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Fish 460 A

Ingestion of Common Microplastics by *Hemigrapsus oregonensis* in a Combined Diet versus Isolated Particles

Introduction

Microplastic materials have become an important topic of study within the field of marine and molecular ecology. The dispersion of such materials throughout the world's oceans has become a subject of contention between scientists, fisheries, and the public in terms of accumulation and biological safety, in terms of the issues posed by their toxicity towards marine species and human safety. (Carbery et al., 2018). The dangers posed by microplastics, or MPs, is numerous, with studies in both human and marine ecophysiology suggesting increased rates of diseases such as cancer, organ failure, and decreased reproductive and metabolic fitness. The Yellow Shore Crab, *Hemigrapsus oregonensis*, is one of many species affected by microplastic toxicity. Debris, in particular those smaller than 5 mm in size, have caused issues in terms of osmoregulatory and respiratory function (Watts et al., 2016) as well as digestive issues related to microplastic reuptake (Watts et al., 2015; Prestholdt and Kemp, 2020). Additional evidence of digestive tract damage has been shown to cause long term issues with nutrient absorption, causing a downstream effect of physiological issues on the microscopic level, in addition to evidence of particulate matter found in crab gills (Zhang et at., 2021).

The aforementioned effects have shown a tendency towards a decrease in the overall fitness of shore crabs, correlated to their size and the amount of microplastics ingested. Because of the smaller size of hairy shore crabs, *H. oregonensis* may be disproportionately affected by the amount of the microplastics ingested, as well as the type of plastics affecting the species' biological functions, including metabolic damage. This study aims to explore the effects of one common type of mixed microplastic found in the environment, High-Density Polyethylene (HDPE) and Polypropylene (PP), typically found in the bottle caps of most manufactured artificial drinks. This microplastic material was added to the water supply of three different crab groups to test whether or not the addition of microplastic debris would significantly impact the crab's metabolic rate and digestive reuptake, and how greatly it would affect each specimen's overall fitness.

Method

Twenty shore crabs were taken from a larger sample pool of adult Yellow Shore Crabs for physiological testing. Out of the selected crabs, all of them were to be held in individual glass jars, at an ambient temperature of around 13 degrees centigrade. Two groups, each with ten crabs each, were held in two 10 gallon tanks, and subsequently submerged in water of the same temperature. The salinity of each jar was set to 30 ppt. and an airstone was added into the larger tanks to provide oxygenated water. The jars themselves were fitted with a mesh covering, in order to prevent any debris from escaping the jars, while shells of small sizes (ranging from 1 to 2.5cm in length) were added to the jars to provide cover for the crabs. Black lids with small openings were fitted to the top of each jar.

Both Group A and Group B were to be subjected to exposure to HDPE/PP plastic. The plastic itself originated from blue plastic Fanta bottles, of which the caps were then filed down using metal graters. A total of 50 mg of plastic (with 2.5 mg of deviation) was collected to be used in the experiment. The material was first soaked with mussel fluids taken from defrosted mussel samples, and then divided into two partitions.

Group A received a total of 25 mg of soaked microplastic material, divided, weighed, and distributed in 2.5 mg portions into each jar. Group A was labeled as the fasting group, so no additional food was to be added into the container of each crab. Group B similarly received 2.5 mg of particulate matter per jar, with each individual sample being mixed with a food sample of oysters to form a combined weight of 2.5 grams of food and plastic sample. These samples were added to the jars and then sealed after.

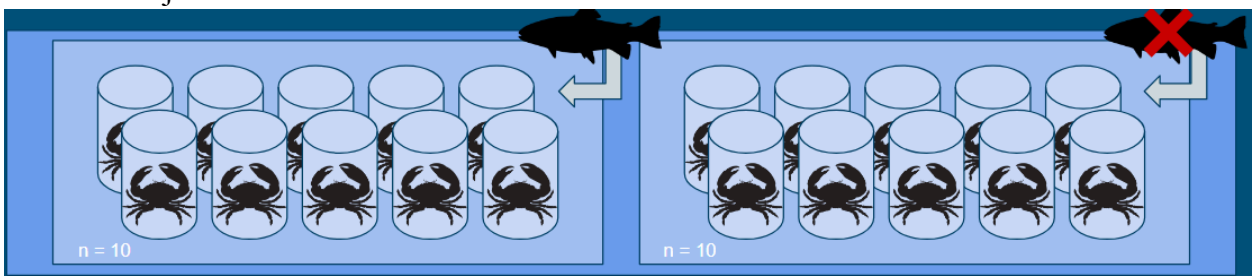

Figure 1. *Two experimental groups, Group A and Group B, contain 10 specimens of H. oregonensis. Both groups featured each crab in isolation within a glass jar, with group B having additional nutritional matter in the form of oyster meat co-administered with MPs. Both jars are then sealed with mesh and a cap, and submerged in a water bath with identical water parameters.*

