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SPECIAL ISSUE-LETTER

Effects of environmentally relevant concentrations of microplastic fibers on Pacific mole crab (*Emerita analoga*) mortality and reproduction

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Scientific Significance Statement

Microplastics are ubiquitous in marine and sandy beach environments, posing a significant threat to the marine organisms that reside therein. The most predominant classification of microplastics found have been microfibers. Although a number of biological effects of microplastics have been measured, with documented effects on growth, little research has examined how microplastic fibers affect reproductive output and subsequent development of offspring. We examined the effects of exposure to microfibers on adult mortality, reproductive output, and embryonic development of the filter feeding Pacific mole crab (*Emerita analoga*), a dominant infaunal organism on sandy beaches. We demonstrate the effects of microplastic ingestion on mole crab mortality and embryonic development, filling a gap in the current knowledge on the impact of microplastics.

Abstract

Microplastics are ubiquitous in marine systems; however, knowledge of the effects of these particles on marine fauna is limited. Ocean-borne plastic debris accumulates in littoral ecosystems worldwide, and invertebrate infauna inhabiting these systems can ingest small plastic particles and fibers, mistaking them for food. We examined the effect of microplastic fibers on physiological and reproductive outcomes in a nearshore organism by exposing Pacific mole crabs (*Emerita analoga*) to environmentally relevant concentrations of microsized polypropylene rope fibers. We compared adult gravid female crab mortality, reproductive success, and embryonic developmental rates between microfiber-exposed and control crabs. Pacific mole crabs exposed to polypropylene rope had increased adult crab mortality, and decreased retention of egg clutches, causing variability in embryonic development rates. These effects of microplastic ingestion on a nearshore prey species have implications for predators such as surf periwinkle and shore birds, as plastic use, and resultant microplastic presence in nearshore environments increases. Microplastics are ubiquitous in marine and sandy beach environments, posing a significant

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Data Availability Statement: Data and metadata are available in the GitHub repository at <https://github.com/cwgrldotty/Sand-Crab-PSU-Data.git> also thru Zenodo 10.5281/zenodo.3564736.

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threat to the marine organisms that reside therein. The most predominant classification of microplastics found have been microfibers. Although a number of biological effects of microplastics have been measured, with documented effects on growth, little research has examined how microplastic fibers affect reproductive output and subsequent development of offspring. We examined the effects of exposure to microfibers on adult mortality, reproductive output, and embryonic development of the filter feeding Pacific mole crab (*E. analoga*), a dominant infaunal organism on sandy beaches. We demonstrate the effects of microplastic ingestion on mole crab mortality and embryonic development, filling a gap in the current knowledge on the impact of microplastics.

Plastic debris in the aquatic environment has increased globally by several orders of magnitude over the past decades, as production continues to outpace the capacity for proper disposal, recycling, or reuse (Rochman et al. 2013; Jambeck et al. 2015). Studies on microplastic debris have identified that microplastic particles are found throughout the water column, in sediments, and are ingested by invertebrate organisms (Cole et al. 2011; Uhrin and Schellinger 2011; Horn et al. 2019). A growing body of research demonstrates that small particles of various plastic (fibers, fragments, nurdles) and polymer (polyethylene (PE), polystyrene (PS), polyvinyl chloride (PVC)) types are accessible to and ingested by a wide range of marine organisms (Bessa et al. 2018; de Sá et al. 2018). Additional research has identified a suite of biological effects of microplastic ingestion by marine organisms (Rochman et al. 2016). Most studies have focused on the effects of particles, rather than on the most commonly identified microplastics, microfibers (Mishra et al. 2019), and much work has utilized high concentrations (not environmentally relevant), leaving significant gaps in our understanding of microfiber ingestion effects on marine organism reproduction and development (Rochman et al. 2016; de Sá et al. 2018). Polypropylene (PP) is one of many polymer types commonly found in marine environments; however, very few studies have investigated its effects on organisms (de Sá et al. 2018) with early studies focusing on ingestion of microspheres or microbeads at environmental irrelevant (high) concentrations (Lenz et al. 2016, Sussarellu, R. et al. 2016, de Sá et al. 2018). Laboratory studies using ambient environmental pollution concentrations and microplastics types are critical to understanding microplastic effects.

