Introduction:

Plastics are more prevalent in the world now than ever in history, and their prevalence will remain a staple in modern society due to their versatility in various applications. Between 1.15-2.41 million tonnes of plastic enter the oceans every year from land and rivers, where it is broken down and degraded (D'Costa, 2022). This degradation forms microplastics (MP), which is a general term that is used to describe any small plastic debris (D'Costa, 2022). Quantitatively, an MP is generally >5mm in length and can be fragments, fibers, films, and spheres, among other objects (Porter et al., 2023). The plastics often pervade trophic chains, due to their ability to become incorporated with sediment, suspended particles, or plankton, thus becoming bioavailable (D'Costa, 2022). Generally, MPs enter the body through two main biological pathways, (1) through the gills, via respiration, which most commonly involves fiber-like particles that are not easily filtered, and (2) orally, whether mistaking an MP particle for a piece of food itself or digesting an organism which has accumulated MP particles (D'Costa, 2022). The ubiquitous distribution of these MPs is likely to impact various organisms of different feeding styles, allowing them to be generalized as a concern for nearly all marine fauna (Porter et al., 2023). An ecologically and economically important example is decapods. MPs have been documented in crustaceans, which act as predators, herbivores, omnivores, saprophytes, and filter feeders, all of which styles at risk of ingesting MPs (D'Costa, 2022).

Hemigrapsus Oregonensis are small shore crabs found off the west coast of North America, occurring from Alaska to California (Rudy Jr et al., 2013). The diet mainly consists of algal debris, as well as supplemental detritivory (Rudy Jr et al., 2013). Among this diet, it is not unlikely that wild crabs would be exposed to various MPs. Birds prey on this species (Rudy Jr et al., 2013), thus, if plastics are likely to accumulate in these crabs, they could be a link to biomagnification within marine birds. Besides local food chain effects, the accumulation of plastics may have several physiological impacts on the crabs themselves. There have been links between plastic ingestion to several negative effects in decapods (D'Costa, 2022). Synergy has been observed between temperatures and MPs in the standard metabolic rate of amphipods, leading to reductions in growth (Marmara et al., 2023). Various invertebrate intestines have had MPs found inside, taking up valuable digestive space (Waite, 2017). Swimming behavior has also been affected in copepods (Kleinschmidt & Janosik, 2021), which may impact phenology. It is likely, then, that *Hemigrapsus oregenesis* could have similar stress interactions with MPs, and thus could further affect the ecological interactions they are woven. For example, hairy shore crabs may compete with the early life stages of the invasive European green crab (*Carcinus maenas).* It is possible that European green crabs are also affected by MPs in the environment, and due to their similar phenology early in life, the question can be raised whether hairy shore crabs could be used as a proxy for plastic accumulation in green crabs. Studies on the plastic ingestion in Hemigrapsus oregenesis can be used to inform the ecological interactions in the littoral food web in the context of warming temperatures, as well as the impact on the globally damaging European green crab.

The goal of this study is to determine the effect of a hard plastic on a physiological parameter in *Hemigrapsus oregensis*. Then, the results of a past study with a different plastic type in a similar experimental design will be discussed to determine if there is a preference. These results will expand the knowledge of plastic ingestion in small shore crabs, possibly with a connection to the invasive European green crab.

Methods:

Twenty *Hemigrapsus oregenesis* of mixed sex were split into two experimental groups, 10 in each experimental group. The plastic used was blue bottle caps from Pepsi soda bottles, which likely contained the plastic type HDPE. A coffee grinder was used to evenly grind plastic particles, which gave a size range from 1-5mm in diameter. The MP group was exposed to 2.5 mg of each plastic soaked in oyster juice for 24 hrs prior to addition. The MP-fed group was exposed to 2.5 mg of plastic each mixed with 2.5 mg of oyster muscle tissue. Each individual crab was placed in an isolated jar, roughly 250ml in volume, with a plastic mesh covering the top, and a lid with a hole screwed over the mesh covering to allow water flow (Fig. 1). Oyster shells were placed in the jars to provide refuge. The temperature was controlled at 25 degrees Celsius with a freshwater bath surrounding the container. Water changes were conducted once a week, for each individual, and were placed in similar conditioned water, barring plastic stressors, during the duration of the water change.

