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Characterization of The Northern Star Coral, (Astrangia poculata) Behavioral and Physiological Response to Microplastics

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Abstract

Preliminary studies have shown that corals ingest microplastics mistaken as food or co-ingested as food, and that ingestion can have effects on physiology. Coral plastic feeding preferences and egestion times are still not well understood. In a series of experiments, I investigated how exposure to microplastics impacts Astrangia poculata, the Northern Star coral, behavior in response to microplastics (feeding and egestion), and whether microplastic exposure and ingestion has any physiology effects. In the first behavioral experiment, I exposed corals to either brine shrimp eggs, or 500 µm polyethylene spheres, and did not find a significant difference in feeding rates. In a second set of experiments, I investigated feeding preferences of different types of plastics, using hand-feeding trials. Polystyrene fragments were ingested at a significantly higher rate than polyethylene fibers, and ocean seasoned fragments were ingested significantly more than unseasoned fragments. Egestion rates were similar across plastic types. I also investigated physiological effects of microplastic exposure, by measuring respiration and growth rates in two separate experiments. Over a six-week period, I did not find significant differences in growth because of microplastic exposure, however microplastic treatments showed high variation. After a 3-week plastic exposure period, effects on respiration rates were not significant. This work adds to a growing body of literature suggesting that corals ingest a variety of microplastic types, and there is a need for further understanding of the energetic and physiological costs of chronic microplastic exposure, ingestion, and egestion.

Introduction

The era of time we are living in now, commonly referred to as the Anthropocene, is characterized by extensive negative human impacts on the natural world. Among these impacts, plastic pollution is now ubiquitously found in formerly pristine environments and has come to represent a stark reminder of detrimental human impacts on the world. Microplastics - broken down, tiny pieces of plastics, and their many impacts on ecosystems, have in turn become a popular focus of scientific inquiry. From the most remote parts of the arctic, to the depths of the ocean, to human blood, these microplastics can be found everywhere. However, a remaining question is do these plastics have any significant effect on the environment and organisms around us that they have infiltrated?

This project attempts to investigate questions surrounding whether plastics have any effects on the health and behavior of the local northern star cup coral, *Astrangia poculata*. Corals are in grave danger for a multitude of Anthropogenic derived reasons. However, *Astrangia* is a temperate coral that provides hope for coral reefs, and other sensitive marine invertebrates, because of its resilient nature and incredible ability to endure harsh conditions. While this thesis examines very narrow questions surrounding one species of temperate coral, I hope to shed light on how nuanced the plastic pollution issue is, and what it might mean for this specific species of coral, and even all benthic marine invertebrates.

I. Microplastics in the Ocean

Microplastics can be defined as any plastic less than 5 mm in diameter (Andrady, 2011). The most common plastic polymers produced are low- or high-density polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), and polyethylene terpathalide (PET) (Sul and Costa 2014). Generally, microplastic types are grouped into categories based on

their structure and composition, of fiber, fiber bundle, fragment, sphere, pellet, film, and foam (Rochman et al., 2019).

The beginning of microplastics in the ocean can be linked to the beginning of mass plastic production in the 20th century, which has only exponentially grown in the present day. In 2010 it was estimated that 4.8 - 12.7 million tons of plastic entered the ocean from land-based sources (Shim and Thompson 2015; Jambeck et al., 2015). Microplastics in the ocean were first identified in 1972, on the surface of the Atlantic Ocean (Carpenter and Smith 1972). A few months later, the same plastics identified on the surface were found inside fish (Carpenter et al., 1972).

Sources of microplastics in the ocean have been divided into two categories: primary and secondary microplastics (Shim and Thompson, 2015). Primary microplastics are microplastics specifically engineered for certain uses, often used in the cosmetics industry or in sandblasting, and are usually introduced through shipping spills. They can also enter the ocean from household sewage, the shipbreaking industry, or from pollutant cosmetic products containing small plastic beads (Lechner et al., 2014; Fendell and Sewell 2009). Secondary microplastics are smaller pieces of larger plastics that have been created from the fragmenting of plastic pieces that break down over time due to weathering from UV radiation, chemical, and thermal degradation. (Sul and Costa 2014).