The filter-feeding crustacean, *Emerita analoga*, (sand crab or Pacific mole crab) is an important inhabitant of the swash zone on many sandy beach ecosystems from British Columbia, Canada to Baja California, Mexico (Veas et al. 2013). On beaches with shallow slopes, fine sediments, and high food availability, larval densities can be greater than 100,000 individuals/m² (Efford 1969; Dugan et al. 2005; Veas et al. 2013) making it a prey item for many shorebirds (MacGinitie 1938). These shorebirds are the terminal host for the acanthocephalan parasites (*Profilicollis altmani*) found in *E. analoga*, that slow its burrowing speed (Kolluru et al. 2011) allowing for higher predation. Marine filter feeders like *E. analoga* can ingest microplastic particles while feeding, with approximately 30% of *E. analoga* in California coastal populations having ingested microplastics (Van Cauwenberghé et al.

2015a2019; Horn et al. 2019). Internalized plastics may become incorporated into an organism's guts, gills, or tissues (Apkan et al. 2014; Watts et al. 2014, 2016). The documented consequences of microplastic internalization include altered endocrine system function in adult fish (Rochman et al. 2014), and changes in physiology, chemistry, and behavior in aquatic organisms such as mussels (*Mytilus edulis*), Japanese medaka (*Oryzias latipes*), and lugworms (Van Cauwenberghé et al. 2015b; Katsnelson 2015). Bioaccumulative toxic compounds such as organic pollutants and heavy metals from seawater and surrounding sediments that adsorb to microplastics are also of concern (Mato et al. 2001; Gouin et al. 2011) and can be transferred to an organism's tissue when microplastics are ingested (Teuten et al. 2009; Cole et al. 2011; Duis and Coors 2016; Lusher et al. 2017).

The most common microplastic types reported in field collection studies are PE (17%), PP (14%), polyester (PES) (13%), polyamide (PA) (10%) and PS (9%) (de Sá et al. 2018), yet most laboratory studies have used PE or PS (de Sá et al. 2018). Though studies have shown increased mortality rates at organismal levels, reproductive and development effects data are lacking. We investigated whether environmentally relevant concentrations of microfibers affect reproductive performance and embryonic development in the filter feeding Pacific mole crab. We collected adult sand crabs from a single beach, to minimize variability in historical environmental microplastics exposure among crabs. We exposed gravid female crabs to field-documented microfiber concentrations to assess effects of microplastic exposure on adult female crab mortality, reproductive success, number of days the females were egg-bearing, number of embryonic development stages progressed through, and whether or not the eggs hatched. We examined whether exposure to PP microfibers (1) increases adult mole crab mortality, (2) inhibits mole crab embryonic development, and (3) reduces adult reproductive success. We hypothesized *E. analoga* crabs exposed to environmentally relevant concentrations of microplastics would have higher adult mortality, that embryonic development stage progression would be slower, and that females would carry eggs for fewer days.

Methods

Microplastic concentration in beach sediments

Marine sediments are likely a sink for microplastics (Cózar et al. 2014; Eriksen et al. 2014; Woodall et al. 2014), and as



Fig. 1. Location map of sand collection sites along the Oregon coast.

such, can indicate the likelihood of historical exposure of sediment-dwelling invertebrate infauna. To assess the extent of microplastic pollution along the Oregon (OR) coast, and to choose a representative site with intermediate levels of microplastic pollution, we characterized microplastic density in sediments across 19 OR beaches (Fig. 1). We identified South Beach, Newport, OR (44.604006, -124.063729) as a site with intermediate sediment microplastic density to collect *E. analoga* females and seawater to determine environmentally relevant concentrations for the laboratory exposure study.

