. The supernatant of the solutions was filtered through the 11 µm filter papers (Whatman grade 1) using a vacuum filter. The filter papers were placed in half-closed glass Petri dishes and oven-dried at 50 ºC for 24 h. Rigorous cross-contamination prevention measures were implemented throughout the study. Cotton lab coats were used to reduce contamination and nitrile gloves were worn at all times from the sampling to the analysis during the study. All tools, equipment, and work surfaces were thoroughly disinfected with alcohol before use, and throughout the experiments. only non-plastic (metal and glass) materials were used. All glassware was placed into a 10% hydrochloric acid bath for 24 h. Before use, glassware and dissection tools underwent triple rinsing with filtered distilled water. Between extractions, glassware was triple-rinsed and covered with aluminum foil when not in use to prevent airborne contamination. Procedural blanks containing only reagents were processed alongside sample batches to monitor potential contamination (Dawson et al., 2021; Gurjar et al., 2021; Hermsen et al., 2018; Wakkaf et al., 2020a).

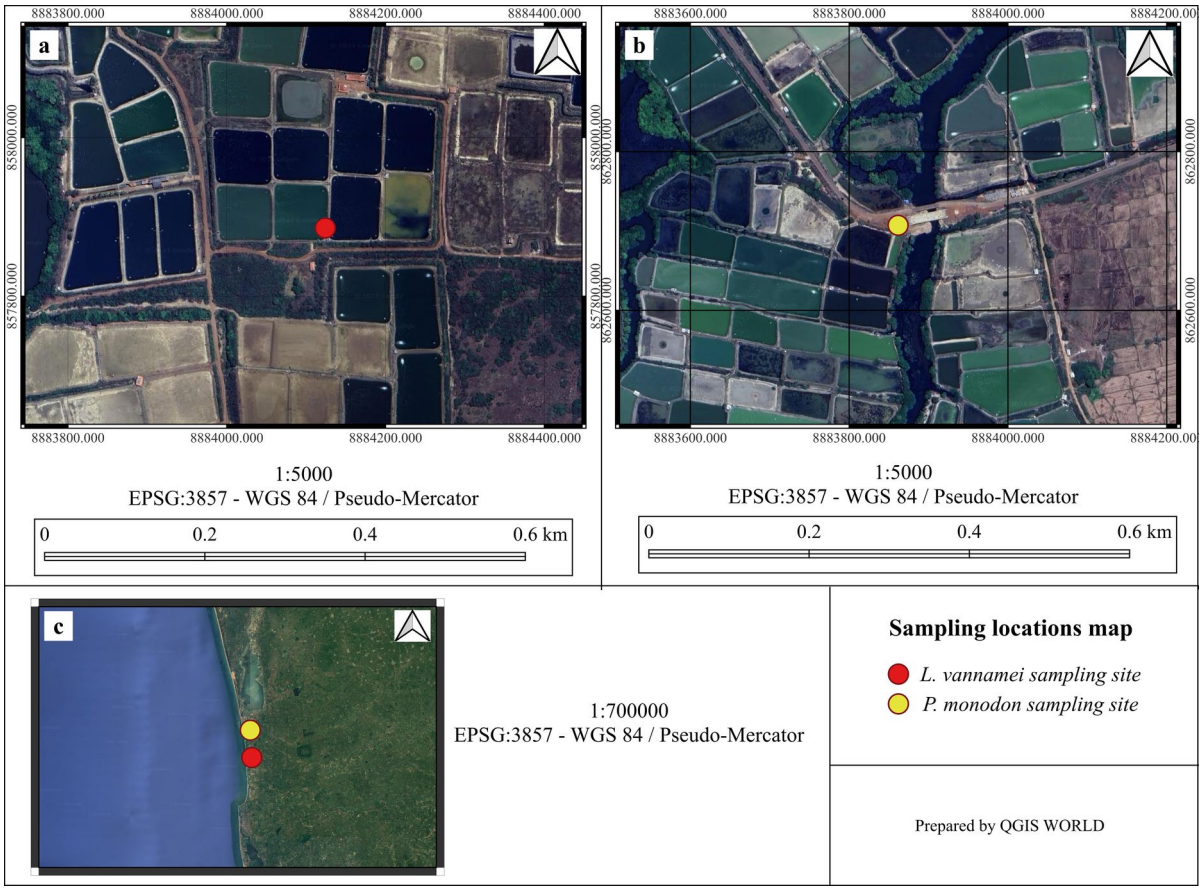

Fig. 1 Sampling locations for *L. vannamei* and *P. monodon* in shrimp farming areas. (**a**) Aerial view of shrimp farming ponds showing *L. vannamei* sampling site (**b**) Aerial view of the adjacent farming area showing *P. monodon* sampling site (**c**) Regional overview map showing the relative positions of both sampling sites along the coastline

2.4 Identification of Microplastics

2.4.1 Microplastic Identification

The samples were visually observed under a stereo microscope (Inskam315w, China), and images of microplastic particles were taken at (×40) magnification. The items were assessed visually and described according to colour, and shape. The hot needle method was used to distinguish microplastics from other particles (undigested shrimp tissue, sand) (Perez et al., 2022) and enumeration was done for each filter paper. The basic principle of this method involves verifying whether a particle is plastic by assessing its melting point, whereby a positive result is identified by melting in response to pin-point pressure from a heated probe or needle, rather than charring or decomposition (Beckingham et al., 2023). The morphotypes of microplastics were classified into fiber, films and fragments (Kooi & Koelmans, 2019) and microplastics were sorted based on their color. The lengths of the microplastic particles were measured using ImageJ software application.

2.4.2 Chemical Identification

The polymer type identification was carried out using the Raman spectroscopy technique. Raman spectra of the prepared samples were acquired using a custom-built, research-grade Raman spectrometer as described in the reference (Rodrigo et al., 2022). A 532 nm laser wavelength, known for its accessibility and effectiveness in Raman studies, was utilized (Chakraborty et al., 2023). The single-frequency continuous wave diode-pumped laser (Cobolt SambaTM 200) provided 60 mW of power at the sample. The laser beam was directed through a long-pass dichroic filter (Semrock LPD02-532RU-25 × 36 × 2.0) and focused onto the sample using a 50 × long-working-distance objective lens (Mitutoyo-MY50X-825).

The Raman signal was collected in a backscattered geometry and directed to the spectrograph, while a notch filter (Semrock LP03-532RU-25) removed unwanted scattered laser light. The spectrograph (Andor Kymera 328i) was equipped with a 600 l/mm diffraction grating and coupled to an electron-multiplying CCD detector (Andor Newton DU970P-BVF) (Gaigalas et al., 2009). Raman spectra were analyzed over the range of 100 cm^{-1} to 3500 cm^{-1}, achieving a spectral resolution of 8 cm^{-1}.

Microplastics collected from shrimp samples were identified individually by analyzing each sample spectrum alongside reference standard spectra. By comparing the characteristic fingerprint regions of standard polymer spectra, sample spectra were characterized, and polymer types were identified. Reference spectra were acquired from commercially available virgin polymers. To enhance the quality and reliability of the obtained Raman spectrum, essential preprocessing steps such as baseline subtraction, smoothing for noise reduction and removal of cosmic rays were conducted. These procedures were executed utilizing a custom Python program developed in the laboratory and Origin Pro software. Baseline subtraction corrects for contributions from fluorescence or scattering from the sample container by fitting a curve to the baseline signal and subtracting it from the raw spectrum. Smoothing techniques (Savitzky-Golay filtering) reduce noise from sources like detector electronics or stray light while preserving Raman peaks which improves the spectrum's signal-to-noise ratio. Additionally, the removal of cosmic rays eliminates artifacts caused by high-energy particles striking the detector ensuring the accurate interpretation of the Raman spectrum.

2.5 Zebrafish Embryonic Toxicity Assay (FET236)

2.5.1 Zebrafish Maintenance and Embryo Collection

Wild-type zebrafish were maintained in separately allocated glass tanks of 3 L capacity (20 × 10 × 15 cm) at 27 °C with a continuous flow of water and a photoperiod of 12L: 12D. The pH (7 ± 0.5) (using pH Test Strips (colorimetric method)) and electric conductivity (470 – 530 µs/m) (via Handheld Conductivity Meter) of the aquarium water were checked daily while nitrates (< 0.009 gm/l) (via API Freshwater Nitrate Test Kit (liquid reagent-based)), nitrite (8—12 mg/l) (API Nitrite Test Kit or Tetra Nitrite Test Kit) and ammonia levels (< 0.05 mg/l) (API Ammonia Test Kit) were checked weekly. These parameters were maintained through biological filtration, weekly water changes, and proper feeding protocols.

To obtain wild-type zebrafish embryos for toxicity experiments, two wild-type adult zebrafish females and one wild-type adult zebrafish male were transferred to 3 L breeding tanks containing a 2 mm mesh insert and a transparent barrier that separates male and female fish. On the following day, the separator was removed from each breeding tank and the zebrafish were allowed an hour for mating, spawning, and fertilization of the eggs (Test #:236, OECD, 2013.). After the spawning period, the adult zebrafish were transferred to their separate culture tanks, the plastic mesh was removed carefully from the breeding tanks, and the fertilized eggs were collected using a sieve and transferred to plastic Petri dishes containing dechlorinated water. The Petri dishes containing the eggs were transferred to the laboratory, rinsed two times with dechlorinated water, and transferred to new Petri dishes. The embryos were incubated at 28 °C in an incubator for 24 h. All animal procedures were performed following the guidelines of the Institute of Biology, Sri Lanka (ERC IOBSL 19907 2019).

2.5.2 Zebrafish Embryo Toxicity Test (FET236)

Four microplastic polymer types were assessed in the study (Nylon 6,6 and low-density polyethylene = LDPE, polyethylene terephthalate = PET, and polystyrene = PS), as they were abundant in the analyzed prawn samples and since they have been frequently detected in marine ecosystems and aquatic organisms (Daniel et al., 2020, 2021; My et al., 2023; Reunura & Prommi, 2022). Stock solutions (1000 mg/L) were prepared by first grinding virgin plastic pellets and filtering them through a sieve to obtain microplastic particles smaller than 500 µm. These filtered microplastic particles were then dissolved in AntiClo-treated water in clean glass bottles.

The microplastic polymer (Nylon 6,6 and LDPE, PET, and PS) combination solutions were prepared for subsequent zebrafish calibration assays. The assessments on the toxicity of zebrafish embryos were carried out employing wild-type zebrafish using a modified version of the OECD Fish Embryo Toxicity (FET) test (Test #:236, OECD, 2013, 2013). In brief, six zebrafish embryos at 24 h post fertilization (hpf) age were introduced into individual wells of a 6-well plate filled with dechlorinated water. Dechlorinated water was removed from the wells and 3 mL of test solutions (Nylon 6,6 and LDPE combinations, PET and PS combinations at 6.25 mg/L, 12.5 mg/L, 25 mg/L, 50 mg/L, and 100 mg/L concentrations) were added to the wells. The zebrafish embryos in 6-well plates were cultured in an incubator at 28 °C, photoperiod of 12L:12D, and 80% relative humidity which remained constant throughout the experiment. The embryo toxicity test was duplicated. Zebrafish embryo hatch and the mortality were evaluated and recorded at 24, 48, 54, 72, and 96 h post-fertilization (hpf). Meichoon Digital Microscope 3-in-1 USB Interface Camera and the 'Hi View' application were used for taking observations. In all the treatment groups and control groups, the number of embryos hatched versus not hatched was expressed as the percentage hatch rate, at each time point. Furthermore, the total number of living embryos versus dead embryos at different exposure concentrations and at each time interval are reported as survival rate percentages.