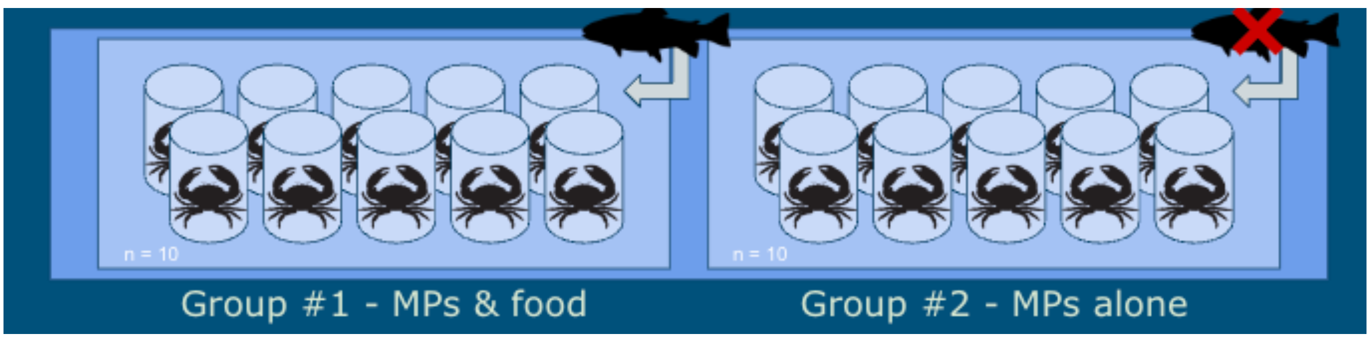

Fig. 1: Experimental design of the study. Here, the fish represents food, which was oyster muscle in this case.

Hemolymph was extracted from soft tissue between the claw and the carapace. Generally, to ensure the survival of the individual, extraction is conducted between a walking leg and the carapace, but due to the terminal nature of this experiment, the larger claw was used to maximize hemolymph extraction. Only one sample was collected, which was at the end of the experiment. This was done to minimize stress response due to extraction, which could have influenced the data. Physiological assay performed with the extracted hemolymph was a lactate analysis, which was done for a total of two trials for each individual.

Dissection was carried out post-mortem, after a period in the freezer for 20 minutes. A small incision was made at the midline, on the posterior side of the carapace, to a degree that the entire superior portion of the carapace could be flipped open, allowing the viewing of the GI tract and respiratory surfaces (Fig. 2). Dissected individuals were viewed under a dissecting scope, and plastics were looked for in the stomach, hindgut, and gill surfaces (Fig. 2).

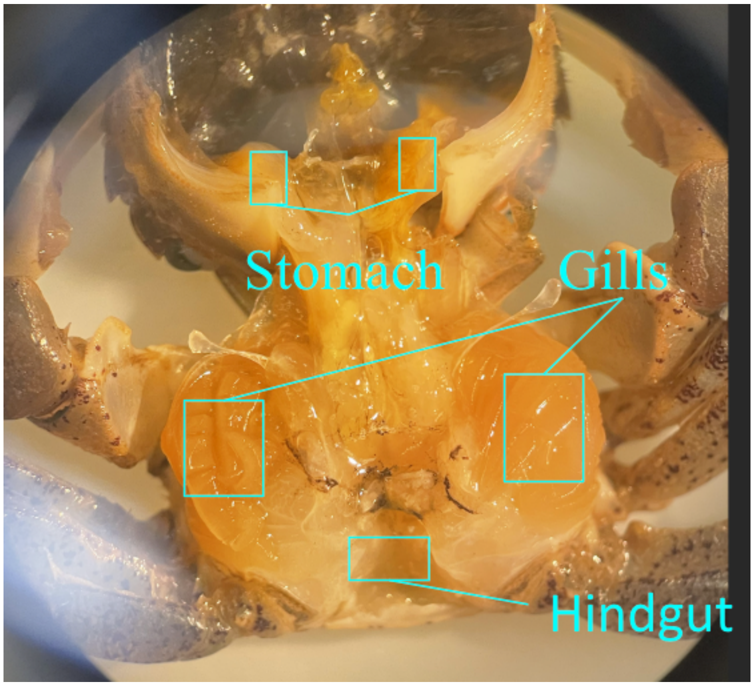

Fig. 2: Dissection from MP group. Portions of anatomy viewed are highlighted with cyan text.

