Lab Experiments on Microplastic Accumulation in *Hemigrapsus oregonensis*

Andy Gardner

Dalton Blackwell, Amelia Ehlers, Kaeya Guevarra, Jasper Nevis,

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1- INTRODUCTION

The threat of microplastics in the bodies of humans and marine life are one of the most topical environmental concerns. Microplastics accumulate in human bodies, disrupting endocrine systems (Chen et al. 2019), stemming from trophic transmission in marine ecosystems (Carbery et al. 2018). Microplastics often co-occur with other threats to marine ecosystems and amplify damages being done to sensitive systems such as polar and coastal regions (Horton & Barnes, 2020).

Among other stressors, invasive species are another threat to our coastal ecosystems. The European green crab (*Carcinus maenas*) has spread rapidly along the coasts of New England and have been attributed as a major cause of shellfish fishery collapse (Tan & Beal, 2015). Models predicting the impacts of European green crabs in Pacific Northwest fisheries warn of similar impacts to fishery stocks (Mach & Chan, 2014). Beyond harvestable catches, green crabs have contributed to the loss of seagrass beds (Neckles, 2015) and outcompete native crab species (MacDonald et al. 2007). Sea grass beds in British Columbia, just north of Puget Sound, have already seen declines related to the invasion (Howard et al. 2019).

Whether European green crabs will outcompete native shore crabs within the Sound or how quickly has yet to be seen, but a variety of physiological factors may help us predict how our competing native populations will respond. Their generalist biology has indicated that they are well adapted to a variety of temperatures, substrates, and salinities and may make their home here (Yamada, 2001), but how they might compete directly with Puget Sound's shore crab species is not yet understood.

Puget Sound, like most of our world's ocean environments, contain free-floating microplastics at relatively consistent densities (Robbins, 2024). Increased human activity near the city of Seattle compared to sparse populations on Washington's peninsula offer a consistent stream of microplastic accumulation (Harris et al. 2022).

Different species and phylogenetic groups of crabs have been studied to assess their physiological responses to microplastics with relatively similar results: chronic exposures cause declines in health (Horn et al. 2020; Zhang et al. 2021; Urbina et al. 2023; Capparelli et al. 2024), including PNW shore crabs (Presholdt & Kemp, 2020). However, no studies have been conducted on crabs within the Sound nor are there available metrics that can be directly compared to European green crab responses to microplastic contamination (Watts et al. 2015; Watts et al. 2016). This project will investigate how the ingestion of microplastics is impacting native shore crabs, *H. oregonensis*, on a physiological scale to better understand the threats of plastic pollution and potential comparisons to invasive species physiology in shore crabs from within the Puget Sound.

2- METHODS

Twenty *H. oregonensis* crabs were collected from Puget Sound near Seattle, WA. The crabs were divided into three treatment groups: one starved control (n=20), one "fed" group (n=10), and one "starved" plastic-only group (n=10). Control crabs were kept in a 30-gallon aquarium at 30ppm salinity and 13°C with an air stone. Both treatment groups were kept at the same salinity and temperature with each individual contained in their own 8-ounce jar. The lids of each container had gaskets covered in plastic mesh to allow water and air flow between the tank and the jar (Figure 1). Each jar contained additional shelter in the form of assorted seashells.

