Comparison of microplastics in feces and pseudofeces of *Mictyris longicarpus* (Soldier Crabs) at One Mile Harbor, Minjerribah

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Note: In consideration of the Traditional Owners of the land, the Quandamooka people, this paper will use the terms Minjerribah (North Stradbroke Island) and Quandamooka (Moreton Bay) to refer to locations used in the study.

Abstract

Plastics pose a major threat to ecosystems worldwide, with continuous production and pollution amplifying environmental degradation. As more plastics enter the marine ecosystem, they begin to break down into microplastics which are then easily ingested by marine organisms. In this experiment we looked at the impacts of microplastics on the soldier crabs in terms of their sediment sorting habits. We were interested in whether they were sorting microplastics out into their pseudofeces or if the microplastics were passing through their digestive system into their feces. This was done through the collection of ten crabs and all pseudofeces off the surface of the sediment in a one by one meter quadrat. Then, a hypersaline solution was used to suspend the microplastics so that we could view and count them under a microscope. We found that there was a higher count of microplastics in feces than pseudofeces. This suggests that soldier crabs are ingesting large amounts of microplastics which could be persisting in their system and tissues.

This may have larger effects on the marine food web through direct or indirect trophic level transfer and, in turn, humans' consumption of microplastics.

Additional Keywords: feeding preference, bioturbation, food web, bioaccumulation

Introduction

Plastic debris in the ocean is a major global concern due to its low biodegradation rate and rapid increase in production worldwide (Gall *et al* 2015). Plastic production has taken a significant increase in the last 70 years. In 1950 there were two million tonnes of plastic produced worldwide; now over 450 million tonnes are produced worldwide (Ritchie *et al* 2023). The increased popularity of plastics has caused serious implications for both terrestrial and marine environments.

Marine debris is one of the major threats to marine biodiversity due to its abundance, durability and persistence in oceans (Gall *et al* 2015). In a study regarding marine debris in 2010 it was found that 192 coastal nations produced 275 million tons of plastic waste. Around 4.8 to 12.7 million metric tons of this plastic ended up in the ocean (Saeedi 2023). As plastic pollution breaks down due to UV exposure, wind, wave action or abrasion it becomes a microplastic, defined as a piece of plastic under 5mm (Devi *et al* 2022). The retention time of microplastics in organisms is longer than the typical time of food digestion and excretion. Because of this, microplastics accumulate in organisms which leads to bioaccumulation in food webs(Saeedi 2023). The small size of microplastics allows them to be easily ingested by small marine organisms such as zooplankton, the base of the marine food web. The microplastics are then transferred up the food chain with many detrimental impacts including humans eating seafood

containing those microplastics (“Lasting Damage” 2023). It is important we begin to understand the retention capacity of microplastics in marine organisms so that we can take action to mitigate these concerns.

The majority of plastic pollution is washed into the ocean by rivers where plastic is trapped in estuaries and coastal ecosystems. One example is Moreton Bay which happens to be one of the largest estuarine bays in Australia (Okoffo *et al* 2024). Microplastics in intertidal zones are actively transported to the backshore leading to large amounts of microplastics in the sand (Tiwari *et al* 2019). This has serious consequences for the multitude of marine organisms that use sediment as their habitat or food source, such as the soldier crab that resides in Quandamooka.

The soldier crab, or *Mictyris longicarpus,* is a small round and blue bodied crab(“Soldier crab” 2020). The soldier crab is a deposit feeder, meaning they eat detritus or microscopic organisms found in the sediment (“Western Soldier Crab” 2017). Soldier crabs play an important role in transporting sediment particles, redistributing and changing the bioavailability of organic matter and oxygenating sediment in a process called bioturbation (Meysman *et al* 2006; Fanjul *et al* 2014). Soldier crabs also have a unique feeding system where they produce both feces, and pseudofeces. Feces is detritus that enters and exits the digestive system, and pseudofeces, is sediment that is not digested for its nutrients but rather packaged and deposited back on the sand in small balls. (Wotton and Malmqvist 2001; Quinn R 2003). Our aim for this experiment is to understand if soldier crabs are ingesting microplastics or are separating the microplastics into their pseudofeces. This issue has serious implications on the marine food chain because if crabs are ingesting large amounts of microplastics, there's a risk of bioaccumulation in the ecosystem. The plastics can leave the crabs' digestive systems and remain in their tissues, potentially passing

to other animals when consumed, further affecting the entire ecosystem (Smith *et al* 2018). This is vital to understand in terms of the health of the soldier crabs, the larger marine ecosystem and human consumption of microplastics.

