Microplastic accumulation in *Hemigrapsus oregonensis* and its effects on oxygen intake

Introduction

Each year millions of tons of plastic enter the environment (Borg et al. 2022). Plastics take hundreds of years to break down into microplastics (MPs) of less than 5 mm in length (Watts et al. 2016). These microplastics accumulate in coastal areas, affecting the species that inhabit coastal environments and can be ingested by organisms in all trophic levels. *Hemigrapsus oregonensis* is a native crab species found abundantly in the Pacific Northwest region of North America. Its reactions to environmental stressors, such as exposure to MPs, can provide insight onto the ecological and physiological effects of MPs on crab species, especially in economically important species such as the *Metacarcinus magister*.

FISH 497C Tire Tread Team (2024) studied whether MP accumulated in the stomach of *H. oregonensis* and its effect on the growth and energetics. The study found that MPs were found to have accumulated in the stomach of the subjects that were given MPs. However, no significant effect of MP presence on the concentration of glucose in hemolymph.

Another way that MPs can enter the body of crabs and other organisms is through ventilation. Watts et al. (2016) found that when *Carcinus maenas* experienced acute inhalation of the polystyrene microspheres, they experienced a small decrease in oxygen intake and ion regulation. However, despite the decrease in oxygen intake, no significant change in osmotic stress was found after acute MP exposure, less information is known about the effect of chronic exposure of MPs Different studies show conflicting evidence on whether more MP accumulation occurs in the stomach or the gills (Zhang et al., 2021; Truchet et al., 2023)

We explore whether the presence of MPs in *H. oregonensis* tissues leads to MP accumulation in the gills, stomach, and hindgut, and if MP accumulation leads to impaired oxygen intake. We hypothesize that: (1) MPs would accumulate in the stomach, gills, and hindgut of the crabs. (2) The presence of MPs causes decreased oxygen uptake. (3) There would be more MP accumulation when the crabs only had access to MPs compared to having access to both food and MPs.

Methods

210 individuals were collected from Lion's Park Boat Launch. We had two trial groups (MP-only and fed) and a control group. Each of the two trial groups had 9 individuals sampled (n=9), and the control group had 20 individuals sampled (n=20).

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Commented [AWE3]: I feel like these two clauses are not contradictory enough to use "although." I feel like instead they are supporting each other because the fact that the study did not find significant results supports the idea that there is little information known about the effects of chronic exposure. Maybe you could clarify these clauses a bit more

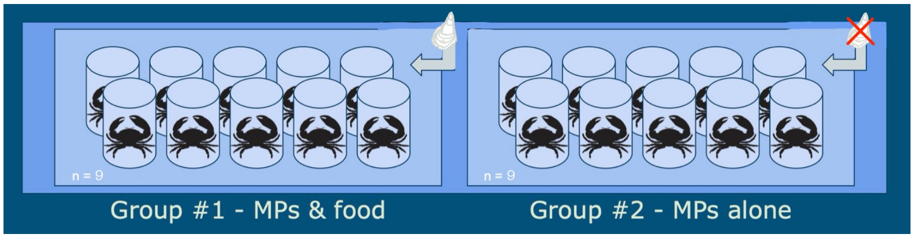

Figure 1. Experimental setup of trial groups. Each trial group had jars for each individual within a larger tank. Trial group #1 (fed group) was fed a mixture of ~1.7g of oyster and 15 mg of MPs. Group #2 (MPs-only group) was fed 15 mg of MPs.

[The first group (fed group) was fed a combination of oyster and MPs (Fig. 1). A single oyster was divided into 9 pieces, measuring at ~1.7 g per fed individual. The individuals were given 15 mg of MPs. The second trial group (MP-only group) were given only MPs (15mg).]

The MPs used in the trials were obtained by shaving blue bottlecaps of mixed plastic type into microplastic shavings with a file. The type of plastic was used to mimic plastic pollution commonly found in *H. oregoneisis* habitat. These MPs were then placed in the residual liquids from the oyster used to feed the fed group. This was to stimulate olfactory senses of the individuals to induce feeding of the MPs (SOURCE).

The individuals were held in individual jars within a larger tank [for each trial group] (Fig. 1). The water was held at 13°C and had a salinity of [30 ppt]. Each jar was covered with mesh and a lid. Each lid had a hole to allow for water flow. The mesh was intended to prevent the MPs from flowing out of the jar. Each jar had an oyster shell placed diagonally in the jar to allow the crabs to access different points of the water column.