Plastics can also change after prolonged time in ocean environments. Chemical degradation makes plastics more susceptible to microbial biofilm formation, which will also change plastic chemical composition (Shah et al., 2008; Andrady 2011). Weathering and biofilm formation can also cause buoyant plastic types to sink and accumulate on the benthos where they are more available for uptake by a range of marine organisms (Lobelle and Cunliffe, 2011). In

addition, as plastics break down to even smaller pieces, the range of organisms able to ingest them increases (Law and Thompson 2014). Recent studies suggest secondary production of microplastics make up the major portion of microplastic abundance in the ocean (Erikson 2014; Andrady 2003). In addition, new threats arise with the identification of nanoplastics, or plastics that have broken down to be sized less than 1 μm, with relatively understudied effects on organisms (Hartmann et al., 2019; Huang et al., 2021).

II. Effect of Microplastic on Marine Wildlife

Many detrimental effects of microplastic ingestion on aquatic wildlife have been identified so far, with studies mainly focusing on major vertebrates like birds, turtles, and fish. A UN report from 2016 estimated that over 800 species are in some way affected by plastic pollution, either from entanglement or ingestion (Harding 2016; Smith et al. 2018). Previous studies have mainly concentrated on plastic ingestion by charismatic marine wildlife like dolphins (Denuncio et al., 2011), sea turtles (Lazar and Gracan 2011), and sea birds (van Franeker et al., 2011). Some of the health effects identified from these studies were digestive tract blockages, false satiation, and starvation, and reduced reproductive output (Wright et al., 2013). Various toxic effects have also been observed in fish as a result of microplastic consumption, ranging from inflammation, increase in enzyme activity, and altered metabolic pathways (Lu et al., 2018; Gola et al., 2021). In addition, the presence of heavy metal and microplastic accumulation together can cause even more issues, such as oxidative damage (Oliviera et al., 2013; Gola et al., 2021). Studies have also focused on major fisheries organisms, such as bivalves, crustaceans, and fish, because of the potential for affecting food safety (Lusher et al., 2016; Smith et al., 2018). There is growing concern that microplastics may travel up the

food chain and, in the end, could have detrimental effects on human health by carrying toxic chemicals and even disease (Lusher et al., 2017; Smith et al., 2018). However, there is still very little data on the quantity of human microplastic consumption correlated with marine organisms (Smith et al., 2018).

III. Effects of Microplastics on the Physiology of Corals

Corals face a number of marine anthropogenic threats to their health and physiology, such as ocean acidification, rising temperatures, and nutrient pollution (Hoegh-Guldberg, O. et al 2007, Riegl et al 2009), and microplastic have emerged as a possible additional threat. Microplastics have been found to pose a variety of threats to corals, and coral reef ecosystems. Larger plastics and micro plastics can entangle, smother, and inhibit structurally complex corals (Huang et al. 2021). This plastic entanglement on reefs has been linked to significant increase in coral pathogens (Lamb et al., 2018; Huang et al., 2021). Microplastic ingestion and exposure is also of high concern. Corals have been shown to ingest microplastics in the laboratory at high rates (Hall et al., 2015). However, ingestion behavior is species specific, with some species ingesting plastics at higher rates or only ingesting plastics when food is present. (Axworthy and Padilla-Gamiño, 2019). Further, the physiological effects on corals and drivers behind microplastic consumption are still relatively understudied. Understanding the scope and significance of this stressor will be significant for coral health and benthic marine invertebrates.

