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Uptake and retention of microplastics in grass shrimp

SIZE- AND SHAPE-DEPENDENT EFFECTS OF MICROPLASTIC PARTICLES ON ADULT DAGGERBLADE GRASS SHRIMP (*PALAEMONETES PUGIO*)

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Abstract

The incidence of microplastics in marine environments has been increasing over the past several decades. The objective of the present study was to characterize the size- and shape-dependent effects of microplastic particles (spheres, fibers, and fragments) on the adult daggerblade grass shrimp (*Palaemonetes pugio*). Grass shrimp were exposed to 11 sizes of plastic: spheres (30, 35, 59, 75, 83, 116, and 165 μm), fragments (34 and 93 μm), and fibers (34 and 93 μm) at a concentration of 2000 particles/400 mL (= 50 000 particles/L) for 3 h. Following exposure, grass shrimp were monitored for survival, ingested and ventilated microplastics, and residence time. Mortality ranged from 0% to 55%. Spheres and fragments <50 μm were not acutely toxic. Mortality rates in experiments with spheres and fragments >50 μm ranged from 5% to 40%. Mortality was significantly higher in the exposure to 93-μm fibers than other sizes tested ($p < 0.001$). The shape of the particle had a significant influence on the number of particles ingested by the shrimp ($p < 0.001$). The residence time of particles in the gut ranged from 27 to 75 h, with an average of 43.0 ± 13.8 h. Within the gills, the residence time ranged from 27 to 45 h, with an average of 36.9 ± 5.4 h. The results suggest that microplastic particles of various sizes and shapes can be ingested and ventilated by adult daggerblade grass shrimp, resulting in acute toxicity.

Keywords: Ingestion, Fiber, Ventilation, Depuration, Residence time

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INTRODUCTION

Plastic debris, in general, is one of the most abundant and persistent contaminants in the marine environment [1]. Approximately 80% of all plastic debris in the marine and coastal environment are from land-based sources, including street litter washed or blown into nearby waterways, public littering, inadequately covered containers, sewage treatment and combined sewer overflows, and people using the seas for recreational fishing [2]. Current estimates suggest that, at a minimum, there are 5.25 trillion plastic particles weighing 268 940 tons afloat in the sea [3]. Plastics fragment in the environment as a consequence of photolytic, mechanical, and biological degradation, resulting in the creation of microplastic particles [4]. This is particularly relevant in estuaries, where physical and biological factors collectively weaken the surface of plastic debris, resulting in the production of microplastic particles in as few as 8 wk [5]. Other microplastic particles can enter estuarine waters through municipal effluents, including spheres (microbeads) found in skin exfoliants and toothpastes [6] as well as fibers produced during the laundering of polyester clothing [7]. In estuarine systems, microplastic concentrations have been reported as high as thousands of particles per cubic meter in surface water [8] and thousands of particles per kilogram in sediment [9]. There is clear evidence that their abundance is increasing [4]. The vast quantity of discarded plastic debris accumulating in marine ecosystems represents an emerging issue that may be capable of destabilizing the earth's normal function at a global scale [10].

Because microplastics occupy the same size fraction as both sediment and planktonic prey items for lower trophic organisms, the potential exists for these particles to be ingested by both benthic and pelagic biota using a wide variety of feeding strategies (reviewed by Wright et

al. [11]). For estuarine species, laboratory studies have demonstrated microplastic ingestion by mollusks, including the eastern oyster (*Crassostrea virginica*), quahogs (*Mercenaria mercenaria*), and the Atlantic sea scallop (*Placopecten magellanicus*) [12–14], and by crustaceans, including the shore crab (*Carcinus maenas*) [15] and copepods (*Acartia tonsa* and *Eurytemora affinis*) [16]. In addition to ingestion, other routes of microplastic uptake are possible. For example, shore crabs (*Carcinus maenas*) take up microplastic particles through inspiration across the gill chamber, where particles adhere to the external surface of the gills for up to 21 d following an aqueous exposure [14].