We collected surface sand samples (<5 cm depth) from the swash zone using a metal hand shovel at 19 beaches along the OR coast (Fig. 1). In the laboratory, a density separation technique, followed by filtration was used to separate plastics from the mineral phase of the sample (Thompson et al. 2004; Horn et al. 2019). We measured 100 mL of sand from each surface sediment sample, placed it into a triple-rinsed glass jar with 400 mL of hyper-salinated solution ($1.2 \text{ kg NaCl l}^{-1}$). After the lid was secured, the jar was agitated for 1 min and then placed on a flat surface to settle (per Thompson et al. 2004, Horn et al. 2019). Once the sand had settled (< 5 min), we poured the supernatant over a vacuum filtration system with a glass fiber

filter (Whatman 1820-047 Glass Microfiber Binder Free Filter, 1.6 Micron, 4.3 s/100 mL Flow Rate, Grade GF/A, 47 mm Diameter) to capture anything separated from the sand. Three controls with just hyper saline solution were run.

Nile Red, a lipid-soluble fluorescent dye that stains hydrophobic materials, can improve the accuracy of microplastic quantification (Shim et al. 2016; Maes et al. 2017). PP, PE, PS, the most commonly identified microplastics on beaches and in surface water (Hidalgo-Ruz et al. 2012), are effectively stained with Nile Red (Shim et al. 2016). The filter from each density-separated sand sample was dyed using Nile Red (Santa Cruz Biotechnology, SC-203747C) prepared as 1 mg mL^{-1} in acetone and diluted in n-hexane (Wiggin and Holland 2019). One milliliter of solution was applied to each glass fiber filter, covered with the lid of the petri dish, and allowed to dry for 2 h. Filters were viewed under illumination by a 455 nm LED light source (Arrowhead Forensics Part No: A-6994FK) and fluorescing microplastic particles and fibers were enumerated using a 10X Leica dissecting microscope with Leica camera connected to a computer running Leica Application Suite X Imaging Software.

Microplastic concentration in seawater

At South Beach in Newport, OR, USA, three 1 L water samples were collected in the swash zone where the crabs were collected. A 1 L DI water blank was run. In the laboratory, each water sample was vacuum filtered through a 47 mm glass fiber filter (Whatman 1820-047 Glass Microfiber Binder Free

Table 1. Pacific mole crab embryonic development stages as defined in Boolootian et al. (1959)

Stage	Description
1	No segmentation observable; yolk circle completely crosshatched.
2	Cleavage has taken place; yolk circle completely crosshatched.
3	A yolk-free (transparent) part becomes apparent. This stage coincides with the appearance of endoderm cells and the beginning of invagination. Yolk circle one-quarter clear.
4	A more distinct division into a yolk-free and a yolk containing part becomes clearly visible. Circle one-third clear.
5	Eye pigment of the embryo becomes visible. Circle one-third clear
6	Pigment bands of the embryo become visible. Circle one-half clear
7	Larvae become strongly pigmented but still contain much yolk. Circle two-thirds clear.
8	The yolk is reduced to two small separate patches. Circle three-fourths clear.
9	Zoea larvae become recognizable. Clear circle.
10	Swimming larvae appear.

Filter, 1.6 Micron, 4.3 s/100 mL Flow Rate, Grade GF/A, 47 mm Diameter). The filter was dyed with Nile Red, covered with a petri dish lid, and allowed to dry for 2 h. The filter was then examined under the dissecting scope using a 455 nm LED Flashlight (Arrowhead Forensics Part No: A-6994FK) to count the number of microplastics per volume of water. The lowest plastic fiber concentration from the three water samples was used as our environmentally relevant treatment level.

Field collection of *E. analoga*

Sand crabs were collected from South Beach, Newport, Oregon ($n = 64$) using a shovel and bucket. Crabs were selected if eggs were visually identified on the exterior of the crab. Selected gravid (egg-bearing) crabs were placed into a bucket with sand and seawater and transported live to the lab. South Beach was selected for collection based on sand crabs availability aggregating at this location during the time of the study.