Early studies have found various physiological effects of microplastic exposure and ingestion, including lower skeletal growth rates, changes in algal-host symbioses, and higher respiration rates (Chapron et al. 2018; Mendrik 2021; Hankins et al. 2021). Other physiology studies have found even more severe effects, like a significant decrease in detoxifying and

immune enzymes (Tang et al. 2018), increased mucus production (Reichert et al. 2018), and even bleaching (Reichert et al. 2018, Syakti et al. 2019). Microplastic ingestion can also result in lesions which then increase the spread of microbial pathogens and promote tissue necrosis and bleaching (Lamb et al., 2016; Page and Willis, 2008, Huang et al., 2021; Syakti 2019).

However, not all studies have found significant physiological effects of microplastics on corals. Hankins et al. (2018) found no difference in calcification after a 48-hour exposure of PE microbeads (90–106, 425–500, and 850–1000 μm) at a concentration of 30 mg/L. Another study (Chapron et al., 2018) found limited effects of PE and PP microbead exposure on coral embryo development, fertilization, and early life stages (Berry et al., 2019; Huang et al., 2021). In addition, Mendrik et al. (2021) found species-specific effects of microplastic exposure, with no significant changes in photochemical efficiency and respiration for the species *Seriatopora hystrix* but significant stress response in *Acropora* sp. Another recent study found no physiological effects on bleached or unbleached corals over a four-week period of plastic feedings combined with food (Boodraj and Glassom 2022).

IV. Effects of Microplastics and the Behavior of Corals

Coral behavior when exposed to or fed plastics, can be quantified by examining feeding rates, egestion times, and specific coral plastic preferences. This line of research could elucidate the drivers behind coral plastic consumption and determine how long plastics might be retained within a coral's gut. This type of work was first established when microplastic consumption rates were quantified by Hall et al. (2015), who found that the scleractinian coral *Dipsastrea pallida* will ingest microplastics at rates as high as ~50 μg plastic cm^{-2} h^{-1}, rates similar to *Dipsastrea*

feeding on regular food particles, *Artemia nauplii* (phytoplankton). Since then, it has been further established that corals ingest a variety of microplastics (Allen et al. 2017).

Other work examining behavioral questions have focused on what type of plastic corals might prefer to ingest. One study indicated that coral microplastic ingestion may be driven by chemoreception, and that non-biofouled, or plastics that have not developed a microbial biofilm from environmental exposure, are preferred (Allen et al. 2017). However, it is also speculated that corals will feed on biofouled plastic at a higher rate compared to non-biofouled plastics, because corals may be less able to distinguish between alien objects and food items when microbial biofilms are present (Corona et al. 2020). In addition, some work has been done to try to quantify *Astrangia poculata* feeding preferences on food compared to microplastics. Previously, Rotjan et al. (2019) performed a 50/50 assay and exposed *A. poculata* to microplastic spheres and brine shrimp eggs in simultaneous suspension feeding chambers and found that plastics were ingested at a higher rate than brine shrimp eggs (Rotjan et al. 2019). This further indicated that corals may in fact choose plastic particles over food – a concerning suggestion.

Another important question to consider is how long corals will retain plastic within their gut, and whether all plastics are fully egested. Retention times could presumably affect the severity of any physiological effects. Studies examining microplastic ingestion and subsequent retention rates by *A. poculata* have so far established that almost all ingested microplastics are egested within 24-48 hours of ingestion, and that most are egested within the first 6 hours (Allen et al., 2019; Rotjan et al., 2019) However, retention rates may vary by plastic type, shape, and size, which has not been thoroughly investigated.