There is a limited body of literature concerning the potential adverse effects resulting from microplastic exposure and uptake in invertebrates. Some studies report that ingested microplastic particles pass through the stomach and intestines of individuals, with little or no observed adverse impacts [17,18]. Other studies suggest that ingested microplastics cause physical disturbances in the digestive system, suppressing feeding as a result of false satiation. For example, exposure to polystyrene spheres resulted in significantly reduced feeding by the copepod *Centropages typicus* [6]. Freshwater amphipods, *Hyalella azteca*, ingesting polyethylene spheres and polypropylene fibers in chronic assays exhibited less growth than controls as a result of a reduction in the egestion of food materials [19]. In 10-d acute toxicity tests, ingested polypropylene fibers were more toxic to the amphipods than the polyethylene spheres [19]. The presence of unplasticized polyvinylchloride at sediment concentrations overlapping those found in the environment resulted in up to a 50% depletion of energy reserves in marine lugworms (*Arenicola marina*) as a result of reduced feeding activity, longer gut residence times, and inflammation [11]. More recently, ingested microplastic polystyrene

particles have been shown to negatively impact energy uptake by adult oysters (*Crassostrea gigas*), resulting in reproductive disruption and developmental effects in offspring [20].

The aim of the present study was to investigate the residence time of microplastic particles in the gut (ingestion) and gills (ventilation) of grass shrimp (*Palaemonetes pugio*). To investigate residence time in the grass shrimp, we exposed the shrimp to various sizes and shapes of microplastic particles to determine the influence various sizes and shapes had on ingestion or ventilation. A secondary objective was to quantify any acute effects of microplastic exposure on grass shrimp. Grass shrimp are an abundant and ubiquitous inhabitant of vegetated estuaries (e.g., salt marshes, mangroves, and seagrass beds) along the eastern coast of North America (reviewed by Key et al. [21]). In its juvenile and adult life stages, it is epibenthic, preferring shallow soft-bottom areas such as sand and mud, where studies have shown microplastic particles accumulate [14,22]. This suggests that grass shrimp, at least in some life cycle stages, are exposed to microplastics.

The usefulness of the grass shrimp in ecotoxicological testing, both in the laboratory and in the field, has been long recognized (reviewed by Key et al. [21]). Grass shrimp are sensitive to a wide variety of estuarine pollutants, including pesticides [21], metals [23], petroleum [24], flame retardants [25], PAHs [26], and contaminant mixtures [27]. Because of their abundance, sensitivity, and ecological importance in southeastern US estuaries, grass shrimp are a useful indicator species to study the effects of pollution.

MATERIALS AND METHODS

Grass shrimp were collected using a dip net from Leadenwah Creek (32.6367°N, 80.2017°W) on Wadmalaw Island, SC, USA. This creek is an unimpacted reference site and a

long-term ecological monitoring site for numerous studies [28,29]. Shrimp were then transferred to the laboratory, where they were held in 5-L glass aquaria containing carbon-filtered brackish water (approximately 28 ppt salinity) at a water temperature of 23 °C. Shrimp were acclimated to laboratory conditions for at least 3 d prior to use in experimental assays (described in the section *Particle exposure assays*).

Shrimp were fed brine shrimp nauplii (*Artemia salina*) twice per day; however, they were not fed 24 h prior to being introduced into the assays. Adult grass shrimp of similar size were selected for the experiment.

Microplastic particles

Eleven different microplastic particles were tested, representing a range of shapes (spheres, fragments, fibers) and sizes (30–165 µm) (Table 1). Fluorescent green polyethylene spheres (sizes 32–38, 53–63, 75–90, 106–125, 150–180 µm) were purchased from Cospheric. Opaque polystyrene spheres (30 and 75 µm) were purchased from Phosphorex. Plastics that were purchased from Cospheric and Phosphorex did not come in any detergent or antimicrobial solutions. The purchased plastics came in dry form with no additives. White polypropylene fragments were obtained by sieving a polypropylene homopolymer powder purchased from TWO H Chem. Fragments within the powder had an average particle size of 40 µm. To obtain size fractions of 30 to 38 µm and 80 to 105 µm for the assays described below, the powder was sieved with deionized water and dried overnight on aluminum foil. Black polypropylene fibers were produced in the laboratory using weathered marine rope that had been sitting on an area of unshaded concrete for 3 yr (obtained from S. Au, Clemson University, Clemson, SC). Previous studies have characterized the physical properties of the rope and have confirmed that it was

composed of polypropylene [19]. The rope was cut into the smallest possible lengths with sharp scissors, then separated into the desired size fractions of 30 to 38 µm and 80 to 105 µm using sieves. For all microplastic particles, only the midpoint values within the range were used to report the results.