Heart Rate The heart rate of zebrafish embryos (beats per minute) was recorded at 72 hpf for each microplastics combination treatments and control treatments using direct microscopic observation. The Meichoon Digital Microscope 3-in-1 USB Interface Camera was used with a magnification range of 50x to 500x, depending on the size and visibility of the zebrafish embryo heart. A magnification of approximately 200x was used to record the heart rate, which provided a clear view of the beating heart while ensuring the entire embryo remained visible within the field of view.

Developmental Abnormalities At 96 hpf, images of zebrafish embryos subjected to combination treatments and control treatments were captured under 200X magnification using the Meichoon Digital Microscope 3-in-1 USB Interface Camera. Subsequently, each zebrafish embryo image was inspected to identify developmental abnormalities, including spinal curvature (SC), pericardial edema (PE), yolk sac edema (YSE), and no structural deformity (NSD). For each treatment well, a binary scoring system (present/absent) was used to record abnormalities, and the percentage of each abnormality per well was calculated.

2.6 Data Analysis

All the statistical tests were performed using GraphPad Prism statistical computing software. Raman spectral analysis was conducted using Origin Pro software. The microplastics were quantified based on shape, color, size, and type for both shrimp species. After confirming normality, parametric tests were performed: Two-way ANOVA to compare the microplastic abundance in the exoskeleton and edible soft tissue parts between the two shrimp species, and Pearson correlation to analyze microplastic abundance relationships with body measurements. Chi-square and Fisher's exact tests were used to analyze relationships between microplastic characteristics and species. In zebrafish toxicity analysis, when the data passed the Shapiro–Wilk's test, Two-way Analysis of Variance (ANOVA) was used to compare the concentration-dependent variation between each combination treatment experiment at varying doses and the control treatment.

All the detailed information regarding the data analysis is included in the Online resource.

3 Results

3.1 Abundance of Microplastics

A total of 40 samples, comprising 20 *Litopenaeus vannamei* and 20 *Penaeus monodon*, were analyzed for microplastic content. The morphometric parameters of the shrimps, body weight, and length of each sample were observed and recorded during the study (Table 1). Microplastic particles were detected in all the tested samples, both in the exoskeleton and edible soft tissue parts of both species. The average abundance of microplastics in the whole body of the two shrimp species was 4.99 ± 1.81 MP particles/g and 1.87 ± 0.55 MP particles/g.

Based on the independent sample t-test at $P < 0.05$, the analysis showed no significant differences between the total number of microplastics items corresponding to the two species ($p = 0.6628$). According to the results of two-way ANOVA followed by Bonferroni's multiple comparison test, there is a statistically significant difference in the microplastic abundance (MP particles/g) of the analyzed body parts (exoskeleton and edible soft tissue parts) between the two shrimp species ($F = 56.06$, $p < 0.0001$) (Fig. 2a). The abundance of microplastics in both the exoskeleton and edible soft tissues of *L. vannamei* is significantly higher than in *P. monodon*. However, two-way ANOVA, which analyzed the microplastic count in the analyzed body parts between the two species, showed no significant difference in microplastic count in analyzed tissues between the two shrimp species ($F = 3.454$, $p = 0.0670$) (Fig. 2b). Moreover, paired t-tests ($P < 0.05$) that were carried out separately for the two shrimp species, analyzed the microplastic abundance per gram weight between exoskeleton and edible soft tissue and found there is no statistically significant difference (*L. vannamei* $P = 0.1129$, *P. monodon* $P = 0.5625$). Pearson correlation results of *P. monodon* revealed that the abundance of microplastics is positively correlated with the total weight ($r = 0.4830$, $p = 0.0309$) and length ($r = 0.5354$, $p = 0.0149$) (Fig. 3), suggesting that species with larger body size is likely to have a higher number of microplastics, and according to the size of shrimp microplastic abundance varies. However, the microplastics in *L. vannamei* did not show any correlation with the lengths ($r = -0.0174$, $p = 0.9418$) and weights ($r = -0.2373$, $p = 0.3137$) of the samples.

3.2 Morphological Characteristics of Microplastics

3.2.1 Colors of Microplastics

The study identified six distinct colors of microplastics. All six colors were detected in *L. vannamei*, while *P. monodon* contained five colors, with gold-colored microplastics being absent (Fig. 4). Overall blue (57.81%), black (34.13%), red (6.62%), green (0.32%), gold (0.08%), and transparent (1.04%) were observed in both species. Based on the Fisher's Exact Test, there is no statistically significant difference in the distribution of colors between the two shrimp species at a 0.05 significance level ($p = 0.2852$).

3.2.2 Size of Microplastics

Microplastics of five different size classes, ranging from 37 µm—4718 µm, were observed in this study (Fig. 5a). All the extracted particles were

Table 1 Morphometric details of the samples and abundance of microplastics

Species	*Litopenaeus vannamei*	*Penaeus monodon*
No. of samples	20	20
Average length (cm)	10.40 ± 0.96	12.86 ± 1.18
Average whole-body weight (g)	6.61 ± 1.02	16.69 ± 3.62
Average soft tissue weight (g)	3.28 ± 0.59	8.36 ± 1.98
No. of MP particles/ individual	32.00 ± 9.72	30.75 ± 9.06
No. of MP particles in exoskeleton/ individual	18.00 ± 8.39	14.75 ± 5.28
No. of MP particles in soft tissue/ individual	14.00 ± 5.23	15.95 ± 5.54
No. of MP particles/g whole weight	4.99 ± 1.81	1.87 ± 0.55
No. of MP particles/g soft tissue	4.39 ± 2.02	1.96 ± 0.71
No. of MP particles/g exoskeleton weight	5.64 ± 2.90	1.82 ± 0.95

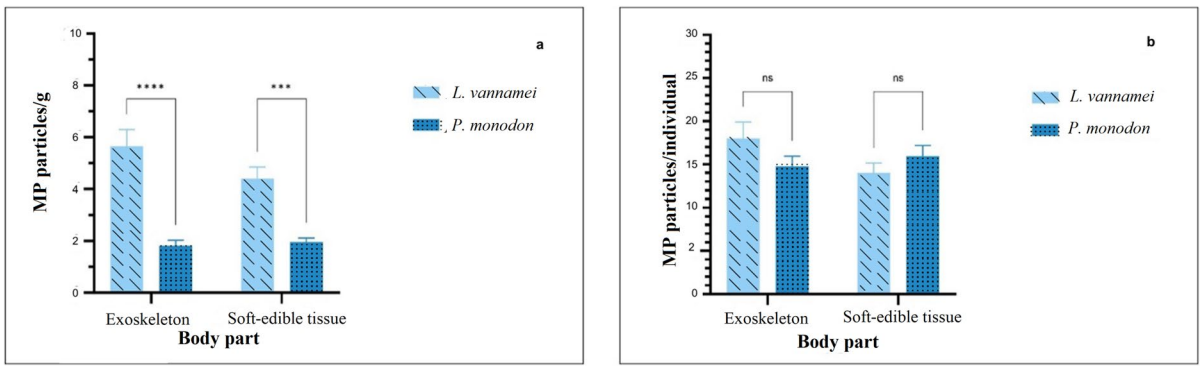

Fig. 2 Average microplastic abundance (Mean ± SEM) in the exoskeleton and edible soft tissue of the two shrimp species according to items/g (**a**) (x-axis- body part, y-axis- MP particles/g) and items/individual(**b**) (x-axis- body part, y-axis- MP particles/individual)

in the defined size range of microplastics; > 1 µm and < 5 µm. In the exoskeleton of *L. vannamei,* 34.44% of microplastics under size 100 µm—250 µm were dominant, while in the edible soft portion of *L. vannamei* predominantly contained microplastics size range of 250 µm—500 µm (29.64%). In contrast, the exoskeleton of *P. monodon* dominated with 500 µm—1000 µm microplastics (41.28%) while microplastics of large size > 1000 µm (39.81%) were predominant in the edible portion. There were no microplastics under 100 µm detected in the edible soft portion of *P. monodon*. Microplastic particles larger than 1000 µm were observed in minimal concentrations in *Litopenaeus vannamei*. Conversely, microplastics smaller than 100 µm were detected in low amounts in *Penaeus monodon.* The smaller size of microplastics dominant in *L. vannamei* is possibly due to their size being smaller (i.e. 6.612 g ± 1.02) when compared with *P. monodon* (i.e. 16.70 g ± 3.62). Further, according to the chi-square test results, the size of microplastics is significantly dependent on shrimp species ($X^2 = 334.7$, df = 12, $p < 0.0001$) (Fig. 5b).

3.2.3 Morphotypes of Microplastics

Fisher's exact test demonstrated that the identified microplastic types: fibers, fragments, and film were not significantly different among the two species (p 0.5) (Fig. 6). Fiber (98.06%) was dominant in the exoskeleton of *L. vannamei* followed by fragments (0.5%) and films (1.3%). While fiber microplastics show predominance (95%) in the edible soft portion of *L. vannamei* followed by fragments (2.86%), and films (2.14%). Similarly, fibers were dominated (96.97%) in the exoskeleton of *P. monodon* followed by fragments (2.34%), and films (0.67%). As well as in the edible portion of *P. monodon*, only two types

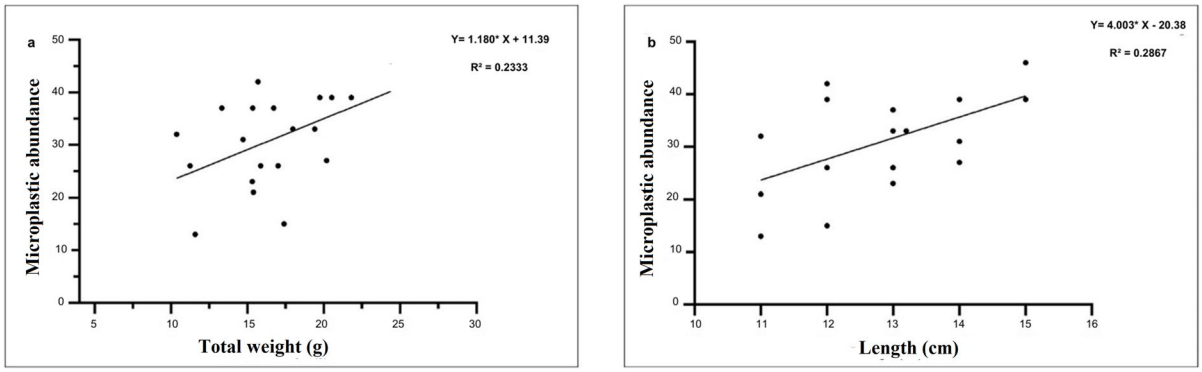

Fig. 3 Relationship of microplastic abundance with shrimp weight (**a**) and body length (**b**) (*P. monodon)* (x-axis- Microplastic abundance, y-axis- Length (cm))