V. The Northern star cup coral, *Astrangia poculata*

The study species used in these experiments is *Astrangia poculata,* a temperate water, facultatively symbiotic, scleractinian coral species. This coral is extremely versatile – with the incredible ability to thrive as far south as the Gulf of Mexico, and as far North as Cape Cod (Grace 2017, Ellis, and Solander 1786). This species is found locally in Long Island sound, often growing on docks or *Crepidula* (slipper limpet) shells as substrate (Patrizzi et al., 2010; Bruce 1989). Seasonal variation in light and temperature in northern areas means that this coral cannot rely on photosynthetic symbionts like tropical corals and must use heterotrophic feeding for means of gaining a large amount of its energy during winter months (Trumbauer et al. 2021). Further, *A. poculata* can serve as a model system for heterotrophic filter-feeders responding to microplastics. This coral has also been identified as a potential bioindicator for plastic pollution in New England waters (Rotjan et al., 2019). Due to its high tolerance for varying environmental conditions, *A. poculata* could serve as a useful organism for identifying where plastics concentrations are high, and a model for how benthic invertebrates react to, and are affected by, plastics exposure and ingestion (Rotjan et al., 2018; Grace 2017; Burmester et al., 2018; Dimond et al., 2013). Due to its hardy and resilient nature, *A. poculata* is also very suitable for laboratory-based experiments. While it may not be as ecologically significant as many of its tropical, reef-building counterparts, *A. poculata* is a fascinating and useful marine invertebrate to use in the context of microplastic physiology and behavior experiments.

Figure 1. The study species used in these experiments, *Astrangia poculata* feeds on *Artemia nauplii*. Taken using Zeiss Stemi 508 Trino dissecting microscope and iPhone XR.

VI. Research Questions

In this project, I aim to address two separate aspects related to microplastic interactions with corals: behavior and physiology. In the first experiment, I ask initial behavior questions, by investigating if there is a difference between *A. poculata* feeding rates on a spherical food item, brine shrimp eggs, and a spherical plastic item, polyethylene, over a two-hour time period. This work continues, within a different set of short-term experiments, in which I ask whether *A. poculata* preferentially feeds on different types of plastics, examining different shape, biofilm,

and size class. I also ask whether retention times vary between plastic types. For the second set of experiments, physiological effects of plastic ingestion and exposure are examined. For the first experiment, effects on growth are assessed by exposing corals to plastics over a six-week time period and measuring growth at three timepoints. In a second physiological experiment, I measured the effects on respiration rates of corals continuously exposed to plastics over a five-week time period and measured rates at three time points during and after exposure.

Chapter I: Comparison of *Astrangia poculata* feeding rates on food versus plastic

Introduction

Previous work has suggested that *Astrangia poculata* may selectively feed on plastics instead of food (Rotjan et al., 2019). By exposing *A. poculata* colonies to both plastics and brine shrimp eggs at the same time in a 50/50 choice assay, Rotjan et al. found that *A. poculata* preferentially fed on plastic. Building off this work, I hypothesized that if exposed to plastic and brine shrimps separately, *A. poculata* colonies would feed on plastics at a higher rate.
This experiment sought to directly compare *A. poculata* feeding rates on plastic versus food, to further understand *A. poculata* feeding selectivity.

Methods

Coral Collection and Maintenance

Corals were collected via SCUBA in Fort Wetherill in Narragansett Bay, RI (41.4778° N, 71.3585° W). Approximately four weeks before experimental use, corals were kept in an

outdoor, flow-through tank in unfiltered seawater at the University of Rhode Island Graduate School of Oceanography. The outdoor system averaged in temperature ranging from 20 - 23 ℃, with salinity of 30 ppt. Corals ranged in size from 0.27 to 2.0 cm^2, at an average of 1 cm^2. Corals were not given any supplemental food in the outdoor tank system.

Experimental setup

Four replicate exposure trials were conducted with two treatments of Polyethylene Microspheres sized 425 -500 μm (Cospheric) and Decapsulated Brine Shrimp Eggs (Brine Shrimp Direct). For each trial, all experimental methods were replicated. Treatments consisted of five colonies, each in separate individual exposure beakers. Trials were conducted for a two-hour period, during which temperature was maintained at 23-25 ℃, and salinity at approximately 30 ppt. A water bath was prepared and filled with unfiltered seawater, which sat on top of a stir plate with 100 mL glass beakers placed on top (Fig. 2). Each beaker contained pieces of egg crate to serve as a pedestal on top of which the corals would sit, so that stir rods would still be able to function. During each trial, the stir plate was set at 130 speed, and two Aqua illumination Prime Series LED lights were mounted on the side of a wooden panel as seen in Figure 1 and set to 60%. Using an Apogee MQ-510 Full Spectrum Underwater LED PAR Meter the light levels of each beaker were measured and recorded at an average of approximately 250 $\mu mol\ m^{-1}\ s^{-1}$.