Particle exposure assays

Particle exposure assays were performed for the following shapes and size fractions: spheres: 30, 35, 59, 75, 83, 116, and 165 µm; fragments: 34 and 93 µm; fibers 34 and 93 µm. Each assay consisted of 40 grass shrimp. Twenty grass shrimp were exposed individually in 600-mL beakers to a nominal concentration of 50 000 particles/L for 3 h, then rinsed and transferred to particle-free brackish water for a 96-h depuration procedure. The other 20 grass shrimp served as the controls and were exposed to the same conditions described, with the exception that the initial 3-h exposure was in particle-free water and depuration was monitored over 96 h. Controls were conducted with each particle assay (total 11 controls). Water quality, measured before and after each assay, did not vary among the various particles tested: dissolved oxygen = 7.39 ± 0.30 mg/L, pH = 7.52 ± 0.09, and salinity = 30 ± 2.45 ppt. Temperature was held constant at 23 °C.

Test chambers consisted of 600-mL beakers containing 400 mL of solution. Particle concentrations for the initial exposures were determined based on weight. For each assay, the average weight of 3 samples consisting of 100 particles each was obtained using an analytical microbalance (CAHN C-33 Microbalance). This average weight was then used to determine the weight of 20 000 particles. To suspend the particles in the brackish water, particles were mixed with ethanol (1 mL), then introduced into the brackish water just prior to the introduction of shrimp. Ethanol was also added to the controls (1 mL). For the fragments and fibers, particles

were kept in suspension during the assays by using gentle aeration. Assays containing spheres did not have gentle aeration because of the particles being neutrally buoyant in the water.

Following the initial 3-h exposure and again every 24 h throughout the remainder of the 96-h assay, the number of particles in the gut and respiratory chambers was determined using a dissecting scope. Mortality was recorded daily. The water was changed at 48 h, and grass shrimp were fed brine shrimp nauplii 1 h after the initial exposure and twice a day thereafter.

Statistics

The influence of size and shape of plastic particles on the number of particles ingested, ventilated, and their residence times were assessed using the general linear model function of SAS Enterprise 4.3. The general linear model was selected because of its flexibility in handling data that do not meet the assumptions of normality. Differences among treatments were determined using least square mean comparisons with a Bonferroni correction. To assess statistical differences in the residence time of plastics, a general linear model was applied with a Ryan-Einot-Gabriel-Welsch multiple range test. The effect of size and shape of plastic particles on mortality was assessed using a Bernoulli (binomial) distribution. Under this distribution, 1 represented survival and 0 represented death. This was also assessed using a general linear model, where differences were determined using Duncan's multiple range test with a Bonferroni correction. Significance was identified at $p < 0.05$.

RESULTS

Microplastic uptake

Most grass shrimp that were exposed to microplastics both ingested and ventilated the particles during the 3-h exposure period (Table 1 and Figure 1). The number of particles found

within the gut following the 3-h exposure period ranged from 2.3 ± 1.7 particles/shrimp in the 93-µm fiber exposure to 28.8 ± 26.4 particles/shrimp in the 75-µm sphere exposure (Figure 2A,B). Regarding shape, the number of fragments (22.23 ± 9.57 particles/shrimp) within the gut was significantly higher than the number of spheres and fibers, and the number of spheres within the gut (9.0 ± 13.55 particles/shrimp) was significantly higher than the number of fibers (4.12 ± 6.27 particles/shrimp) ($p < 0.001$; Figure 3). Within the gills, the numbers of fragments (9.26 ± 7.39 particles/shrimp) and spheres (7.31 ± 7.84 particles/shrimp) were significantly higher than that of fibers (2.22 ± 2.15 particles/shrimp) ($p < 0.001$; Figure 3). Significantly higher levels of particles were ingested by shrimp exposed to the 340- and 93-µm fragment exposures and the 75-µm sphere exposure, whereas the lowest levels of particle ingestion occurred in those shrimps exposed to the 35-, 116-, and 165-µm spheres and the 93-µm fibers (Figure 2). The number of particles found within the gills following the 3-h exposure period ranged from 1.3 ± 1.6 particles/shrimp in the 75-µm sphere exposure to 12.5 ± 9.4 particles/shrimp in the 116-µm sphere exposure (Figure 2B). Significantly higher levels of particles were ventilated by shrimp exposed to 59-, 83-, and 116-µm spheres and the 34-µm fragments, whereas the lowest levels of particle ventilation occurred in those shrimps exposed to 38- and 75-µm spheres and the 34- and 93-µm fibers (Figure 2B). Ten of the 11 treatments had at least 80% of ingested plastics present in guts, and 9 of the 11 had at least 85% particles present in the gills after exposure (Table 2).