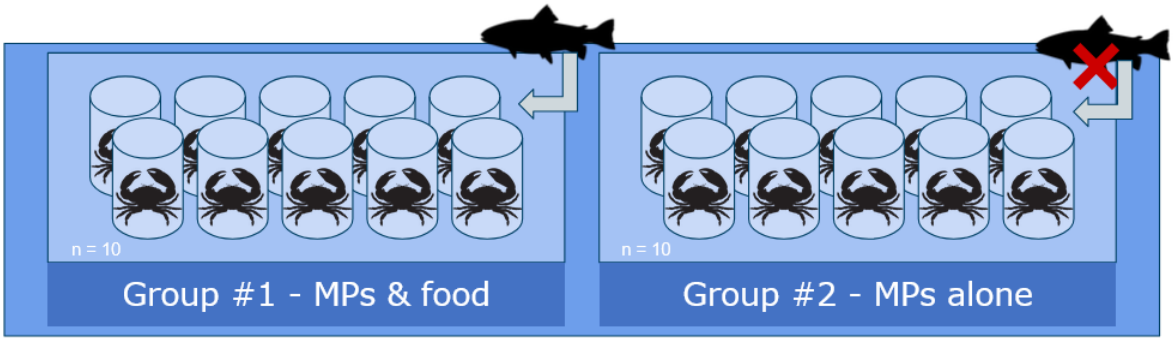

Figure 1 – Treatment Group #1 was fed a combination of microplastics shavings and food (defrosted shellfish) while Group #2 was fed marinated microplastic shavings alone. Individual crabs were contained in 8-oz jars with mesh-covered holes in the lids to maintain water and oxygen flow.

Treatments were prepared by taking blue, recycled plastic bottlecaps from Fanta bottles and grating the plastic material into 1-2mm shreds with a store-bought zester. The plastic shreds were weighed on an analytical balance, measuring about 500mg total.

A deceased oyster and other mollusks from previous experiments conducted at the University of Washington were used as feed. Ice containing juices from clams were combined with the 500mg of plastic shreds and left to soak for 48hrs. On feeding day, the soaked plastic shreds were divided into servings for each crab: the food treatment group received ~2.5mg of shredded plastic combined with ~1.5g of defrosted oyster meat while the plastic-only treatment group received 25mg of shredded plastic. Initial methods aimed to combine food with about 1% microplastic shreds by weight, however handling defrosted tissues and wet shredded plastic proved too difficult to get precise measurements.

Crabs in the "fed" group (Group #1, n=10) were fed prepared servings of 1.5g of oyster combined with ~25mg of shredded plastic. The lids on the jars were then closed and each jarred crab was placed within the treatment tank. Crabs in the "starved" group (Group #2, n=10) were provided with 2.5g of marinated plastic.

Crab mortalities that occurred before the end of the study period that could not be assayed were held in a standard home freezer (~0°C) until dissections took place. Crabs were individually dissected and examined for the presence of plastic shreds in the gut, gills, and

hindgut. The carapace of each crab was carefully removed, revealing a top-down view of the internal organs under a dissection scope (x10-30 magnification).

3- RESULTS

An unexpectedly high number of crabs died within the first week of the experiment; 90% of the fed group and 60% of the starved group were deceased by the first check-in. Dissections done on both groups revealed virtually no accumulation of microplastics in the gills, gut, or hindgut of the crabs (Figure 2).

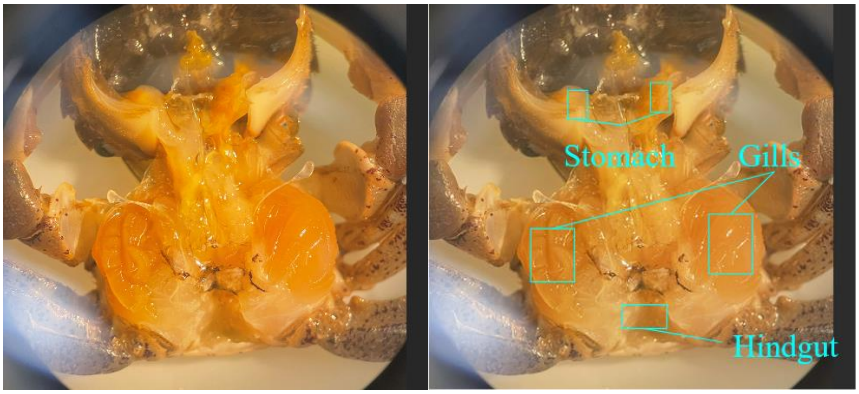

Figure 2 – The stomach, gills, and hindgut of dissected crabs revealed no accumulation of plastic particles.