Prior to the start of each trial, corals were collected from outdoor tanks and kept in a bin of unfiltered seawater in the laboratory room for about an hour before experimental start. Two 1 L 0.2 μm filtered sea water stock solutions containing either plastic spheres or brine shrimp eggs were prepared prior to experimental use. Glass beakers were then filled with 75 mL of 0.2 μm filtered seawater using serological pipettes. Beakers were sitting in a plastic tub which was filled with unfiltered seawater to the same level as the beakers to serve as a temperature water bath.

Corals were placed in each beaker, and using separate serological pipette tips, 20 mL of either brine shrimp egg or plastic sphere stock solution was administered haphazardly to each beaker to achieve a desired concentration of 2000 particles L^{-1}. Two beakers were filled with stock solution but did not contain corals to serve as a particle control.

Ingestion Measurements

After each trial, three 25 mL samples were collected from each beaker using a serological pipet, and the solution was applied to a mesh filter, where particles could easily be counted under a dissecting microscope.

Statistical analysis and calculations

The three sample counts from each beaker were recorded, averaged, and subtracted from the known estimated particle count within each beaker. Ingestion rates were calculated per hour and normalized by average surface area (cm^2). All four trials were average, and full data sets for each treatment were combined. A Welch Two-sample t-test was performed (GraphPad Prism)

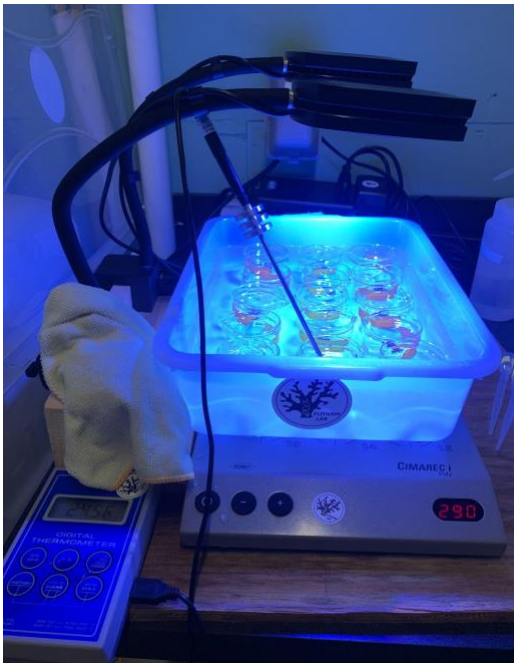


Figure 2. Stir bar exposure set up used for PE sphere and Brine Shrimp egg trials

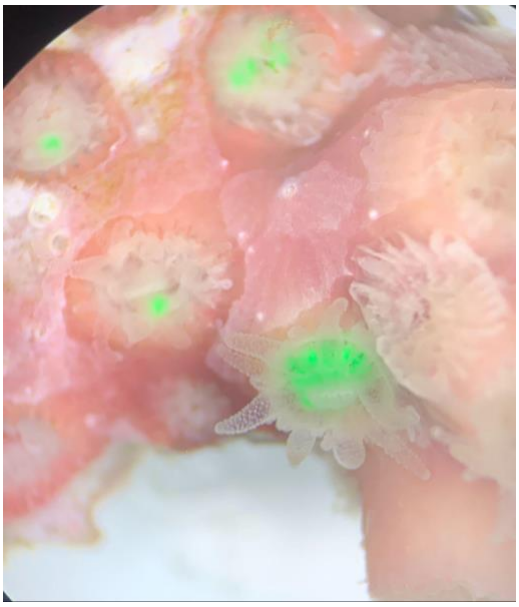


Figure 3. Image of polyps after ingesting polyethylene spheres, taken using dissecting scope and iPhone XR.