Results:

There was a mass mortality (88 percent) in the fed group within the first week of the two-week experimental design. After discovery of death, the crabs were frozen for 1 week for later analysis. Post-mortem dissection suggested crabs died of hypoxia, which was determined visually with the color of the gills, being grey or black. The likely cause for this oversight was limited water flow in individual chambers, causing a limited oxygen supply. The original design was created to control the stress of interaction between crabs, which may vary from individual to individual. Future studies attempting to replicate this study should incorporate a bubbler or some other flow system for the individual holding containers.

The average lactate levels for the 5 individuals sampled are summarized in Table 1. Blood lactate levels varied largely over MP individuals, as seen in Fig. 3, (STDev = 108.1). Despite this variation, there is a notable difference between the MP individuals and the surviving MPFed individual. The lack of a sufficient sample size and thus standard deviation for the MPFed group means no statistical significance; however, if the standard deviation was low among MPFed individuals, these data could suggest that the presence of plastic particles in the water column induced higher lactate levels than the presence just in food (Fig. 4).

Table 1: Average lactate levels for each individual. “Number” refers to the number of successful lactate assay runs.

	MP1 n = 2	MP2 n = 2	MP3 n = 2	MP4 n = 1	MPFed n =1
Lactate (uM)	170.24	82.86	335.37	250.03	36.857

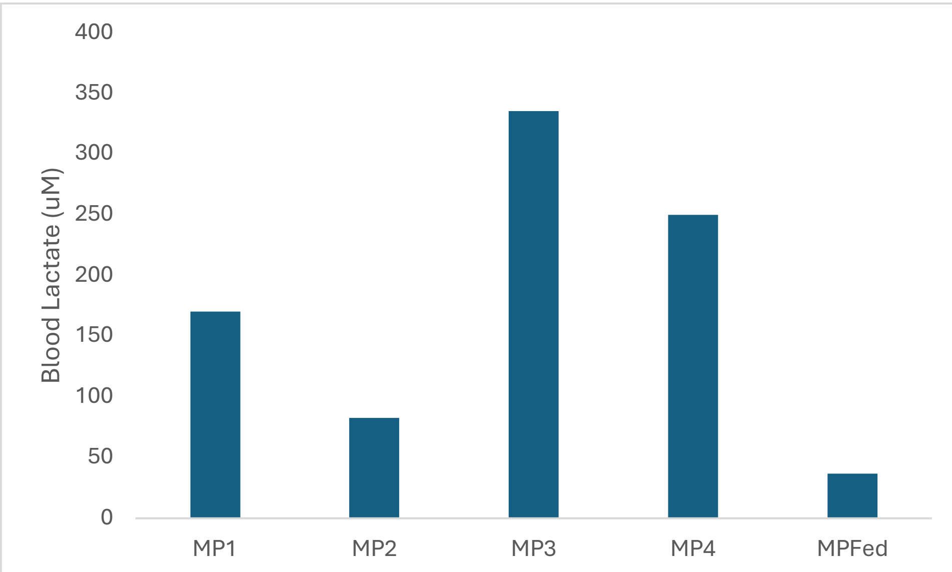

Fig. 3: Bar plot of each individual. No significance between individuals.

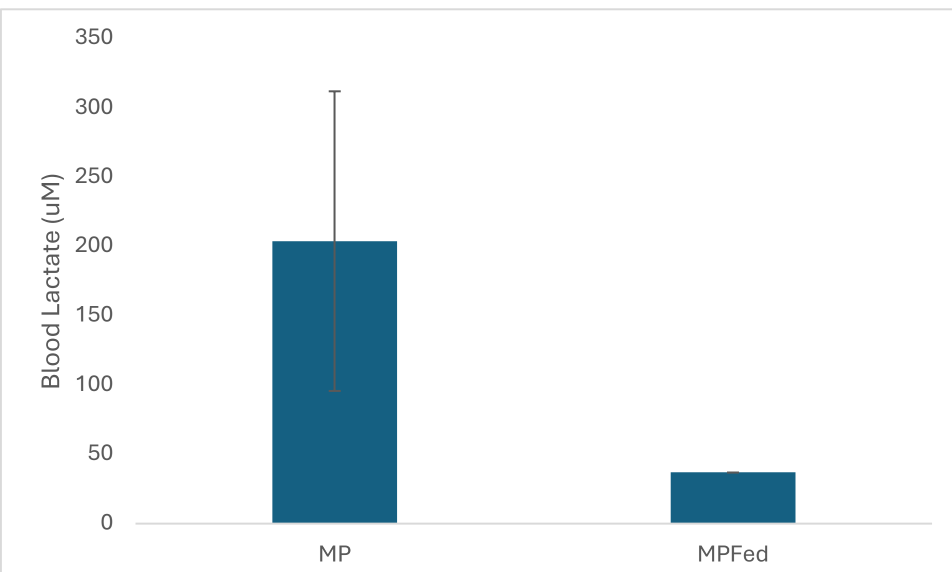

Fig. 4: Bar plot of averages of blood lactate between trials. Note that there is a large difference between groups, but no statistical significance is present.