Mescocosm exposure of *E. analoga* to microplastics

In the laboratory, we measured carapace length (from the tip of the rostrum to the end of the carapace where it meets the top of the abdomen in mm) (range: 13.9–25.4 mm) and width (across the carapace at the widest spot between the

second and third walking leg) of each crab. Each crab was placed in a cleaned 1 L glass jar with 4 cm depth of sand collected from Newport, OR. Artificial sea water (Instant Ocean) maintained at 35 ppm, filled the rest of the jar with a lid with aerator placed on top (Supporting Information Fig. S1). Jars were randomly numbered to identify organisms and placed in a water bath maintained at 11°C. Crabs were randomly assigned to either control ($N = 32$) or treatment ($N = 32$) groups. Controls were considered any mesocosm without added microplastics (Green et al. 2016; Tosetto et al. 2016). There was no significant difference in carapace length between crabs exposed to microplastics (19.00 ± 0.54 [mean \pm S.E.]) and controls (19.28 ± 0.56) ($t = 0.34$, $df = 62$, $p = 0.73$). In each treatment jar, three 1 mm pieces of bright yellow polypropylene rope were added to the water every 4 d for 71 d, or until female crab mortality occurred. The PP rope was purchased from a local marine supply store, the diameter of the rope was <0.1 mm and the pieces were cut into 1 mm lengths using micro-scissors. The selected experimental time frame (71 d) allowed for two full embryonic development cycles as *E. analoga* has an incubation cycle of 29–32 d (Booolootian et al. 1959; Efford 1969). The microplastic

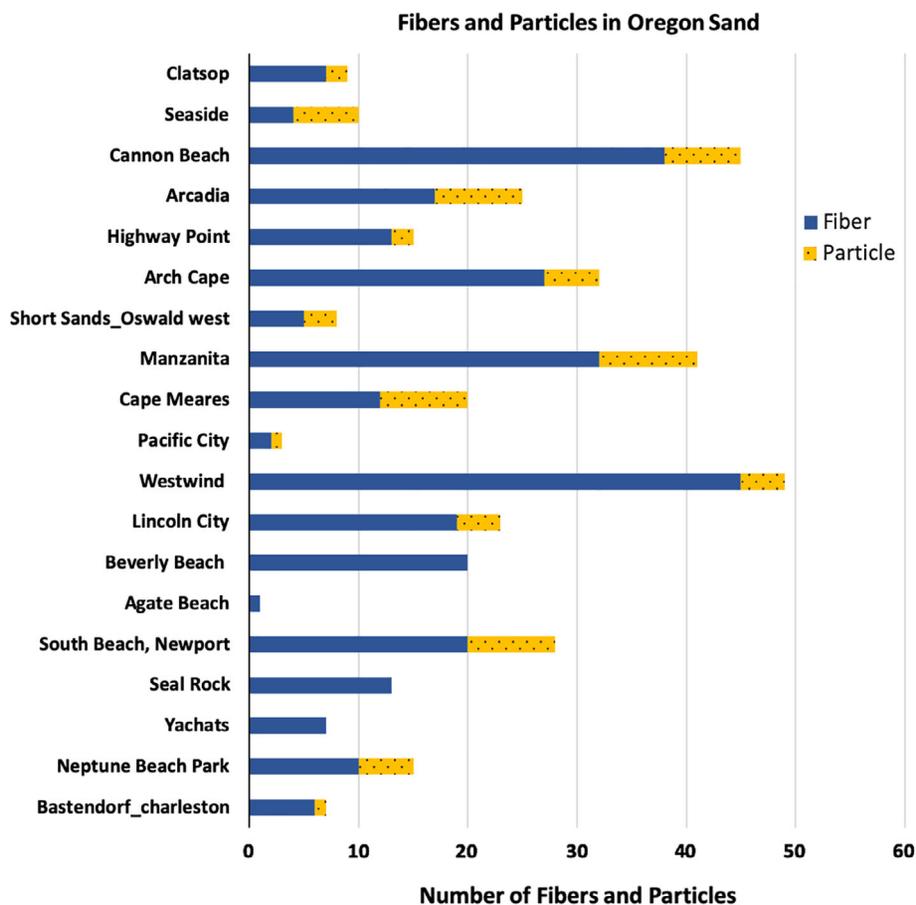


Fig. 2. Beach sand collection sites (north to south) with numbers of microplastic fibers and particles per 100 mL of sand collected. See location map (Fig. 1).

