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Ecological Exposure and Effects of Microplastics in Crabs Along the Pacific Coast

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by

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Abstract

Microplastics have been documented across the global oceans as an ubiquitous pollutant. Found in the water column, sediment, shorelines, estuaries, freshwater streams and rivers along with terrestrial soils, flora and fauna. The continuous input of plastic waste into the marine environment doesn't seem to be slowing as the amount of plastic created each year increases globally.

This study investigated (1) the effects of microplastic ingestion in the indicator species, the Pacific mole crab (Emerita analoga), testing the predator avoidance behavior, reproductive output and parasitism effects when an adult female gravid crab had ingested microplastics (2) adult mortality, hatching success and growth time of indicator species, the Pacific mole crab (Emerita analoga), when exposed to an environmentally relevant amount of polypropylene microplastic fibers and lastly (3) the presence of microplastic ingestion in the important commercial fishery organism the Dungeness crab *(Metacarcinus magister).*

Conclusions show that there are deleterious effects of microplastic ingestion on Pacific mole crabs across testing parameters, including increased mortality, slower predator avoidance behaviors and significant effects on reproductive output and success. Within Dungeness crabs, we found that these crabs ingested microplastics across locations as well as different body parts investigated. However, Dungeness crabs were found to have the lowest amount of microplastics per gram of body tissue compared to other fishery organisms researched in the Pacific Northwest such as clams and oysters.

Dedication

I dedicate this dissertation to my wife who has stood by me through many long days and nights, living in a cold rainy place so that I could chase this goal.

Acknowledgments

I want to say thank you first to my friends, who I would not have made it through this journey without their support. To the long days, crazy times and fun that we had I will always remember and hold the memories close. I also want to say a huge thank you to Catherine de Rivera, who mentored me through this journey when I was ready to quit. Your mentorship and professionalism is something that other advisors should strive to attain.

To all of the people who I got to work with, learn from and adventure through the beaches with, I hope we get to keep working together. Lastly to the National Science Foundation for granting me the graduate research fellowship that allowed me to succeed in earning my doctorate.

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Introduction

Microplastics in the marine environment have been documented in the scientific literature starting in the 1970's (Carpenter & Smith 1972, Carpenter et al. 1972, S. Rothstein 1973, Venrick et al. 1973) but in the last decade there has been a large uptick in the scientific literature (Klingelhöfer et al. 2020) documenting not only presence in the environment but also ingestion by organisms.

In the last decade, researchers have documented the global distribution of plastics, including microplastic pollution, and its presence in and impacts to many varied organisms (Elgarahy et al. 2021, Karbalaei et al. 2018, Kerkshaw & Rochman 2015) . Plastic ingestion was first documented in seabirds in the 1960's; since then over 600 organisms have been documented as affected by marine debris or microplastics (Carbery et al. 2018). On their own have been deemed "biochemically inert" (Carbery et al. 2018); however, the additives in plastics to make them safer, more pliable etc. are cause for concern (Galloway 2016). These chemicals are added during the manufacturing process for a variety of reasons such as pliability and durability when exposed to UV light and temperature changes. Plastics have chemicals that resist microbial growth as well as making them opaque and colorful. These additives also have an effect in the marine environment (da Costa et al 2018, Law 2017). In addition to additives, plastics accumulate chemicals from the environment. The high surface area to volume ratio of most plastic pellets and items can concentrate contaminants onto the plastics up to 6 orders of magnitude or higher than the surrounding sea water (Mato et al 2001). Not only do microplastics absorb chemicals from the surrounding water and sediment, the older

the plastic or longer it has been in the environment, the higher the concentrations of persistent organic pollutants are found (Mato et al 2001).

Documentation of the evidence of Persistent organic pollutants such as polychlorinated bisphenols (PCBs), dichlorodiphenyl dichloroethylene (DDE) and polyaromatic hydrocarbons (PAHs) (Mato et al 2001) as well as dichlorodiphenyl trichloroethane (DDT) (Ashton et al. 2010) have all been found on plastics collected from the sand and the water in marine environments globally. Not only are persistent organic pollutants (POPs) found on plastics, but so are heavy metals such as cadmium and lead (Ashton et al. 2010) that we know are toxic to humans, wildlife and fish and are especially harmful at lower concentrations to aquatic organisms (Mohlenberg and Jensen 1980, Eisler 1979). Studies across different trophic levels have shown biomagnification of organic pollutants to fish through microplastic ingestion (Kelly et al. 2007) by amphipods, polychaetes, mussels and other fish (Chua et al. 2014; Besseling et al. 2013; Browne et al. 2013; Avio et al. 2015; Oliveira et al. 2013).

Ingestion of microplastics has been documented in several different organisms to cause a variety of effects such as inflammation (Von Moos et al. 2012, Wright et al. 2013), reduction in feeding ability/activity (Browne et al. 2008) offspring impacts (Sussarellu et al. 2016) and energy reserve depletion (Wright et al. 2013; Watts et al. 2015). There have also been studies showing increased mortality rates when organisms are exposed to plastics (Browne et al. 2013, Oliveira et al. 2013). The breakdown into smaller micro- (1mm -5mm) (Andrady, 2011, Cózar et al., 2014, Ter Halle et al., 2016, Gigault et al.

2018) and nano-(< 1mm)(Gigault et al. 2018) plastic research has documented evidence of nano-plastics that are capable of crossing the cell membranes, the blood brain barrier and the placenta that have shown effects of cell damage (Avio et al 2015), inflammation, negative effects on energy storage and oxidative stress (Vethaak and Leslie 2016; Carbery et al 2018) as well as impacts on offspring by slowing development and reducing the number of successful larvae (Sussarellu et al 2016).

History of Plastics

Plastics have become incorporated into all aspects of our lives from household and personal goods to packaging, clothing and construction materials, continually expanding their reach, ever since their mass production started in the 1930's (Van Cauwenberghe et al. 2015). Plastics were originally designed to be "pliable and easily shaped" derived from the natural polymer found in plant cell walls as cellulose. Over the last century humans have developed long chain polymers with the mass amounts of carbon produced in petroleum driving further research and eventually the development of the synthetic polymer chains that we call plastic (Frienkel 2017). John Wesley Hyatt created the first synthetic polymer in 1869, motivated by a $10,000 reward posted by a New York firm for anyone who could create a viable substitute for natural ivory (Frienkel 2017). This was the first time in history that humans could create new materials that not only helped people but also saved the elephants from continuing to be exploited for their ivory. In 1907 Leo Baekeland developed the first fully synthetic plastic, made of molecules developed in the lab and never found in nature (Fendall & Sewell 2009). Throughout World War II and the years that followed, plastics were developed into everything from

household items to parts of weapons used in the military (Frienkel 2017). It was not until the 1960's and the initial rumblings of what would grow into the Environmental movement that the wider public became aware of the possible dangers of something that never degraded (Frienkel 2017). Today this is true as we see plastic of all shapes and sizes everywhere and comprising one of the largest categories of debris we find on our beaches and in our oceans (Barnes et al. 2009).

Plastics in the marine environment

The discovery of the Great Pacific garbage patch as well as the 5 gyres around the world have given visual evidence of the gravity of the plastic waste problem. We have found that most of the marine debris, approximately 80% (Jambeck et al. 2015), comes from land based sources travelling down watersheds and making its way into coastal ecosystems. Much of this is entering the oceans due to littering as well as inadequate waste management. Citizen science as well as traditional researchers have brought to light the enormous amounts of plastics littering our oceans.

Plastics are ubiquitous in the marine environment. "By 2050, there will be more plastic than fish in the ocean" (Geyer 2017). The total amount of plastic debris is distributed at the scale of kilo- to megameters across ocean basins from both terrestrial and marine sources. Every year, 8 million metric tons of plastic enter the ocean on top of the estimated 150 million metric tons that currently circulate in marine environments (Jambeck et al. 2015). It is estimated that the ocean surface currently contains between

7,000 – 25,000 tons of plastic. Plastics production ramped up from 1.5 million tons in 1950 to ~322 Million tons in 2015. It is estimated that 2500 million tons of plastics—30% of all plastics ever produced—are currently in use. Between 1950 and 2015, cumulative waste generation of primary and recycled plastic waste amounted to 6300 million tons(Jambeck et al. 2015). Of this, approximately 800 million tons (12%) of plastics have been incinerated and 600 million tons (9%) have been recycled, only 10% of which have been recycled more than once. Around 4900 million tons—60% of all plastics ever produced—were discarded and are accumulating in landfills or in the natural environment (Kerkshaw & Rochman 2015).

The density of plastics varies based on polymer composition (Kerkshaw & Rochman 2015). Within 48 hours, these polymers start to attract microorganisms that create biofilms as well as physically break down and degrade from exposure to seawater, UV light and temperature changes (Webb et al. 2013, Kerkshaw & Rochman 2015).These exposures break the chemical bonds causing physical degradation, eventually causing all plastics to sink as their density changes (Webb et al. 2013, Kerkshaw & Rochman 2015).

Size definition of plastics

Research on plastics currently uses five size classifications . Mega- plastics are anything larger than 100mm, Macro-plastics range from 20mm to 100mm, Meso-plastics span 5mm to 20mm, Micro-plastics are any plastics less than 5mm, and lastly Nano-plastics are any plastics smaller than 1mm (Kerkshaw & Rochman 2015). Within each size class

there is a range of impacts to different marine organisms, ranging from entanglement to ingestion and even crossing through the blood-brain barrier in the case of nano-plastics (da Costa et al 2016).

Diversity of Microplastics

Not all plastics are the same. Similar to the array of chemicals and other pollutants found in the marine environment, each piece of microplastic is made with a set of different polymers and additives along with each being a different shape and size. This is why we cannot say all microplastic is the same, but we can categorize the size of these plastics into 'microplastics' (Rochman et al. 2019).

Primary microplastics are manufactured such as microfibers (clothing), microbeads (for personal care products) or nurdles (used to melt into other products i.e. cell phone cover). Then there are secondary microplastics which are fragments of larger items such as pieces of plastic toys, buoys, tire particles and many more. Microplastics can be many shapes and colors, this is how researchers assign categories when combing through samples (Helm 2017). Overall there are 7 groupings of microplastics (fibers, fiber bundles, fragments, spheres, pellets, films and foams) that researchers use to categorize the pieces they find (Rochman et al. 2019). The morphology of each of these categories helps organize findings for comparison in microplastics research. Nanoplastics (<1um) are likely the most numerically abundant items of plastic debris in the ocean today, and quantities will inevitably increase, in part because large, single plastic items ultimately degrade into millions of smaller pieces. Microplastics are created when larger pieces of

plastic debris undergo degradation or fragmentation to secondary microplastic particles (< 5mm) (Andrady 2011), or they occur as primary microplastics (such as fibers from clothing or beads from abrasives in personal care or industrial products), directly entering the marine environment because wastewater and storm-water treatment only remove up to 90% of them (Talvitie et al 2017).

The most commonly found plastics in the marine environment are Polypropylene (PP), Polyethylene (PET), Polystyrene (PS) and Polyvinyl Chloride (PVC) (Rochman et al. 2013, de Sa et al. 2018). All plastics are made with a similar range of harmful toxins such as plasticizers, flame retardants, dyes, microbial deterrents and chemicals to increase their durability that are all harmful to organisms they come into contact with (de Costa et al. 2019; Law 2017, Yang et al. 2011). All of these types of plastics can concentrate contaminants from the environment onto their surface up to 6 orders of magnitude higher than the surrounding water/sediment (Mato et al. 2001). The longer the plastics are in the environment, the higher the concentration of persistent organic pollutants is found on their surface (Mato et al. 2001). Lastly synthetic fibers from clothing are common. A single synthetic piece of clothing can create up to 1900 microfibers per wash cycle (Brown et al., 2011). The majority of microplastics found are synthetic microfibers (Acharya et al. 2021).

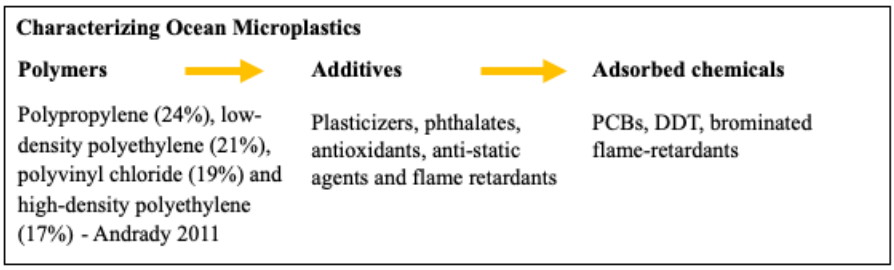


Figure 1. Ocean Microplastic Characterization. Ocean microplastics found as the top 4 polymers (Andrady 2011), with possible additives and adsorbed chemicals.

Microfibers

Microfibers are the predominant type of debris found in most sediment and organism field studies (Gago et al. 2017). Many but not all microfibers are plastic as some are derived from cellulose, but still may impact organisms that ingest them. Individual beaches in the Great Lakes and Pacific Islands have had some of the highest concentrations of microplastics, specifically in the category of microfibers (Earn et al. 2020). Microplastics were even found in remote areas of Alaska (Whitmore and Van Bloem 2017). Apostle Island National Lakeshore (Wisconsin), National Park of American Samoa (American Samoa) and Kalaupapa National Historical Park (Hawaii) had the highest abundances of microplastics, averaging between 170 and 225 pieces of microplastics per kg of sand (Whitmore and Van Bloem 2017).

Microplastics impacts on Marine organisms

All plastics, regardless of their size and composition, have the potential for causing harm whether through entanglement, smothering or being ingested. Microplastics have been found throughout the water column, in sediments and have been ingested by a variety of organisms (Cole et al. 2011,Lusher et al. 2015). Microplastics are of environmental concern because their size (millimeters or smaller) renders them accessible to a wide

range of organisms at least as small as zooplankton, coral, copepods, marine worms, filter feeders, fish, and other organisms that serve as prey for larger species (Cole et al. 2013, Rochman et al. 2014, Wright et al. 2013, Setala et al. 2014). Harmful pollutants incorporated into plastic products or absorbed by plastics from the environment can be transferred into the tissue of organisms that have internalized the plastic (Rochman et al. 2016). This is not surprising since microplastics are often the same size as food particles for these organisms.

Many studies investigating the effect of microplastics on organisms have shown risks due not only directly to ingestion but also toxicological effects (Browne et al. 2013, Wright et al. 2013, Farrell and Nelson 2013, Setala et al. 2014, Rochman et al. 2014, Avio et al. 2015). Plastics alone are manufactured with chemicals that are known to be carcinogenic, endocrine disruptors and cause other sub-lethal effects. For example, phthalates, which are used for flexibility in the plastic, and Bisphenol-A, which is added to polycarbonate and plastic resins (Barboza et al. 2018), can cause these lethal and sublethal effects. The overall concern for the implications of microplastics and the effects they may have on organisms as well as any implications for coastal food webs has led to future concern over human ingestion of these organisms and the possible plastic they contain. However, some studies have had negative effects of organisms while other studies have shown neutral or no effects (Foley et al. 2018). The difference in effects could be due to the diverse array of physical and chemical makeups of plastics that is found in the environment (Rochman et al. 2019) as well as the mechanism and length of exposure.

Sandy Beach Ecosystem

Sandy beaches are fundamental to our coastal economy and culture. Beaches provide protection for homes along the coastline, recreation for locals and tourists as well as habitat for birds, invertebrates and some marine mammals. Beaches have unique ecosystems as they have food webs "highly reliant on imported subsidies" (Schlacher 2015) and an "extreme malleability of habitats" (Schlacher 2015). The sandy beach ecosystem is ever changing, molded hourly by the tide, surf and water temperature. It helps to mineralize nutrients as well as affording a recreational fishing area (Schlacher 2015).

One of the defining characteristics of a beach ecosystem is its dependence on nutrients and material inputs washed ashore by the surf (Schlacher 2105). This materialization affects invertebrates in the ecosystem and their functioning in the beach ecosystem. The sandy beach ecosystem food web is the connection between the marine and terrestrial ecosystems (Schlacher 2105). Many different taxa on the beach including shore birds, raptors, fish, turtles and invertebrates that burrow into the sand. These beach invertebrates are highly mobile and able to adapt to a constantly changing habitat (Schlacher 2105). Because of the highly diverse food web found on the beach that is linked to the marine and terrestrial ecosystems, study of the resident species provides insight on the broader ecosystem.

Crabs as an ecosystem indicator organism for microplastic pollution in nearshore environments

Investigations into pollution in nearshore environments have traditionally been done using environmental samples such as water and soil, to test for pollutants (Giblock and Crain 2013). However, over the last decade, the influx of plastic debris into these systems has created a trend of investigations, not only into the environmental pollution aspect but within an array of organisms (Provencher et al. 2020). For decades, crabs have been used across systems as indicator species for all types of pollution, chemical pollutants (Arya et al. 2014), as well as salinity fluctuations within estuaries (Shirley et al. 2004, Giblock and Crain 2013) and overall habitat quality (Amaral et al. 2009). Crab gills trap pollutants (Arya et al. 2014) but biological effects such as carapace growth (Márquez & Idaszkin 2021) have been used to track heavy metal pollution (Márquez & Idaszkin 2021) as well as microplastics in multiple species of crabs in China (Zhang et al. 2021). Therefore, I chose to investigate the microplastic pollution effects on the Pacific mole crab *(Emerita analoga),* as a continuation of previous research I completed (Horn et al. 2019) as well as lay the groundwork for an important fishery species of crabs, the Dungeness crab *(Metacarcinus magister)* in the Pacific northwestern United States.

Pacific mole crabs or sand crabs (Emerita analoga)(Anomura, Hippidae) collected in California

The indicator crustacean *Emerita analoga,* also known as the Pacific Sand Crab and Pacific mole crab, is of the super family Hippidae and an important part of sandy beach

ecosystems regionally. Sand crabs are found along the coast from British Columbia to Magdelena Bay, Baja California. They are the dominant macrofaunal species found in sandy beaches along the North American continent (Veas 2013). These crustaceans are one of the most successful invertebrates that live in the sandy intertidal zone (Efford 1969). They live in the swash zone where the waves crash on the beach and feed by filtering out plankton from the water (Veas 2013). Higher densities of *E. analoga* tend to be found on beaches with lesser slopes and finer sediment and (Veas 2013). The sand crab has been found in high abundance, over 100,000 individuals per meter squared of shoreline in some places (Dugan et al. 1994).

The life cycle of the sand crab starts with the mating season in late spring and summer before the asynchronous release of eggs during the summer months. During the summer months (ambient temperature of 21℃-23℃) all females are found to have eggs (Barnes & Werner 1968). An incubation period of 29 to 32 days was confirmed in the laboratory (Dudley and Cox 1968) as well as a "re-berrying" of eggs in females as many as four times in one season (Barnes & Werner 1968). The larval stage of the sand crab is about 3-4 months, in Oregon as low as 10℃ (Sorte et al 2001) up to 23°C moving south across Pacific(Barnes and Wenner 1968, Dawson et al. 2011). The highest numbers of larvae (zoeae) were found in August; almost all of the larval population in the southern California range is late stage zoeae by mid-December (Barnes & Werner 1968). The larvae go through approximately 5 molts as a zoea before they metamorphosize to a megalopa and find their place in the sandy beach where they spend another month eating before they molt into juvenile crabs (Barnes & Werner 1968). Throughout the season the

larger female sizes along with the absence of females in smaller size classes suggest a sex reversal (protandric hermaphrodism) (Barnes & Werner 1968).