In addition to dissections, the lab used measures of lactate levels in the musculature to assess stress. The starved control group had the highest levels of lactate (367.0 ± 65.1µM/L; n=4), followed by plastic only (205.0 ± 106.2µM/L; n=4), then ranked last came the fed group (36.9µM/L; n=1).

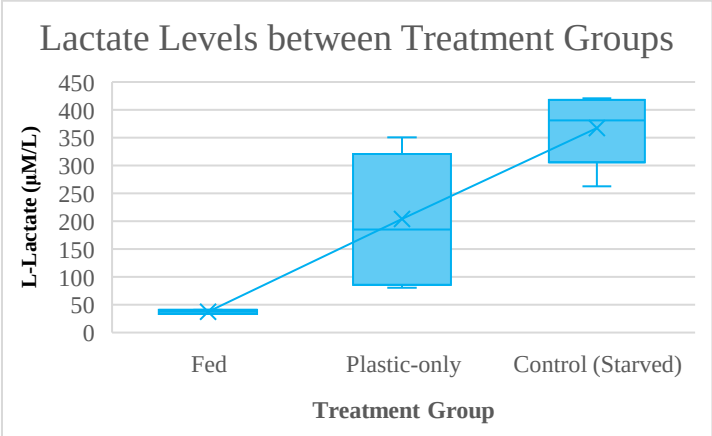

Figure 3 – L-Lactate tests conducted at the University of Washington graphed with an upward trend between Fed, Plastic-only, and Control (Starved) crabs.

CONCLUSION

The study aimed to identify whether microplastic accumulation was occurring in native *H. orogenesis* shore crabs via trophic transmission or passive feeding and respiration and whether those plastics were contributing to physiological stress. We found that microplastic shavings from recycled plastic bottlecaps did not accumulate in the body. As such, we found no difference between crabs that ate plastic shavings as a part of their food compared to crabs strictly exposed to microplastics in the water column and their starved but unexposed counterparts.

Our conclusions, however, are extremely limited by several factors. Our initial sample size was small and suffered a mass mortality event in the first week of the study. None of the crabs had accumulated plastic in their bodies, leading the authors of this study to believe that the deaths were caused by external factors such as ammonia build up within the jars. Another possibility is that the food source provided may have contained unknown toxins or bacteria harmful to the crabs, given the fed group had the greatest die-off.

Given the mass-mortality event, the authors opted not to conduct other assays. Our initial methods consisted of running resazurin assays to measure changes in respiration due to hypothesized accumulation of plastics in the gills, but because of our limited sample size and existing stressful conditions, the crabs were not tested.

Dissections revealed that no accumulation of plastic shavings occurred. Literature regarding microplastics found in crab bodies often mention “fibers” from fishing nets or synthetic textiles (Horn et al. 2020; Zhang et al. 2021). It is possible that one reason the microplastic shavings in this experiment did not appear during dissection is that the shape is not conducive to staying in the crabs’ body and is easily passed or avoided while eating.

A major driver for this study was to compare the physiological response of native shore crabs to microplastics relative to documented responses displayed by invasive species such as European green crabs (*Carcinus maenas*). Microplastics are a known anthropogenic issue in Puget Sound (Robbins 2024) compounding with the threat of European green crab invasions which have wiped out native crab populations elsewhere in America (Ens et al. 2022). European green crabs experimentally fed plastics may have slowed growth and will accumulate plastic in their gills, but short exposure periods resulted in limited measurable impacts (Watts et al. 2015; Watts et al. 2016). This lack of physiological responses raises alarm; if European green crabs are more resilient to microplastic contamination than native species in the Pacific Northwest, increased plastic pollution as a function of human activity (Harris et al. 2022) may spell disaster for Puget Sound’s native shore crabs. While no solid conclusions regarding the comparison between European green crabs and Puget Sound’s native shore crabs could be drawn, our failure to upkeep living conditions highlight the necessity of pilot studies and improved husbandry guidance for student-run experiments involving live animals.

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