Microplastic residence time

Residence times for microplastic particles in the digestive tract of grass shrimp ranged from 27.6 to 76.0 h with an average across all treatments of 43.0 ± 13.8 h (Figure 2C). Residence times were a function of size ($p < 0.001$), with the longest residence times observed for the 75-

µm spheres (75.9 ± 13.3 h) and the 30-µm spheres (60.6 ± 28.5 h). The shortest residence time was observed for the 116-µm spheres (27.6 ± 8.57 h). There was no significant difference observed in residence time based on shape for the digestive tract ($p = 0.0564$, $n = 192$; Figure 3C).

Residence times for microplastic particles in the gills ranged from 27.3 to 45.6 h, with an average across all treatments of 36.9 ± 5.4 h (Figure 2D). Residence times in the gill chambers were also a function of size ($p < 0.001$), with the longest residence time observed for the 75-µm spheres (45.6 ± 23.9 h). The shortest residence time was observed for the 116-µm spheres (27.27 ± 8.24 h). There were no significant differences observed in the residence time based on shape ($p = 0.6734$, $n = 192$) (Figure 3D).

Acute toxicity

At the time of death, microplastics were present in the gut, the gills, or both (Table 3). Following the initial 3-h exposure period, morality across the various treatments ranged from 0% to 55% (Figure 4). Mortality of the control shrimp (those not exposed to microplastic) was 0% in all assays. Some shrimp that died (19% overall) were unable to clear microplastic particles from their gut during the 3-h initial exposure. Across all treatments, mortality was a function of particle size ($p < 0.001$) but not shape ($p = 0.1547$). For those treatments involving spheres, shrimp exposed to the smallest size fractions (30 and 38 µm) experienced 0% mortality, whereas shrimp exposed to size fractions ≥75 µm experienced mortality ranging from 20% to 40%. A similar pattern was observed with those treatments involving fragments. Shrimp exposed to the smallest size fraction (34 µm) experienced 0% mortality. Shrimp exposed to the 93-µm fragments experienced 20% mortality. By contrast, all size fractions of fibers resulted in

mortality, ranging from 35% to 55%.

DISCUSSION

Previous studies have investigated microplastic ingestion by mollusks, including eastern oysters (*Crassostrea virginica*), quahogs (*Mercenaria mercenaria*), and Atlantic sea scallops (*Placopecten magellanicus*) [12–14], and crustaceans, including shore crabs (*Carcinus maenas*) [15] and copepods (*Acartia tonsa* and *Eurytemora affinis*) [16]. Results from previous studies have demonstrated that some marine species can select for certain sizes of microplastics, which in turn can be transferred into the food web via lower trophic organisms to higher trophic organisms. These smaller sizes of microplastics, identified as nanoplastics, can have longer gut retention than larger ones and pose a potential toxicological risk as they have been shown to be taken up in nonfilter-feeding species via ventilation into the gills. The aim of the present study was to assess whether grass shrimp would ingest and ventilate microplastic particles when exposed and the residence time of these microplastics once ingested or ventilated. Grass shrimp ingested microplastics of all sizes and shapes to which they were exposed during the assay, and residence times of the microplastic particles varied. An interesting finding we did not expect with ingestion was the associated mortality in the shrimp during the 3-h exposure and 96-h depuration procedure. In grass shrimp, acute exposure (3 h) to a wide range of microplastic sizes and shapes resulted in mortality. Regarding shape, ingested fibers were more toxic to shrimp, particularly those fibers <50 μm in length. The increased toxicity of these smaller fibers may be related to inability to completely egest this type of particle. Murray and Cowie [30] found that lobsters (*Nephrops norvegicus*) were unable to completely egest polypropylene fibers, leading to the retention of fibers within the foregut of the animal. Retained fibers may become entangled

within the intestinal tract over time, leading to a nonbiodegradable gut blockage, resulting in death [23]. It is also possible that toxicity related to fiber ingestion is the result of internal structures becoming damaged as the entangled fibers pass through the gut.