Results

Ingestion rates between the two treatments were not statistically different (Welch two-sample t-test, df = 36.09, $p > 0.5$) (Fig. 3). Over the four trials, average ingestion rates of microplastics (26.630 ± 27.480, N = 20 particles/hr), were lower than brine shrimp ingestion rates (40.980 ± 34.740, N = 20 particles/hr), with a large amount of variation. Many colonies did not exhibit consumption of any particles, and some colonies egested plastic before the two-hour trial period was over.

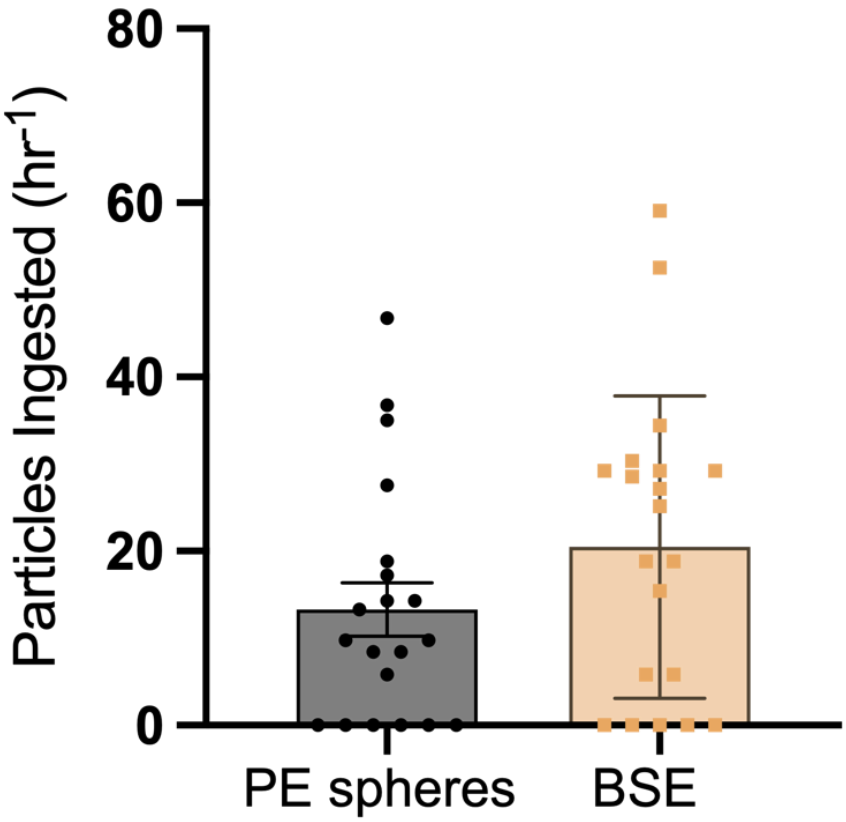


Figure 4. 500 µm polyethylene sphere and 200 µm brine shrimp egg ingestion rate comparison. Bars represented standard deviation.

Discussion

These results indicate that *Astrangia poculata* feeds at a similar rate on plastic and brine shrimp eggs, as there was not a statistically significant difference between the average ingestion rates from the two treatments (Fig. 4). However, there was substantial variation, as some corals did not have polyps active, and thus did not feed on either type of particle at all.

These data do not support the hypothesis that plastics are fed on at a higher rate than food particles. In fact, based on overall averages, brine shrimp eggs were fed on at a higher rate.