It is thought that there are two distinct groups of sand crabs that create the populations along the beaches. The first is the intertidal reservoir that produces pelagic larvae throughout the summer and into the fall. The second is the pelagic reservoir that supplies many beaches with megalopae in the fall, winter and spring (Barnes & Werner 1968). Between these two groups it has been shown that the intertidal reservoir is fairly empty during the winter months and the pelagic reservoir is fairly empty during the summer months creating a continuous flow of sand crabs that supplies the sandy beach populations (Barnes & Werner 1968).

Dungeness crab (Metacarcinus magister)(Brachyura, Cancridae) collected in Oregon

Dungeness crabs make up a billion dollar commercial fishery that ranges from Alaska to Santa Barbara, California (Rasmuson 2013). Once adult male crabs molt, they head inshore and find females that are about to molt. Mating usually occurs between recently molted females and males that have already molted (Hartnoll 1969). A Dungeness crab reaches sexual maturity around 100 mm carapace width, which occurs between 2-3 yrs of age, depending on temperature and location. Northern California populations mature earlier than Alaskan populations (Shirley et al. 1987). Male crabs will start to track into the nearshore as females are close to their molting time and perform a 'premating embrace' to protect and guard the females and mate (Snow & Neilsen 1966). Both female and male crabs extend their abdomens, and using pleopods the male deposits sperm into

the females gonopores. There is a sperm plug that hardens from male seminal fluid to block other males from mating with the same female (Jensen et al. 1996). *Brood production* - a few months after copulation has occurred, eggs are extruded (Wild & Tasto1983) and the eggs are inseminated.

Females are berried (aka gravid, ovigerous) in California from September to November; in Oregon/Washington from October to December; in BC from September to February; and in Alaska from September to November (Rasmuson 2015). *Release of larvae* - Timing of hatching depends on location, as hatching occurs earlier in warmer waters and later in cooler waters, towards Alaska (Rasmuson 2013). Prezoea are sometimes released and live in the water column before quickly transforming into the first zoeal stage (Rasmuson 2013). There are 5 zoea stages - Water temperature and salinity can alter the rate of development throughout the five zoea stages (Rasmuson 2013). When zoeae are released during the winter months in the California current system they typically are transported north until the spring transition of the currents, when the Davidson current slows and more zoeae are found off of the continental shelf and flowing southward with the California current (Shanks and Eckert 2005, Rasmuson 2013). This is when the zoeae molt into megalopae then begins to migrate inshore and settle (Rasmuson 2013).

Salinity changes in the estuary based on freshwater influences tend to drive crabs in or out of estuaries. When there is a rain event, the fresh water influx drives down salinity in the estuary and crabs retreat to the ocean (Rasmuson 2013). Cardiac stress is hypothesized to be the driver for this movement (McGaw & McMahon 1996).

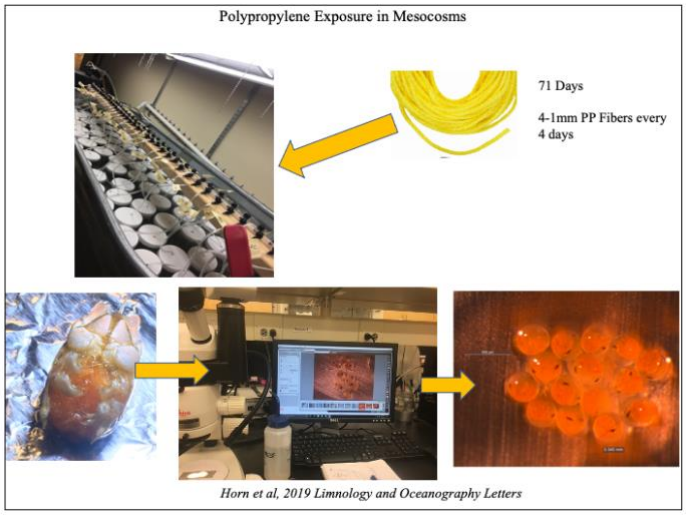
Movement to and from estuarine environments also depends on hypoxic events and both of these stressors are tied to whether or not the crab has had enough to eat (Bernatis et al. 2007). If a crab was satiated, their tolerance was high and they stayed put, if not, they travelled out of the estuary up to 1370m within 6 hours to avoid the stressors (Bernatis et al. 2007). There are also correlations with the neap tide cycle and larval recruitment into estuaries (Roegner et al. 2007)

Since these crabs hatch in the winter near the coast and take a few months to mature, the zoeae are pushed offshore by the California current, past the continental shelf (Shanks and Roegner 2007). In order for megalopae and adults to survive, they have to settle to the ocean floor, so they start to move inward to more shallow waters. The recruitment of these megalopae is facilitated by some tidal mechanisms over the continental shelf (Shanks and Roegner 2007; Johnson and Shanks 2002). The California current system is changed by the atmosphere: in winter, downwelling conditions emerge with winds from the south and a warmer water movement occurs. The Davidson current moves north across the continental shelf and the California current flows south on the outer edge of the continental shelf (Peterson et al. 2010). In the spring, the atmosphere changes with movements of high and low pressure shifting the wind direction, blowing south. Hence, in spring, the Davidson current switches to flow south and the winds cause coastal upwelling. All of this change brings the megalopae back to the nearshore along with lots of food availability due to upwelling (Shanks and Roegner 2007)

Current dissertation research

The problem is global microplastic pollution in the marine environment. Daily reports show plastics washing along our coastlines as well as continued reports of entanglement and ingestion by marine species. In this dissertation, I investigated an indicator species, the Pacific mole crab, and the effects of microplastic ingestion. I hypothesized that polypropylene microplastics would be ingested and that they would negatively affect the reproductive output of Pacific mole crabs. I conducted a laboratory study to determine if microplastic ingestion affected their mortality, reproductive output, and hatching success in a laboratory study by feeding adult female gravid Pacific mole crabs polypropylene rope microfibers over two reproductive cycles (Figure 2) to measure their mortality rates and hatching success when exposed to microplastic fibers. In the second chapter, we tested the hypothesis that mole crabs that have ingested high levels of fibers and other anthropogenic particles will burrow more slowly, suffer from greater prevalence of parasites, and produce fewer eggs. This study was conducted in the field, by measuring burying speed for adult female gravid Pacific mole crabs to assess their predator avoidance success. Once crabs were tested in the field, we moved to the lab to investigate their reproductive output and parasitism rates if the crabs were found to have ingested microplastics (Figure 2).

a.



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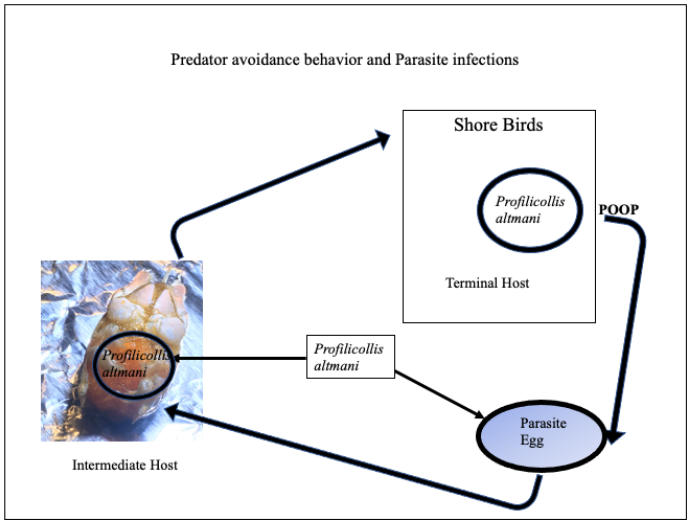

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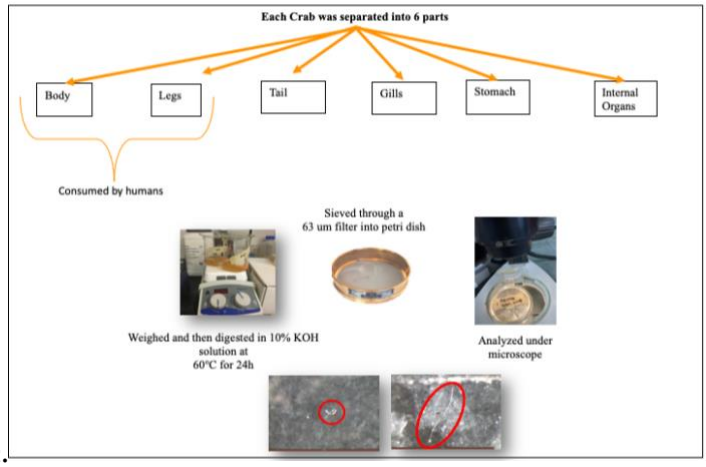

Figure 2. Conceptual Models(a) conceptual model for chapter one, investigating the effects of exposure to polypropylene microfibers in adult female gravid Pacific mole crabs. **(b)** conceptual model of the terminal host of the parasite *Profilicollis altmani* in shore birds, with the Pacific mole crab as its secondary host.

(c)conceptual model of chapter 3, investigating microplastics in Dungeness crabs, dividing body into six parts, digesting and then investigating under the microscope.

Moving to a larger crab, the Dungeness crab, we investigated whether this highly sought after species is in fact ingesting microplastics. We hypothesized they would be exposed to microplastics and have ingested pieces as adults. We hypothesized that crabs collected from estuaries will accumulate more microplastics per size than those collected offshore as estuaries have been shown to be sinks for microplastics (Vermeirem et al. 2016, Kaiser et al. 2017) creating a higher bioavailability to Dungeness crabs in the estuary. We also hypothesized that most of the microplastics found would be trapped in the gills of these crabs similar to other crabs (Zhang et al. 2021, Lusher et al. 2020) and that the Dungeness maybe able to egest them through feces or stop the microplastics from entering via its gills. I also looked at six parts of the Dungeness crab to determine the microplastic per gram of body tissue load to compare it to other organisms in the Pacific northwest that microplastic ingestion has been documented in. The main question was investigating whether Dungeness crabs ingested microplastics, but also where those microplastics aggregated within the body of each crab. I also investigated if the parts of the crab we eat, body and leg tissue had microplastics and if the amount of microplastics ingested by Dungeness crabs was more or less than other organisms investigated within the Pacific Northwest.

My research investigated the prevalence of microplastics in the sandy beach environment as well as the biological effects of microplastic ingestion in the sand crab *(Emerita analoga).* The first chapter, published in Limnology and Oceanography Letters, identified the effects on polypropylene fibers in sand crab mortality and hatching success(Horn et

al. 2020). This data will begin to fill the gap in the effects on marine organisms caused by plastic pollution. In the last decade, there has been an exponential increase in the number of publications on microplastic presence and ingestion across marine and freshwater organisms. However, we are just starting to uncover the effects of ingestion and exposure to plastics and the associated chemicals. The last two chapters are laying the baseline work for studying the impacts of microplastics on two important fishery species of crabs along the Pacific coast. Neither of these crabs have been documented to be exposed to or ingest plastic pollution. My work will investigate the exposure of microplastics in Dungeness crab (*Metacarcinus magister*) in Oregon. I investigated whether or not this species of crab ingest or internalize microplastics, as determined if they are able to egest the microplastics or if the microplastics are found in the parts of the Dungeness crab used for human consumption. This project will set the groundwork for future research on Dungeness crabs and microplastics pollution. In addition, this research allows documentation of whether there are any evident patterns of distribution of crabs with versus without ingested plastics. The research into Dungeness crabs also allows examination into how plastics are distributed throughout a crab, including the parts people eat. By studying the prevalence and effects of microplastic ingestion in these crabs, we can assess the impacts of plastic pollution as well as its possible food web consequences.

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Chapter 1 Effects of Environmentally-Relevant Concentrations of Microplastic Fibers on Pacific Mole Crab *(Emerita analoga)* Mortality and Reproduction

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DAH and CLS developed the research question, and DAH and EFG designed the laboratory study. DAH collected the specimens, conducted the laboratory study, and ran the statistical analyses. DAH, EFG, and CLS jointly authored and edited the manuscript.

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.

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

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, mistaking them for food. Investigations have shown that the predominant type of microplastic in the sandy beach ecosystems are microfibers. 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 micro-sized 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 nearshore predators such as surf perf and shore birds, as plastic use, and resultant microplastic presence in nearshore environments increases.

Keywords: *Polypropylene, Food Web, Sandy Beach, Reproductive Success, Development*

Introduction

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 Sa 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, 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 Sa 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 Sa 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 Sa 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 2013). On beaches with shallow slopes, fine sediments, and high food availability, larval densities can be greater than 100,000 individuals/m^2 (Efford 1965, Dugan et al. 2005, Veas 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 (Kollaru 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 Cauwenberghe 2015, Horn et al. 2019). Internalized plastics may become incorporated into an organism's guts, gills, or tissues (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 (Katnelson 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 2011) and can be transferred to an organism's tissue when microplastics are ingested (Teuten 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 Sa et al. 2018), yet most laboratory studies have used PE or PS (de Sa 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 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 (Figure 1). We identified South Beach, Newport, OR (44.604006, -124.063729) as a site with intermediate sediment microplastic density to collected *E. analoga* females and seawater to determine environmentally relevant concentrations for the laboratory exposure study.

We collected surface sand samples (<5cm depth) from the swash zone using a metal hand shovel at 19 beaches along the OR coast(Figure 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 100mL of sand from each surface sediment sample, placed it into a triple-rinsed glass jar with 400mL of hyper-salinated solution (1.2kg NaCl l^{-1}). After the lid was secured, the jar was agitated for one minute and then placed on a flat surface to settle (per Thompson et al. 2004, Horn et al. 2019). Once the sand had settled (< 5 minutes), 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/100mL Flow Rate, Grade GF/A, 47mm Diameter) to capture anything separated from the sand. Three controls with just hyper saline solution were run.

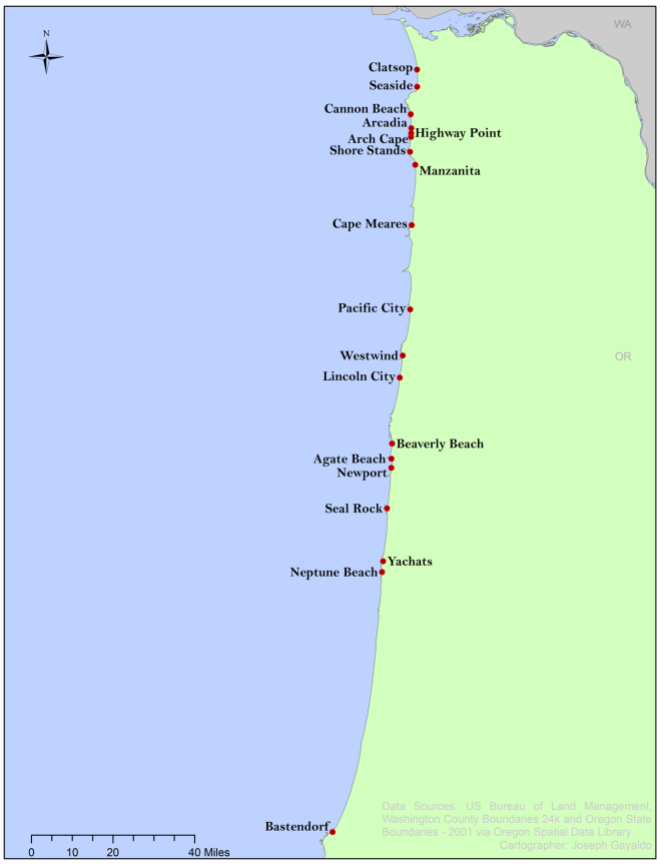


Figure 1. Location Map of sand collection sites along the Oregon coast

Nile Red, a lipid-soluble fluorescent dye which 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 in acetone and diluted in n-hexane (Wiggin & Holland 2019). One ml of solution was applied to each glass fiber filter, covered with the lid of the petri dish, and allowed to dry for 2 hrs. Filters were viewed under illumination by a 455nm 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 1L water samples were collected in the swash zone where the crabs were collected. A 1L DI water blank was run. In the laboratory, each water sample was vacuum filtered through a 47mm glass fiber filter (Whatman 1820-047 Glass Microfiber Binder Free Filter, 1.6 Micron, 4.3 s/100mL Flow Rate, Grade GF/A, 47mm Diameter). The filter was dyed with Nile Red, covered with a petri dish lid, and allowed to dry for 2 hours. The filter was then examined under the dissecting scope using a 455nm 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 1L glass jar with 4cm depth of sand collected from Newport, OR. Artificial sea water (Instant Ocean) maintained at 35ppm, filled the rest of the jar with a lid with aerator placed on top (Supplemental Figure 1). 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(Tosetto et al 2016;Green et al 2017). There was no significant difference in carapace length between crabs exposed to microplastics (19.00±0.54 (mean ±S.E.)) and controls (19.28±0.56)($t=0.34$, $df=62$, $p=0.73$). In each treatment jar, three 1mm pieces of bright yellow polypropylene rope were added to the water every four days for 71-days, or until female crab mortality occurred. The PP rope was purchased from a local marine supply store, the diameter of the rope was <0.1mm and the pieces were cut into 1mm lengths using micro-scissors. The selected experimental time frame (71 days) allowed for two full embryonic development cycles as *E. analoga* has an incubation cycle of 29-32 days (Boolootian 1959, Efford 1969). The microplastic 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, 300mL of the 800mL of seawater was removed from each jar and replaced with fresh artificial seawater (Instant Ocean) and

food. Food (ATLMSPD4 Marine Snow Plankton Diet) (conc. 5ml/liter 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

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.

Four to ten eggs were collected from gravid females every fourth day to assess embryonic stage (1-10) and photographed using a 10X 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 24hrs at 40°C (Rochman et al. 2016, Baechler et al. 2019). The solution was filtered through a 63µm steel mesh, then the residue was transferred into a glass petri dish triple-rinsed with filtered deionized water, and examined under a 10X 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 6 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.

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). 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, 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 +/- 2.8) and 0-9 particles (average 4 particles +/- 0.7) per 100mL of sand sampled (Figure 2.) The 1L water samples collected at South Beach contained 3-7 microfibers (average of 4.6 fibers/L +/- 1.7) and no particles were identified. The fiber sizes in the water and sand samples ranged from 0.03mm - >6mm in length. These findings guided the protocol of three PP fibers per treatment in the mesocosm study to maintain environmental relevance.