exposure concentration was based on the lowest density of microplastics in seawater at Newport, OR when crabs were collected (three microplastic fibers/L). This concentration was applied to the experiment to maintain environmental relevance. Daily, 300 mL of the 800 mL of seawater was removed from each jar and replaced with fresh artificial seawater (Instant Ocean) and food. Food (ATLMSPD4 Marine Snow Plankton Diet) (conc. 5 mL/L of saltwater) was mixed with fresh Instant Ocean saltwater; nitrates and pH were monitored daily to maintain a controlled environment for the 64 crabs. Every fourth day, four to ten live eggs were retrieved from each crab and frozen for subsequent analysis of embryonic stage. At the end of the experiment or upon adult mortality, crabs were frozen whole in individual containers for subsequent digestion and assessed for the presence of internalized microplastics.

Egg development stage identification

Four to ten eggs were collected from gravid females every fourth day to assess embryonic stage (1–10) and photographed using a 10× Leica dissecting microscope connected via a Leica camera to a computer running Leica Application Suite X Imaging Software (Table 1). Crab embryonic development stage (1–10) was determined using methods from Boolootian et al. (1959).

Assessment of microplastic internalization by *E. analoga*

To analyze whether *E. analoga* had internalized the PP fibers used in the treatment, frozen adult crabs were transferred to a clean glass container triple-rinsed with filtered deionized water and thawed. The carapace was peeled back and the number of acanthocephalan parasites (*Profilicollis altmani*) was recorded, as this parasite slows the sand crabs' burrowing speed to increase predation of the intermediate host by the definitive host, shore birds (Kollaru et al 2011). Then each crab was digested in a 10% KOH solution for 24 h at 40°C (Rochman et al. 2016; Baechler et al. 2019). The solution was filtered through a 63 μm steel mesh, then the residue was transferred into a glass petri dish triple-rinsed with filtered deionized water, and examined under a 10× Leica dissecting microscope to determine whether the yellow polypropylene fibers had been ingested. A blank of just DI water and KOH was run for every six sand crabs digested.

Field and laboratory controls

To minimize contamination, 100% cotton clothing was worn during field collection and lab work and new nitrile gloves were worn for each sample. Each piece of glassware and any dissection tools were rinsed three times with filtered deionized water and covered before and between use.