Results of the present study are consistent with previous studies involving the amphipod *Hyalella azteca* [19], where microplastic fibers were more toxic than spheres. In studies involving *Daphnia magna* exposed to a wide range of fiber sizes (length range 62–1400 µm, width 31–528 µm, thickness 1–21.5 µm), mortality was dependent on both the feeding regime and concentration [31]. Similar to the results found in the present study, Jemec et al. [31] found that ingested fibers were more toxic to *D. magna* following a period of starvation. Reduced toxicity following feeding is likely the result of reduced feeding activity and consequently a reduction in the amount of ingested fibers. In the present study, grass shrimp were exposed to microplastic particles following a period of starvation. Providing food prior to the microplastic exposures in the present study could have resulted in a reduction in the observed toxicity. In the field, grass shrimp and other organisms are likely to encounter periods of both high and low food abundances, making the study of both fed and starved conditions environmentally relevant. The grass shrimp being exposed for 3 h as part of the experimental design was to assess the uptake and residence time of plastics once ingested. The mortality was unexpected considering such a short exposure time; however, it does provide insight into the potential effects chronic exposure may have on grass shrimp in the environment.

Particle size has also been found to be a critical determinant in toxicity [32,33]. Researchers found that the optimum size class of plastic for filter-feeder ingestion appears to be <1 mm in diameter [33]. In grass shrimp, exposure to spheres and fibers resulted in no

discernable trends in acute mortality across the size fractions tested (30–165 μm diameter). It is interesting to note that all spheres and fragments ≤75 μm in diameter had similar mortality, despite differences in the amount of particles being ingested and ventilated. This demonstrates that microplastic particles of certain shapes and sizes can have similar acute effects on grass shrimp and possibly other marine organisms.

Another noteworthy finding was the ability of grass shrimp to inspire microplastic particles and have them adhere to their gill filament. These findings are similar to those reported by Watts et al. [15], who investigated the uptake and retention of microplastic particles in the green crab (*Carcinus maenas*). The residence time for removal of microplastics in the gills of green crabs differed from that found for grass shrimp. Residence times in green crabs ranged from 2 to 3 wk, whereas in grass shrimp residence times ranged from 27 to 45 h (36.9 ± 5.4 h), suggesting that grass shrimp are able to remove particles faster. In the present study, we were unable to ascertain the relative contribution of the presence of these particles in the gills to the overall toxicity of microplastics. Certainly, further research regarding the adverse effects of the presence of these particles in the gills of invertebrates is warranted.

In the present study, there were no discernable trends in the residence times of the various sizes and shapes of tested particles in the gut of grass shrimp. By contrast, Au et al. [19] found that fibers had longer clearance times than spheres, which was attributed to the shape of the plastic particle. Limited evidence in the present study suggests that polymer composition of microplastic spheres may influence residence time in grass shrimp. Polystyrene spheres with a 30- or 75-μm diameter had significantly longer residence time than similarly sized polyethylene spheres. Such differences in residence time may be attributed to interactions between the spheres

themselves (aggregation within the gut) or between the spheres and the surface lining the gut. In any event, prolonged resident times of spheres in the gut can result in decreased food consumption because of false satiation, leading to reduced fitness.

One limitation of the present study is the exposure time of the assays. Although we have demonstrated uptake with a short exposure time, it is important to consider the potential chronic effects of such prolonged exposures, including alterations of the energetics of the grass shrimp resulting in reduced growth, development, reproduction, and fitness. For example, microplastic exposure to the marine copepod *Acartia tonsa* resulted in microplastics adhering to appendages and hindering locomotion [6,34]. Furthermore, the ingested particles led to a decrease in ingestion rate, growth, and fecundity, probably because of energetic deficiencies [6,34]. Recent studies have shown that further degradation of plastic debris leads to even smaller particles, called "nanoplastics," which have the ability to translocate across the gut epithelium into the tissues of the organism [35,36].

Environmental relevance

The concentrations of microplastics tested in the present study (50 000 particles/L) were higher than the maximum concentrations found in the intertidal sediments of Charleston Harbor (2524 particles/m^2) [14]. However, the levels tested in the present study are on the same order of magnitude as the highest levels reported in the literature for estuarine sediments (92 217 particles/m^2) and surface water (132 particles/L) **<ZAQ;1>**[37]. It is also important to note that concentrations of microplastics in water near intertidal areas are occasionally likely to be higher than those reported for other habitats as a result of their resuspension through wave action caused by natural phenomena (e.g., wind) and anthropogenic factors (e.g., wakes from boating activity).