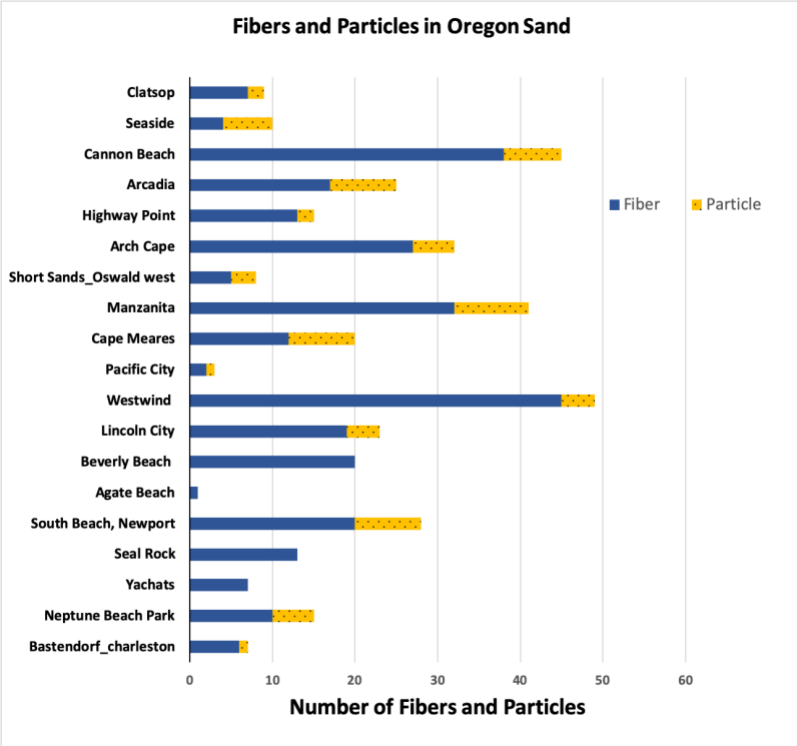
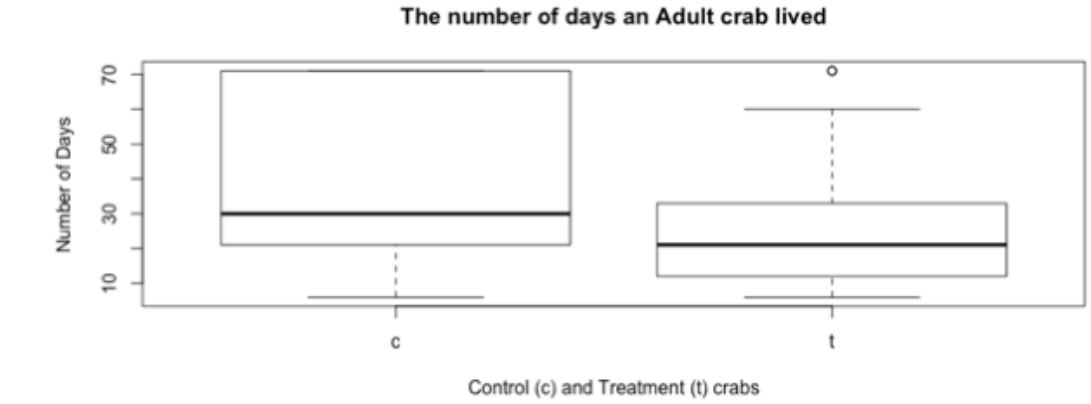


Figure 2. Beach sand collection sites (north to south) with numbers of microplastic fibers and particles per 100mL of sand collected. See location map Figure 1.

Adult Sand Crab Mortality

Crabs experimentally exposed to PP had significantly higher mortality than the control group (Chi Sq ($\mathcal{X}^2$) = 45.83, df = 30, p = 0.03)(Figure 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 ~5.5 days ±2.1 SE. (Table 2)



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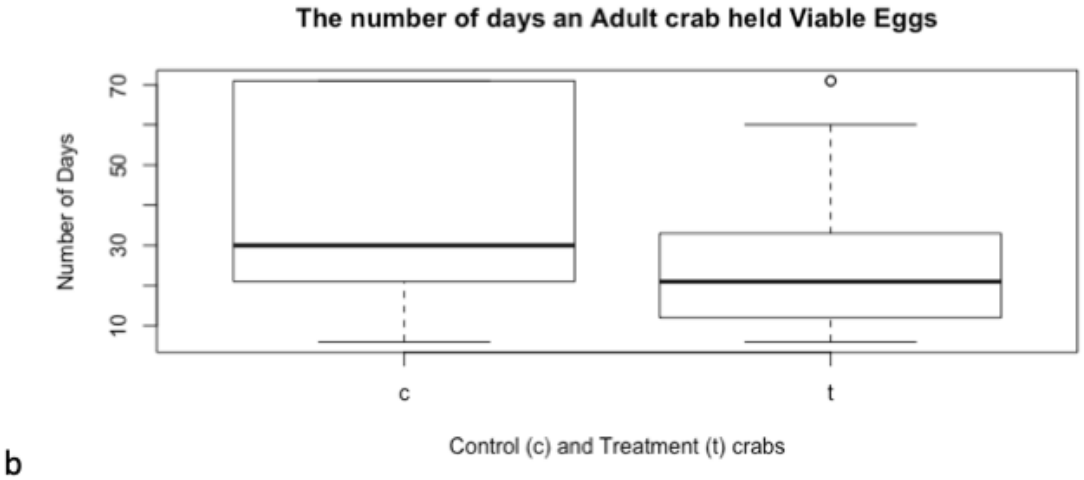

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Figure 3a. Boxplot of Mortality displaying Adult crabs experimentally exposed to polypropylene microplastics had higher mortality than control group crabs (Chi Sq ($\mathcal{X}^2$) = 45.83, df = 30, p = 0.03) **Figure 3b. Boxplot of Viable eggs**; showing the number of days an adult sand crab held live/viable eggs between control and treatment groups.

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)Chi sq ($\mathcal{X}^2$) = 9.55, df = 4, p = 0.04)(Figure 3b). We found that number of PP fibers internalized, decreased the number of days that a crab held live/viable eggs(LRT)$\mathcal{X}^2(1) = 27.54$, $p<0.001$), decreasing by ~4.46 days ±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)$\mathcal{X}^2(1) = 11.825$, $p<0.001$). 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 ~5.06 fewer days ±1.7 SE (LRT) ($\mathcal{X}^2(1) = 39.72$, $p<0.001$) . Crabs *captured with eggs at later embryonic stages*, and exposed to PP held viable/live eggs ~13.3 fewer days ±2.7 SE than control crabs (LRT) ($\mathcal{X}^2(1) = 4.72$, $p = 0.02$).

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)$\mathcal{X}^2(1) = 24.32$, $p<0.001$) (Table 2). Later embryonic

stages experienced ~0.5 fewer stages ±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 ~0.73 stages ±0.7 SE, (LRT)$\mathcal{X}^2(1) = 8.13$, p = 0.004). Embryonic stages were reduced by ~0.33 stages ±2.5 SE (LRT)$\mathcal{X}^2(1) = 8.61$, p = 0.03) during crab molting. The number of parasites in a crab decreased the number of embryonic stages ~ 0.19 stages ±0.19 SE (LRT)$\mathcal{X}^2(1) = 10.82$, p = 0.01). The number of PP fibers internalized by the crab increased the number of embryonic stages ~1.04 stages ±0.5 SE (LRT) $\mathcal{X}^2(1) = 11.53$, p = 0.04). In crabs with egg clutches starting at *stage eight* of embryonic development, crab size increased the number of embryonic stages ~ 0.6 stages ±0.22 (SE) ($\mathcal{X}^2(1) = 8.37$, p = 0.015), crab molting increased the number of embryonic stages ~ 1.46 stages ±0.5 (SE) ($\mathcal{X}^2(1) = 8.74$, p = 0.03), the number of parasites decreased the number of embryonic stages ~0.08 stages ±0.05 (SE) ($\mathcal{X}^2(1) = 8.4$, p = 0.03), and number of PP fibers internalized by crabs increased the number of embryonic stages ~0.07 stages ±0.17 (SE) ($\mathcal{X}^2(1) = 9.58$, p = 0.04).

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**	**Standard Error**
Adult Mortality	Number of PP Fibers internalized	Increased mortality	$\mathcal{X}2(1) = 30.1$, p<0.001	~5.5	2.1
Reproductive Output	stage of embryonic development	Stage 2 of embryonic development effects outcome	$\mathcal{X}^2$= 9.55, df = 4, p = 0.04	N/A	N/A

Reproductive Output	number of PP microplastic fibers internalized	decreased the number of days an adult crab held the egg clutch	$\mathcal{X}^2(1) = 27.54$, $p<0.001$	~ 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	$\mathcal{X}^2(1) = 39.72$, $p<0.001$	~ 5.06	1.7
Reproductive Output	eggs at **later** embryonic stages at the start of the experiment	decreased the number of days an adult crab held the egg clutch	$\mathcal{X}2(1) = 4.72$, $p = 0.02$	~13.3	2.7
Embryonic Development in later start stages (7-9)	later (7-9)egg start stage of embryonic development at the start of the experiment	*fewer* embryonic stages	$\mathcal{X}^2(1) = 24.32$, $p<0.001$	~0.5	0.01
Embryonic Development in start stage 2	Adult crab size	*fewer* embryonic stages	$\mathcal{X}2(1) = 8.13$, $p = 0.004$	~0.73	0.7
Embryonic Development in start stage 2	Adult crab molting during experiment	*fewer* embryonic stages	$\mathcal{X}^2(1) = 8.61$, $p = 0.03$	~0.33	2.5
Embryonic Development in start stage 2	Number of parasites in adult crab	*fewer* embryonic stages	$\mathcal{X}^2(1) = 10.82$, $p = 0.01$	~0.19	0.19
Embryonic Development in start stage 2	Total number of PP fibers internalized	*Increased* embryonic stages	$\mathcal{X}^2(1) = 11.53$, $p = 0.04$	~1.04	0.5
Embryonic Development in start stage 8	Adult crab size	*Increased* embryonic stages	$\mathcal{X}^2(1) = 8.37$, $p = 0.015$	~0.6	0.22
Embryonic Development in start stage 8	Adult crab molting during experiment	*Increased* embryonic stages	$\mathcal{X}^2(1) = 8.74$, $p = 0.03$	~1.46	0.5
Embryonic Development in start stage 8	Number of parasites in adult crab	*fewer* embryonic stages	$\mathcal{X}^2(1) = 8.4$, $p = 0.03$	~0.08	0.05

Embryonic Development in start stage 8	Total number of PP fibers internalized	*Increased* embryonic stages	χ^2 (1) = 9.58, p = 0.04	~0.07	0.17

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 2016).

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, Van Cauwenberghe et al 2015(b), Watts et al 2015, de Sa 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 versus 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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Appendix A

Data Analysis Information

Supplemental Statistical information for reproducible analysis:

To report the impact of each fixed effect, we use the intercept from the coefficient table and the standard error to show how each fixed effect change on the component we are testing. The reporting will read as a positive or negative intercept value ± standard error for each significant fixed effect on the model. This is based on the Wilk's Theorem, which has proofed this approach (Winter 2013). When a model had a possible interaction between fixed effects, we used an interaction function (Winter 2013) to see if effects were interdependent on each other and were not able to be separated effects within the model. We created random slope models, where each crab could have different intercepts as well as slopes for the effect of polypropylene microfiber exposure. By including random slope models, we are able to reduce our Type I error rate (Winter 2013).

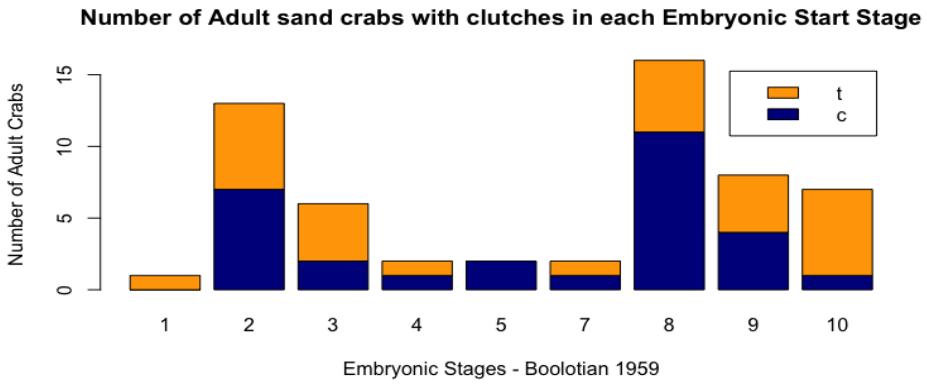

Figure 4. Egg Stages by sample size;The number of adult sand crabs (separated into control and treatment) in each embryonic start stage (Boolootian et al 1959).

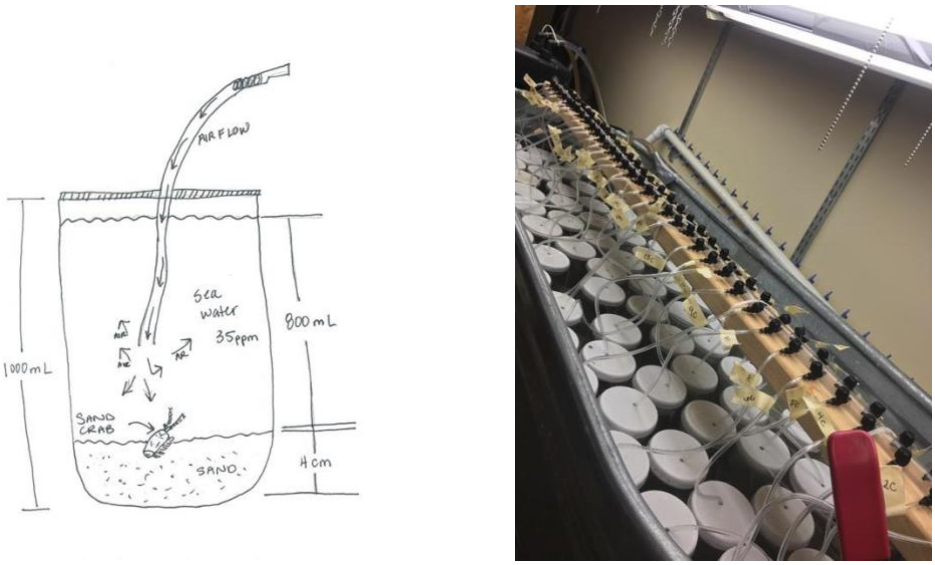

Figure 5. Experimental set up; Drawing of a single mesocosm for scale as well as the full laboratory set up of 64 (32 control, 32 treatment) mesocosms each with one *E. analoga* female either exposed to microplastics or used as a control for up to 71 days. The jars were randomly placed within the water bath and replicates were randomly assigned.

Chapter 2 Field study of the microplastic pollution effects on Pacific mole crabs (Emerita analoga) predator avoidance behavior, reproductive output and parasitism

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Introduction

The abundance of microplastics found in marine environments is increasing daily as more and more plastic enters the oceans as waste (Borelle et al. 2020). As more studies are conducted, the prevalence of these small plastics is being revealed globally. Scientists have worked to identify a diverse suite of marine organisms that ingest these microplastics, the effects of the microplastics, their associated pollutants and how these toxins may enter human food systems (Van Cauwenberghe and Janssen 2014). Even with the increased trend in research, there are still many questions about sources and sinks of plastic pollution, as well as how organisms are affected by their prevalence and ingestion among other questions. Coastal sediments in particular, have been identified as a sink for microplastics (Lusher et al. 2021). As Pacific mole crabs *(Emerita analoga)* call the sandy beach their home, they have been deemed an indicator species for coastal pollution issues such as paralytic shellfish toxins, as well as oil spills (Dugan et al. 1994), making them an excellent choice to study the microplastic pollution problem along the coastline (Horn et al. 2020).

A number of field and laboratory studies have already identified a suite of marine organisms that ingest microplastics and may be impacted by them. Pelagic fish adjacent to the North Pacific Subtropical Gyre have ingested microplastics (Davison and Asch 2011), Norway lobsters (*Nephrops norvegicus*) from the Clyde Sea had plastic fibers in their digestive tracts (Murray and Cowie 2011) and Pacific mole crabs from California ingested microplastics (Horn et al. 2019). In laboratory studies, mysid shrimp, copepods, cladocerans, rotifers, polychaete larvae, and ciliates all ingested fluorescent polystyrene beads (Setala et al. 2013). The added concern for this pollutant is that plastics and the chemicals used in manufacturing can leach into an organism's tissue (Hermabessiere et al. 2017, Gunaalan et al. 2020) as well as attract other pollutants already in the environment, such as heavy metals and persistent organic pollutants (Mato et al. 2001). These chemicals can be transferred to the organism's tissue (Teuten 2009, Hermabessiere et al. 2017) causing an array of negative effects.

The effects of microplastic ingestion have also already been documented in some marine organisms (Guzetti et al. 2018), such as intestinal blockages in copepods (Cole et al. 2015), increased respiration in oysters (Green 2016), changes in fecundity in oysters (Susarellu et al. 2016), and alteration in endocrine systems in fish (Rochman et al. 2014). In Pacific mole crabs, exposure to and internalization of polypropylene fibers caused increased adult mortality, variation in embryonic development and a lower clutch retention rate (Horn et al. 2020). More recently, in shore crabs, the effects of increased environmental temperature and microplastic ingestion have shown a decrease in crab size

and their ability to camouflage against predators as well as prey items (Watson 2021). However, only a handful of these studies have examined the behavioral and trophic consequences of microplastic ingestion. Therefore, we aimed to investigate the effects of the internalization of anthropogenic fibers, particles or microplastics affecting the predator avoidance behavior, reproductive output and the parasitism of this indicator species, the Pacific mole crab.

We investigated whether internalization of anthropogenic microdebris by gravid, female Pacific mole crabs affects their predator avoidance behavior (burying in the sand), reproductive output (number of eggs), or parasitism of these crabs. The Pacific mole crab has been deemed an indicator species (Dugan et al. 1994, Bretz et al. 2002) for paralytic shellfish poisoning caused by harmful algal blooms as well as petroleum toxicity after oil spills (Donahoe et al. 2021). The Pacific mole crab resides in the swash zone along the beaches ranging from Alaska to Baja California (Veas 2013) where microplastics have been found to accumulate (Horn et al. 2019, Lusher et al. 2021). These crabs burrow into the sand at the water line, moving with the tidal changes (Efford 1965) and filter feeding as the waves move across the sand.

Mole crabs have just a few main predators and parasites, and their interactions with these could be affected by microplastics. Their main predators are shorebirds and nearshore fish (Efford 1965). To escape predation, the crabs burrow quickly into the sand as the water recedes after each wave (Kollaru et al. 2011). Environmental aspects may change burrowing speeds in the crab, such as beach slope and sand coarseness (Dugan et al.

2000, Kollaru et al. 2011), where crabs burrow faster in coarse sand than in fine grained sand (Kollaru et al. 2011). Pacific mole crabs can become infected with the acanthocephalan (*Profilicollis altmani Perry, 1942)* parasite (Bhaduri et al. 2018) and the trematode (*Microphallus nicolli* Cable & Hunninen, 1938) as both parasites intermediate host (Bhaduri et al. 2018) to be predated on by shorebirds, both parasites terminal host (Bhaduri 2020). In addition, greater parasite loads slow burrowing, allowing the parasite to reach its terminal host, the shorebird (Kollaru et al. 2011, Bhaduri et al. 2018). We know that there are ecologically joint effects, as parasites slow burrowing speed (Kollaru et al. 2011) and have been found not to affect reproductive success (Bhaduri 2020), however, egg-bearing females do have a higher instance of parasites and the more eggs in a clutch, the more parasites present (Bhaduri 2020).