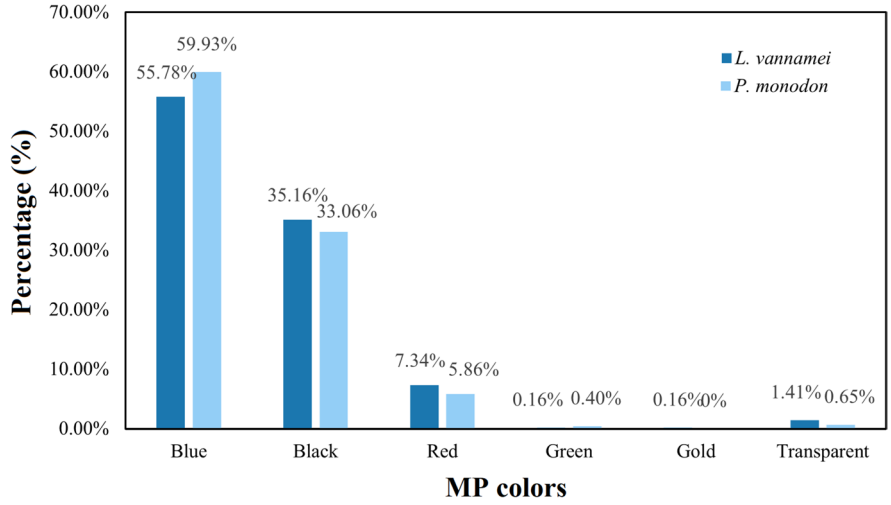

Fig. 4 Microplastic colors observed in both exoskeleton and edible soft tissues of individual shrimp species (x-axis- Percentage and y-axis- the type of the shrimp)

of microplastics were observed, and fibers were also predominant (99.37%) followed by films (0.63%) and no fragments were detected. Further, no other types of microplastics including pellets, or foams, were observed in the sample species (Fig. 7 a-i).

3.3 Chemical Composition of Isolated Microplastics

The chemical composition of suspected 25 particles with a margin of error of ± 19.6% (at a 95% confidence level) (Cowger et al., 2024; Kedzierski et al., 2019) was validated using Raman spectroscopy. The polymer types including PS, nylon 6,6, and PE were identified (Fig. 8). Out of 25 particles analyzed, 23 were confirmed as microplastics, and 2 remained unidentified. The most abundant polymer was nylon 6,6 (40%) followed by PS (32%) and PE (20%). The unidentified microplastics (8%) were excluded from the quantification of microplastics.

3.4 Zebrafish Embryonic Toxicity Assessment Assay

The present study was designed to gain insights into the acute toxicity effects of combined microplastic exposure by utilizing a sensitive model organism; *Danio rerio*. In comparison with microplastic levels found in aquatic environments, the microplastic concentrations selected for this analysis were relatively high. However, the selected concentrations are following the OECD test protocol 236, Fish embryo toxicity test (FET) (Malafaia et al., 2020; Test #:236,

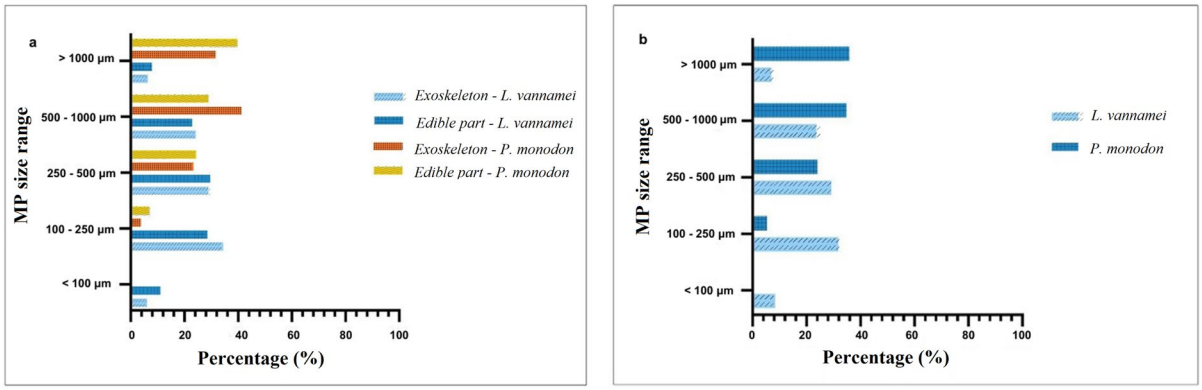

Fig. 5 (**a**) Microplastic size distribution between the exoskeleton and edible soft portion of two shrimp species (**b**) Microplastic size distribution between two shrimp species (x-axis- Microplastic size range and y-axis- Percentage (%))

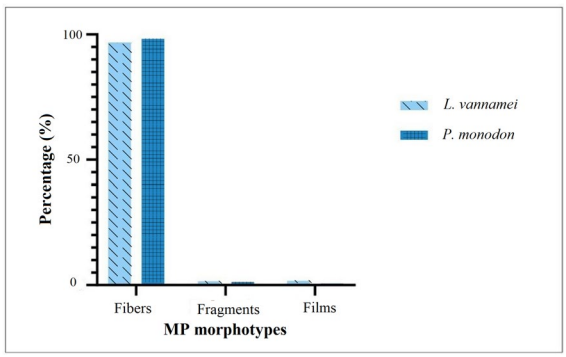

Fig. 6 Morphotype percentages in two shrimp specimens (x-axis- Microplastic morphotypes, and y-axis- Percentage (%)

OECD, 2013, 2013.), and also compatible with the findings of this study on microplastics abundance. Furthermore, the size of the microplastic particles (< 500 µm) used for the toxicity analysis is compatible with the findings of this study and previous studies as well (Daniel et al., 2020; Hossain et al., 2020; Yoon et al., 2022).

3.4.1 Hatch Rate

In all control treatments, embryos developed and hatched normally, with complete hatching observed at the 72—hour time point.

Hatch Rate of Nylon 6,6 and LDPE Combinations The treatment combination of nylon 6,6 12.5 mg/L with LDPE 25 mg/L, showed a significantly altered hatching pattern compared to the control ($P \leq 0.0001$) at 54, 72, and 96 hpf time points. Similar to combination treatments, LDPE individual treatment showed a significant effect on the hatching pattern at 54 hpf at the doses of 6.25, 12.5, 25, and 50 mg/L (Online resource Fig. S1.VII). Contrastingly, nylon 6,6 did not show significant effects on hatching when individually exposed to zebrafish embryos, while nylon 6,6 combination with LDPE showed a significant effect on hatching mainly at 12.5 and 25 mg/L combinations.

Hatch Rate of PS and PET Combinations The treatment combinations of PS and PET did not show significant alterations with the controls at the time points of 24, 48, 54, 72, and 96 hpf. Further, the individual treatment of PET showed significant effects at the concentration of 50 mg/L and 12.5 mg/L at the time points of 72 hpf and 96 hpf (Online resource Fig. S2.V). The individual treatment of PS significantly altered at the time points of 72 hpf and 96 hpf under 6.25, 12.5, 25, 50, and 100 mg/L concentration levels. (Online resource Fig. S2.VI).

3.4.2 Survival rate

In all the control treatments, embryos survived normally throughout the assessed time points.

Survival Rates of Nylon 6,6 and LDPE Combinations and PS and PET Combinations The survival rate of zebrafish embryos exposed to

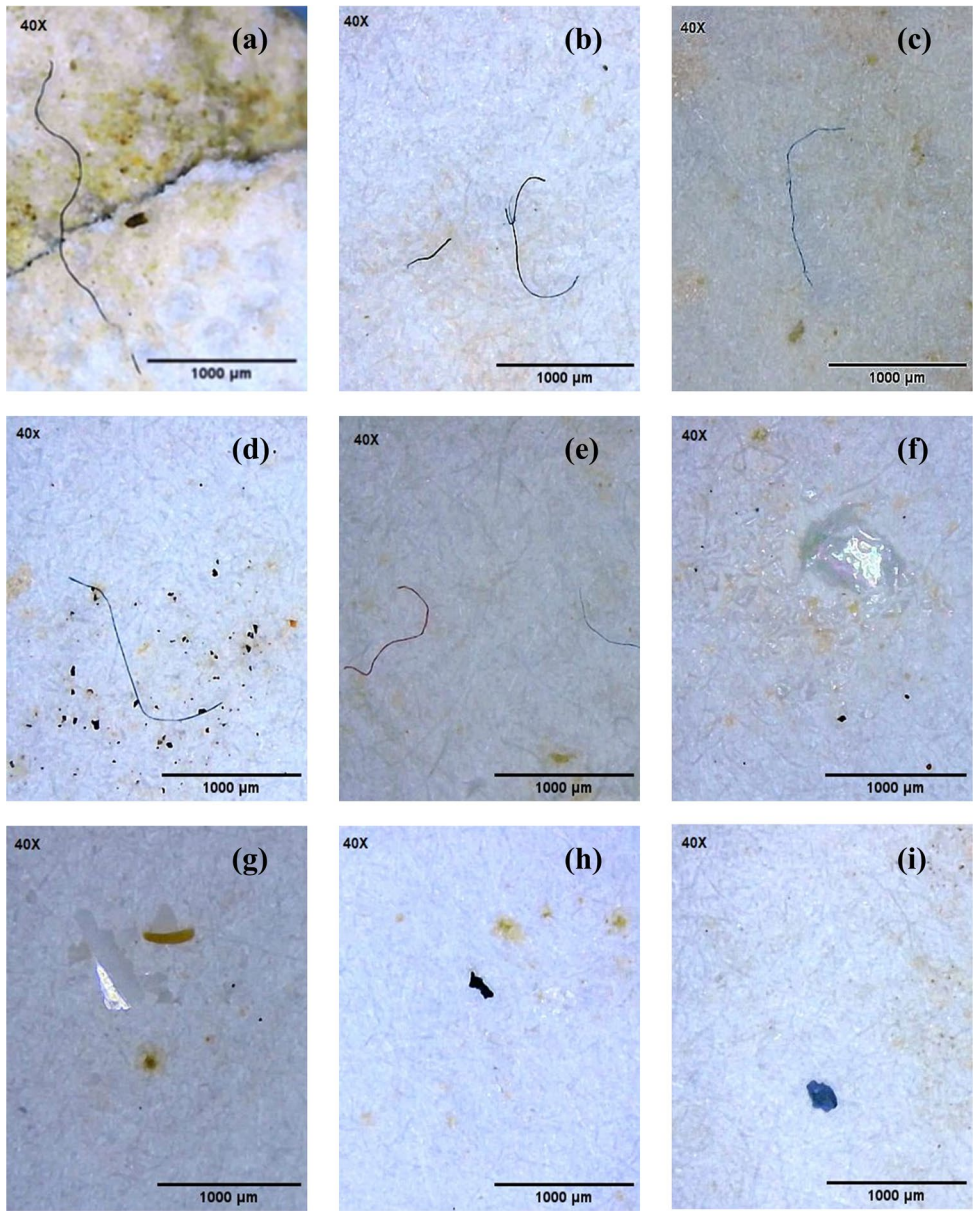

Fig. 7 Images of different morphotypes and colors of microplastics obtained from two shrimp species (**a**), (**b**) black fibers; (**c**), (d) blue fibers; (**e**)red fiber; (**f**), (**g**) transparent film; (**h**) black fragment; (**i**) blue fragment

combination treatments of nylon 6,6 6.25 mg/L with LDPE 6.25, 12.5, 25, 50 and 100 mg/L, nylon 6,6 12.5 mg/L with LDPE 6.25, 25, and 50 mg/L, was significantly affected at 54,72 and 96 hpf time points (Online resource Fig. S3. I, II). In all the treatments, controls were observed to be at 100% survival rates along the time points. In individual treatments of nylon 6,6 and LDPE, the survival rate of the embryos did not show any significant difference from the controls at any of the tested concentrations or time point (Online resource Fig. S3. VI, VII). In contrast, the survival rate of the embryos exposed to combination treatments of PS and PET did not show any significant difference from controls at any of the tested concentrations or any time point (Online resource Fig. S4).