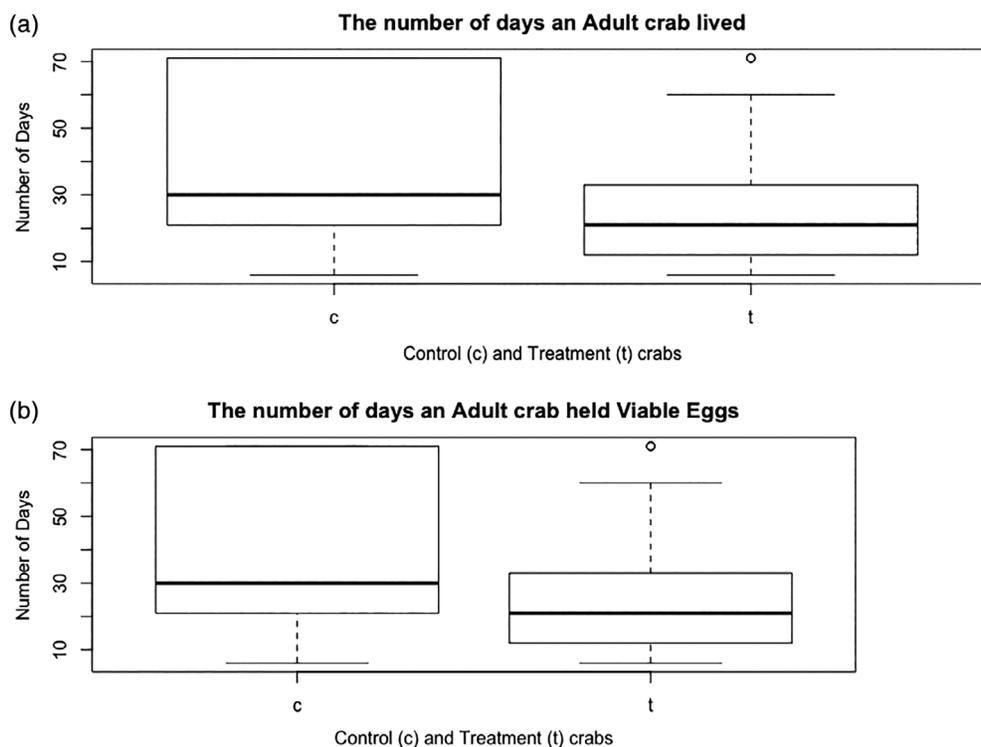


Fig. 3. (A) Boxplot of control and treatment groups displaying adult crabs experimentally exposed to polypropylene microplastics had higher mortality than control group crabs (chi Sq [χ^2] = 45.83, df = 30, $p = 0.03$). (B) Boxplot showing the number of days an adult sand crab held live/viable eggs between control and treatment groups.

Data analysis

To assess the effect of microplastic exposure on adult crab mortality, a chi-squared test was performed on the number of days each crab survived during the experiment. To examine the effect of microplastics on the number of days each adult crab held viable/live eggs in her clutch, we used a chi-squared test. To further analyze the data and test for effects of PP fibers exposure on embryonic development we performed a linear mixed effects model (lme) examining the relationship between exposure to microplastic fibers and adult mortality used R (Version 1.0.153) and lme4 (Bates, Maechler & Bolker 2012; Bates et al. 2014b). Fixed effects included the number of

PP fibers internalized by each adult crab, adult sand crab size, whether the sand crab went through a molt during the experiment, the number of parasites in the adult sand crab gut, and the starting stage of the eggs each sand crab was carrying. Interdependence of fixed effects are further discussed in the results. Random effects were intercepts for control and treatment, as well as by-control and by-treatment random slopes for the effects of microplastic fibers. No obvious deviations from homoscedasticity or normality were evident upon visual inspection of residual plots. Full models were compared to the reduced model using a Likelihood Ratio Test (LRT) (Winter 2013). This allows examination of significant fixed effects,

Table 2. Linear mixed effects model outputs for adult mortality, reproductive output, and embryonic development stages. Model effects and outcomes of the internalization of polypropylene (PP) fibers. Results from the likelihood ratio test (LRT) comparing the mortality, reproductive output and embryonic development between interaction and null models in a linear mixed effects model.

	Model effect	Outcome of PP fibers internalized	LRT	Output	Std. error
Adult mortality	Number of PP fibers internalized	Increased mortality	$\chi^2(1)=30.1, p<0.001$	~5.5	2.1
Reproductive output	Stage of embryonic development	For stage 2 of embryonic development fibers effects outcome	$\chi^2=9.55, df=4, p=0.04$	N/A	N/A
Reproductive output	Number of PP microplastic fibers internalized	Fibers decreased the number of days an adult crab held the egg clutch	$\chi^2(1)=27.54, p<0.001$	~4.46	0.75
Reproductive output	Embryonic development stage at the start of the experiment	Decreased the number of days an adult crab held the egg clutch	$\chi^2(1)=39.72, p<0.001$	~5.06	1.7
Reproductive output	Eggs at later (7–9) embryonic stages at the start of the experiment	Decreased the number of days an adult crab held the egg clutch	$\chi^2(1)=4.72, p=0.02$	~13.3	2.7
Embryonic development later start stages (7–9)	Later (7–9) egg start stage of embryonic development at the start of the experiment	Fewer embryonic stages	$\chi^2(1)=24.32, p<0.001$	~0.5	0.01
Embryonic development start stage 2	Adult crab size	Fewer embryonic stages	$\chi^2(1)=8.13, p=0.004$	~0.73	0.7
Embryonic development start stage 2	Adult crab molting during experiment	Fewer embryonic stages	$\chi^2(1)=8.61, p=0.03$	~0.33	2.5
Embryonic development start stage 2	Number of parasites in adult crab	Fewer embryonic stages	$\chi^2(1)=10.82, p=0.01$	~0.19	0.19
Embryonic development start stage 2	Total number of PP fibers internalized	Increased embryonic stages	$\chi^2(1)=11.53, p=0.04$	~1.04	0.5
Embryonic development start stage 8	Adult crab size	Increased embryonic stages	$\chi^2(1)=8.37, p=0.015$	~0.6	0.22
Embryonic development start stage 8	Adult crab molting during experiment	Increased embryonic stages	$\chi^2(1)=8.74, p=0.03$	~1.46	0.5
Embryonic development start stage 8	Number of parasites in adult crab	Fewer embryonic stages	$\chi^2(1)=8.4, p=0.03$	~0.08	0.05
Embryonic development start stage 8	Total number of PP fibers internalized	Increased embryonic stages	$\chi^2(1)=9.58, p=0.04$	~0.07	0.17

using an LRT to obtain a chi-squared value, degrees of freedom, and p -value.