To explore the potential consequences of consumption of microplastics, we conducted a field experiment to see if gravid, female, Pacific mole crabs (*Emerita anolaga*) displayed a change in their predator avoidance behavior (burrowing into the sand) if they had internalized microplastics. In this paper we test the hypothesis that mole crabs that have ingested high levels of fibers and other anthropogenic particles will burrow more slowly, suffer from greater prevalence of parasites, and produce fewer eggs. To test this hypothesis, we gathered mole crabs from beaches that varied in their microplastic exposure, measured their burying time in experimental arenas, then checked their microplastic and parasite loads in the lab.

Methods

Field testing effects of microplastics on behavior and reproductive output of Pacific mole crabs

To evaluate any potential effects of microplastics on burrowing performance and reproductive output, we collected gravid adult female Pacific mole crabs from two beaches in southern California, Solimar Beach, Ventura (n=92), and Silver Strand Beach, Oxnard (n=25) in the summer of 2017. We selected these two beaches based on prior quantification of microplastic ingestion rates across 38 California beaches that showed Solimar had the lowest prevalence of microplastic ingestion (10%) whereas Silver Strand had high prevalence (80%; Horn et al., 2019).

These crabs tend to aggregate in large numbers in specific areas across the beach (Dugan 2000) rather than having a more homogenous or random distribution across the sand. From each beach we randomly collected gravid adult female Pacific mole crabs of similar sizes from visible aggregating sections on the beach (Dugan 2000). Then we placed the crabs into a holding bucket with sand and fresh sea water to settle. The field burying experiment entailed placing four identical testing chambers in the swash zone (Figure 1; Kolluru et al. 2011). Each chamber consisted of a 4-L plastic tub, filled with 6cm of sand that we collected from the beach in the same locations as the crabs, then homogenized before dividing into the four tubs. We then added seawater to three cm above the sand. Burrowing time into the sand in the chamber was measured to the nearest 0.1 second (Kolluru et al. 2011) for each of the 117 Pacific mole crabs, 92 Pacific mole crabs from

Solimar and 25 from Silverstrand. Due to larger visible aggregate groups at Solimar Beach our collection numbers were uneven between beaches. Burrowing time was defined as the time between beginning of digging activity to the point when the carapace was entirely submerged below the sediment (Kolluru et al. 2011). Each crab was put through a burying trial twice and the times were averaged for analysis. Trials were run on a single Pacific mole crab at a time, with at least 30 seconds separating trials within a chamber (Kolluru et al. 2011). Each Pacific mole crab tested was then euthanized by placing each crab individually in a labelled container in a cooler with dry ice for later dissection in the lab to assess the presence of any anthropogenic fibers or particles (microplastics) that had been internalized.

Figure 1. Field experiment set up; Field predator avoidance set up for Pacific mole crabs: On the beach nearest to the swash zone, we set up the four identical 4-L plastic tubs as testing chambers (Kolluru et al. 2011) filled with six cm of sand from the beach and seawater to three cm above the sand. (photo credit Dorothy Horn)

Laboratory Quantification

Contamination control and cleaning procedures

All surfaces and glassware were cleaned with DI water and kept covered to avoid contamination. Cotton lab coats, clothing, and nitrile gloves were worn during all

laboratory investigations. All tools, glassware and microscope platforms were cleaned with DI water and ethanol in between processing each crab.

Pacific mole crab size

In the laboratory each Pacific mole crab was evaluated under the dissecting scope. Crab length was measured using digital calipers (from the tip of the rostrum to the edge of the carapace where it meets the abdomen, in mm) (Dugan et al. 2000).

Reproductive output

To measure the number of eggs or reproductive output in each gravid crab, we removed all of the eggs from the crab's clutch and placed them onto a glass slide to take a weight measurement. Next, we collected a subsample of eggs from the clutch to reach the desired weight of 0.04g. Then using the dissecting scope we counted how many eggs were included in the 0.04g. To estimate the total number of eggs, we used the number of eggs counted in 0.04g sub sample and the total weight of the eggs to determine the approximate total number of eggs in each clutch. For example if Pacific mole crab x had 150 eggs in the 0.04g measurement and the total weight of eggs measurement was 0.08g, then that Pacific mole crab was estimated to have 300 total eggs in her clutch.

Parasite quantification

In the laboratory, we removed each Pacific mole crab's carapace for enumeration and identification of parasites using a 10X Leica dissecting microscope with Leica camera connected to a computer running Leica Application Suite X Imaging Software. Within

each crab we identified and quantified all parasites; only *Acanthocephalans* were found. These thorny headed worms, once ingested by the sand crab, live in its intestines throughout its lifetime (Kollaru et al. 2011). Following this procedure, each Pacific mole crab and carapace were immediately moved into a pre-cleaned glass beaker for digestion.

Digestion of crabs

Each Pacific mole crab was placed in a 100 mL pre-cleaned glass beaker with 60 mL of 10% KOH solution at 60° C for 24 hours (Rochman et al.2014, Horn 2019) then sieved over a 63 mm copper filter into a pre-cleaned petri dish for analysis under microscopy. Each petri dish was analyzed for any possible suspected microplastics or other anthropogenic micro-debris. Any suspected fibers or particles were placed into a clean 1mL vial with deionized water and sent to the University of California at Davis lab for Raman spectroscopy analysis.

Raman Identification of fibers at University of California Davis

Sample preparation

All tools, equipment, and nearby lab surfaces were thoroughly cleaned using Milli-Q water or filtered isopropyl alcohol. Laboratory blanks containing filtered deionized water were left open during the entire duration of sample processing each day. Samples were filtered onto aluminum-coated polycarbonate filters with a 5um pore diameter. Once

dried, each filter was mounted to a steel ring and stretched over the flat surface of a disc-shaped magnet to provide a smooth surface for imaging.

Raman Analysis

Raman spectroscopic analysis was performed using a Horiba XploRA^{TM} PLUS Raman confocal microscope combined with an internal video camera, a thermoelectrically cooled charge-coupled device (CCD) detector, and operated using LabSpec6 software. The system was calibrated using zero-order correction of the 600mm and 1200mm grating with a silicon wafer and the band at 520 cm^{-1}. A mosaic image of the entire filtration area for each filter was acquired and particles of interest were identified. Particle spectra were acquired using a 532 (25 mW) or 785 nm (100mW) excitation laser wavelength coupled with a 50x or 100x objective. To prevent sample burning and improve spectral quality, laser intensity varied between 0.1 and 100% and acquisition time varied between 0.5 and 90 seconds. If necessary, baseline correction was performed using a polynomial regression model in LabSpec. Spectral matching was performed using Bio-Rad's KnowItAll Raman spectral library. Spectra from contamination in blanks were compared to spectra from each sample prepared on the same day to identify likely contamination in samples.

Statistical analysis

R Studio (version 1.4.1717) was used as the statistical program for analysis.

An ANCOVA was used to measure the main effect of the presence of microplastics, as none of them had more than one fiber or particle internalized (categorical: present or absent), and the parasite presence or absence (categorical), the interaction between microplastic and parasite, carapace length (continuous), estimated number of eggs (by their weight, Continuous), the beach where collected (Categorical), and the tub the burying test was used (categorical), on average burying time (the average of the two trials of a crab) to determine if any of these variables affects the predator avoidance behavior. Because size, beach, and parasite load were significant in the test of collinearity (VIF), we divided the size variable into categories and performed simple regressions to determine the relationship between the categories (size class, beach location or parasite presence) within each independent variable and the response variable. A fully crossed model was run but was reduced due to high VIF. The final model was selected based on its (AIC. The model was then checked using variance inflation factor (VIF), and confirmed none of them had high collinearity. Once all the predictor variables were categorical save for egg weight none of them had high collinearity.

A generalized linear model with Poisson distribution was used to determine the effects of the independent variables of size of each crab (continuous), microplastic presence, parasite presence and beach location of collection on the reproductive output response variable of the estimated number of eggs in each clutch. Again, factors in the model were chosen using AIC then checked using variance inflation factor (VIF), and confirmed none of them had high collinearity.

A second generalized linear model with Poisson distribution was used to determine the effects of the independent variables of size of each crab(continuous), microplastic presence(categorical), total egg number(continuous) and beach location(categorical) of collection on the response variable of parasitism(continuous). A fully crossed model was run but was reduced due to high VIF. The final model was selected based on its (AIC. The model was then checked using variance inflation factor (VIF), and confirmed none of them had high collinearity.

Gradistat software was used to determine the mean grain size of sand for each beach.

Results

Fibers and particles

Of the 117 Pacific mole crabs collected for the field experiment, 22 had internalized some anthropogenic debris, 11 from each beach location, 44% of the Pacific mole crabs from Silverstrand Beach, the site known to have high prevalence of microplastics (Horn et al. 2019), had internalized anthropogenic micro-debris, whereas only 12% of the Pacific mole crabs from Solimar Beach had internalized anthropogenic micro-debris. The RAMAN spectroscopy analysis on the composition of each particle or fiber found that 10 of the 22 microfibers and particles (45%) were cellulose, and we recorded six of those having synthetic dye following Athey et al. 2020's methods. The cellulose microfibers with dye were categorized as anthropogenic; those without dye were not. 12 microfibers and particles (55%) were found to be a type of five types of polymer, as listed below in Table 1. The length and width of each particle or fiber was recorded and shown in Table 1.

Table 1. Anthropogenic particle composition; 22 of the 117 Pacific mole crabs collected in the field experiment from two beaches in southern California (Silverstrand, Solimar) had internalized anthropogenic particles or fibers. 44% of the Pacific mole crabs from Silverstrand Beach and 12% of the Pacific mole crabs from Solimar Beach had internalized anthropogenic micro-debris (fibers or particles). As seen in the table below, there is a mixture of polymers and cellulose components. Any fiber or particle made from cellulose that also had dye present is marked as anthropogenic (Athey and Erdle 2021) as many fibers that are cellulose based with dye or mixtures come from cigarette butts, rayon clothing or baby wipes (Athey and Erdle 2021).

Beach	Type	Length (µm)	Width (µm)	Composition	Plastic	Anthropogenic	Dye Present
Silverstrand	Fiber	2148	14	Polyacrylonitrile	Y	Y	Y
Silverstrand	Fiber	548	11	cellulose	N	Y	Y
Silverstrand	Fiber	281	10	cellulose	N	Y	N
Silverstrand	Fiber	579	82	polycarbonate	Y	Y	N
Silverstrand	Fiber	2498	17	cellulose	N	Y	N
Silverstrand	Fiber	730	16	cellulose	N	Y	Y
Silverstrand	Fiber	1360	33	cellulose	N	Y	Y
Silverstrand	Particle	166	46	polystyrene	Y	Y	N
Silverstrand	Particle	521	224	polycarbonate	Y	Y	N
Silverstrand	Particle	131	83	polycarbonate	Y	Y	N
Silverstrand	Particle	113	23	cellulose	N	Y	Y
Solimar	Fiber	326	11	cellulose	N	Y	Y
Solimar	Fiber	527	7	Nylon	Y	Y	Y
Solimar	Fiber	2210	12	cellulose	N	Y	N
Solimar	Fiber	421	11	cellulose	N	Y	N
Solimar	Fiber	479	20	polycarbonate	N	Y	Y
Solimar	Fiber	2321	12	Polyacrylonitrile	Y	Y	Y

Solimar	Fiber	1359	12	cellulose	N	Y	Y
Solimar	Particle	88	62	polycarbonate	Y	Y	Y
Solimar	Particle	104	46	polystyrene	Y	Y	N
Solimar	Particle	203	66	polycarbonate	Y	Y	N
Solimar	Particle	148	76	Acrylonitrile-acrylic acid	Y	Y	Y

Potential effects of particles and fibers on crabs

Predator Avoidance behavior

The predator avoidance behavior, measured as averaged burying speed, was significantly affected by the presence of microplastics (ANCOVA:F=1.73, df = 1, p=0.02). Within the ANCOVA, parasite presence, beach and carapace length are shown to correlate with each other, but are not collinear, therefore the post hoc test of simple regressions was done to determine the relationship between plastic presence and average burying speed between the two beaches; Silverstrand Beach (1) (linear regression: F=0.84, df 23, p=0.37, R squared (-0.006)) Solimar Beach (2) (F=0.05, df=1, p=0.8, R squared (-0.01)) showing no significant effect of beach. A simple regression was used to determine the relationship between plastic presence and average burying speed with the presence(linear regression; F=2.13, df-23, p=0.15, R squared 0.06) or absence of parasites (linear regression: F=2.19, df=37, p=0.14, R squared=0.03) showing no significant effect of parasites on plastics. Finally a simple regression was used to determine the relationship between plastic presence and average burying speed within the different carapace size groups (linear regression: (A) Crabs 25mm or larger F=0.16, df-11,p=0.69, R squared (-.07), (B) Crabs

22.1 to 24.99mm F=7.63, df=33,p=0.01 (BB) Crabs 20-22mm F=2.84, df=26, p=0.1, R squared (0.06), (C) Crabs 18-19.99mm F=0.43, df=28, p=0.52, R squared (-0.02)) (D) Crabs 17.99mm or smaller had no microplastics found. The relationship between average burying time and microplastic internalization in crabs between 22.1 to 24.99mm in length was significant (Table 2). Mean grain size of Silverstrand beach was 650um and Solimar beach was 275.3um.

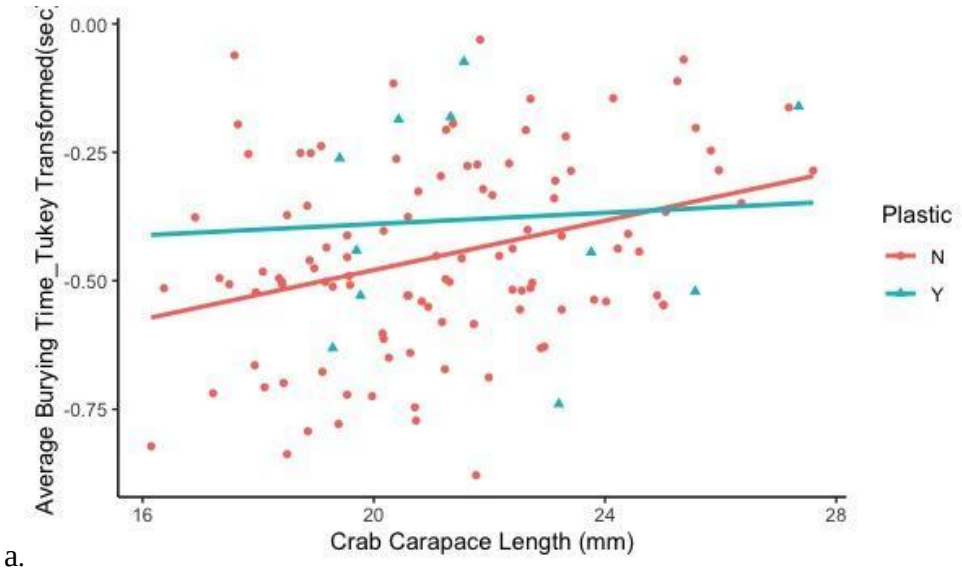
Table 2. ANCOVA output; Analysis for the response variable of Average Burying Time (Tukey transformed).

Analysis of Variance Table

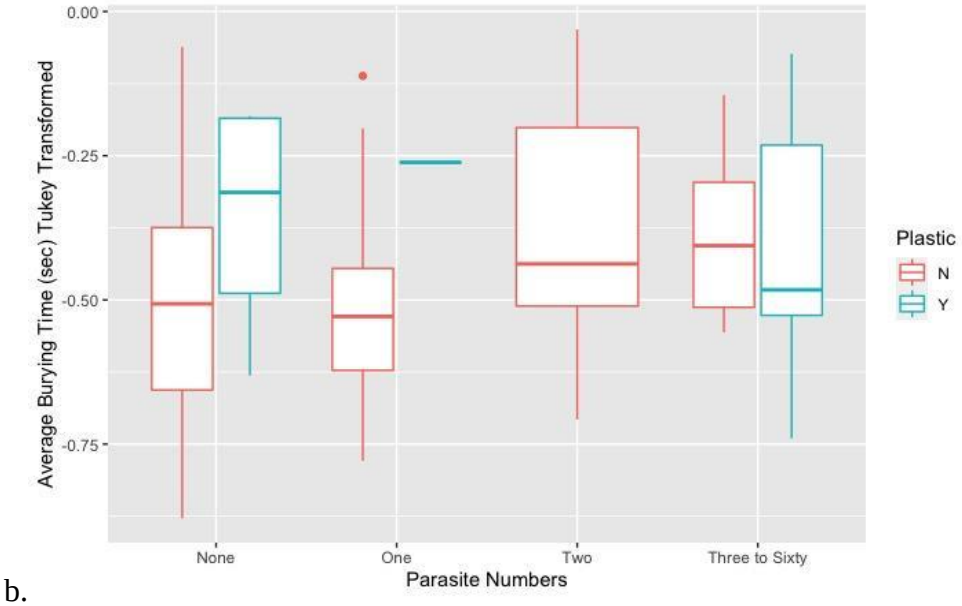
Response: Average Burying Time (tukey transformed)

	Df	Sum Sq	Mean Sq	F Value	Pf(>F)
Plastic	1	0.05	0.05	1.7	0.19
Beach	1	0.13	0.13	4.6	0.03
Size	4	0.34	0.08	2.8	0.03
ParaPres	1	0.17	0.17	5.8	0.02
TotalEggWeight	1	0	0	0.16	0.69
tub	1	0	0	0.03	0.86
Plastic:Beach	1	0	0	0.21	0.64
Plastic:Size	3	0.3	0.1	3.3	0.02
Beach:Size	3	0.02	0	0.31	0.81
Plastic:ParaPres	1	0.01	0.01	0.49	0.48
Beach:ParaPres	1	0.05	0.05	1.66	0.2

SizeGrp:ParaPres	3	0.02	0.01	0.25	0.85
Plastic:TotalEggWeight	1	0.11	0.11	3.7	0.05
Beach:TotalEggWeight	1	0	0	0.29	0.58
SizeGrp:TotalEggWeight	4	0.2	0.05	1.7	0.16
ParaPres:TotalEggWeight	1	0.03	0.03	1.1	0.3
TotalEggWeight	1	0.02	0.02	0.81	0.37
Plastic:Beach:Size	1	0	0	0.3	0.58
Beach:Size:ParaPres	2	0.16	0.08	2.6	0.07
Plastic:Beach:TotalEggWeight	1	0.01	0.01	0.56	0.45
Plastic:SizeGrp:TotalEggWeight	1	0.01	0.01	0.53	0.46
Beach:SizeGrp:TotalEggWeight	3	0.15	0.05	0.66	0.18
SizeGrp:ParaPres:TotalEggWeight	2	0.05	0.02	0.94	0.39
Beach:Size	3	0.05	0.01	0.62	0.6
SizeGrp:ParaPres	2	0.02	0.01	0.47	0.62
Beach:TotalEggWeight	1	0.1	0.1	3.4	0.06
SizeGrp:TotalEggWeight	4	0.07	0.01	0.62	0.64
ParaPres:TotalEggWeight	1	0.01	0.01	0.52	0.47
Beach:SizeGrp:TotalEggWeight	1	0.02	0.02	0.84	0.36
SizeGrp:ParaPres:TotalEggWeight	2	0.07	0.03	1.2	0.29
Residuals	56	1.6	0.03		



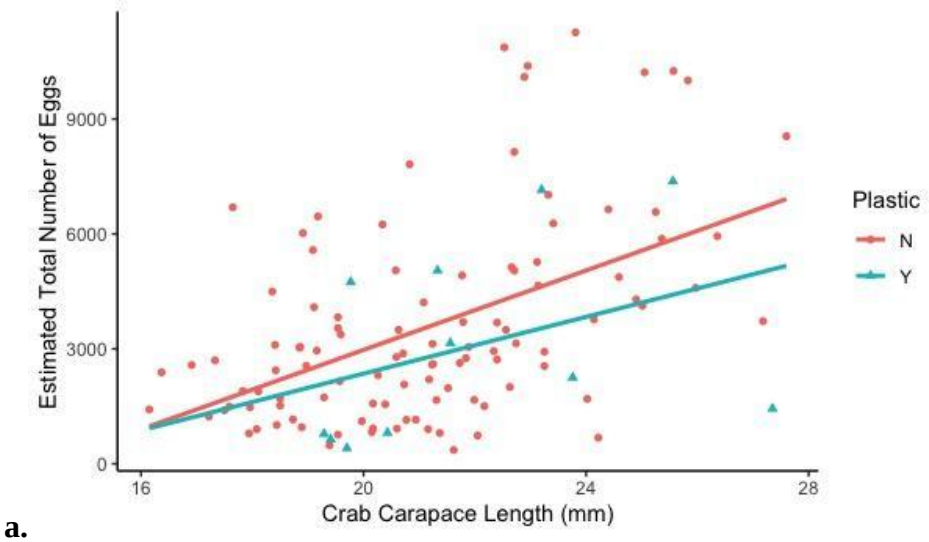
a.