Materials and methods

Sample Collection

Our study began by collecting soldier crabs and pseudofeces outside of the Moreton Bay Research Station at One Mile Harbor (27.49497° S, 153.40033° E) at low tide. It is important that we collected data during the falling tide because this is when the soldier crabs are most actively performing bioturbation(Takagi *et al* 2010). We created ten one by one meter quadrats horizontally along the coastline, as depicted in Figure One. After laying down the quadrat, we collected all the pseudofeces in the quadrat using spoons and then placed the sediment in a bucket. We then collected ten crabs within the same quadrat and placed them in a separate container. Pseudofeces and crabs for all ten quadrats were collected on the same day. We returned the crabs to the research station and rinsed them in filtered seawater to remove excess sediment, then waited 24 hours for complete digestive system excretion (Takagi *et al* 2010). After 24 hours passed the crabs were removed from the containers and returned to the beach. These steps were repeated for all ten quadrats that we sampled, keeping each quadrat sediment in a separate container.

Microplastic Extraction from Sediment

In order to extract the microplastics from the pseudofeces we created a hypersaline solution consisting of eight grams of flaky salt in 100 mL of freshwater. We subsampled 200 grams of pseudofeces from each pseudofeces quadrat container and submerged that subsample of

sediment in 300 mL of the hypersaline solution. After two hours, the microplastics were floating on the surface due to the density of microplastics being lower than that of the saline solution (Qinglan *et al* 2019). We then took the top 60 mL of the solution and removed it with a syringe. Using a suction filtration system, the 60 mL solution was dried on filter paper (Pichardo *et al* 2024). Due to resource limitations, we had to use cellulose filter paper, Whatman 1 Filter papers(110 mm diameter), but would recommend using fiberglass in future experiments (Chia *et al* 2022). We then rinsed the filter paper with freshwater onto individual petri dishes and counted the amount of microplastics per petri dish using a dissecting microscope. We identified a microplastic as a white filamentous strand 5 mm and under, equally thick through the entire fiber with clear and homogeneous color ("Guide to Microplastic Identification").

Microplastic Extraction from Feces

After the crabs had been in the containers for 24 hours, we removed and returned them to the beach and then weighed each container with the feces. Then using a hypersaline solution (eight grams of flaky salt to 100mL of freshwater) we weighed out 60 mL of this solution in grams and added it to the container with the feces. After the feces was completely dissolved into solution, the feces saline solution was removed and poured into another preweighed container. In order to find out how much feces was in the container we used the weight of the feces saline solution and its container, then subtract the weight of the container prior to the addition of the feces saline solution and the weight of the saline solution to determine the weight of the feces. After two hours we removed 20 mL of the solution using a syringe and used the dissecting microscope to count the number of microplastics using the same identification process as pseudofeces, repeating for the following 40 mL in 20 mL increments.

Data Analysis

We standardized the data to be the number of microplastics per gram for both pseudofeces and feces. We then used this data for the following analysis.

Because we filtered the pseudofeces and did not filter the feces we used a Welch two-sample T-test to deduce if this impacted the count of microplastics. Using a paired T-test, we compared the feces and pseudofeces data from each quadrat. This allowed us to compile statistical data to understand if the microplastics were higher in feces or pseudofeces.

Results

The mean amount of pseudofeces collected from each quadrat was 680.57 grams with a standard error of 82.68, which was then subsampled to 200 g for analysis. The mean amount of feces collected was 1.241 grams with a standard error of 0.433. We found an average higher number of microplastics strands per gram in the feces compared to pseudofeces, see Table 1. There was a mean of 0.37 pieces of microplastics per gram of pseudofeces with a standard error of 0.0586 and a mean of 160.70 pieces of microplastics per gram of feces with a standard error of 67.28.