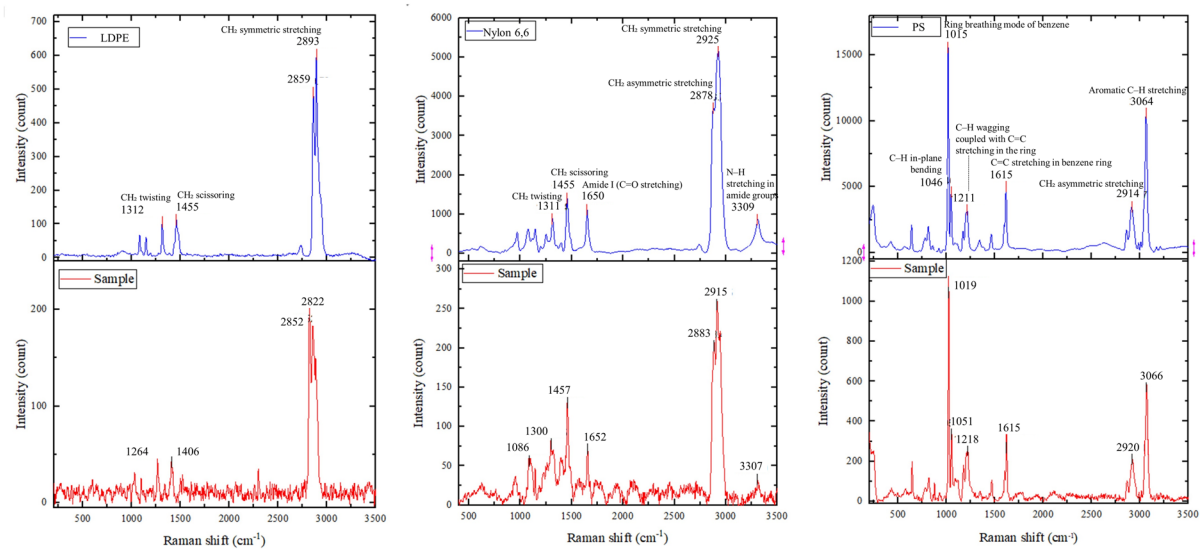

Fig. 8 Raman spectra of different microplastics obtained from farmed two shrimp species including polyethylene, nylon 6,6, and polystyrene (x-axis- Raman shift and y-axis- Intensity (count))

3.4.3 Heart rate

Heart Rates of Nylon 6,6 and LDPE Combinations At the 95% confidence level, most combination treatment groups showed significantly altered heart rates compared to the controls. (Online resource Fig. S5. I, II, III, IV, V). In individual treatments, significant differences from the controls were not observed in any of the LDPE concentrations, while nylon 6,6 single treatment showed significantly reduced heart rates compared to the control ($p \leq 0.05$) (Online resource Fig. S5.VI).

Heart Rates of PS and PET Combinations Most concentration combinations did not show significant differences with controls. However, PET 6.25 mg/L with the treatment of PS 12.5 mg/L showed a significantly dropped heart rate compared to the control ($p \leq 0.05$). See the (Online resource Fig. S6).

3.4.4 Development Abnormalities

The structural deformities of wild-type zebrafish exposed to combination treatments and individual treatments were observed to account for treatment toxicity in the early development stages of zebrafish (Online resource Fig. S7, Fig. S8, Fig. S9). No abnormal development of zebrafish was found in the control group.

Development Abnormalities of Nylon 6,6 and LDPE Combinations Nonstructural deformities were the most abundant in all the combined and individual treatments. PE = Pericardial edema was not observed at any of the combination or individual treatments. SC = spinal curvatures and YSE = yolk sac edemas were reported in both combination and individual treatments at very low levels (Online resource Fig. S7).

Development Abnormalities of PS and PET Combinations None of the structural deformities were reported at significant levels. Only YSE was detected in a few concentration combinations (Online resource Fig. S8).

4 Discussion

This is a pioneer study reporting the ingestion of microplastics in farmed shrimp *L. vannamei* and *P. monodon* from Puttalam, Sri Lanka. The results show that all the sampled shrimps of the two species had accumulated microplastics in both their exoskeleton and edible soft tissues. The average abundance of microplastics in the two species of the present study is comparatively higher than in previous studies (Hossain et al., 2020; Yoon et al., 2022). In a

previous study that analyzed farmed shrimps in central Vietnam, microplastic abundance in *L. vannamei* was reported as 8.6 ± 3.5 items/individual (1.1 ± 0.4 item/g-ww) and *P. monodon* as 7.7 ± 3.5 items/individual (0.5 ± 0.3 item/g-ww) (My et al., 2023). Furthermore, the microplastic levels found in the wild-caught seafood varieties harvested from the Western coast of Sri Lanka are lower than the level found in this study, *P. monodon* (1.7 ± 0.29 MP particles/g), (Kandeyaya et al., 2023).

The microplastic level in marine and freshwater organisms correlates with the level of microplastic pollution in the habitat environment, feeding strategy and the preference for synthetic material intake (Daniel et al., 2020). As this study revealed comparatively high microplastic abundance in *L. vannamei* and *P. monodon,* compared to other studies of the same species, it indicates considerable microplastic pollution in the culture ponds where samples were collected. The sampling sites, Aarchchikattuwa and Udappuwa, located on the coast of north-western, Sri Lanka. The Dutch Canal, a semi-enclosed coastal water body which connects Puttalam to Colombo via Negombo also lies in this area, connecting to the water sources in the sampling site. According to Corea et al. (1995), majority of the prawn farms rely on the Dutch Canal as the primary water source for their aquaculture, and as an effluent discharge point. Recent research demonstrates that the water in the Dutch Canal contains the highest microplastic accumulation (2.48 ± 0.43 microplastic pieces/L) highlighting the potential impact of microplastic-contaminated water on shrimp habitats (Sasangi et al., 2024). Furthermore, due to poor pond design and management, inlet and outlet canals of neighboring farms are located closely, resulting in discharged water flowing between farming facilities. Since there is less water exchange with the Dutch Canal, effluents and plastic waste accumulate in farming pond water, leading to high microplastics abundance in aquaculture samples. Shrimp meal and fishing gear are the possible sources of microplastic transfer to the farming ponds (Reunura & Prommi, 2022).

A previous study that analyzed both farmed and wild-caught *L. vannamei* in Cau Hai Lagoon, Vietnam, found that the number of microplastics in farmed shrimp is significantly higher than the wild-caught shrimp (My et al., 2023) suggesting that differences in water qualities and stagnated water in farms and wild environments may be the reason behind this. Notably, when compared with the study conducted by Kandeyaya et al. (2023), which analyzed wild-caught seafood varieties from the Western coast of Sri Lanka, confirms that farmed shrimps have a higher microplastic content than wild-caught shrimp. The high concentration of microplastics found in aquaculture sites compared to the ocean could be due to the limited space in culture ponds. Unlike the open ocean where waves can disperse microplastics, the stagnant water of ponds allows microplastics to accumulate. A study on microplastics in surface water in Southern Sri Lanka found that fishery harbor sites have the highest abundance of microplastics among all the analyzed sites, due to intense gear-handling activities (Bimali Koongolla et al., 2018). This confirms that fishing gear contributes to microplastic pollution, and subsequently affects the water quality of the shrimp ponds as well. Further studies are required in microplastic contamination in the North-west area and in water sources of the sampling sites to confirm the findings.

The paired t-test analysis of microplastics in the exoskeleton and edible soft tissues of the two species revealed that there is no statistically significant difference according to the body parts. A previous study revealed the presence of microplastics in the digestive tract, head, or gills but not in the abdominal muscle tissue, which is the edible part (soft tissue in this study) (Devriese et al., 2015). Contrastingly, this study revealed that a considerable number of microplastics are present in the abdominal muscle, which usually is the edible part that humans consume. Similar to the finding of this study, 36 microplastic were found in the muscle tissue of 12 sampled *Penaeus semisulcatus* (Abbasi et al., 2018) and 0.360 items/g muscle tissue of *P. semisulcatus* (Akhbarizadeh et al., 2019), confirming the presence of microplastics in the edible soft portion. Furthermore, previous studies have found a significantly high abundance of microplastics in the gut (My et al., 2023), which confirms the potential transfer of microplastics to humans when consuming smaller shrimps like Whiteleg shrimp, where the gut is not removed before consumption. This highlights the risk of microplastic transfer to humans, even when the exoskeleton is removed, due to the similarity of microplastic abundance in both exoskeletons and edible soft tissue.

Additionally in this study, blue, black, red, green, gold, and transparent (white) colored microplastics were observed in the two shrimp species. In both species, blue-colored microplastics were dominant with 57.81% of the total microplastics. These observations are comparable to previous studies by Lawan et al. (2024) and Yoon et al. (2022) showing a higher percentage of blue color microplastics in shrimps. The color distribution of microplastics in an organism depends on the source, study sites, and resemblance of microplastics to natural food items or prey (Gurjar et al., 2021; Vo & Tran, 2022).

Microplastics with five different size classes ranging from < 100 µm, 100—250 µm, 250 µm—500 µm, 500 µm—1000 µm, and > 1000 µm were detected in the present study. The size range of > 1000 µm (39.81%) microplastics was predominant in the edible soft portion of *P. monodon* while 250 µm—500 µm was foremost (29.64%) in the edible soft portion of *L. vannamei*. Furthermore, microplastics in the range of 500 µm—1000 µm were highly contained in the exoskeleton of *P. monodon; in contrast,* this size range was lowest (6.39%) in *L. vannamei*. A similar result was reported by My et al. (2023) with microplastics larger than 250 µm were dominant in the gastrointestinal tract (GI tract) samples, while the majority of microplastics found in the tissue samples were smaller than 500 µm. Further Hossain et al. (2020) investigated that microplastics size range of < 1000 µm (70%) in the GI tract of tiger shrimp (*P. monodon*) are dominant while microplastics with 500 µm – 1000 µm size range (40%) were predominant in brown shrimp (*M. monocerous*). They indicated that the size of ingested microplastics varies according to the species and the sample collection location. The difference in microplastic sizes between the edible soft portion and exoskeleton could be attributed to the selective filtration and retention of microplastics by the gastrointestinal system in the edible soft portion. The larger microplastics may have difficulty permeating through the gastrointestinal system and therefore tend to accumulate in the gastrointestinal tract (My et al., 2023). Conversely, smaller microplastics may be easily permeable and can accumulate in the exoskeleton. Previous studies have shown that microplastic ingestion and egestion in shrimps have a size-dependent effect, microplastics with smaller sizes causing more tissue damage (Zhou et al., 2023).