Grass shrimp inhabit intertidal, salt marsh habits and are epibenthic in nature. As such, they are likely to be exposed to microplastics. Eriksen et al. [3] estimated that there are more than 5.25 trillion plastic particles around the world that are currently afloat in the sea, not including the number of particles that are suspended in the water column or in the sediment. Because the concentrations of microplastic particles are likely to increase with time, understanding the hazard that they pose to invertebrates, such as shrimp, is critical in an overall risk assessment of these particles in coastal habitats.

CONCLUSION

The present study demonstrates that grass shrimp ingest and ventilate microplastic particles upon exposure. Ventilation as a route of uptake of microplastics in decapod crustaceans has received little attention, and assessing their contribution to overall toxicity following exposure warrants further investigation. The residence times of these microplastics in grass shrimp vary and may be influenced by the ability of individual grass shrimp to remove the particles. Nonetheless, the present results demonstrate that exposure to a wide variety of microplastic sizes and shapes in grass shrimp results in acute toxicity. Although not addressed in the present study, chronic toxicity resulting from exposure may also be of concern. Rochman et al. [38] suggested that exposure during critical early life stages of organism development may impair reproductive success and harm wildlife populations. Grass shrimp play a critical role in energy transfer within their respective habitats, and exposure to microplastics has the potential to negatively impact their population.

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Data availability—Data, associated metadata, and calculation tools are available on request (adgray2@uncg.edu).

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Figure 1. Light micrographs of adult grass shrimp exposed to fluorescent green polyethylene spheres (83 µm). Yellow arrows indicate microplastic particles present in the gut (**A**, **B**) and gills (**B**).

Figure 2. Graphs depicting the influence of size on average number of particles ingested (**A**), average number of particles ventilated (**B**), average residence time in the gut (**C**), and average

residence time in the gills (**D**). Bars containing the same letters are not significantly different. Error bars represent +1 standard deviation.

Figure 3. Graphs depicting the influence of size on average number of particles ingested (**A**), average number of particles ventilated (**B**), average residence time in the gut (**C**), and average residence time in the gills (**D**). Bars without letters represent no significant difference. Error bars represent +1 standard deviation.

Figure 4. Graph depicting mortality between the sizes (11) and shapes (3) of microplastics tested. Mortality was recorded as a percentage.

<<ENOTE>> AQ1: Ref 38 was deleted because it was a copy of ref 37; ref 39 was renumbered as 38. Citation in text also renumbered.

<<ENOTE>> AQ2: Ref 29: Please complete entry: year, book editors, publisher, publisher location.

Table 1. Overview of designated size, shapes, and polymers used in the present study

Size (μm)	Shape	Polymer type
30	Sphere	PS
34	Fragment	PP
34	Fiber	PP
35	Sphere	PE
59	Sphere	PE
75	Sphere	PS

83	Sphere	PE
93	Fragment	PP
93	Fiber	PP
116	Sphere	PE
165	Sphere	PE

PE = polyethylene; PP = polypropylene; PS = polystyrene.

Table 2. Table comparison of shrimp (n = 20) from each treatment[a]

Particle type (size/shape/polymer)	Both gut and gill (%)	Gut (%)	Gill (%)
30/sphere/PS	100	0	0
34/fiber/PP	80	15	5
34/fragment/PP	95	5	0
35/sphere/PE	100	0	0
59/sphere/PE	90	0	10
75/sphere/PS	50	50	0
83/sphere/PE	85	15	0
93/fiber/PP	75	10	15
93/fragment/PP	90	10	0
116/sphere/PE	75	5	15
165/sphere/PE	85	5	10

[a] The table displays microplastic particles present in both the gut and the gills, the gut only, and the gills only following the initial exposure.

PE = polyethylene; PP = polypropylene; PS = polystyrene.

Table 3. Comparison of treatments at the end of the study that had mortality with microplastic particles present in the gills and guts and those with no particles present at time of death

Particle type size (μm)/shape/polymer	Percent dead with plastics in gut	Percent dead with plastics in gills	Percent dead with no plastics
30/sphere/PS	N/A	N/A	N/A
34/fiber/PP	100	100	0
34/fragment/PP	N/A	N/A	N/A
35/sphere/PE	N/A	N/A	N/A
59/sphere/PE	100	100	0
75/sphere/PS	0	0	100
83/sphere/PE	37.5	62.5	37.5
93/fiber/PP	42.85	42.85	57
93/fragment/PP	75	100	0
16/sphere/PE	60	60	20
165/sphere/PE	50	50	50

N/A = not applicable (applied to treatments that had no mortality at the end of the study); PE = polyethylene; PP = polypropylene; PS = polystyrene.