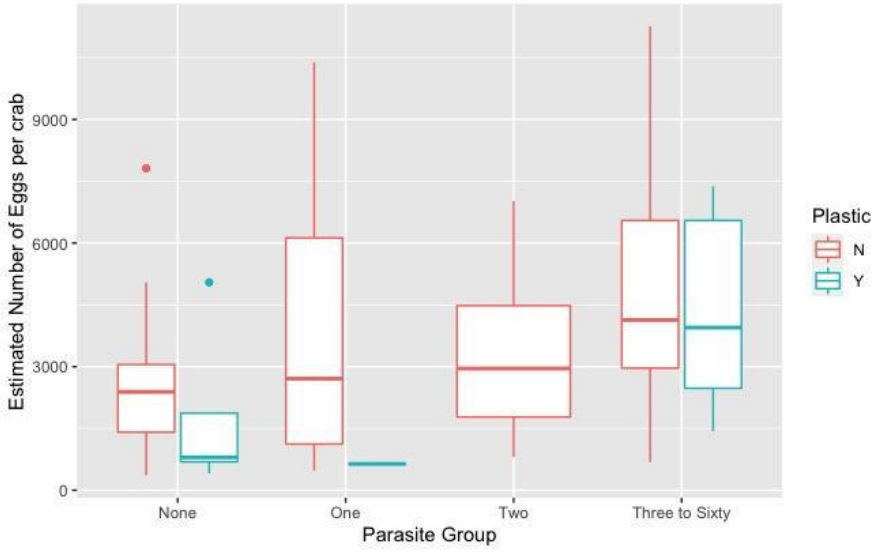
b.

Figure 2. (2a)Crab size burying time; Scatterplot of Average Burying Time (sec), normalized using the Tukey transformation versus Crab size (carapace length (mm)), with plastic presence or absence as a categorical variable. **(2b)Parasite effect on burying**; Box plot with the Y axis as the response variable of Average Burying Time(sec), normalized using the Tukey transformation and the x axis for Parasite number groups (No parasites, One parasite, Two Parasites and 3 or more Parasites). Plastic presence or absence as a categorical variable.

Reproductive output



a.



b.

Figure 3. (3a)Reproductive output and sizeA scatterplot with the Y axis as the response variable of estimated total number of eggs, and X axis as the crab carapace length in mm with the color code as plastic presence (y) or absence (n). **(3b)Reproductive output and parasites**Has the x axis for Parasite groups (none, one, two, and 3 to sixty). The variable parasite was split beyond simple presence and absence into four abundance categories to better show how the relationship among parasites, eggs and plastic changes across parasite loads. Each of the plots also has plastic presence or absence as a categorical variable.

There a significant effect on reproductive output measured by the response variable of estimated number of eggs by any of the independent variables we measured, Carapace Length ($p < 0.05$), plastic presence ($p<0.05$), parasite presence ($p<0.05$) and beach of collection ($p<0.05$; Table 3).

Table 3. GLM output for reproduction; The output for the general linear model with Poisson distribution for the effects of Plastic ingestion, size (Carapace Length), and presence of parasites, and also beach, on the response variable of estimated number of eggs.

Deviance Residuals					
	Min	1Q	Median	3Q	Max
	-84.2	-31.34	-9.66	19.39	80.2
Coefficients					
	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	4.57	0.01	241.2	<0.05	
Plastic Ingestion	-0.18	0	-32.3	<0.05	
Carapace Length	0.13	0	194.9	<0.05	
Parasite Presence	0.19	0	45.06	<0.05	
Beach	0.27	0	62.91	<0.05	

(Dispersion parameter for Poisson family taken to be 1), Null deviance: 206007 on 116 degrees of freedom. Residual deviance: 143302 on 112 degrees of freedom, AIC: 144450, Number of Fisher Scoring iterations: 5

Effects of microplastics on parasitism

There was a significant effect on parasitism by the presence of microplastics (p=<0.05)carapace length for each size category (Group B(22.1-24.99mm) (p=<0.05), GroupBB (20-22mm) (p=<0.05), Group C (18-19.99mm) (p=<0.05) and Group D (17.99mm or smaller) (p=<0.05), as well as the location that crabs were collected, Beach (p=0.05). Plastic has an effect on parasitism on crabs smaller than 25mm.

Table 4. GLM output for parasitism; The output for the general linear model with Poisson distribution for the effects of plastic ingestion, size groups divided into categories (25mm and up, (22.1 to 24.99mm, 20-22mm, 18-19.99mm, 17.99mm or smaller) as well as total egg mass effects on the response variable of parasitism (continuous).

Deviance Residuals					
	Min	1Q	Median	3Q	Max
	-6.48	-1.31	-0.53	0.58	4.73
Coefficients					
	Estimate	Std. Error	z value	Pr)<|z|)	
Intercept	-1.59	0.4	-3.9	<0.05	
Plastic Ingestion	0.53	0.17	3.7	0.001	
Size Group (22.1-24.99mm)	-1.2	0.14	-13.5	<0.05	
Size Group (20-22mm)	-2.58	0.22	-10.6	<0.05	
Size Group (18 -19.99mm)	-2.02	0.22	-12.4	<0.05	
Size Group (<17.99mm)	-3.06	0.4	-8.6	<0.05	
Beach	1.98	0.2	10.4	<0.05	
Total Egg Mass g	0.91	0.2	0.8	0.0003	

(Dispersion parameter for Poisson family taken to be 1), Null deviance: 808.43 on 116 degrees of freedom, Residual deviance: 307.23 on 109 degrees of freedom, AIC: 553.54, Number of Fisher Scoring iterations: 5

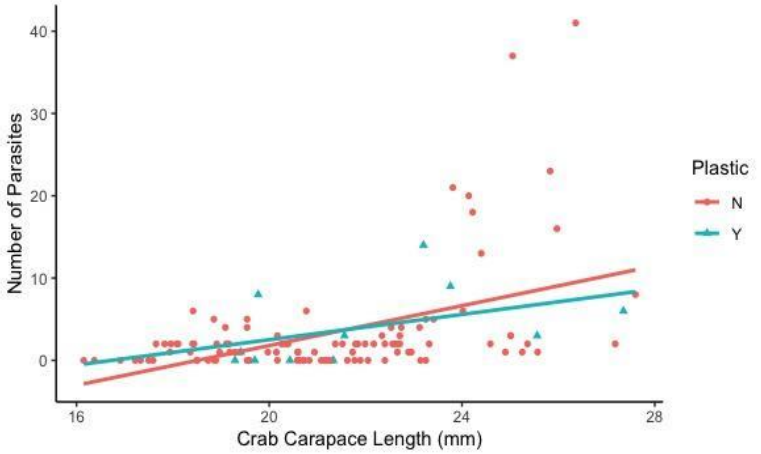


Figure 4. **Crab size on parasitism**; The box and whisker plots with the Y axis as the response variable of Parasitism, the x axis for Carapace group sizes (A 25mm or larger, B 22.1-24.99mm, BB 20-22mm, C 18-19.99mm and D 17.99mm or smaller), with plastic presence or absence as a categorical variable.

Pacific mole crab size

Pacific mole crab length ranged from 16.15mm to 27.6 mm, mean 21.25mm (+/- 2.56) and weight ranged from 1.33mg to 9.14 mg, mean weight of 4.15mg (+/- 1.44). Crabs with no plastic ranged from 27.18mm to 16.15 mm, mean length of 21.09mm (+/- 2.55), with a maximum weight of 9.14mg and a minimum weight of 1.33mg, mean weight of 4.15mg (+/- 1.44). Crabs with plastic ranged from 27.35mm to 21.84mm and a mean length of 19.29 (+/-2.99), with a maximum weight of 8.03mg, a minimum weight of 2.91mg and a mean weight of 4.41mg (+/-1.8).

Discussion

Although there is an increase in studies that have looked at the consequences of microplastic ingestion in marine invertebrates (Foley et al. 2018) most of these studies have been done in the laboratory and have not looked at effects beyond mortality in the

effects of microplastic ingestion (Foley et al. 2018). Especially rare have been studies examining the interaction of microplastic ingestion and other factors such as parasitism (Pennino et al. 2020).

This was the first field experiment done to determine effects of microplastics on the important indicator species *Emerita analoga,* the Pacific sand crab (Dugan et al. 1994). We found a measurable correlative effect between microplastic ingestion and predator avoidance behavior (burrowing speed), showing that intermediate-sized crabs (size classes of 20-21.99 & 22-24.99mm) burrowed slower when they had ingested microplastics. This increases their chance of being consumed by their predators when they have ingested microplastics and could lead to less sand crabs in a population over time due to a higher rate of predation. We have seen that other toxins released into the water column can also affect crab behavior and predator avoidance (Peters et al. 2017).

We also found measurable correlative effects on parasitism of Pacific mole crabs when they ingested microplastics, leading to more parasites in crabs with microplastics in crabs smaller than 25mm and less parasites in crabs without microplastics. From previous research we know that metabolism slows in Pacific mole crabs when they are parasitized (Figueroa et al. 2019), and that crabs with a higher rate of fecundity have more parasites (Bhaduri 2020). However, this is the first study to look at effects of microplastics and parasites in Pacific mole crabs and there could be a few explanations for this correlative effect. It is possible that heavier parasite loads make it more difficult to egest microplastics; however, this will need to be investigated further in future studies. As this is a field study we aren't able to see the entire response curve of possible effects.

There was also a correlative effect on the reproductive output of Pacific mole crabs, decreasing the number of eggs in a clutch when microplastics were internalized, compared to those crabs without microplastics. In a recent laboratory study, polypropylene microplastic fibers were found to affect the hatching success and mortality of adult Pacific mole crabs (Horn et al. 2020) but not the reproductive output. In the current study, the numerous gravid crabs on the beach with less plastic pollution but many fewer on the beach with more plastic pollution is consistent with those earlier findings of plastic reducing reproductive success and mortality of crabs. In addition, the crabs used in the current study had a relatively low microplastic load, with just one piece of plastic per crab and only 12-44% of the crabs with any fibers or microplastics at all.

We know microplastics have an array of effects on marine organisms, but there is much that is still unknown. As the sandy beach ecosystem is complex and the abundance of environmental microplastics has been documented for the last 60 years, it is possible there are other factors that we did not account for in this study. These crabs ingest microplastics along the California coastline (Horn et al. 2019) as well as other shorelines (Miller et al. 2018) in the region, but the total effect of this pollutant is not known. Our findings in this field study, that microplastic ingestion is significantly correlated with slower predator escape, for crabs between 20 and 24.99mm in length, lower reproductive output, and higher parasitism, all relative to similar sized crabs without plastic continues to add to our knowledge of the research on microplastics effects in our sandy beach ecosystem. These findings point to food web aspects given the joint effects we found of

microplastic ingestion and parasitism. This highlights the range of impacts even a tiny piece of plastic can have on a small species. As Pacific mole crabs are an indicator for the sandy beach ecosystem, it is important to pay attention to these small effects as they most likely lead to larger population and food web effects as the continued amount of plastic pollution is not slowing down across marine systems.

We did see trends in line with recent research on other aspects of sand crab biology. For example, there were more parasites in larger female crabs as there is more room for parasites to grow and more food availability (Bhaduri et al. 2018, Bhaduri 2020) for these intermediate hosts. We also saw that crabs that had more than 14 parasites present did not have any microplastics internalized. This could be a sampling effect given that the percentage of crabs with plastic was only 2% of the sample collection, or it could be another correlative effect of parasitism. For example, if crabs are affected This is a question to be investigated moving forward.

We know that trophic transfer of plastics occurs across many marine organisms (Steinbarger et al. 2021, Wang et al. 2021, Gouin 2020, Miller et al. 2020). As Pacific mole crabs are an intermediate host for parasites, it is likely that microplastics are also being transferred into their shorebird (Macginitie 1938) and nearshore fish (Perry 1980) predators. In addition, we know that plastics do affect mole crabs in additional ways (Horn et al. 2020). There are still many questions to be answered about clearing times, possibly nano-plastic (<1um) ingestion effects, as well as joint effects of temperature changes and other ecosystem changes we are seeing with climate change.

Conclusion

Plastic pollution is everywhere and there doesn't seem to be a current stop gap happening for the input into the global marine systems. As more and more plastics enter the oceans and sandy beach ecosystems, organisms will continue to be affected. This study is a red flag warning for continued effects we will see in organisms across the marine environment. The Pacific mole crab has been deemed an indicator species for a reason and we should pay attention to the findings in this study that directly indicate detrimental effects to the species and its population.

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Chapter 3 Diversity of microplastic types and partitioning within body parts of Dungeness Crab *(Metacarcinus magister)*

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Introduction

The presence of microplastics has been documented as "ubiquitous" and raises concerns about the human food supply, including in our oceans (Lusher et al. 2017). More than 690 marine species have been impacted by plastic (Carbery et al. 2018). The largest commercial fisheries are finfish and crabs, with about 260 million people dependent on these fisheries for jobs (Lusher et al. 2017, Teh and Sumalia 2013), but these fisheries could be impacted by microplastic pollution. Indeed, fish and invertebrates vital to our food systems such as crabs, mussels and clams comprise over half of the 220 marine species documented to ingest microplastics (Lusher et al. 2017). We investigated the bioavailability of microplastics to one of the largest fishery items in the Pacific northwestern United States (Norton et al. 2020), the Dungeness crab *(Metacarcinus magister).*

Dungeness crabs (*Metacarcinus magister*) are one of the most economically important fisheries in the Pacific Northwest (Norton et al. 2020). With the growing pressures in the ocean, this fishery is repeatedly closed from pollution, domoic acid outbreaks, ocean

acidification not allowing for proper carapace growth in critical stages of development and a change in currents and temperatures changing larval dispersal along the coast. Previous studies found microplastics distributed throughout Oregon's coastline (Horn et al. 2020) along populated and unpopulated areas of the coast. In Oregon, microplastics have been ingested by Pacific mole crabs *(Emerita analoga)* (Horn et al. 2020), Razor clams (*Siliqua patula)* (Baechler et al. 2020), and Oysters *(Magallana/Crassostrea gigas)* (Baechler et al. 2020). However, no work has investigated the possibility of microplastic ingestion in Dungeness crabs. We aim to fill this gap in the research by creating a baseline for this species.

The Dungeness crab lives most of its life in the benthos of the ocean where microplastics accumulate (Wang et al. 2020, Zhang et al. 2020, Pagter et al. 2020). As all plastics in the ocean eventually sink as their density changes in seawater after 48 hours of UV light exposure(Wang et al. 2020). They likely are consistently exposed to microplastics in their environment and through the food they eat (bivalves, smaller crustaceans and dead fish (Rasmussen 2013))as these organisms have all been shown to ingest microplastics themselves (Ward et al. 2019, Bour et al. 2018,Van Cauwenberghe & Janssen 2014). Many species of crabs trap pollutants and other unwanted debris in their gills (Lusher et al. 2020, Watts et al. 2016). Plastic pollution makes up 95% of all the waste found on beaches and marine coastal areas (Andrady 2011). Estuaries, the secondary habitat for Dungeness crabs (Rasmussen 2013), are a sink for microplastics (Vermeirem et al. 2016, Kaiser et al. 2017). Crabs hunt for food by kicking up or digging up bivalves and/or they are filter feeding through the moving water. Microplastics occupy the same size range as

sand and plankton, making them bio-available to various organisms across a range of feeding strategies (Setälä et al. 2016, Erikson et al 2014). Organisms ingest these plastic particles unknowingly, or mistake them for food as they filter feed large quantities of water and/or sediment for nutrients (Browne et al. 2015; Cole et al. 2013, Farrell and Nelson 2013, Horn et al. 2019).

As Dungeness crabs live their adult lives in the benthos, we hypothesized they would be exposed to microplastics and have ingested pieces as adults. We hypothesized that crabs collected from estuaries will accumulate more microplastics per size than those collected offshore as estuaries have been shown to be sinks for microplastics (Vermeirem et al. 2016, Kaiser et al. 2017) creating a higher bioavailability to Dungeness crabs in the estuary. We also hypothesized that most of the microplastics found would be trapped in the gills of these crabs similar to other crabs (Zhang et al. 2021, Lusher et al. 2020). We collected adult Dungeness crabs from multiple sites along the coast. Our research questions are whether or not Dungeness crabs are ingesting microplastics or if they are able to trap them in their gills or remove them through their feces. We investigated the microplastic load of near shore collected adult crabs as well as crabs from two estuarine systems along the Oregon coast.

Methods

Field Collection

Adult Dungeness crabs (24) were collected from six different sites along the Oregon coast (Figure 1) by Oregon Fish and Wildlife scientists. These locations are used to monitor domoic acid levels in crabs across the region. Crabs were randomly selected using crab pots at different depths depending on locations (Table 1). Crabs were bagged and frozen for transport to the Portland State University laboratory. Each crab was rinsed with deionized water, body condition was recorded as well as weight, sex and carapace width. Carapace width was measured using digital calipers across the largest part of the carapace(measured at the widest spine(10th).