In the paired t-test we found a p-value of $p=0.04$, which was used to compare the mean amount of microplastics in pseudofeces and feces. In the Welch two sample t-test we found a p-value of $p=0.58$ which was used to compare the filtered and unfiltered process.

Discussion

Based on our standardized data and statistical analysis we found that there is a difference in the amount of microplastics in feces and pseudofeces. Soldier crabs are excreting more microplastics in feces than the amount found in pseudofeces, therefore, a large amount of

microplastics are passing through the soldier crabs digestive system. Based on the paired t-test we found that there was a statistically significant difference in the amount of microplastics in feces and pseudofeces. The results of the two sample t-test showed that there was no statistically significant difference between filtering the pseudofeces and not filtering the pseudofeces therefore not impacting the methodology or results of this experiment.

The results of this study suggest that the soldier crabs may be preferentially eating the microplastics. This could be due to the fact that algae grows on plastic, which is soldier crabs' preferable food in the sediment (Ingestion 2021; Quinn 1986). Microplastics have the capability to translocate from the digestive system into the circulatory system and surrounding tissue allowing for microplastic persistence and bioaccumulation in organisms (Smith *et al* 2018). Existing experimental studies found that the toxic effects of microplastics on decapods most commonly include oxidative stress, immunotoxicity and reproductive and developmental toxicity (D'Costa 2022). These impacts all alter the normal function of crustaceans and may affect development and survival (D'Costa 2022).

The findings from our study not only have implications on the soldier crab system, but the larger food web through direct consumption or indirect trophic transfer in the marine food web, and in turn the human consumption of microplastics from seafood. Humans' digestive systems can be impacted by microplastics due to physical irritation. This leads to inflammation, which impacts the intestinal microbiome and can result in unbalanced bacteria levels causing various gastrointestinal symptoms (Lee *et al* 2023). In terms of the respiratory system, microplastics can cause oxidative stress when inhaled leading to inflammation and damage in the lungs(Lee *et al* 2023). Microplastics can also act as carriers of other environmental toxins, such as primary polystyrene, and higher exposure to primary polystyrene leads to increased risk of

chronic obstructive pulmonary disease(Lee *et al* 2023). Microplastics have a multitude of interferences with hormonal pathways leading to various endocrine disorders such as metabolic disorders, developmental disorders and reproductive disorders (Lee *et al* 2023)**.** While the long term effects of microplastics on organisms is largely unknown, microplastics still pose significant risks to marine and human health and it is vital that we begin to understand more about microplastics in order to take steps to mitigate their potential long-term impacts. We suggest that further research be done on soldier crabs through dissection after excretion period to understand the concentration of microplastics that are persistent in the tissue and soldier crab system. This information will add to the understanding of the quantity of bioaccumulation in the food web and in turn the amount of microplastics humans are at risk for consuming. Without further research, we risk detrimental impacts on marine ecosystems, food webs and human health as these plastics break down and persist in the environment leading to unpredictable consequences for future generations.

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Figure Legend:

Figure 1: One by one meter quadrat locations outside of the Moreton Bay Research Station at One Mile Harbor(27.49497° S, 153.40033° E), horizontal to the tideline.

Table 1: Standardized data including number of microplastics per gram of feces and pseudofeces, including the average of microplastics for all of the quadrats for pseudofeces and feces.

Figure 1.

Table 1.

Microplastic Strands Per Gram

	Pseudofeces	Feces
Q1:	0.2448163877	92.59259259
Q2:	0.2894644907	44.79166667
Q3:	0.2639573684	43.40659341
Q4:	0.4595863723	650
Q5:	0.1398391849	9.803921569
Q6:	0.6837009682	100
Q7:	0.2098321343	7.1278826
Q8:	0.2786762876	18.56287425
Q9:	0.4527813713	406.6666667
Q10:	0.6442269277	234.0425532
Mean:	0.3666881493	0.3666881493