Figure 1.

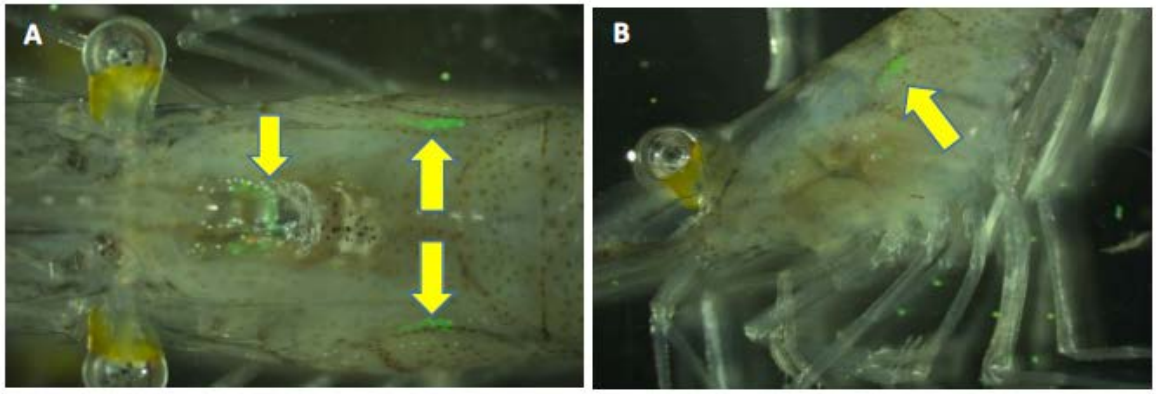

Figure 2.