At the end of the first week, we experienced a mass mortality event. To ensure that the remaining individuals survived, the remaining individuals were released into the larger tank. The dead individuals were frozen during the period between when they were taken out and when the tests occurred at the end of the two weeks.

After two weeks, hemolymph samples were taken from the surviving individuals of each group to perform a lactate assay. A t-test was performed on the two trial groups. The individuals were then frozen for 20 minutes to induce mortality before being dissected under a dissection scope. The focus of the dissection was to look at the amount of MPs found in the body, with a focus on the stomach and gills.

Results

The MP-only group experienced a 56% mortality rate. The fed group experienced a 89% mortality rate. All mortalities occurred in a mass mortality event in the first week of the study.

Dissections:

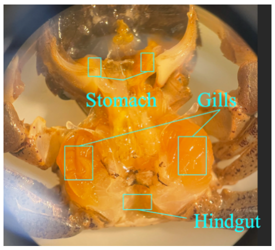

Figure 2. Dissection of surviving crab from the MP-only group showing the stomach, gills, and hindgut, as areas of focus.

A dissection was performed on each crab under a dissection scope focusing on the stomach, gills, and hindgut (Fig. 2). No MPs were found in any of tissue samples in individuals from either trial group. The samples that had died during the mortality event showed signs of greying gill color symptomatic of hypoxic conditions.

Lactate levels:

The average lactate concentration in the MP-only trial group was 205.04 µmol/L from the 8 samples taken from 4 living individuals. The average lactate concentration in the fed trial group was 32.97 µmol/L from two samples taken from the singular surviving individual in the trial. A t-test run on the data from both trial groups

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Discussion

No MPs were found in either trial group. This refutes our hypothesis, that accumulated MPs would be found in the gills, stomach, and hindgut. This is different from the findings of FISH 497C Tire Tread Team (2024), which found significant accumulation in the stomach. Although they did not investigate MP presence in the gills of *H. oregonensis.* The cause of the difference in MP accumulation in this study compared to the previous performed by FISH 497C Tire Tread Team (2024) is likely the type of plastic used. The plastic in this study largely floated, making it more difficult for the crabs to readily access. Additionally, the tire tread used by FISH 497C Tire Tread Team (2024) is more porous and was likely able to hold more of the liquids and oils of a food source. More research on MP accumulation of different types of plastic would be able to provide more insight as to why no MPs were found in this study as compared to previous studies. Further studies may focus on colorless polyamide, polypropylene, and polyethylene terephthalate fibers of 1–5 mm, which have been found to be the most common form of MP in metadata studies of other crab species (Truchet et al., 2023)

Given that no MPs were found in the dissections of either trial group, MPs are unlikely to be the cause of the differing lactate concentrations. The higher level in lactate in the MP-only group may be related to the crabs' interactions with other crabs after the mortality event. When the crabs were released into the tanks after the mass mortality event, the four MP-only crabs were put together in one tank as compared to the singular fed crab that was alone in the larger tank.

The higher level of lactate could be due to interactions between MP individuals causing higher levels of activity, such as fighting. Short bursts of activity in other intertidal crabs leads to higher levels of lactate accumulation in the hemolymph (Burke, 1979). The increased levels lactate in the hemolymph could also be attributed to a sampling error as there was only one individual from the trial group and four individuals from the MP-only trial group that could be accurately sampled for lactate.

The biggest limitation of our study was the mortality event that occurred in the first week of the study. The cause of the mortality event likely was due to the lack of water flow between the jars and the tank due to the mesh on top of the hole in the jar lid. This likely caused hypoxia in the jars, leading to the mortality event. Additionally, the fed crabs were given too much food leading to the oyster rotting in the jars, causing a build-up of ammonia in the jars. This caused the amount of fed individuals that died in the mortality event to be greater than the amount of MP-only individuals that died in the mortality event. Future studies would investigate more effective methods of experimental setup that would decrease the mortality rate of the subjects. One way that this could be done is by avoiding types of MPs that float and not using mesh over the jars, increasing the flow of water and oxygen.

Our study found that no MPs were found in the tissues of individuals from either group. No significant physiological damage was caused by the presence of MPs, regardless if the subject had access to alternative forms of food or not. This shows that *H. oregonensis* and other crab species may not be as affected by this biological stressor as other types of organisms. However, this study conflicts with previous findings of studies investigating accumulation of MPs in the gills and stomach of crabs (FISH 497C Tire Tread Team, 2024; Truchet et al. 2023). More research is needed on both the accumulation of MPs in *H. oregonensis* and its effects on the oxygen intake of these crabs.

Sources:

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