Over the course of two weeks, the crabs would be monitored at minimum twice weekly, during every Tuesday and once in between lab dates to monitor proper water parameters, perform water changes,and perform regular feedings of Group B. During the first week of the experiment, a Resazurin assay was scheduled to be taken for each group of crabs, intended to be 5 crabs each. (The Resazurin Assay was not conducted due to a mass extinction event). During the second week of the experiment, a haemolymph extraction was conducted via fine-needle aspiration of the claws of each crab on the final day of the experiment. Any crabs that perished prior to this date were to be collected, sealed, and frozen for preservation. Upon collection of all relevant data, the remaining crabs were sealed in the freezer for a 20 minute period to terminate all remaining specimens. Afterwards, each crab was dissected under an optical microscope to check for any visual evidence of microplastic matter.

Results

During the course of the first week, the crabs were monitored per protocol, however after the weekend had passed by during the first week, a mass mortality event occurred, with 9 out of the 10 crabs in group B having perished, and 6 out of the 10 crabs in group A having perished. Upon closure of the first week of testing, the crabs, and their plastic contents were released into the greater tank to prevent further mortality. All 4 Group A crabs were placed into the larger 10 gallon tank, and likewise was done with the sole Group B crab. During week 2, results were summarily collected via haemolymph extraction and dissection, with the lost crabs being frozen and disposed of due to unreliable visual data due to the decaying crab corpses.

Physiological assay data was analyzed and compiled, with an end result for lactate being measured across the remaining crab specimens. Lactate data was averaged across 2 replicates for each specimen, and our experimental results were compared with control data from two other groups running experimental conditions with no manipulated variables.

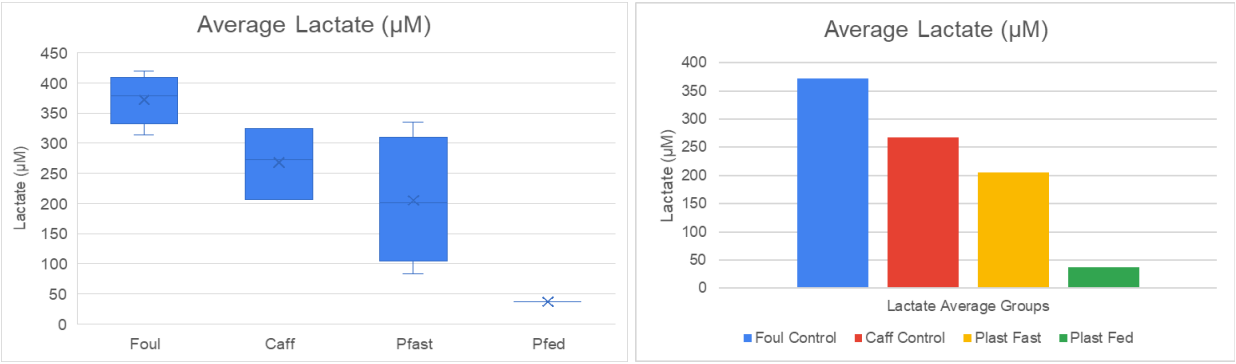

Figure 2. *Average measured lactate (µM) for treatment and experimental groups. Groups from other experimental tests labeled "Foul" and "Caff" signify control groups. These groups have no experimental changes, and also follow the same water parameters as "Pfast" (Group A) and "Pfed" (Group B). Each bar in graph A represents the measured average of lactate found across a select number of crabs (Foul = 4 crabs, Caff = 3 crabs, Pfast = 4 crabs, Pfed = 1 crab). The upper and lower extremes in Graph A represent the variability in the lactate date spread. Graph B represents the absolute average amount of lactate found in each group.*

After completion of the haemolymph extraction, all remaining crabs were terminated and dissected. Preliminary results showed no visible particulate matter on all crab specimens from Group A and Group B. No special imaging was taken due to a lack of available visual data from both microscopic inspection (30x magnification) of the digestive tract and the gill area.