Table 1. **Descriptors of the Dungeness crabs**; Dungeness crab information within this study and their collection sites along the Oregon coast, including: Date of collection, crab ID, crab weight(g), carapace width(mm), crab sex, collection in Nearshore marine or Estuarine location, Latitude and Longitude of Pot and depth of pot for capture.

Date collected	Crab ID	Crab weight (g)	Carapace width (mm)	Sex	NearShore or Estuarine	Latitude (DDM)	Longitude (DDM)	Miles Offshore	Pot Depth (fa)
7/2/18	50-I_1	496.94	154.61	male	Near Shore	43 57.5	124 12.5	2 to 3	30
7/2/18	50-I_2	597.65	154.17	male	Near Shore	43 57.5	124 12.5	2 to 3	30
7/2/18	50-I_3	563.46	153.89	male	Near Shore	43 57.5	124 12.5	2 to 3	30
6/11/18	ABUMB_1	418.22	135.95	male	Estuarine	44 46.2	124 055	0	2.5
6/11/18	ABUMB_2	572.56	151.27	male	Estuarine	44 46.2	124 055	0	2.5
6/11/18	ABS25_1	386.49	140.91	male	Estuarine	44 42.8	124 069	0	2.5
6/11/18	ABS25_2	430.12	137.69	male	Estuarine	45 42.8	125 069	0	2.5
6/11/18	ABS25_3	419.53	143.37	female	Estuarine	46 42.8	126 069	0	2.5
6/1/18	ABS25_4	342.64	138.58	female	Estuarine	47 42.8	127 069	0	2.5
7/1/18	50-A_1	513.99	154.7	male	Near Shore	46 00.0	124 03.6	2 to 3	30

7/1/18	50-A_2	462.94	154.9	male	Near Shore	46 00.0	124 03.6	2 to 3	30
7/1/18	50-A_3	569.27	163.2	male	Near Shore	46 00.0	124 03.6	2 to 3	30
2/12/18	YBP9_1	316.31	137.58	male	Estuarine	44 13.3	124 01.9	0	2.5
2/12/18	YBP9_2	369.77	131.27	male	Estuarine	45 13.3	125 01.9	0	2.5
2/12/18	YBP9_3	375.75	132.08	male	Estuarine	46 13.3	126 01.9	0	2.5
2/12/18	YBP9_4	321.43	130.72	male	Estuarine	47 13.3	127 01.9	0	2.5
2/12/18	YBP9_5	517.9	146.58	male	Estuarine	48 13.3	128 01.9	0	2.5
2/12/18	YBP9_6	177.27	69.03	male	Estuarine	49 13.3	129 01.9	0	2.5
7/11/18	50-B_1	493.45	160.02	male	Near Shore	45 33.0	124 02.0	2 to 3	30
7/11/18	50-B_2	478.55	153.23	male	Near Shore	45 33.0	124 02.0	2 to 3	30
7/11/18	50-B_3	431.69	155.8	female	Near Shore	45 33.0	124 02.0	2 to 3	30
7/12/18	50-H_1	544.52	153.7	male	Near Shore	43 36.5	124 15.3	2 to 3	30
7/12/18	50-H_2	572.34	155.34	male	Near Shore	43 36.5	124 15.3	2 to 3	30
7/12/18	50-H_3	537.5	151.2	male	Near Shore	43 36.5	124 15.3	2 to 3	30

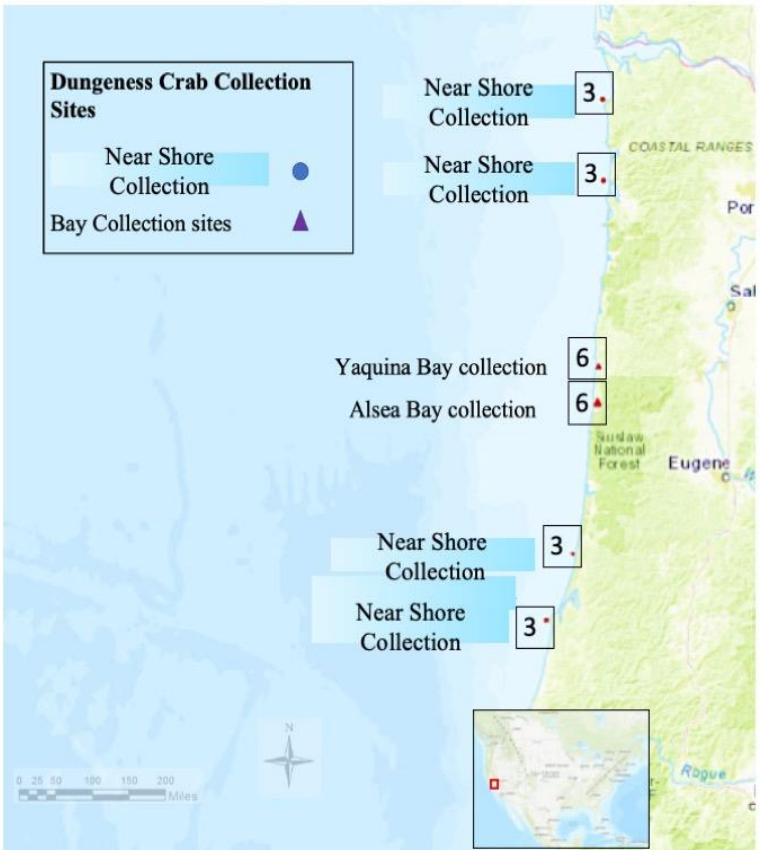


Figure 1. Collection site map; Dungeness collection sites across Oregon.

Laboratory Analysis

Contamination control and cleaning procedures

All surfaces and glassware were cleaned with DI water and kept covered to avoid contamination. Cotton lab coats, clothing, and nitrile gloves were worn during all laboratory investigations. All tools, glassware and microscope platforms were cleaned with DI water and ethanol in between processing each crab.

Crab dissection and digestion

To investigate the presence or absence of microplastics in crabs as well as to determine if microplastics move across tissue barriers to different parts of Dungeness crabs, we

dissected each crab and separated them into 6 distinct parts. (1) Legs - all swimming legs and front claws (2) Body tissue - all muscle tissue located behind the joint where the legs connect to the underside of the carapace (3) Gills - under the carapace, the filtration organ known as gills were collected (4) Cardiac heart - attached to the inside of the shell as a sac, this was dissected out without opening the stomach to ensure contents were included in digestion (5) Telson - tail/feces was removed from the carapace just past the end of the top of the carapace to collect any feces (6) Innards - the rest of any organs within the main body of the crab (gonads, gastric muscles, reproductive organs, pericardial sac, digestive oscipels and midgut cecum).

Each crab was dissected under the fume hood and a procedural blank was also processed with each crab to identify possible contamination. Previous studies have shown that crabs trap microplastics in their gills (Watts et al. 2014) guiding the separation of gills in this study. The cardiac stomach was removed and placed into its own jar to determine if microplastics were actually ingested. The telson was removed and separated into its own jar to determine if these crabs were able to remove any microplastics in their excrement. We separated the body tissue and leg tissue to investigate whether microplastics are present in parts of this crab that are ingested by humans. Lastly the innards were separated and digested to analyze for any microplastics.

Each distinct part (gills, stomach, body tissue, leg tissue and innards; Figure 2) was separately placed into a 1000mL pre-cleaned glass beaker with 400mL of 10% KOH solution at 60 degrees C for 24 hours (Horn 2019) then sieved over a 63 mm copper filter into a pre-cleaned petri dish for analysis under microscopy. Each petri dish was analyzed

for any possible suspected microplastics or other anthropogenic micro-debris. Any suspected fibers or particles were placed onto a clean concave microscope slide, then covered and sealed by a secondary glass slide for FTIR analysis. The suspected fiber or particle was circled on the secondary glass slide for direction in FTIR analysis and to avoid any identification errors.

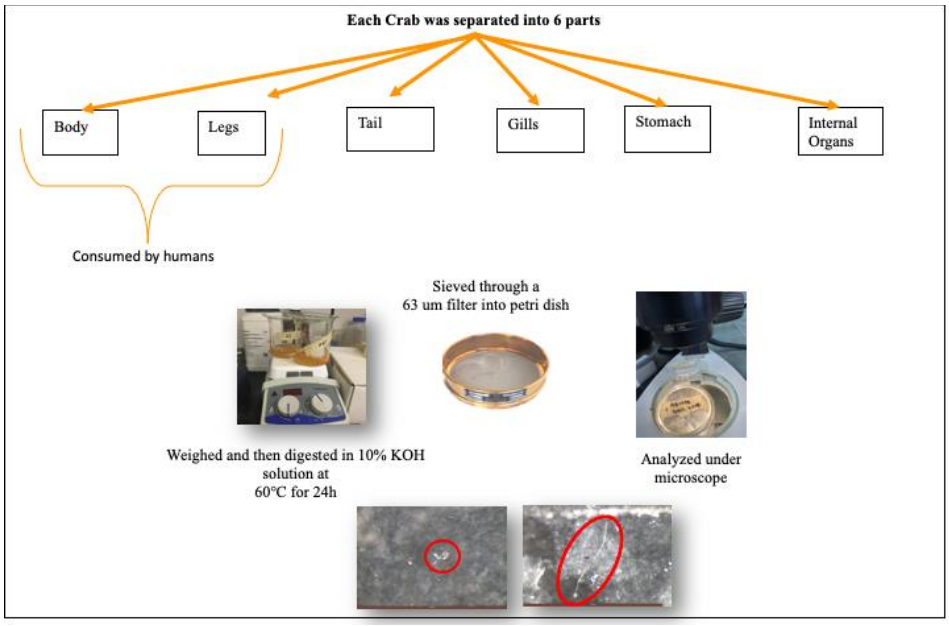


Figure 2. Conceptual model; pathway used for Dungeness crab analysis for microplastics

Microplastic Analysis using Fourier Transforming Infrared Reflectance (FTIR)

Sample slides were placed one at a time in the viewfinder of the micro-FTIR on a Thermo Fisher Nicolet iN10 MX, equipped with a germanium crystal for attenuated total reflectance, with a spectral range of 7800 - 450cm^{-}1 (LaDTGS) or 670cm^{-}1(MCT) and analyzed using the OMNIC Picta (2017 1.7.208, Driver Version: 9.11.0.693). Sample spectra were collected with 16 scans at the resolution of 4cm^{-}1 over the range of 400-4000cm^{-}1. Each spectra was compared against a robust library of spectra within the Cal

State Channel Islands collective database. As a secondary comparison, each spectra was saved and analyzed on Open Specy, open source spectra database for comparison and identification. Results were reported when the match was at least 80% or higher.

Statistical analysis

In order to identify any differences between location of crab collection, types of microplastics found in each crab and any differences in the number or type of plastics found in the six different body parts of the crab an anova was performed. All tests were conducted in the R statistical program (v. 1.4.1717) using the aov function and differences determined with Post Hoc Tukey tests.

Results

Field collection of Dungeness crabs

Sizes ranged from 69.03mm to 163.2mm(Mean 145.1mm +/- 16.7). Crab weights ranged from 177.27g to 597.65g(mean 452.6g +/- 93.1). Half of the crabs (12) were collected at near shore sites (range 151.2mm to 163.2mm, mean carapace size 155.7mm +/-17.9, range 431.7g to 572.3g, mean weight 514g +/- 40.2) and half (12) were collected within estuarine sites(range 69mm to 151.3mm, mean carapace size 134mm +/-3.15,range 177.3g to 572.6g, mean weight 388.6g +/-89.5).

Crab dissection and digestion

Procedural blanks were run with every crab digestion and any contamination was recorded (Appendix Table 1.). Expected procedure is to report any contamination during

analysis for procedural blanks - 10% KOH solution with DI water was put through the same procedure as crab parts for digestion, as well as microscope work -scope control- open glass petri dish was placed next to microscope to track any environmental contamination during analysis. Not all contamination was able to be analyzed under the FTIR. Every crab dissected had at least one anthropogenic particle or fiber in a part of its body. We separated each crab into seven distinct parts: Gills, Body Tissue(collected last after the claws, carapace, innards and gills are removed, we accessed the body cavity to collect tissue that is sought after when eating these crabs) Leg Tissue(from front claws and back legs), Cardiac Stomach, Telson (feces) and Innards. 64% of the collected crabs trapped some anthropogenic fibers or particles in their gills and 64% of the crabs were able to remove anthropogenic fibers and particles through their feces. 48% of the crabs collected had anthropogenic fibers or particles in their cardiac stomachs, 52% of the crabs had anthropogenic fibers or particles in their leg tissue, 36% had anthropogenic fibers or particles in their body tissue and 36% of crabs had anthropogenic fibers or particles in their innards (including gonads, liver, and other organs).

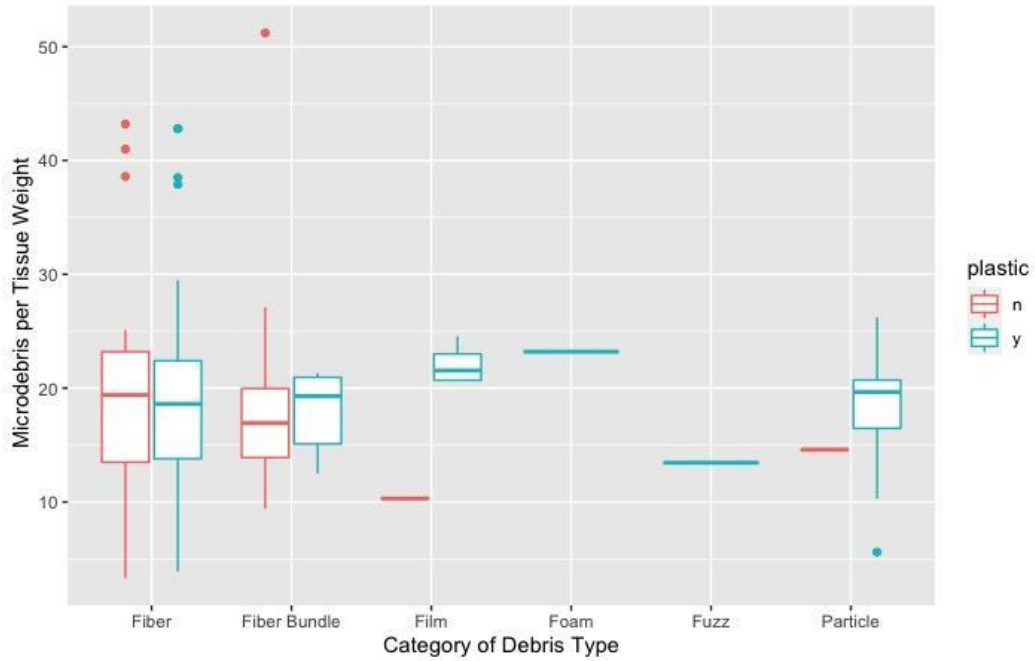


Figure 3. Debris Categories; Box and whisker plot showing the microplastics per gram of tissue over body weight, found in crabs, based on FTIR analysis. On the Y axis is the tissue weight of body parts found with each type of microdebris, the x-axis is the category (Fiber, Fiber bundle, Film, Foam, Fuzz or Particle) and the legend shows whether the FTIR analysis found it to be plastic (teal) or not (red).

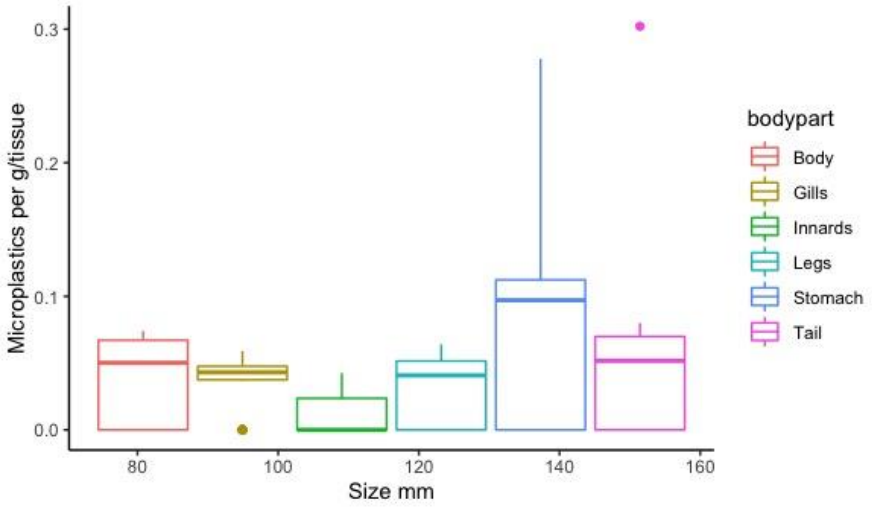


Figure 4. **Microplastics in body parts**; The x axis shows the weight of each crab in grams, and the Y axis shows the microplastics per g/tissue, with body part colored in the box plot.

Microplastic Analysis using Fourier Transforming Infrared Reflectance (FTIR)

124 pieces of debris were collected from the crab parts to be analyzed under the FTIR. 14 of those were cellulose, 30 rayon, 15 nylon and 11 polyester along with an array of other polymers (Table 2; Figure 5).

Table 2. **FTIR Identification**; Source and identification of the 125 pieces of microdebris found in the Dungeness crabs collected, including the crab identity code (see Table 1), the body part in which it was found, the structural type, color and length of the microdebris as well as its chemical composition from FTIR spectroscopy analysis.