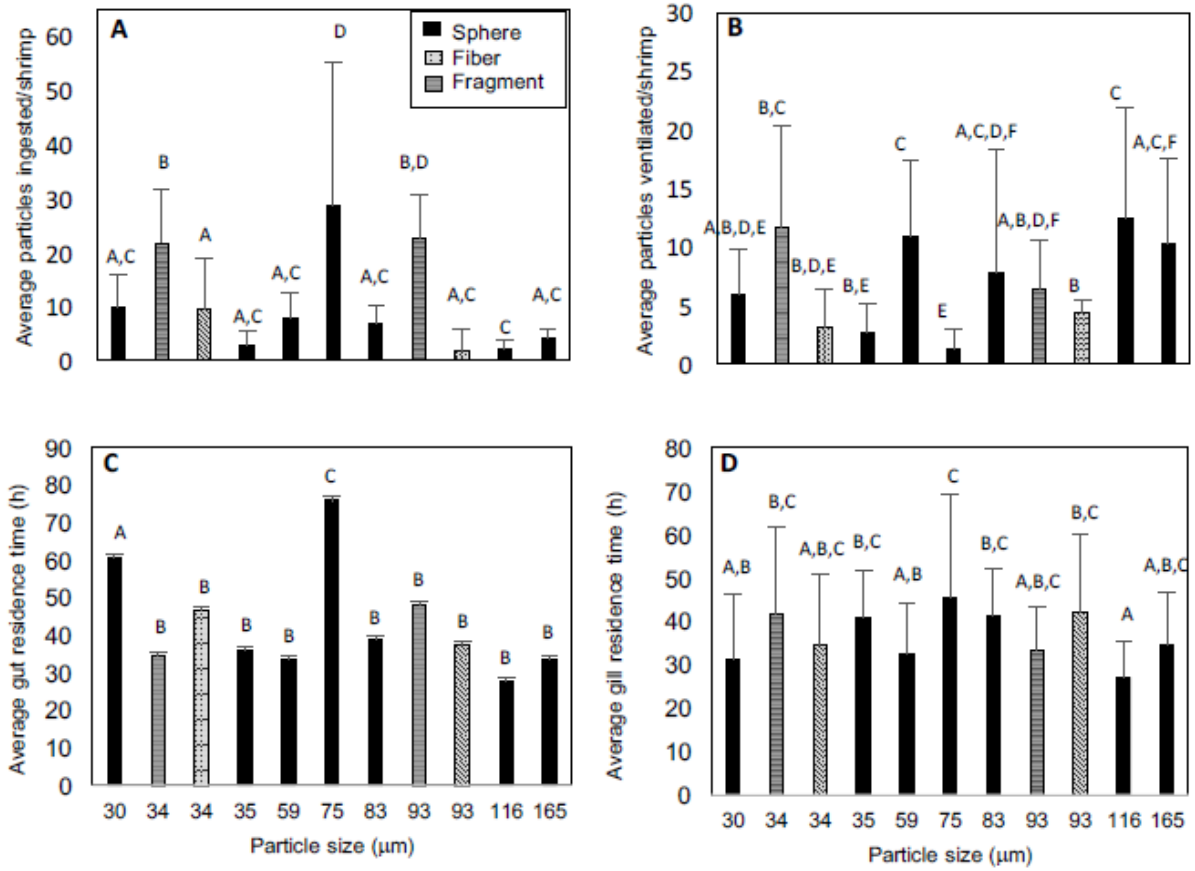

Figure 3.

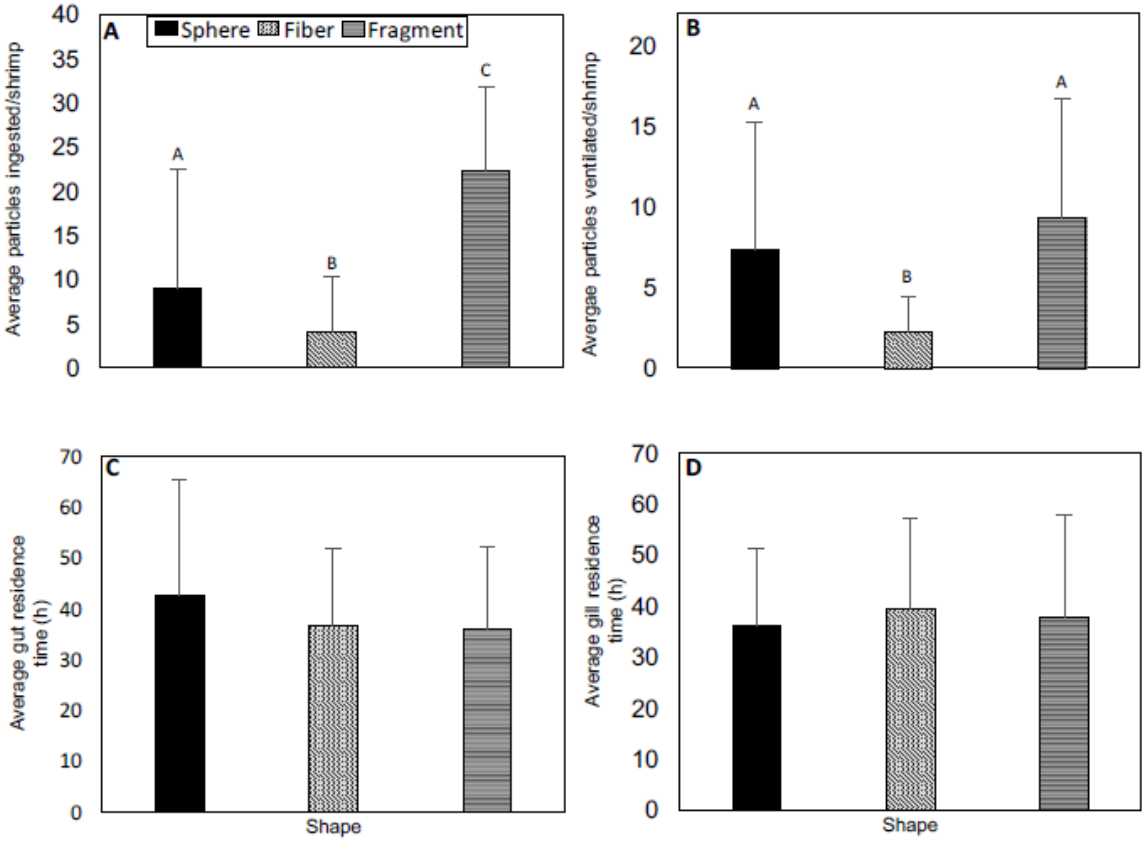

Figure 4.