Results

Microplastic density in beach sediments and seawater

Sediment samples from all sites contained microplastic fibers and particles (identified by fluorescence with Nile Red dye), with 1–45 microfibers (average 15 fibers \pm 2.8) and 0–9 particles (average 4 particles \pm 0.7) per 100 mL of sand sampled (Fig. 2.) The 1 L water samples collected at South Beach contained 3–7 microfibers (average of 4.6 fibers/L \pm 1.7) and no particles were identified. The fiber sizes in the water and sand samples ranged from 0.03 mm to 6 mm in length. These findings guided the protocol of three PP fibers per treatment in the mesocosm study to maintain environmental relevance.

Adult sand crab mortality

Crabs experimentally exposed to PP had significantly higher mortality than the control group (Chi Sq [χ^2] = 45.83, df = 30, p = 0.03) (Fig. 3A). Crab mortality increased with number of PP fibers internalized (LRT), $\chi^2(1) = 30.1$, $p < 0.001$, independent of other fixed effects. For each PP microfiber a crab internalized, the number of days it lived decreased by \sim 5.5 d \pm 2.1 SE. (Table 2).

Reproductive output

Duration viable eggs were held by adult sand crabs

The number of days a crab held live/viable eggs in her clutch was negatively affected by PP exposure when those eggs were at stage two of embryonic development at the study start (LRT) (χ^2) = 9.55, df = 4, p = 0.04) (Fig. 3B). We found that number of PP fibers internalized, decreased the number of days that a crab held live/viable eggs (LRT) ($\chi^2(1) = 27.54$, $p < 0.001$), decreasing by \sim 4.46 d \pm 0.75 SE. The embryonic stage of the eggs a crab was carrying correlated with the number of microfibers internalized and the number of days a crab held live/viable eggs in her clutch (LRT) ($\chi^2(1) = 11.825$, $p < 0.001$). Additionally, embryonic development stage at the start of the experiment affected the number of days a crab held the egg clutch, such that egg clutches at later embryonic stages carried live/viable eggs for \sim 5.06 fewer days \pm 1.7 SE (LRT) ($\chi^2(1) = 39.72$, $p < 0.001$). Crabs captured with eggs at later embryonic stages, and exposed to PP held viable/live eggs \sim 13.3 fewer days \pm 2.7 SE than control crabs (LRT) ($\chi^2(1) = 4.72$, $p = 0.02$).

Number of embryonic development stages for *E. analoga*

The number of embryonic stages a crab egg clutch went through during the experiment was affected by starting stage ((LRT)² (1) = 24.32, $p < 0.001$) (Table 2). Later embryonic stages experienced \sim 0.5 fewer stages \pm 0.01SE. Crabs with egg clutches starting at stage two of embryonic development, crab size reduced the number of embryonic stages the egg clutch by \sim 0.73 stages \pm 0.7 SE, (LRT) ($\chi^2(1) = 8.13$, $p = 0.004$). Embryonic