Discussion:

Plastic types used in this study were ignored by crabs, despite being soaked in clam juice. This is opposed to FISH 497C Tire Tread Team 2024, who saw ingestion of tire material, with a similar soaking period. Several factors could have caused this. The particles used were positively buoyant, which may have reduced the number of feeding opportunities each individual crab had (Hinz et al., 2001). This was especially seen in the first week of the experiment, where limited water flow in the individual containers caused only a slight minority of particles to be within reach of the crabs. This is opposed to the negatively buoyant tire particles (FISH 497C Tire Tread Team 2024). A greater flow and thus prevalence in the water column that was present in week two, though this didn't have much of an effect on the outcome. The porosity of plastic bottle caps is also much less than that of rubber (Vermeiren et al., 2023). With this in mind, it is plausible to assume that plastic particles were less perfumed than the tire tread and thus saw less consumption. Other factors like size and color may have played a role in the lack of ingestion as well. Despite circulating in the water column, no plastic was found in the gills after dissection. These crabs live in highly sedimented environments, which commonly include particles of similar size to these plastics (Rudy Jr et al., 2013). Typically, fibers are more likely to intrude into the gill tissue than beads (Waite, 2017), so more experimentation with plastic types is necessary to determine if the respiratory systems of *Hemigrapsus oregenesis* are at risk in polluted areas.

Despite a lack of significance, all MP individuals had higher lactate levels than the remaining MP-fed trial. A higher lactate level is a byproduct of anaerobic cellular respiration, indicating a higher stress level in normal conditions. High concentration of particles in MP trials may have induced stress. Plastics are known to deteriorate in seawater, so there may have been a level of plastic derivatives in the water column (D'Costa, 2022). There were also four total individuals in the MP enclosure by the end of the experiment, whereas the fed individual had only a single individual, meaning competitive behavior and stress may have had an influence. A lack of feeding also may have reduced the lactate response, as the MP group was fasted during the whole experiment. Another consideration is that the single fed individual may have been unusually hardy due to the ability to survive a mass mortality event, thus exhibiting less stress under similar conditions.

Sources:

D'Costa, A. H. (2022). Microplastics in decapod crustaceans: Accumulation, toxicity and impacts, a review. *Science of the Total Environment*, *832*, 154963.

Hinz, S., Sulkin, S., Strom, S., & Testermann, J. (2001). Discrimination in ingestion of protistan prey by larval crabs. *Marine Ecology Progress Series*, *222*, 155–162.

Kleinschmidt, J. M., & Janosik, A. M. (2021). Microplastics in Florida, United States: A case study of quantification and characterization with intertidal snails. *Frontiers in Ecology and Evolution*, *9*, 645727.

Marmara, D., Katsanevakis, S., Brundo, M.-V., Tiralongo, F., Ignoto, S., & Krasakopoulou, E. (2023). Microplastics ingestion by marine fauna with a particular focus on commercial species: A systematic review. *Frontiers in Marine Science*, *10*, 1240969.

Porter, A., Godbold, J. A., Lewis, C. N., Savage, G., Solan, M., & Galloway, T. S. (2023). Microplastic burden in marine benthic invertebrates depends on species traits and feeding ecology within biogeographical provinces. *Nature Communications*, *14*(1), 8023.

Rudy Jr, P., Rudy, L. H., Shanks, A., & Butler, B. (2013). Hemigrapsus oregonensis. *Oregon Estuarine Invertebrates, Second Edition*.

Vermeiren, P., Ikejima, K., Uchida, Y., & Muñoz, C. C. (2023). Microplastic distribution among estuarine sedimentary habitats utilized by intertidal crabs. *Science of The Total Environment*, *866*, 161400.

Waite, H. (2017). *Investigating the Quantity and Types of Microplastics in the Organic Tissue of Oysters and Crabs in the Indian River Lagoon*.