In terms of microplastic type abundance, fibers were the most prevalent category in two shrimp species: *L. vannamei* (96.70%), and *P. monodon* (98.21%) followed by fragments and films. Fibers are predominant in two shrimp species due to intensive shrimp feeding, slower digestion of fibers, and human activities (Li et al., 2019; Reunura & Prommi, 2022; Zhang et al., 2021), transportation of microplastic fibers through water surface runoff, rivers, and the atmosphere (Wang et al., 2022). The entanglement of fibers with food particles increases the likelihood of organisms ingesting them. Hossain et al. (2020) and Patterson et al. (2021) reported that almost 57% of fiber microplastics are predominant in giant tiger prawns while My et al. (2023) identified fiber as the most predominant microplastics type (46.06%) in the GI tract of *L. vannamei*. There is another study that was done around Negombo Lagoon found that fibers (73.0%) were the most abundant microplastic (Athukorala et al., 2023). Some other comprehensive studies conducted globally, such as Nan et al. (2020) in Australia, investigated fibers as the most dominant microplastic type in farmed shrimp species. Although some studies reported different predominant microplastic types in *L. vannamei* compared to our findings (Curren et al., 2020; Vitheepradit & Prommi, 2023).

Raman spectroscopy is a powerful analytical technique utilized across diverse scientific disciplines which involves irradiating a sample with monochromatic light (typically by a laser source) and analyzing the inelastically scattered light that generates as a result of the interaction of incident light with the sample. This process reveals valuable insights into the vibrational and rotational modes of molecules within the sample. The resulting Raman spectrum exhibits peaks corresponding to specific vibrational energies facilitating deductions about molecular structure, chemical composition and physical properties (Chakraborty et al., 2023; Iri et al., 2021).

The chemical structures of microplastics such as nylon 6,6, LDPE, and PS dictate their unique properties and can be elucidated through Raman spectroscopy. Nylon 6,6 is characterized by amide linkages in its backbone which exhibits Raman peaks indicative of amide stretching vibrations around 1650 cm^{-1} and 1550 cm^{-1}, alongside peaks associated with alkyl chain vibrations near 1450 cm^{-1}. LDPE, with its long chains of ethylene monomers and branching, displays Raman peaks corresponding to symmetric and

asymmetric stretching vibrations of CH_2 groups at 1460 cm^{-1} and 2850 cm^{-1}, as well as vibrations of CH_2 groups around 720 cm^{-1} and 1300 cm^{-1}. PS, composed of chains of styrene monomers, shows Raman peaks attributed to aromatic C–C stretching vibrations at 1600 cm^{-1}, and aromatic C-H bending vibrations at 1000 cm^{-1}. Understanding these chemical structures and associated Raman peaks facilitates the identification and characterization of microplastics for diverse applications. Additionally, plastics can be colored through several methods, including surface printing, surface coating (painting), application of decorative films, or by incorporation of colorant additives into the polymer mass via compounding. Colorfulness in plastic products does not solely originate from the material itself. Therefore, when a Raman spectrum exclusively indicates the presence of colorants, it becomes impossible to determine the composition of the polymer (Kiran et al., 2022; Nava et al., 2021).

In the present study, 4 types of polymers including nylon 6,6 (40%), PS (32%), and PE (20%) were mainly identified. These polymers are commonly used as raw materials in fishing nets, floats, fish baskets/bags, ropes, and clothing materials that are used in fishing activities in Dutch Canal (Cheung et al., 2018; Wang et al., 2019). Most microplastic fibers were identified as nylon 6,6. Similar to the observations of the present study, Lawan et al. (2024) found PS, nylon 6,6, polyester, polypropylene, and rayon as the most available microplastic polymers. In another study most abundant polymers found in the GI tract of *Metapenaeus monoceros*, *Parapeneopsis stylifera*, and *Penaeus indicus* from the trawling grounds of the northeastern part of the Arabian Sea were PE, polypropylene, nylon 6,6, polyester, and PET (Gurjar et al., 2021). Therefore, the current research shows that microplastic pollution in the studied area is largely caused by laundry and domestic wastewater, fishing equipment, and food packaging materials, as confirmed by the chemical analysis of polymers.

4.1 Toxicity Assessment of Combined Microplastics Using Zebrafish

In the present study, the potential acute toxicity of the most abundant microplastic polymer combinations of Nylon 6,6 with LDPE and PET with PS were assessed in zebrafish embryo development evaluating hatch rate, mortality rate, heart rate, and abnormal disabilities after 24 hpf, 48 hpf, 54 hpf, 72 hpf, and 96 hpf. In the early developmental life cycle of zebrafish, the hatching process marks a critical point. Hatching is initiated when the embryo reaches a size that requires a greater oxygen supply than can be provided by diffusion through the egg envelope and perivitelline fluid (Muller et al., 2015). Chorion softening is a critical step in the hatching process, and it is facilitated by a proteolytic enzyme also known as Zebrafish hatching enzyme (ZHE1) (Muller et al., 2015). In untreated zebrafish embryos, hatching occurs between 48—96 hpf with the majority hatching at the 72 hpf time point. In a few combinations, hatching was significantly affected compared to controls, possibly due to microplastic adsorption on the chorion surface. This can block the release of secretory chorinase by hatching glands, which initiates hatching gene expression (Bashirova et al., 2023). The size of microplastic affects on chorionic adhesion and penetration. While this study used larger microplastics compared to previous studies, yielding different results for chorionic adhesion and penetration, earlier research using smaller microplastics showed reduced embryo hatching rates with increasing microplastic concentrations in single microplastic treatment assays (Zhao et al., 2021). Dunn's multiple comparisons test compared the effects of individual treatments versus combined treatments and found out that the hatching rate is not significantly different between combined treatments and single treatments at the evaluated concentrations. Overall, this suggests that microplastics combinations and individual exposure of microplastics have similar toxicity effects on the hatching process, at the evaluated size and concentrations of microplastics. The effects of combination treatments on hatch rate were also confirmed by the results of De Guzman et al. (2020), which used PE microbeads at 200 and 2000 µg/L (diameter = 300—355 µm), similar to the size of microplastics used in this assay. These findings suggest that the size, morphotype, and concentration of the microplastics affect toxicity results, as they influence microplastic penetration through the chorionic membrane of the embryo. Overall, the typical hatching pattern of zebrafish embryo was interrupted at combination treatments of Nylon 6,6 and PE, especially when both the microplastic concentrations are low, while in PS and PET combinations when the concentrations are comparatively high.

Mortality observed during the early stages of development emphasizes the ability of toxicants to disrupt the proper functioning of various organs, ultimately leading to death as a result of acute toxicity. During the assay, coagulation and lack of heartbeat were considered as toxicity endpoints. According to the literature, the mechanism behind coagulation upon toxicant exposure is not entirely clear, but it supposes that coagulation is induced by the lack of specific metabolic enzymes which are required to metabolize harmful chemicals during the early developmental stages (De Guzman et al., 2020). Disruption of the central nervous system and inhibition of acetylcholinesterase activity may also result in complications in the heart and can be observed as a lack of visible heartbeat (De Guzman et al., 2020). Individual treatments and most of the combination treatments did not have a significant effect on embryo survival at the evaluated concentrations. Confirmable results were reported by de Souza Freire et al. (2023) with no increase in mortality after PE microplastic (diameter = 53—73 µm) exposure on zebrafish. A similar lack of mortality results was reported from zebrafish with PS nanoplastic exposure, even though they were able to penetrate the chorion (Pitt et al., 2018). This is also contrary to the previous observations, in which significant mortality was observed after PE microplastic exposure (De Guzman et al., 2020; Malafaia et al., 2020), and exposure to higher concentrations of PET (100 ppm, 200 ppm) led to a decrease in the survival rate of zebrafish embryo and have an impact on liver functioning, oxidative stress, and cellular membrane integrity (Bashirova et al., 2023). Importantly, a study has shown that oxidative damage to the intestine is mainly dependent on microplastic size rather than on chemical composition, which investigated the toxic effects of nylon 6,6, polyethylene, polypropylene, polystyrene, and polyvinyl chloride microplastics (Lei et al., 2018). The combination treatments which had a significant effect on the survival were reported (in PA and PE combinations), microplastics may be able to penetrate the chorion or enter via oral uptake and cause subsequent toxicities.

The cardiovascular system is the first organ to develop during embryogenesis in vertebrates (Zaffran & Frasch, 2002). This is a complex process involving a variety of signaling molecules and cellular mechanisms (Lawson & Weinstein, 2002), that have sensitivity toward environmental pollutants that may develop cardiovascular abnormalities during early development (Manjunatha et al., 2018). In this study, heart rate was measured to investigate the potential cardiovascular toxicity of microplastic exposure on zebrafish embryos. Notably, this study found that heart rates at 72 hpf were significantly decreased by the exposure to combination treatments of nylon 6,6 with LDPE, when the concentration combination is lower, while PET and PS combination treatments did not report significant alterations at any of the concentration combinations. At lower concentration combinations, there appears to be less particle aggregation between the two polymers, which is attributed to the surface chemistry of the polymers. Under these conditions, smaller microplastics may be able to penetrate the chorion and elicit toxicity effects. Additionally, oral uptake of microplastics could potentially be greater at lower concentration combinations due to increased particle dispersion. These observations are comparable to previous findings where heart rates were significantly decreased in a dose-dependent manner and suggested that changes in heart function occur at even relatively low concentrations of microplastic exposure (Pitt et al., 2018; Zhang et al., 2020). According to collective literature abnormal heart rates- tachycardia and bradycardia are sensitive indicators of heart function and cardiovascular disease (Bhagat et al., 2020; Park & Kim, 2022; Pitt et al., 2018). Studies have shown that altered heart functionality may result directly from microplastic interaction with cardiac sarcomeres and indirectly from oxidative stress-induced responses (Pitt et al., 2018). Taken together, these results emphasize the potential cardiovascular toxicity of microplastic exposure and highlight the need for further investigations.