Discussion

The data from the physiological assays showed that on average, the control crab groups featured a relative level of lactate levels, with values ranging from between ~400 micromolar lactate to ~200 micromolar. In comparison, both plastic groups had a range from anywhere between ~300 to 100 micromolars in the fasted group and a lone sub 50 micromolar measurement from the fed group. From this data, we can extrapolate that our crabs, fed with contaminated microplastics, produce significantly less lactate on average in comparison to the two control groups shown in the charts. In addition ,the fed crab had the lowest measurement of all the crabs within the data compilation.

On a microbiological scale, this supports the idea that the addition of microplastic contamination resulted in a lowered capacity to carry out metabolic functions, and can cause issues in terms of metabolic stress and interference within the digestive systems and metabolic pathways of shore crabs, similar to species in other studies (Urbina et al., 2023). Crabs exposed to microplastics over greater periods of time decreased the overall energy budget of crabs who ingested MPs over a long period of time, contributing to weight loss and nutritional damage. The consumption of MPs causes macroscopic and microscopic damage to the intestinal tracts of crabs, and subsequently results in lowered uptake of carbohydrates, causing a shift in energy substrate usage.

It is important to note, however, that the results for both Group A and Group B feature an abnormally small sample size, due to an unforeseen mortality event that occurred over the course of the first week of experimentation. The small sample size means that the data collected from the crabs samples is prone to other factors or variables other than just MP contamination.

The mass mortality proved to be a major setback realized over the course of the experiment. While water changes were managed during each biweekly check during the first week, the sudden collapse of both group ecosystems can likely be attributed to two main reasons. The first, and most likely reason is due to hypoxia in each individual jar, since the glass jars used were small, measuring roughly 2.5 inches in diameter. The small size, in conjunction with the mesh and lid throttled sufficient water flow, preventing a suitable amount of oxygen from being saturated in each of the crabs' water supply. The other likely reason, and why Group B suffered a greater mortality rate in comparison to Group B, is the accumulation of waste products from the decaying food and waste products made from the fed crabs themselves. Waste products and biological material break down into ammonia, and when the jars for Group B were extracted after the first week, the liquid was noted to be thick and murky, with an unpleasant odor. Ammonia and other nitrogenous compounds accumulated within the jars, contributing to toxic shock and death.

According to an article relating to hypoxia within Chasmagnathus granulatus species (Maciel et al., 2008), Neohelice crabs were subjected to a study measuring the oxidation of lactate as an energy substrate during periods of differing oxygen levels, including during hypoxia and normoxia, and found that lactate levels were typically four times higher during periods of normoxia. These crabs are typically found in estuaries, similar to yellow shore crabs, who are found in intertidal zones including estuaries and other similar events with varying degrees of water oxygenation. It is possible that the lack of lactate, in regards to our experiment, may have also been due to a last ditch effort by our crabs to use lactate as an energy substrate to sustain vital functions.

Some ideas were postulated on how our overall crab mortality could have been reduced. One such method was to avoid the use of glass jars entirely, and to instead allocate the crabs directly into the 10 gallon tanks. Indeed, after the first week, the remaining crabs were transitioned to this setup to prevent further mortality. This would have provided greater oxygenation for the crabs, due to an increased surface area exposed to air, as well as a more direct line towards the airstone to prevent hypoxia from occurring. In addition, the open chamber design would also stifle the accumulation of toxic nitrogenous compounds, as more diluted concentrations of ammonia and other compounds would lessen overall exposure and would contribute to a lowered mortality rate.

The lack of available visual data was another point of contention during experimentation. While numerous experiments were conducted with MP matter of less than 5 mm, and indeed our plastic used was scraps in sizes of 3 mm or less, the overall size of the shore crabs was not taken into account, and it likely might have been difficult for crabs of smaller sizes to ingest MPs and uptake them into their system. In order to remedy this, a different MP substrate may be used, or another method to create MP matter can be used in order to create more fine particles that shore crabs can ingest, in order to create accurate visual data.

In conclusion, with the data we do have available however, we can conclude that it is likely that MP consumption does contribute to overall decreased metabolic fitness, rejecting our null hypothesis that MPs would have no effect, and supporting prior studies focusing on the physiological effects of shore crabs. Future studies towards MP consumption could include variations in the level of MPs or types of MPs used, in particular MPs such as Teflon or precursor materials such as C8 to address the differing effects these MPs have on both the aforementioned tested systems, or other related functions, such as the nervous system.

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