Crab ID	Body Part	Type of Microdebris	Color	Length (mm)	FTIR analysis data
YBP9-1	Innards	Fiber	blue	1.56	rayon
YBP9-1	Body	Fiber	clear	2.61	cellulose
YBP9-1	Body	Fiber	brown	3.53	cellulose
YBP9-1	Tail	Fiber	blue	0.49	olefin
YBP9-1	Tail	Particle	blue	0.37	acrylonitrile butadiene styrene
YBP9-1	Gills	Film	brown	0.89	aluminum silicate
YBP9-1	Gills	Fiber	black	0.13	methyltrichlorosilane
YBP9-1	Gills	Fiber	black	0.14	cellophane
YBP9-1	Stomach	Fiber	blue	0.67	nylon
YBP9-1	Stomach	Fiber	clear	0.82	aramid
YBP9-2	Body	Particle	blue	0.14	olefin
YBP9-2	Tail	Fiber Bundle	clear	1.48	rayon
YBP9-2	Tail	Fiber	blue	0.82	cellulose
YBP9-2	Gills	Fiber	clear	1.24	PDMS
YBP9-2	Gills	Particle	orange	1.16	polyethylene high density

YBP9-2	Gills	Particle	black	0.58	aramid
YBP9-3	Legs	Fiber	clear	2.96	cellulose
YBP9-3	Legs	Fiber	clear	1.26	polyvinyl chloride
YBP9-3	Gills	Film	black	0.34	acrylonitrile butadiene styrene
YBP9-3	Gills	Fiber	black	0.91	polyester
YBP9-3	Tail	Fuzz	brown	1.4	polyethylene high density
YBP9-3	Stomach	Fiber	blue	4.96	nylon
YBP9-3	Stomach	Particle	blue	0.23	polyethylene
YBP9-4	Legs	Fiber	clear	0.84	nylon
YBP9-4	Tail	Fiber Bundle	clear	2.35	rayon
YBP9-5	Body	Fiber	blue	2.97	cellulose
YBP9-5	Body	Fiber	blue	0.33	resin dispersion
YBP9-5	Stomach	Fiber	red	0.09	Polypropylene with silicate mix
YBP9-5	Stomach	Fiber	red	2.48	cellulose
YBP9-5	Stomach	Fiber	clear	1.42	polyacetal
YBP9-5	Legs	Fiber	clear	0.71	resin dispersion
YBP9-5	Legs	Fiber	clear	0.8	rayon
YBP9-6	Stomach	Fiber	blue	2.9	rayon
ABUMB-1	Legs	Fiber	clear	0.81	cellulose
ABUMB-1	Legs	Fiber	blue	1.74	rayon
ABUMB-1	Stomach	Fiber	clear	2.1	polyacrylamide
ABUMB-1	Stomach	Fiber Bundle	clear	1.53	rayon
ABUMB-1	Stomach	Fiber	clear	1.27	cellulose

ABUMB-1	Stomach	Fiber Bundle	clear	1.71	rayon
ABUMB-1	Body	Particle	blue	0.24	rayon
ABUMB-1	Innards	Fiber	black	2.38	rayon
ABUMB-1	Tail	Fiber	red	1.8	rayon
ABUMB-1	Tail	Fiber	black	1.61	polyester
ABUMB-2	Innards	Fiber	clear	0.64	cellulose
ABS25 1	Gills	Fiber	black	1.55	styrene maleic anhydride
ABS25 1	Legs	Fiber	clear	1.84	Methyl laurate
ABS25 1	Legs	Fiber	clear	1.51	polyester
ABS25 1	Innards	Fiber	black	0.56	hydroxypropyl methyl cellulose
ABS25 1	Body	Fiber	clear	1.31	PET
ABS25 2	Gills	Fiber	clear	2.4	rayon
ABS25 2	Stomach	Fiber	clear	2.28	naphthalene
ABS25 2	Body	Fiber	clear	2.74	hydroxypropyl methyl cellulose
ABS25 3	Tail	Fiber Bundle	clear	4.3	2-component polysulfide
ABS25 3	Legs	Fiber	clear	3.9	chlorinated rubber
ABS25 3	Stomach	Fiber	blue	0.55	cellulose
ABS25 3	Stomach	Fiber	black	0.42	nylon
ABS25 3	Stomach	Fiber	clear	0.71	rayon
ABS25 4	Tail	Fiber Bundle	clear	2.3	polycarbonate
ABS25 4	Legs	Fiber	clear	1.04	Polypropylene with silicate mix
ABS25 4	Legs	Fiber	clear	1.07	hydroxypropyl methyl cellulose
ABS25 4	Innards	Fiber	blue	2.65	nylon

ABS25 4	Gills	Fiber	blue	0.62	polyvinyl chloride
50I-2	Innards	Fiber	blue	0.67	polyester
50I-2	Innards	Fiber	black	0.17	acrylonitrile butadiene styrene
50I-2	Legs	Fiber	clear	1.39	polyester
50I-2	Tail	Fiber	clear	1.12	polystyrene
50I-2	Tail	Fiber	brown	0.78	polyester
50I-2	Body	Fiber Bundle	clear	1.14	rayon
50I-3	Body	Fiber	clear	1.54	rayon
50I-3	Body	Fiber Bundle	clear	2	polyacrylamide
50I-3	Gills	Particle	blue	0.29	polyvinyl chloride #4
50I-3	Tail	Fiber	clear	1.21	nylon
50I-1	Innards	Fiber	black	0.53	nylon
50I-1	Tail	Fiber Bundle	clear	0.25	di-methyl formamide
50I-1	Gills	Particle	black	0.19	cellophane
50I-1	Legs	Fiber Bundle	clear	1.24	Thionyl Bromide
50I-1	Legs	Fiber Bundle	clear	1.41	polyethylene chlorosulfonated
50B-1	Stomach	Fiber	clear	1.3	polystyrene
50B-2	Gills	Fiber Bundle	clear	3.1	rayon
50B-3	Tail	Fiber	blue	0.82	cellulose
50B-3	Stomach	Fiber	clear	1.47	cellulose
50H-3	Gills	Fiber Bundle	clear	3.1	rayon
50H-1	Innards	Fiber Bundle	clear	1.2	rayon
50H-1	Gills	Fiber	clear	2.3	cellulose acetate butyrate

50H-1	Stomach	Fiber	clear	0.7	polyvinyl acetate
50H-2	Tail	Fiber	red	0.23	nylon
50H-2	Legs	Fiber	red	2.64	cellulose
50H-2	Gills	Fiber	clear	2.1	polyester
50H-2	Gills	Particle	black	0.43	polyacrylamide
50A-1	Gills	Fiber	black	1.57	polyester
50A-1	Gills	Fiber	red	2.38	rayon
50A-1	Gills	Fiber	black	1.75	nylon
50A-1	Gills	Fiber	clear	1.46	nylon
50A-1	Gills	Fiber	clear	1.3	polyester
50A-1	Stomach	Fiber	yellow	1.39	polyvinyl chloride
50A-1	Stomach	Fiber	clear	1.11	nylon
50A-1	Stomach	Film	clear	3.14	rayon
50A-1	Stomach	Fiber	white	1.11	nylon
50A-1	Stomach	Fiber	red	0.53	cellulose
50A-1	Stomach	Particle	black	0.15	cellophane
50A-1	Stomach	Fiber	clear	1.8	nylon
50A-1	Tail	Fiber	clear	1.3	rayon
50A-1	Tail	Fiber	clear	3.4	rayon
50A-1	Tail	Fiber Bundle	clear	1.24	rayon
50A-1	Body	Fiber	clear	13.3	rayon
50A-1	Body	Fiber	clear	1.75	rayon
50A-1	Body	Fiber	clear	0.76	polyester

50A-1	Legs	Fiber	blue	1.14	rayon
50A-1	Legs	Fiber	clear	1.77	Styrofoam
50A-1	Legs	Fiber	blue	0.87	nylon
50A-1	Innards	Fiber	clear	4	rayon
50A-2	Gills	Particle	yellow	0.2	poly(vinyl alcohol:vinyl ethyl carbonate) 5:3
50A-2	Gills	Film	clear	1.08	poly(tetrafluoroethylene)
50A-2	Gills	Film	clear	0.45	Polypropylene with silicate mix
50A-2	Gills	Particle	red	0.15	Indene
50A-2	Gills	Film	clear	1.28	hydroxypropyl methyl cellulose
50A-2	Body	Fiber	clear	0.8	nylon
50A-3	Gills	Fiber	clear	2.24	rayon
50A-3	Gills	Foam	white	0.29	Phenoxy resin #6
50A-3	Gills	Fiber	clear	2.86	HDPE
50A-3	Gills	Fiber	clear	1.67	rayon
50A-3	Gills	Film	clear	0.32	barium metaborate
50A-3	Gills	Fiber	clear	1.42	cellulose acetate butyrate
50A-3	Stomach	Fiber	clear	5.8	polyester
50A-3	Legs	Fiber	clear	3.14	rayon

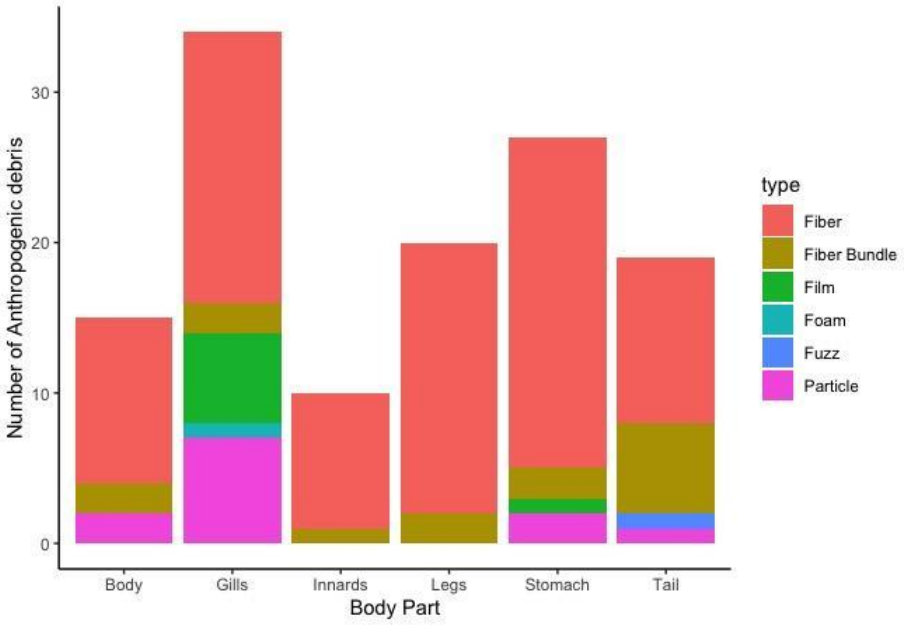


Figure 5. Anthropogenic debris across body parts; The distribution of anthropogenic debris found across different body parts of each of the Dungeness crabs. On the Y axis is the number of anthropogenic debris found, on the X-axis is the body part and the legend fill is the type of debris found (Fiber, Fiber bundle, Film, Foam, Fuzz or Particle)

Microplastics found in Dungeness crabs

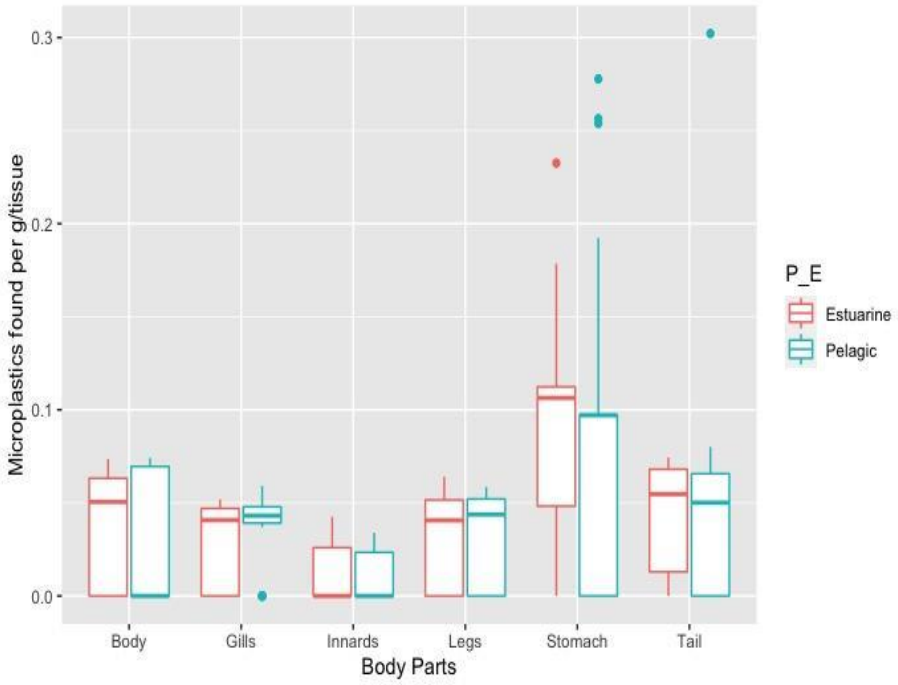
124 (+/-0.48) suspected anthropogenic pieces were collected from the 24 Dungeness crabs and analyzed under the FTIR. 94 of those were single fibers, 15 fiber bundles, 12 particles, 6 films, 1 fuzz, and 1 piece of foam. (Figure 5, Table 3) Cellulose, rayon, nylon and polyester fibers were found across body parts of Dungeness crabs. There was a significant difference in the type of plastic (Fibers, fiber bundles, particles, films, fuzz and foam) found within Dungeness crabs ($F=2.3, df=5, p = <0.05$) with the most being fibers. Individual Dungeness crabs had between one and 22 pieces of microplastic total, with most plastics found trapped in the gills and then the stomach. The highest number of plastics were found trapped in the crabs gills with the second highest trapped in the body tissue ($F=2.3, df-5, p=0.05$). Only fibers and fiber bundles were found in all six types of tissue examined, and film was only found in the gills and stomach, foam was only in the

gills, and fuzz was only in the tail (Fig 5). The source of the crab, whether open ocean or the estuary did not seem to influence the number or type of plastics found in crabs ($F=0.15, df=1, p=0.7$).

Table 3. Average microplastic number per gram of body tissue; Microplastics were not distributed evenly across the six body parts we isolated (Body tissue, Leg tissue, Cardiac stomach, Gills, the feces from the Telson and the innards).

		Average # of MP/gram of tissue by Body Part						Total # MP/body part					
Site	ID	Gills	Stomach	Body	Leg	Telson/ Feces	Innard	Gills	Stomach	Body	Legs	Telson /Feces	Innards
Yaquina Bay	1	0.13	0.27	0.1	0	0.1	0.04	3	2	2	0	2	1
	2	0.15	0	0.07	0	0.11	0	3	0	1	0	2	0
	3	0.08	0.35	0	0.08	0.07	0	2	0	0	2	1	0
	4	0	0	0	0.04	0.06	0	0	0	0	1	1	0
	5	0	0.17	0.1	0.08	0	0	0	3	2	2	0	0
	6	0	0.04	0	0	0	0	0	1	0	0	0	0
Alsea Bay	1	0	0.42	0.06	0.1	0.14	0.02	0	4	1	2	2	1
	2	0	0	0	0	0	0.02	0	0	0	0	0	1
	3	0.04	0	0.07	0.11	0	0.03	1	0	1	2	0	1
	4	0.04	0.2	0.06	0	0	0	1	1	1	0	0	0
	5	0	0.33	0	0.06	0.05	0	0	3	0	1	1	0
	6	0.05	0	0	0.1	0.07	0.02	1	0	0	2	1	1
Near Shore	I -1	0.04	0	0	0.09	0.08	0.03	1	0	0	2	1	1
	I-2	0	0	0.07	0.04	0.12	0.04	0	0	1	1	2	2
	I-3	0.05	0	0.1	0	0.07	0	1	0	2	0	1	0

B-1	0	0.25	0	0	0	0	0	1	0	0	0	0
B-2	0.06	0	0	0	0	0	1	0	0	0	0	0
B-3	0	0.27	0	0	0.3	0	0	1	0	0	1	0
H-1	0.04	0.19	0	0	0	0.02	1	1	0	0	0	1
H-2	0.07	0	0	0.05	0.07	0	2	0	0	1	1	0
H-3	0.03	0	0	0	0	0	0	0	0	0	0	0
A-1	0.23	0.65	0.22	0.16	0.15	0.02	5	7	3	3	3	1
A-2	0.24	0	0.06	0	0	0	5	0	1	0	0	0
A-3	0.21	0.25	0	0.04	0	0	5	1	0	1	0	0



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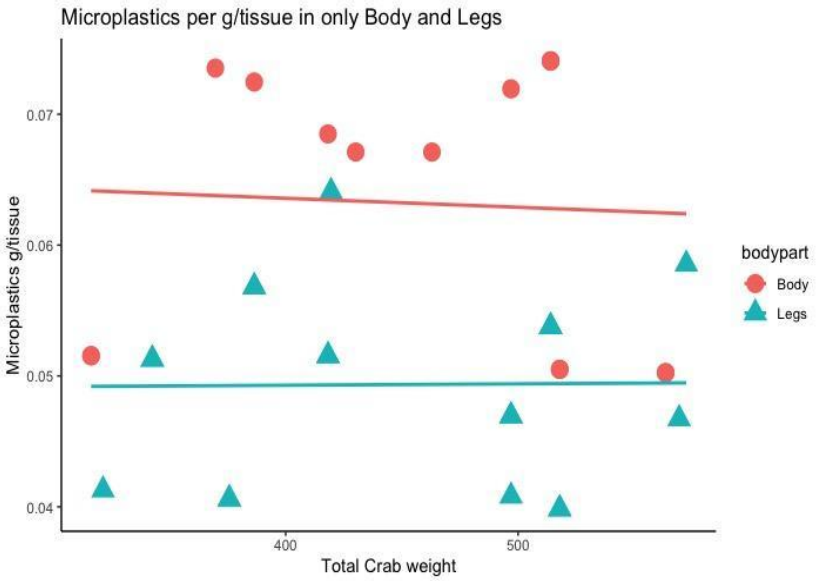

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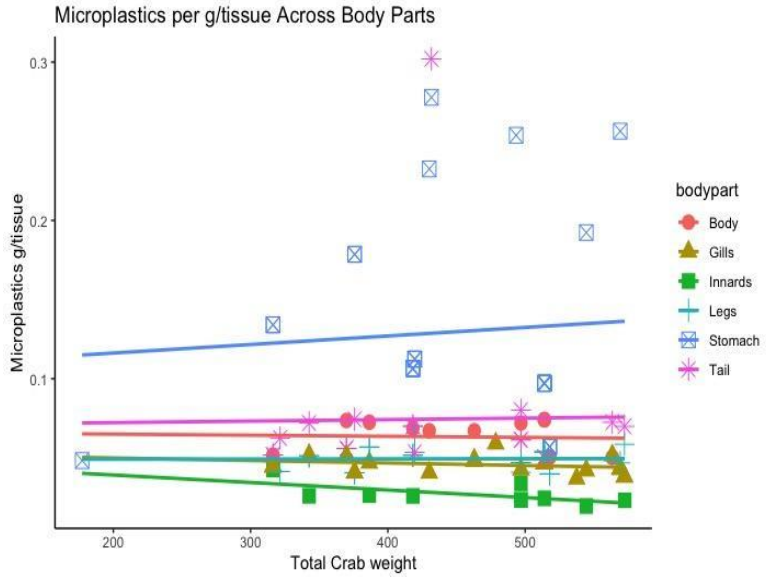

c.

Figure 6. (6a)Microplastics per g/tissue; Box and whisker plot showing the microplastics per g/tissue on the (Y-axis) found in each body part (x-axis) of the Dungeness crabs. The legend codes the collection location of crabs (P=Near shore, E= Estuary), using the FTIR analysis. **(6b)Microplastics increase with body size**; scatterplot showing the number of microplastics per gram of tissue increases with body size for microplastics found in the parts of the crab used for human consumption (Legs and Body tissue) Y axis is microplastics g/tissue and X axis is Total Crab weight. **(6c)Microplastics found across all body parts;** scatterplot showing the number of microplastics per gram of tissue increases with body size for microplastics found in all parts of the crab, Y axis is microplastics g/tissue and X axis is Total Crab weight.