This study investigated three types of early developmental abnormalities including pericardial edema, yolk sac edema and spinal curvature. In this study, exposure to combination treatments did not result in significant levels of developmental abnormalities ($p \geq 0.05$), but a few yolk sac edema and spinal curvature were observed suggesting lower levels of toxicity effects. Similar results were shown in a study that exposed PS nanoplastics at 0.1, 1 and 10 ppm levels (Trevisan et al., 2019) and PE microplastics (58 µm) at 6.25, 12.5, 50, and 100 mg/L. Furthermore, contrasting observations were reported in a study which exposed PE microplastics (diameter = 300—355 µm) at 20, 200, and 2000 µg/L concentration series, showing

that number of malformities increased upon exposure concentration increment (De Guzman et al., 2020). Embryonic developmental abnormalities in zebrafish have been extensively studied and have identified osmotic balance plays a crucial role in maintaining physiological features and metabolic mechanisms. As the gills and digestive system are responsible for maintaining the osmotic balance during early development until the kidney becomes functionally active, due to the presence of microplastics in the digestive system, internal water diffusion barriers may have been negatively affected (Malafaia et al., 2020). Also, previous studies of microplastic exposures have reported reduced *cdx4 tnnc2* gene expression (Zou et al., 2020), reduced expression of cysteine-rich motor neuron 1 (*crim1*) gene or mutations in polycystine-2 (pkd2) (De Guzman et al., 2020) which may be able to cause spinal malformations such as spinal curvature. Overall, a significant effect on structural development was not observed at any combination treatments and individual treatments at the analyzed size and concentration ranges. However, the occurrence of spinal curvatures and yolk sac edema suggests that combined exposure to microplastics may have an underlying effect on the zebrafish embryos causing hypoxia, structural damage, and stress-induced responses. To understand the underlying mechanisms of developmental abnormalities due to microplastic exposure requires further investigations. Overall, combinations of nylon 6,6 (nylon 6,6) and LDPE had significant effects on embryo hatching, survival, and heart rate at lower concentration combinations, while PET and PS showed significant effects on embryo hatching at comparatively higher concentrations, suggesting that there is not a simple linear relationship between co-exposure and toxicity. Instead, it may underscore the presence of multiple factors in the response, including microplastic size, concentration, and surface chemistry.

However, prolonged consumption of contaminated shrimps could lead to potential health risks including oxidative stress, metabolic disorders, cytotoxicity, and systemic inflammation (Timilsina et al., 2023). In addition, recently microplastics have been detected in the human placenta raising concerns regarding fetal exposure (Alberghini et al., 2023). Therefore, further studies are vital for developing mitigation strategies to address the growing concerns associated with microplastic contamination and ensure the safety of seafood consumption.

5 Conclusion

This is a pioneering study reporting the ingestion of microplastics in farmed shrimps *L. vannamei* and *P. monodon* in Puttalam, Sri Lanka. The results indicated that both shrimp species are contaminated with microplastics, and their presence was observed in both the exoskeleton part and the edible soft tissue of all the analyzed samples. The average microplastic abundance in this study is greater than in previous studies, indicating a heightened level of microplastic pollution in the culture ponds. Notably, when compared to the previous studies that analyzed wild-caught seafood, farmed shrimps used in this study showed a higher microplastic level, suggesting the stagnant conditions of aquaculture ponds promote the accumulation of microplastics, resulting in higher exposure for farmed organisms compared to their wild counterparts in the open ocean. The abundance similarity of microplastics in both exoskeletons and edible soft tissue underscores the possibility of microplastic transfer to humans through consumption, even when the exoskeleton is removed. Furthermore, the study suggested that microplastics found in the edible soft tissues may be mainly accumulated in the gastrointestinal tract, highlighting the potential of microplastic transfer to humans when consuming smaller shrimps such as whiteleg shrimp, where the gut is not removed before consumption. As the microplastic level in these assessed shrimps is notably higher and owing to the anticipated rise in environmental microplastic pollution, there might be a potential threat to seafood safety in future. This study provides for the first time the potential synergistic acute toxic effects of combined microplastics using zebrafish embryos at the developmental stage. Although the tested concentrations revealed lower levels of acute toxicity in zebrafish embryos exposed to combined microplastics, the enhanced accumulation of microplastics on organisms over time may lead to concerning health issues. Future studies should aim at providing additional evidence on the synergistic acute toxicity effects of microplastic exposure, assessing different sizes, concentrations, and polymers. This highlights the importance of further research to fully understand the impacts of microplastics.

Acknowledgements The authors express their gratitude to the Director and staff of the Institute of Research and Development, for the facilities provided during the entire research

work. The authors are also grateful to Dr. Neranga Abeyasinghe for his help and support in sample analysis using Raman Spectroscopy, and the members of the Department of Physics, Department of Chemistry, University of Colombo for their help and support.

Author Contributions All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Yureshi Umanda Jayaweera, Hennayaka Mudiyanselage Amasha Induwari Hennayaka. The first draft of the manuscript was written by Yureshi Umanda Jayaweera, Hennayaka Mudiyanselage Amasha Induwari Hennayaka and Undugodage Dulanjali Rodrigo. Herath Mudiyanselage Lalinak Priyashan Bandara Herath, Gajanayake Mudalige Pradeep Kumara, Mahagama Gedara Yohan Lasantha Mahagamage, Undugodage Dulanjali Rodrigo, and Danushika Charyangi Manatunga were involved in the reviewing of the drafted manuscript. Further, Herath Mudiyanselage Lalinak Priyashan Bandara Herath, Gajanayake Mudalige Pradeep Kumara, Mahagama Gedara Yohan Lasantha Mahagamage were involved in the supervision, conceptualization, and management of the project. All authors read and approved the final manuscript.

Funding The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Data Availability The data that support the findings of this study are available in the SI as well as can be provided on request from the corresponding author (Manatunga D.C).

Declarations

Competing Interests The authors have no relevant financial or non-financial interests to disclose.

Human and Animal Studies All animal procedures were performed in accordance with the guidelines of Institute of Biology, Sri Lanka (ERC IOBSL 19907 2019).

Consent to Participate Not applicable.

Consent to Publish Not applicable.

References

Abbasi, S., Soltani, N., Keshavarzi, B., Moore, F., Turner, A., & Hassanaghaei, M. (2018). Microplastics in different tissues of fish and prawn from the Musa Estuary, Persian Gulf. *Chemosphere, 205*, 80–87. https://doi.org/10.1016/j.chemosphere.2018.04.076

Akhbarizadeh, R., Moore, F., & Keshavarzi, B. (2019). Investigating microplastics bioaccumulation and biomagnification in seafood from the Persian Gulf: A threat to human health? *Food Additives & Contaminants: Part A*, *36*(11), 1696–1708. https://doi.org/10.1080/19440049.2019.1649473

Ashrafy, A., Liza, A. A., Islam, M. N., Billah, M. M., Arafat, S. T., Rahman, M. M., & Rahman, S. M. (2023). Microplastics pollution: A brief review of its source and abundance in different aquatic ecosystems. *In Journal of Hazardous Materials Advances, 9*, 100215. https://doi.org/10.1016/j.hazadv.2022.100215

Athukorala, A., Amarathunga, A. A. D., De Silva, D. S. M., Bakir, A., McGoran, A., & Sivyer, D. (2023). Occurrence and abundance of microplastics in surface waters and sediment from the Negombo Lagoon, Sri Lanka [abstract]. In *Proceeding of the 16th International Conference on the Environmental Effects of Nanoparticles and Nanomaterials, Sri Lanka.* https://www.researchgate.net/publication/374229238

Alberghini, L., Truant, A., Santonicola, S., Colavita, G., & Giaccone, V. (2023). Microplastics in fish and fishery products and risks for human health: A review. *In International Journal of Environmental Research and Public Health, 20*(1), 789. https://doi.org/10.3390/ijerph20010789

Auta, H. S., Emenike, C. U., & Fauziah, S. H. (2017). Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions. *In Environment International, 102*, 165–176. https://doi.org/10.1016/j.envint.2017.02.013

Beckingham, B., Apintiloaiei, A., Moore, C., & Brandes, J. (2023). Hot or not: Systematic review and laboratory evaluation of the hot needle test for microplastic identification. *Microplastics and Nanoplastics, 3*, 8. https://doi.org/10.1186/s43591-023-00056-4

Bandara HMNN, De Silva HSDR, Wijayathilaka HD, Kodikara KAS, De Silva PMCS (2023) Microplastic pollution in Dondra Lagoon, Southern Coast of Sri Lanka: Influence on habitat and feeding modes of selected fish species [abstract]. In *Proceeding of the PGIS Research Congress 2023. Postgraduate Institute of Science, University of Peradeniya, Sri Lanka.* https://www.researchgate.net/publication/375893432

Bashirova, N., Poppitz, D., Klüver, N., Scholz, S., Matysik, J., & Alia, A. (2023). A mechanistic understanding of the effects of polyethylene terephthalate nanoplastics in the zebrafish (*Danio rerio*) embryo. *Scientific Reports, 13*, 1891. https://doi.org/10.1038/s41598-023-28712-y

Bhagat, J., Zang, L., Nishimura, N., & Shimada, Y. (2020). Zebrafish: An emerging model to study microplastic and nanoplastic toxicity. *In Science of the Total Environment, 728*, 128707. https://doi.org/10.1016/j.scitotenv.2020.138707

Bhathiya, M. A. M., Udagedara, S., Dissanayake, P. A. K. N., & De Silva, P. M. C. S. (2024). Determination of microplastics accumulation in seagrass beds in the Puttalam lagoon, Sri Lanka [abstract]. In *Proceeding of the National Aquatic Resources Research and Development Agency (NARA) Scientific Sessions 2023: Sustainable Fisheries for Economic Prosperity and Food Security, Colombo, Sri Lanka.* https://www.researchgate.net/publication/382877977

Bhuyan, M. S. (2022). Effects of microplastics on fish and in human health. *In Frontiers in Environmental Science, 10*, 827289. https://doi.org/10.3389/fenvs.2022.827289

Bimali Koongolla, J., Andrady, A. L., Terney Pradeep Kumara, P. B., & Gangabadage, C. S. (2018). Evidence of microplastics pollution in coastal beaches and waters in southern Sri Lanka. *Marine Pollution Bulletin*, 137, 277–284https://doi.org/10.1016/j.marpolbul.2018.10.031

Chakraborty, I., Banik, S., Biswas, R., Yamamoto, T., Noothalapati, H., & Mazumder, N. (2023). Raman spectroscopy for microplastic detection in water sources: A systematic review. *In International Journal of Environmental Science and Technology, 20*, 10435–10448. https://doi.org/10.1007/s13762-022-04505-0

Cheung, L. T. O., Lui, C. Y., & Fok, L. (2018). Microplastic contamination of wild and captive flathead grey mullet (*Mugil cephalus*). *International Journal of Environmental Research and Public Health, 15*(4), 597. https://doi.org/10.3390/ijerph15040597

Corea, A. S. L. E., Jayasinghe, J. M. P. K., Ekaratne, S. U. K., & Johnstone, R. (1995). Environmental impact of prawn farming on Dutch Canal: The main water source for the prawn culture industry in Sri Lanka. *Ambio*, 24(7), 423–427. https://www.researchgate.net/publication/279908841_Environmental_impact_of_prawn_farming_on_Dutch_Canal_the_main_water_source_for_the_prawn_culture_industry_in_Sri_Lanka

Cowger, W., Markley, L. A. T., Moore, S., Gray, A. B., Upadhyay, K., & Koelmans, A. A. (2024). How many microplastics do you need to (sub)sample? *Ecotoxicology and Environmental Safety, 275*, 116243. https://doi.org/10.1016/j.ecoenv.2024.116243

Curren, E., Leaw, C. P., Lim, P. T., & Leong, S. C. Y. (2020). Evidence of marine microplastics in commercially harvested seafood. *Frontiers in Bioengineering and Biotechnology, 8*, 562760. https://doi.org/10.3389/fbioe.2020.562760

Daniel, D. B., Ashraf, P. M., & Thomas, S. N. (2020). Abundance, characteristics and seasonal variation of microplastics in Indian white shrimps (*Fenneropenaeus indicus*) from coastal waters off Cochin, Kerala India. *Science of the Total Environment, 737*, 139839. https://doi.org/10.1016/j.scitotenv.2020.139839