stages were reduced by \sim 0.33 stages \pm 2.5 SE (LRT) (χ^2 (1) = 8.61, $p = 0.03$) during crab molting. The number of parasites in a crab decreased the number of embryonic stages \sim 0.19 stages \pm 0.19 SE ((LRT)² (1) = 10.82, $p = 0.01$). The number of PP fibers internalized by the crab increased the number of embryonic stages \sim 1.04 stages \pm 0.5 SE (LRT) (χ^2 (1) = 11.53, $p = 0.04$). In crabs with egg clutches starting at stage eight of embryonic development, crab size increased the number of embryonic stages \sim 0.6 stages \pm 0.22 (SE) ($\chi^2(1) = 8.37$, $p = 0.015$), crab molting increased the number of embryonic stages \sim 1.46 stages \pm 0.5 (SE) (χ^2 (1) = 8.74, $p = 0.03$), the number of parasites decreased the number of embryonic stages \sim 0.08 stages \pm 0.05 (SE) (χ^2 (1) = 8.4, $p = 0.03$), and number of PP fibers internalized by crabs increased the number of embryonic stages \sim 0.07 stages \pm 0.17 (SE) (χ^2 (1) = 9.58, $p = 0.04$).

Discussion

Microplastics in sediments

Globally, microplastics are common in littoral and marine sediments (Barnes et al. 2009; Browne et al. 2011; Cole et al. 2011, Uhrin and Schellinger 2011, Horn et al. 2019), potential sinks sequestering microplastics (Cózar et al. 2014; Eriksen et al. 2014; Woodall et al. 2014). Sediments from all 19 Oregon beach sites sampled had microplastics. As in prior coastal studies (Abayomi et al. 2017; Miller et al. 2017; Barrows et al. 2018; Horn et al. 2019), fibers are the dominant microplastic type along the Oregon coast. Sediment-dwelling suspension and deposit feeders, such as *E. analoga* show an inability to differentiate between plastic and food items (Graham and Thompson 2009; Cole et al. 2013; Sussarellu et al. 2016; Lusher et al. 2017).

Effects of ingestion

Of the crabs exposed to PP microplastics, all individuals internalized at least one yellow PP fiber. Our findings align with studies that found internalization of plastics at high concentrations (Watts et al. 2014; Hall et al. 2015; Watts et al. 2015; Van Cauwenberghe et al. 2015b; de Sá et al. 2018), but here we demonstrate that even at much lower concentrations, ingestion is extremely likely. The sand crabs exposed to PP rope experienced variance in embryonic stages, particularly interesting in the difference in effects depending on embryonic start stage. We found that there was a slight decrease in embryonic development when adult crabs experienced natural biotic events such as molting, but when exposed to PP, embryonic development increased. The size of the adult crab had an effect on embryonic development depending on the starting stage. Later embryonic stage clutches had increased development in larger crabs, but decreased development when the egg clutch was in an early stage. There was marginal decrease in days of carrying viable eggs no matter the embryonic start stage when adult crabs were exposed to PP fibers as well as increased adult mortality when exposed to PP

microfibers. Adult mortality when exposed to PP microfibers is an important finding as many papers have focused on other plastics. Although we are unable to distinguish the effects of the microplastics themselves from those of the yellow dye in the plastics, many environmental microplastics are dyed (Phuong et al. 2018), so dye exposure frequently goes hand in hand with microplastic exposure. This is one of the limitations of the study, as we cannot separate the effects of exposure to the plastics themselves, the dyes and any additives adsorbed from the sediment or water (Tosetto et al. 2016). We also face the challenge that there are plastics throughout the ecosystem and therefore the control is simply one that was not exposed to additional PP fibers.

Population- and ecosystem-level consequences

Given the role sand crabs play as a prey item for shorebirds such as sandpipers, sanderlings, and godwits (MacGinitie 1938), nearshore fish such as barred surf perch (Perry 1980), and some marine mammals (Kvitek and Bretz 2005), increased mortality and decreased reproductive performance following microplastic exposure may affect the communities to which these crabs belong with potential effects on higher trophic level species (Perry 1980).

Conclusion

This study increases our understanding of the effects exposure to environmentally relevant microplastics concentrations can have on marine invertebrates, specifically adult crab mortality and embryonic development. As plastic use and resultant release into aquatic systems increases, the potential for microplastic exposure rises. Additional research into how microplastic contamination in prey items such as sand crabs affects higher trophic level species such as seabirds, surf perch, and marine mammals constitute important next steps. Additionally, further research to distinguish effects of microfibers vs. the dyes that color them will assist in understanding drivers of decreased physiological and reproductive outcomes. Finally, these findings highlight the need to address sources and reduce inputs of microplastics into sandy beach and marine ecosystems.

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