Discussion

Findings

Microplastics were present in every Dungeness crab (*Metacarcinus magister*) we collected. The amount of microplastics per gram of tissue for the whole crab averaged at 0.24 mp g/tissue. Our findings had the lowest amount of microplastics found in current studies of organisms used for human consumption in the Pacific Northwest compared to other studies. For example, the amount of mp g/tissue ranged from 0.62 mp g/tissue in whole Pacific Oysters (Baechler et al. 2020), 0.50 mp/g tissue in whole Razor clams (Baechler et al. 2020), as well as 0.9 mp g/tissue in whole Manilla clams (Davidson & Dudas 2016) and 0.3 mp g/tissue in whole Manilla clams (Covernton et al. 2019).

Table 4. Comparison of microplastic load to other fishery items; Average number of microplastics per gram of tissue in whole organisms investigated in the Pacific northwest, showing Dungeness crabs to have the least amount of microplastics per gram of tissue than other organisms collected as fishery items.

Name	**Body Parts**	**Average # of Microplastics per gram of tissue**	**Publication**
Manilla Clams	Whole Organism	0.9 mp g/tissue	Davidson & Dudas 2016
Pacific Oysters	Whole Organism	0.62 mp g/tissue	Baechler et al. 2020
Razor Clams	Whole Organism	0.50 mp g/tissue	Baechler et al. 2020b
Manilla Clams	Whole Organism	0.3 mp g/tissue	Covernton et al. 2019
Dungeness Crabs	**Whole Organism**	**0.24 mp g/tissue**	**This study**

Intake areas (stomach and gills) had high plastic loads: In addition, plastic foam and films were only found in these organs. The lowest amount was in the innards, which includes all organs except the stomach and gills, and so provide some measure of clearing. The majority of microplastic prevalence studies focus on ingestion of microplastics into the

intestines or stomach of organisms(Pinheiro et al. 2020) so we are unable to compare our findings across multiple anatomical areas of Dungeness crabs to other benthic crustacean studies. The amount of microplastics does seem to be in correlation with the area in which the organism resides (benthos) and how they feed (deposit, filter) (Pinheiro et al. 2020) and what type and the number of microplastics are present in their digestive tracts. Although few studies have investigated these questions and hypotheses in the field, over 80% of the studies so far have been conducted in the laboratory to track the destination of microplastic ingestion and possible effects(Pinheiro et al. 2020).

Similar findings in a laboratory study of the shore crab *Carcinus maenas,* showed uptake of microbeads in the gills, foregut and removal in fecal samples (Watts et al. 2014). These shore crabs (*Carcinus maenas),* have also been shown to accumulate microplastics via trophic transfer from predation on mussels *(Mytilus edulis)* (Farrell and Nelson 2013) and the uptake of microplastics into their gills has caused deleterious effects (Watts et al. 2016) as well as issues with food consumption and energy balance (Watts et al. 2015). Given the effects of microplastics on shore crabs, further studies are needed to examine the possible effects of microplastics uptake on Dungeness crabs to assess possible population effects and consequences.

Within the sample of crabs, we found gills, stomach and feces to be the highest anatomical divisions of microplastics retention. However, the leg tissue and body tissue that is normally the part used for human consumption also contained microplastics. These findings are similar to the microplastic load for other commercial fishery items such as

Razor clams (*Siliqua patula)* (Beachler et al. 2020) and Oysters *(Magallana/Crassostrea gigas)* (Baechler et al. 2020) that found microplastics in the anatomical tissue for human consumption. In working with agencies that regulate the Dungeness crab fishery, we can better inform them of the possibility of microplastic ingestion by human consumption of these crabs. To allow the fishery to stay open and thrive, evisceration orders can be issued to only consume leg and body tissue for these crabs to avoid higher microplastic loads of ingestion for humans. These are similar steps taken when other pollution issues such as domoic acid that concentrates in the innards of the Dungeness crabs, allow the fishery to stay open, but keep the public safe.

The bioavailability of microplastics in the marine environment is high for Dungeness crabs as they live in the benthos where plastics settle. Our findings of 100% presence coincides with the investigation of the Atlantic crab (*Panopeus herbstii)* collected in Florida that had 100% presence of microplastics in the sample population(Waite et al. 2018). Globally benthic marine organisms have ingested high amounts of microplastics (Bour et al. 2018), in the south China sea microplastics were found in all of the benthic organisms sampled(point-head flounder (*Cleisthenes herzensteini*); decorator crab (*Oregonia gracilis*); *Cancer gibbosulus*; anglerfish (*Lophius litulon*); Starfish (*Luidia quinaria*); Ophiuroid (*Ophiura sarsii*); Snailfish (*Liparis tanakae*); Sand shrimp (*Crangon affinis*); Acila (*Acila mirabilis*)), with the decorator crab (*Oregonia gracilis*), having the highest concentration of microplastics (Wang et al. 2019).As well in the southeastern Arabian sea, two benthic invertebrates (*Sternaspis scutata, Magelona cinta)* and (*Tellina* sp.) were both found to have ingested microplastics (Naidu et al. 2018).

These findings highlight the need for further research into microplastic retention time, and whether these crabs are able to clear microdebris through their feces. Mussels have been found to clear upwards of 80% of the microplastics over a six day period in the laboratory (Fernández & Albentosa 2019), however, mussel clearance rates decreased by 62% (Harris & Carrington 2020) in the presence of microplastics in the water pointing to an increase in stress and issues with clearing pollution and the amount of microplastics in these environments continue to increase. We could not find any studies to date regarding retention time or clearance of plastic pollution in crabs. Questions about anatomical loads, juvenile versus adult crab population ingestion as well as laboratory studies to determine if or how microplastics can be egested or passed through in feces need to be answered. Microplastic retention time is important as in other marine species as it can affect physiological functions (Lee et al. 2019).We do not know if there is possible accumulation of microplastics over time, or if these crabs have short gut retention times, similar to other crabs that have been investigated in the laboratory (McGoran et al. 2020). Microplastics being ingested or trapped in gills was similar to other studies on microplastic ingestion in species of crabs (Lusher et al. 2017, Piarulli et al. 2019, McGoran et al. 2020). The gills are highly susceptible to microplastic exposure (Villegas et al. 2021) as they are the primary organs exposed to any contaminants in the Dungeness crab habitat.

Estuaries have been shown to be sinks for microplastics (Vermeirem et al. 2016, Kaiser et al. 2017) so we had expected that crabs in estuaries would accumulate more microplastics than those collected offshore. However, there was no difference in the number of plastics or the types of plastics we found between crabs collected within the estuary or in open ocean pots. There have not been any studies to date comparing ingestion differences in a marine organism between estuarine systems and the open ocean. Dungeness crabs move in and out of estuaries, often spending time in them as juveniles, which might account for the lack of difference, along with the fact that plastics are washed asea even while estuaries retain many. Fibers were the most abundant category of microdebris found, tracking with the most current research, showing that fibers are the primary microdebris found in studies of ingestion (Gusmão et al. 2016, Kroon et al. 2018).

As this study is the first to document the presence of microplastics in Dungeness crabs , there is no comparison available from other populations. Given that this is an economically and culturally important fishery in the Pacific northwestern United States. This study has created a baseline for microplastic ingestion and a stepping stone into possible effects research on the Dungeness crab population and any food web effects of trophic transfer.

As this is a highly sought after fishery item commercially, recreationally and culturally we see this investigation as a stepping stone to assist in driving policy (Provencher et al. 2020) on the prevention of microplastic pollution in our oceans. Finding microplastics in the environment is a common theme over the last decade in ecotoxicology research as the

exponential increase in peer reviewed papers has shown (Provencher et al. 2020). However, existing in the environment does not necessarily mean that organisms ingest it or incorporate it or, when they do, that it negatively impacts the organism. This research is the first verifying that Dungeness crabs have microplastics in them, not only in their stomachs but also that it has moved into tissue elsewhere in the body, including tissue humans consume. Hence, there are possible policy implications (Provencher et al. 2020) and there is an importance to act on plastic manufacturing and recycling to avoid later policy or management that could affect the crabbing industry. As more of these studies become mainstream scientific information, the push for policies to address the issue has increased (Connors et al 2017).

Microplastic pollution is a clear threat to these crabs as a species and has secondary economic consequences to the fishery. Much more research is needed to determine the full nature and severity of the threat and possible management options. Not only is there a continuous source of plastic debris entering our oceans daily (Borelle et al. 2020) studies have shown hundreds of marine species are routinely ingesting plastics. We know that with the possible deleterious effects on organisms, the already stressed population of Dungeness crabs could be highly affected by this pollutant. Dungeness crabs are already susceptible to paralytic shellfish poisoning (Isbister & Kiernan 2005), which has unfortunately closed the commercial and recreational fishery causing economic hardships across the Pacific Northwest as well as a strain on the tribal populations depending on the sustenance harvest each year.

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Appendix B

Control output for blanks

Table 5. Control Information; The reported blanks, procedural and microscope controls. Standard procedure is to report any contamination during analysis (procedural blanks - 10% KOH solution with DI water was put through same procedure as crab parts for digestion) as well as microscope work (scope control- open glass petri dish was placed next to microscope to track any environmental contamination during analysis). Not all contamination was able to be analyzed under the FTIR due to mechanical restraints.

Control Code	Contamination Category	Color	Length(mm)	FTIR
Scope Control	none	none	0	none
Lab Procedural Control for YPB9-1/2	Fiber	clear	4.88	cellulose
Scope Control	Fiber	clear	2.3	not analysed
Lab Procedural Control YPB9-3/4	Fiber	clear	2.47	cellulose
Scope Control	Fiber	black	3.4	not analysed
Scope Control	Particle	red	0.1	not analysed
Scope Control	none	none	0	none
Lab Procedural Control YPB9-5	none	none	0	none
Lab Procedural Control YBP9 5/6	none	none	0	none
Scope Control	Particle	purple	0.52	not analysed
Scope Control	none	none	0	none
Scope Control	Fiber	purple	3.1	not analysed
Scope control	Particle	black	0.3	not analysed
Scope Control	none	none	0	rayon

Scope Control	Particle	black	0.23	not analysed
Scope Control	Fiber	yellow	1.3	not analysed
Scope Control	Particle	black	0.34	not analysed
Scope Control	Particle	purple	0.43	not analysed
Scope Control	Particle	black	0.14	not analysed
Scope Control	Particle	blue	0.4	not analysed
Scope Control	Fiber	blue	4.3	not analysed
Scope Control	Particle	pink	0.3	not analysed
Scope Control	fiber	red	4.3	not analysed
Lab Procedural Control 50B	none	none	0	none
Lab Procedural Control 50B-1/3	none	none	0	none
Scope Control	Particle	red	0.34	not analysed
Scope Control	Particle	blue	0.31	not analysed
Scope Control	Fiber	red	2.5	not analysed
Scope Control	none	none	0	none
Scope Control	Fiber	red	4.2	not analysed
Scope Control	Fiber	blue	1.2	not analysed
Lab Procedural Control 50H	none	none	0	none
Scope Control	none	none	0	none
Scope Control	none	none	0	none
Lab Procedural Control 50H2	Fiber	clear	1.3	not analysed
Scope Control	Fiber	blue	0.89	none

Scope Control	Fiber	blue	1.2	none
Lab Procedural Control 50A1/50A2/50A3	Fiber	blue	0.17	polyester
Scope Control	none	none	0	none
Scope Control	Fiber	red	0.4	Thionyl Chloride
Scope Control	Fiber	blue	1.4	cellulose

Conclusion

The continuing amount of plastic pollution entering the global marine systems is a threat not only to the organisms that live in this environment, but also a threat to human health (Borelle et al 2020) as many people rely on sustenance from the oceans (Landrigan et al. 2020). Over the course of the last decade, an increase in research into microplastic pollution (Provencher et al. 2020) has shown that we have a large gap in our knowledge of the prevalence of plastic debris, specifically microplastic debris, as well as any effects within marine ecosystems. This knowledge gap has led to more and more investigations, not only into the amount of microplastics, the types and the associated chemicals as well as the ingestion of these microplastics (Provencher et al. 2020).

Crabs have been used across marine and freshwater habitats as indicator species of all types of pollution, including oil spill toxicity (Dugan et al 2004) and paralytic shellfish poisoning (Donahoe et al. 2021). As well as indicators of salinity fluctuations within estuaries (Shirley et al. 2004,Giblock and Crain 2013), other chemical pollutants such as heavy metals (Arya et al. 2014) and general habitat quality (Amaral et al. 2009). The mechanisms in which crab gills trap pollutants (Arya et al. 2014) is ideal for the investigation into microplastic pollution using a biological species. Some studies have shown that long term pollutants do cause biological effects on carapace size (Márquez & Idaszkin 2021) when crabs are exposed to heavy metal pollution (Márquez & Idaszkin 2021). In China, a recent investigation using multiple species of crabs as bioindicators was done for the prevalence of microplastics (Zhang et al. 2021).

The work I have completed in this dissertation directly addresses the gap in research not only on effects of microplastic pollution on one indicator species, the Pacific mole crab *(Emerita analoga),* but lays the groundwork for continued research into a very important fishery crab species, the Dungeness crab *(Metacarcinus magister)* in the Pacific northwestern United States.

In the published laboratory study on Pacific mole crabs(Horn et al 2020), we found that even with low, environmentally relevant exposure to polypropylene microfibers, hatching success decreased significantly and adult mortality increased. We found that each crab had variance in embryonic stages, specifically in the effects depending on the embryonic starting stage. There was a decrease in embryonic development when adult crabs experienced natural biotic events such as molting but when also exposed to polypropylene microfibers, embryonic development increased. The size of each adult crab had an effect of embryonic development depending on the starting stage where later embryonic stage clutches had increased development in larger crabs, but decreased development when the egg clutch was in an earlier stage. We also saw a negative trend in the days that each crab carried viable eggs when crabs were exposed to polypropylene microfibers.

In the field study, we found that the ingestion of microplastics had a correlative effect on the predator avoidance behavior (burying in the sand) within a distinct size class (20-24.99mm), as well as the overall parasitism of crabs smaller than 25mm, and a significant effect on the reproductive output of Pacific mole crabs. It is interesting to note that in

both the laboratory study and the field study, the size of these crabs had significant effects on how microplastics play a role in reproductive measurements as well as predator avoidance and parasitism.

In the Dungeness crab study, we found that every one of the crabs we collected had some type of microplastic in one of the six body parts we investigated. There are no other publications to date investigating an entire crab in this manner as most research concentrates solely on the digestive tract and gills. As this is a very important fishery item in the Pacific northwest, it is imperative that we continue this research on the possible effects on Dungeness crabs individually and as a population going forward.

Physiological and toxicological mechanisms of low versus high concentrations of microplastic pollution

Studies on organisms that have ingested microplastics were originally documented at high concentrations in the lab, to show the standard dose response curve. What this did however, was create a gap in knowledge of environmentally relevant concentrations found in marine environments that did not equal the lab testing being conducted. Because of this, it's very hard to convince the general public and policy makers that there is a problem with plastic pollution and ingestion of those plastics. The next step were calls for studies with low concentrations - considered to be environmentally relevant. Plastics are made with known endocrine disruptors such as plasticizers and other synthetic chemicals that interact with cellular hormone receptors. Rochman's (2016) paper showed us that the chemicals in or attached to those plastics can transfer those

toxins into the tissue of marine organisms. Moreover, even nano-particles with environmental toxins can pass into and invade cellular structure (Mato 2001, Verma et al. 2008). Studies on juvenile gobies did show that very low concentrations of microplastics (18.4 ug/L) caused an inhibition of AChE activity and the mixture of microplastics and pyrene significantly reduced IDH activity showing that a mixture of microplastics and environmental toxins can have synergistic effects at environmentally relevant concentrations.(Olivera et al. 2013) Studies on lobster, showed that long term low level exposure to microplastics caused toxic effects and decreased food intake, slowed growth and reduced nutritional status (Welden and Cowie 2016). These results follow the concept of non-monotonic dose response curves, which are harder to track than traditional toxicological monotonic response curves that show an organism's response increases as chemical concentrations increase. Instead, a non-monotonic curve can show an increase in response over time with lower chemical concentrations such as the studies I have mentioned. Laboratory studies showed higher concentrations of microplastics causing mortality from satiation or physical stomach blockage (Cole et al 2013). Measured physiological responses (sub-lethal) include reproduction (Sussarellu et al 2016) and developmental processes (Browne et al 2008) but not direct mortality at lower dosages. We have learned through other chemical response tests that estrogen disrupting chemicals (EDCs) (Eggen et al 2004) are diverse and enter the water through Wastewater treatment plants, runoff from agriculture and roads causing problems for aquatic organisms. Reduced reproduction is an impact of endocrine disrupting chemicals (Susullaru et al 2016, Eggen et al 2004). Because pollutants interact initially at the cellular level, we must increase the types of low level/environmentally relevant studies

being done that are just with microplastics as well as microplastics plus known environmental chemicals. To do this we can focus our efforts on early biological effects (Eggen et al 2004).

Microplastics are creating a serious toxicological issue for these marine organisms. Evidence of pseudo food particles and reduced food consumption have been shown as well as sub-lethal acute and chronic effects (Cole et al. 2011). Evidence of lethal effects have been documented at environmentally relevant doses (Horn et al 2020). Evidence shows behavioral effects, fecundity and maturity impacts, hormonal physiology changes, and effects on genes responding to vital physiological processes(Yin et al. 2021, Stienbarger et al. 2021). We do not have all of the evidence yet, but each study completed adds to the pile of evidence showing that microplastics and their adherence of POP's are very dangerous for individuals as well as populations over time(Rodrigues et al. 2019). This is a gap in the research and needs to be further investigated.

Individual and population effects of microplastics

Sub-Lethal Individual effects have been documented and population effects are only speculative at this point. As of 2017 there is no direct evidence for any negative effects on populations or communities of organisms from microplastic exposure (Lusher et al 2017). We do know that effects such as hatching success (Susullaru 2016, Horn et al. 2020) , fecundity impacts (Susullaru 2016) and male reproductive health are known to have population level impacts (Eggen et al 2004). Overall, if there is less hatching success (Horn et al 2020), there will be fewer crabs in the overall population. We may

not see a population "crash" but a change in community structure is likely and would need to be studied. Like other community structure changes we have seen in the marine environment, such as the impact of sea star wasting disease, the decimation of multiple species of sea stars that changed the community structure of the rocky intertidal habitat over the last few years. If we see a decline in other species populations, we may not even realize it's happening because they are not monitored, as well we may not understand the community level effects as we are lacking in that knowledge as well. Marine species are hard to study at the population level. We can however, study effects of microplastics and other low level pollutants at the cellular and individual level to create hypotheses and modeling to the population effects that may occur. Other anthropogenic contamination studies have shown population impacts such as herbicide impacts in low concentrations (Belden & Lydy 2001) was found to be an effective inhibitor of AcHE. This particular study found results of a lack of acute toxicity at very high concentrations but an increase in toxicity at lower levels as well as synergistic effects with other toxins at that low level. These types of laboratory experiments just haven't been done with plastics yet and are a gap in the research.

My work specifically starts to fill this gap answering multiple questions about mortality, reproductive success, hatching success and the stress of microplastic ingestion on top of normal biotic factors such as molting. Given the state of the oceans today, we need to not only look at single pollutant factors, but also investigate multiple stressors that these crabs face currently to mitigate and prepare the fishery management for future generations.

Conclusion References

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