Daniel, D. B., Ashraf, P. M., Thomas, S. N., & Thomson, K. T. (2021). Microplastics in the edible tissues of shellfishes sold for human consumption. *Chemosphere, 264*, 128554. https://doi.org/10.1016/j.chemosphere.2020.128554

Das, J., & Mishra, H. N. (2023). A comprehensive review of the spoilage of shrimp and advances in various indicators/sensors for shrimp spoilage monitoring. *In Food Research International, 173*, 113270. https://doi.org/10.1016/j.foodres.2023.113270

Dawson, A. L., Santana, M. F. M., Miller, M. E., & Kroon, F. J. (2021). Relevance and reliability of evidence for microplastic contamination in seafood: A critical review using Australian consumption patterns as a case study. *In Environmental Pollution, 276*, 116684. https://doi.org/10.1016/j.envpol.2021.116684

Dayal, J. S., Ponniah, A. G., Khan, H. I., Babu, E. P. M., Ambasankar, K., & Vasagam, K. P. K. (2013). Shrimps-a nutritional perspective. *Current Science*, 104(11), 1487–1491. https://www.researchgate.net/publication/287283789

De Guzman, M. C., Anne Chua, P. P., & Sedano, F. S. (2020). Embryotoxic and teratogenic effects of polyethylene microbeads found in facial wash products 2 in Zebrafish *(Danio rerio)* using the fish embryo acute toxicity test. *bioRxiv*. https://doi.org/10.1101/2020.09.16.299438

De Silva, M. L. I., Ranjula, M. A. S., Thanuja, M., Katuwawala, D. M., & Sumanapala, A. P. (2021). Review on impacts of *Litopenaeus vannamei* on aquaculture. *Wildlanka*, 9(1),149–170. https://www.researchgate.net/publication/353760282

de Souza Freire, I., Fascineli, M. L., Piau, T. B., Pereira, B. F., & Grisolia, C. K. (2023). Multilevel toxicity evaluations of polyethylene microplastics in zebrafish (*Danio rerio*). *International Journal of Environmental Research and Public Health, 20*(4), 3617. https://doi.org/10.3390/ijerph20043617

Devriese, L. I., van der Meulen, M. D., Maes, T., Bekaert, K., Paul-Pont, I., Frère, L., Robbens, J., & Vethaak, A. D. (2015). Microplastic contamination in brown shrimp (*Crangon crangon*, Linnaeus 1758) from coastal waters of the Southern North Sea and Channel area. *Marine Pollution Bulletin, 98*(1–2), 179–187. https://doi.org/10.1016/j.marpolbul.2015.06.051

Digka, N., Tsangaris, C., Torre, M., Anastasopoulou, A., & Zeri, C. (2018). Microplastics in mussels and fish from the Northern Ionian Sea. *Marine Pollution Bulletin, 135*, 30–40. https://doi.org/10.1016/j.marpolbul.2018.06.063

Ditlhakanyane, B. C., Ultra, V. U., Mokgosi, M. S., & Mokopasetso, T. (2023). Microplastic abundance in the surface water, clams (*Corbicula fluminea*), and fish species (*Oreochromis niloticus* and *Coregonus kiyi*) from the Gaborone Dam, Botswana. *Water, Air, & Soil Pollution*, *234*(9), 1–11. https://doi.org/10.1007/s11270-023-06617-w

Gaigalas, A. K., Wang, L., He, H.-J., & DeRose, P. (2009). Procedures for wavelength calibration and spectral response correction of CCD array spectrometers. *Journal of Research of the National Institute of Standards and Technology, 114*(4), 215–228. https://doi.org/10.6028/jres.114.015

Gurjar, U. R., Xavier, M., Nayak, B. B., Ramteke, K., Deshmukhe, G., Jaiswar, A. K., & Shukla, S. P. (2021). Microplastics in shrimps: A study from the trawling grounds of North Eastern part of Arabian Sea. *Environmental Science and Pollution Research, 28*(35), 48494–48504. https://doi.org/10.1007/s11356-021-14121-z

Hermsen, E., Mintenig, S. M., Besseling, E., & Koelmans, A. A. (2018). Quality criteria for the analysis of microplastic in biota samples: A critical review. *In Environmental Science and Technology, 52*(18), 10230–10240. https://doi.org/10.1021/acs.est.8b01611

Hossain, M. S., Rahman, M. S., Uddin, M. N., Sharifuzzaman, S. M., Chowdhury, S. R., Sarker, S., & Nawaz Chowdhury, M. S. (2020). Microplastic contamination in Penaeid shrimp from the Northern Bay of Bengal. *Chemosphere, 238*, 124688. https://doi.org/10.1016/j.chemosphere.2019.124688

Hurley, R. R., Lusher, A. L., Olsen, M., & Nizzetto, L. (2018). Validation of a method for extracting microplastics from complex, organic-rich, environmental matrices. *Environmental Science and Technology, 52*(13), 7409–7417. https://doi.org/10.1021/acs.est.8b01517

Iri, A. H., Shahrah, M. H. A., Ali, A. M., Qadri, S. A., Erdem, T., Ozdur, I. T., & Icoz, K. (2021). Optical detection of microplastics in water. *Environmental Science and Pollution Research*, *28*(2), 1–7. https://doi.org/10.1007/s11356-021-12358-2

IUCN. (2012). An Environmental and Fisheries Profile of the Puttalam Lagoon System. *Regional Fisheries Livelihoods Programme for South and Southeast Asia (GCP/RAS/237/SPA) Field Project Document 2011/LKA/CM/06* (*xvii*+237 pp). https://openknowledge.fao.org/handle/20.500.14283/ar443e

Jayavel, S., Govindaraju, B., Michael, J. R., & Viswanathan, B. (2024). Impacts of micro and nanoplastics on human health. *Bulletin of the National Research Centre, 48*, 110. https://doi.org/10.1186/s42269-024-01268-1

Kandeyaya, K. B. K. D. K., Ranatunga, S., & Ranatunga, R. R. M. K. P. (2023). Occurrence of microplastics in some commercially important seafood varieties from Negombo, Sri Lanka. *Regional Studies in Marine Science, 62*, 102958. https://doi.org/10.1016/J.RSMA.2023.102958

Kedzierski, M., Villain, J., Falcou-Préfol, M., Kerros, M. E., Henry, M., Pedrotti, M. L., & Bruzaud, S. (2019). Microplastics in Mediterranean Sea: A protocol to robustly assess contamination characteristics. *PLoS ONE, 14*(2), e0212088. https://doi.org/10.1371/journal.pone.0212088

Kiran, B. R., Kopperi, H., & Mohan, S. V. (2022). Micro/nano-plastics occurrence, identification, risk analysis and mitigation: Challenges and perspectives. *Reviews in Environmental Science and Bio/technology, 21*(1), 169–203. https://doi.org/10.1007/s11157-021-09609-6

Kooi, M., & Koelmans, A. A. (2019). Simplifying microplastic via continuous probability distributions for size, shape, and density. *Environmental Science and Technology Letters, 6*(9), 551–557. https://doi.org/10.1021/acs.estlett.9b00379

Korez, Š, Gutow, L., & Saborowski, R. (2020). Coping with the "dirt": Brown shrimp and the microplastic threat. *Zoology, 143*, 125848. https://doi.org/10.1016/j.zool.2020.125848

Lawan, P. L. M. J. H., De Silva, D. S. M., Amarathunga, A. A. D., McGoran, A., Bakir, A., Sivyer, D. B., & Reeve, C. (2024). Microplastic contamination in shrimps from the Negombo lagoon—Sri Lanka. *Water (Switzerland)*, 16(3). https://doi.org/10.3390/w16030447

Lawson, N. D., & Weinstein, B. M. (2002). In vivo imaging of embryonic vascular development using transgenic zebrafish. *Developmental Biology, 248*(2), 307–318. https://doi.org/10.1006/dbio.2002.0711

Lei, L., Wu, S., Lu, S., Liu, M., Song, Y., Fu, Z., Shi, H., Raley-Susman, K. M., & He, D. (2018). Microplastic particles cause intestinal damage and other adverse effects in zebrafish Danio rerio and nematode Caenorhabditis elegans. *Science of the Total Environment, 619–620*, 1–8. https://doi.org/10.1016/j.scitotenv.2017.11.103

Li, J., Lusher, A. L., Rotchell, J. M., Deudero, S., Turra, A., Bråte, I. L. N., Sun, C., Shahadat Hossain, M., Li, Q., Kolandhasamy, P., & Shi, H. (2019). Using mussel as a global bioindicator of coastal microplastic pollution. *In Environmental Pollution, 244*, 522–533. https://doi.org/10.1016/j.envpol.2018.10.032

Li, Y., Tao, L., Wang, Q., Wang, F., Li, G., & Song, M. (2023). Potential health impact of microplastics: A review of environmental distribution, human exposure, and toxic effects. *In Environment and Health, 1*(4), 249–257. https://doi.org/10.1021/envhealth.3c00052

Malafaia, G., de Souza, A. M., Pereira, A. C., Gonçalves, S., da Costa Araújo, A. P., Ribeiro, R. X., & Rocha, T. L. (2020). Developmental toxicity in zebrafish exposed to polyethylene microplastics under static and semi-static aquatic systems. *Science of the Total Environment, 700*, 134867. https://doi.org/10.1016/j.scitotenv.2019.134867

Manjunatha, B., Park, S. H., Kim, K., Kundapur, R. R., & Lee, S. J. (2018). Pristine graphene induces cardiovascular defects in zebrafish (*Danio rerio*) embryogenesis. *Environmental Pollution, 243*, 246–254. https://doi.org/10.1016/j.envpol.2018.08.058

Muller, E. B., Lin, S., & Nisbet, R. M. (2015). Quantitative adverse outcome pathway analysis of hatching in Zebrafish with CuO nanoparticles. *Environmental Science and Technology, 49*(19), 11817–11824. https://doi.org/10.1021/acs.est.5b01837

My, T. T. A., Dat, N. D., & Hung, N. Q. (2023). Occurrence and characteristics of microplastics in wild and farmed shrimps collected from Cau Hai lagoon Central Vietnam. *Molecules, 28*(12), 4634. https://doi.org/10.3390/molecules28124634

Nan, B., Su, L., Kellar, C., Craig, N. J., Keough, M. J., & Pettigrove, V. (2020). Identification of microplastics in surface water and Australian freshwater shrimp *Paratya australiensis* in Victoria Australia. *Environmental Pollution, 259*, 113865. https://doi.org/10.1016/j.envpol.2019.113865

Nava, V., Frezzotti, M. L., & Leoni, B. (2021). Raman spectroscopy for the analysis of microplastics in aquatic systems. *In Applied Spectroscopy, 75*(11), 1341–1357. https://doi.org/10.1177/00037028211043119

OECD. (2013). *OECD guidelines for the testing of chemicals: Fish Embryo Acute Toxicity (FET) Test.* https://www.oecd-ilibrary.org/environment/test-no-236-fish-embryo-acute-toxicity-fet-test_9789264203709-en

Öztekin, A., Üstün, F., Bat, L., & Tabak, A. (2024). Microplastic contamination of the seawater in the Hamsilos Bay of the Southern Black Sea. *Water, Air, & Soil Pollution*, *235*, 325. https://doi.org/10.1007/s11270-024-07138-w

Park, S. H., & Kim, K. (2022). Microplastics induced developmental toxicity with microcirculation dysfunction in zebrafish embryos. *Chemosphere, 286*, 131868. https://doi.org/10.1016/j.chemosphere.2021.131868

Parolini, M., Stucchi, M., Ambrosini, R., & Romano, A. (2023). A global perspective on microplastic bioaccumulation in marine organisms. *Ecological Indicators, 149*, 110179. https://doi.org/10.1016/j.ecolind.2023.110179

Patterson, J., Jeyasanta, K. I., Laju, R. L., & Edward, J. K. P. (2021). Microplastic contamination in Indian edible mussels (*Perna perna* and *Perna viridis*) and their environs. *Marine Pollution Bulletin, 171*, 112678. https://doi.org/10.1016/j.marpolbul.2021.112678

Paul, S., Nath, S., Bhattacharjee, S., & Mukherjee, S. (2024). Unveiling the effects of microplastics pollution on marine fauna. *Blue Biotechnology, 1*(1), 6. https://doi.org/10.1186/s44315-024-00006-6

Perez, C. N., Carré, F., Hoarau-Belkhiri, A., Joris, A., Leonards, P. E. G., & Lamoree, M. H. (2022). Innovations in analytical methods to assess the occurrence of microplastics in soil. *Journal of Environmental Chemical Engineering, 10*(3), 107421. https://doi.org/10.1016/j.jece.2022.107421

Pourebrahimi, S., & Pirooz, M. (2023). Microplastic pollution in the marine environment: A review. *Journal of Hazardous Materials Advances, 10*, 100327. https://doi.org/10.1016/j.hazadv.2023.100327

Pitt, J. A., Kozal, J. S., Jayasundara, N., Massarsky, A., Trevisan, R., Geitner, N., Wiesner, M., Levin, E. D., & Di Giulio, R. T. (2018). Uptake, tissue distribution, and toxicity of polystyrene nanoparticles in developing zebrafish (*Danio rerio*). *Aquatic Toxicology, 194*, 185–194. https://doi.org/10.1016/j.aquatox.2017.11.017

Reunura, T., & Prommi, T. O. (2022). Detection of microplastics in *Litopenaeus vannamei* (Penaeidae) and *Macrobrachium rosenbergii* (Palaemonidae) in cultured pond. *PeerJ, 10*, 12916. https://doi.org/10.7717/peerj.12916

Rodrigo, U. D., Jayawardhana, S., Jayaweera, H., Abeyasinghe, N., Fernando, V., Abeywickrama, U., ... & Gunewardene, S. (2022). Designing and construction of a custom-built Raman spectrophotometer at a modest budget to detect Chronic Kidney Disease of unknown etiology (CKDu) related biomarkers using SERS substrates. *American Chemical Society SciMeetings*, *3*(1). https://doi.org/10.1021/scimeetings.2c0026

Sasangi, W. A. J., Gunathilake, A. B. S., Kumarasiri, H. N. T. M., Jayathilake, K. G. A. H., Tissera, P. M. W. S. R., Mahagamage, M. G. Y. L., McGoran, A., Jayakody, S., & Ekanayake, M. S. (2024). Microplastic contamination in different sources of water feeding into accelerated the natural regeneration of mangroves site in the Anawilundawa Ramsar sanctuary [abstract]. In *Proceedings of the 1st International Symposium on Microplastics Pollution (SYMP 2024)* (pp. 16). Postgraduate Institute of Science, University of Peradeniya, Sri Lanka. https://www.researchgate.net/publication/386223485

Sevwandi Dharmadasa, W. L. S., Andrady, A. L., Kumara, P. B. T. P., Maes, T., & Gangabadage, C. S. (2021). Microplastics pollution in marine protected areas of Southern Sri Lanka. *Marine Pollution Bulletin, 168*, 112462. https://doi.org/10.1016/j.marpolbul.2021.112462

Shamsuyeva, M., & Endres, H. J. (2021). Plastics in the context of the circular economy and sustainable plastics recycling: Comprehensive review on research development, standardization and market. *Composites Part c: Open Access, 6*, 100168. https://doi.org/10.1016/j.jcomc.2021.100168

Thammatorn, W., & Palić, D. (2022). Potential risks of microplastic fomites to aquatic organisms with special emphasis on polyethylene-microplastic-glyphosate exposure case in aquacultured shrimp. *Applied Sciences (Switzerland), 12*(10), 5135. https://doi.org/10.3390/app12105135

Timilsina, A., Adhikari, K., Yadav, A. K., Joshi, P., Ramena, G., & Bohara, K. (2023). Effects of microplastics and nanoplastics in shrimp: Mechanisms of plastic particle and contaminant distribution and subsequent effects after uptake. *In Science of the Total Environment, 894*, 164999. https://doi.org/10.1016/j.scitotenv.2023.164999

Trevisan, R., Voy, C., Chen, S., & Di Giulio, R. T. (2019). Nanoplastics decrease the toxicity of a complex PAH mixture but impair mitochondrial energy production in developing zebrafish. *Environmental Science & Technology, 53*(14), 8405. https://doi.org/10.1021/ACS.EST.9B02003

Vital, S. A., Cardoso, C., Avio, C., Pittura, L., Regoli, F., & Bebianno, M. J. (2021). Do microplastic contaminated seafood consumption pose a potential risk to human health? *Marine Pollution Bulletin, 171*, 112769. https://doi.org/10.1016/j.marpolbul.2021.112769

Vitheepradit, A., & Prommi, T. O. (2023). Microplastics in surface water and tissue of white leg shrimp, *Litopenaeus vannamei,* in a cultured pond in Nakhon Pathom Province Central Thailand. *AIMS Environmental Science, 10*(4), 478–503. https://doi.org/10.3934/environsci.2023027

Vo, V. C., & Tran, T. B. H. (2022). Microplastic fiber accumulation in some wild and cultured shrimp species. *Dalat University Journal of Science,* 13(2), 52–62. https://doi.org/10.37569/dalatuniversity.13.2.1061(2023)

Wakkaf, T., El Zrelli, R., Kedzierski, M., Balti, R., Shaiek, M., Mansour, L., Tlig-Zouari, S., Bruzaud, S., & Rabaoui, L. (2020a). Characterization of microplastics in the surface waters of an urban lagoon (Bizerte lagoon, Southern Mediterranean Sea): Composition, density, distribution, and influence of environmental factors. *Marine Pollution Bulletin, 160*, 111625. https://doi.org/10.1016/j.marpolbul.2020.111625

Wakkaf, T., El Zrelli, R., Kedzierski, M., Balti, R., Shaiek, M., Mansour, L., Tlig-Zouari, S., Bruzaud, S., & Rabaoui, L. (2020b). Microplastics in edible mussels from a southern Mediterranean lagoon: Preliminary results on seawater-mussel transfer and implications for environmental protection and seafood safety. *Marine Pollution Bulletin, 158*, 111355. https://doi.org/10.1016/j.marpolbul.2020.111355

Wang, J., Li, Y., Lu, L., Zheng, M., Zhang, X., Tian, H., Wang, W., & Ru, S. (2019). Polystyrene microplastics cause tissue damages, sex-specific reproductive disruption and transgenerational effects in marine medaka (*Oryzias melastigma*). *Environmental Pollution, 254*, 113024. https://doi.org/10.1016/J.ENVPOL.2019.113024

Wang, W., Ge, J., & Yu, X. (2020). Bioavailability and toxicity of microplastics to fish species: A review. *Ecotoxicology and Environmental Safety, 189*, 109913. https://doi.org/10.1016/j.ecoenv.2019.109913

Wang, X., Bolan, N., Tsang, D. C. W., Sarkar, B., Bradney, L., & Li, Y. (2021). A review of microplastics aggregation in aquatic environment: Influence factors, analytical methods, and environmental implications. *Journal of Hazardous Materials, 402*, 123496. https://doi.org/10.1016/j.jhazmat.2020.123496

Wang, X., Zhu, L., Liu, K., & Li, D. (2022). Prevalence of microplastic fibers in the marginal sea water column off southeast China. *Science of the Total Environment, 804*, 150138. https://doi.org/10.1016/j.scitotenv.2021.150138

Yee, M. S. L., Hii, L. W., Looi, C. K., Lim, W. M., Wong, S. F., Kok, Y. Y., Tan, B. K., Wong, C. Y., & Leong, C. O. (2021). Impact of microplastics and nanoplastics on

human health. *In Nanomaterials, 11*(2), 1–23. https://doi.org/10.3390/nano11020496

Yoon, H., Park, B., Rim, J., & Park, H. (2022). Detection of microplastics by various types of whiteleg shrimp (*Litopenaeus vannamei*) in the Korean Sea. *Separations,* 9(11). https://doi.org/10.3390/separations9110332

Zaffran, S., & Frasch, M. (2002). Early signals in cardiac development. *In Circulation Research, 91*(6), 457–469. https://doi.org/10.1161/01.RES.0000034152.74523.A8

Zhang, R., Wang, M., Chen, X., Yang, C., & Wu, L. (2020). Combined toxicity of microplastics and cadmium on the zebrafish embryos (*Danio rerio*). *Science of the Total Environment, 743*, 140638. https://doi.org/10.1016/j.scitotenv.2020.140638

Zhang, Y. Q., Lykaki, M., Alrajoula, M. T., Markiewicz, M., Kraas, C., Kolbe, S., Klinkhammer, K., Rabe, M., Klauer, R., Bendt, E., & Stolte, S. (2021). Microplastics from textile origin-emission and reduction measures. *In Green Chemistry, 23*(15), 5247–5271. https://doi.org/10.1039/d1gc01589c

Zhao, J., Rao, B. Q., Guo, X. M., & Gao, J. Y. (2021). Effects of microplastics on embryo hatching and intestinal accumulation in larval Zebrafish *Danio rerio*. *Huanjing Kexue/Environmental Science*, 42(1), 485–491. https://doi.org/10.13227/J.HJKX.202003199

Zhou, N., Wang, Z., Yang, L., Zhou, W., Qin, Z., & Zhang, H. (2023). Size-dependent toxicological effects of polystyrene microplastics in the shrimp *Litopenaeus vannamei* using a histomorphology, microbiome, and metabolic approach. *Environmental Pollution, 316*, 120635. https://doi.org/10.1016/J.ENVPOL.2022.120635

Ziani, K., Ioniţă-Mîndrican, C. B., Mititelu, M., Neacşu, S. M., Negrei, C., Moroşan, E., Drăgănescu, D., & Preda, O. T. (2023). Microplastics: A real global threat for environment and food safety: A state of the art review. *In Nutrients, 15*(3), 617. https://doi.org/10.3390/nu15030617

Zou, W., Xia, M., Jiang, K., Cao, Z., Zhang, X., & Hu, X. (2020). Photo-oxidative degradation mitigated the developmental toxicity of polyamide microplastics to Zebrafish larvae by modulating macrophage-triggered proinflammatory responses and apoptosis. *Environmental Science and Technology, 54*(21), 13888–13898. https://doi.org/10.1021/acs.est.0c05399