Further, this does not match with the findings from the 50/50 choice assay performed by Rotjan et al (2019). It is possible that this discrepancy could be due to a difference in methodology – and that exposing *A. poculata* to plastic and food at the same time yields different results. This does suggest that on its own, plastics are not preferred to food by corals. However, this data still does imply that corals are not discriminately feeding on food over plastic and will feed on plastics at a high rate. As plastic concentrations in the oceans increase this raises concern of physiological effects of this high rate of plastic consumption.

Chapter II: Effects of Microplastics on Ingestion and Egestion of the Northern Star Coral, *Astrangia poculata*

Introduction

A huge range of microplastic types exist in ocean environments, and while some types are more common than others, it is important to consider how shape, size, and chemical composition may have varying effects on coral physiology and behavior. For example, while microbeads are often used in microplastic related coral studies, microfibers make up a large amount of ingested or adhesion plastics in corals. In field *Astrangia poculata* colonies collected from Narragansett Bay, RI, fibers made up 73.4% of total microplastics characterized, while round plastic particles were 15.6% (Rotjan et al. 2019). Characterizing the comparative preferences, rates of ingestion/egestion and physiological effects of microfibers compared to microspheres and fragments, is still understudied. In a study exposing *Acropora* sp. and *Seriatopora hystrix* to microspheres and microfibers, fibers decreased photosynthetic ability in

Acropora. However, the responses to microplastics were species-specific and dependent on microplastic type (Mendrik et al., 2021). Thus far, fiber and fragment retention rates are suggested to be similar (Hankins et al., 2021), but here we expand on that work and test feeding and egestion of fibers and fragments to organic items like sand and food particles.

Building off the previous data, these experiments further targeted behavioral questions related to corals and plastic consumption. This work attempted to shed light on the biological drivers behind plastic ingestion and egestion on *A. poculata.* Further, these experiments encompassed two central themes: ingestion and egestion behavior of *A. poculata* when fed different shapes, sizes, and types of microplastics. A series of short-term hand-feeding trials were conducted to target these questions.

Methods

Coral Collection and Maintenance

Prior to use in these experiments, corals were collected by SCUBA from the dock adjacent to the Marine Biological Laboratory in Woods Hole, Massachusetts (41.5256° N, 70.6724° W). Corals were laboratory acclimated in the MBL Marine Resource Center at a temperature of 21˚C in ambient light (12-hour light dark cycle) prior to use in experimentation.

Plastic Preparation

Low density Polystyrene and low-density Polyethylene Terpathalide (PET) were used in this experiment, in collaboration with the MBL EAGER Ocean Flux project. Micro Fragments were prepared by sanding Polypropylene sheets using P100, P220, and P320 sanding paper, and a metal file. This created small particulate shredded plastic pieces. The pieces were sorted into separate size classes using geological sieves, which ranged in size from 53µm, 106µm, 250µm, and 1000µm. A 70% Ethanol solution was squirted onto the sieves so that the plastics would sink

and fall through the sieve openings. Particles were counted and weighed to determine stock solution concentrations.

Fibers were prepared by cutting LDPE PET strands into small pieces under a Zeiss 2000 dissecting scope using forceps and micro clippers. Fibers were imaged using Stemi SV-11 stereo microscope equipped with an Olympus DPZ3 (17.2M) camera. Fiber lengths were determined using Image J software. Fibers were measured to be 100μm - 2 mm. Prior to experimental use, plastics were washed in a 50 mL 2% micro soap solution. They were then rinsed and dried overnight and stored in glass scintillation vials.

Feeding Trials

The corals were presented particles by hand with surgical forceps to quantify feeding and capture rate. A total of 20 corals were used and were kept in individual containers in the lab and maintained at ambient light (12 hr L:D) and temperature between 17-19 ˚C. Colonies were haphazardly chosen for each trial, but never used more than once a day. While *A. poculata* can be aposymbiotic (no photosynthetic algae) or symbiotic, it is presumed that aposymbiotic colonies would rely more on heterotrophs because of the lack of energy from photosynthesis. Further, Coral colonies used in these experiments had aposymbiotic and symbiotic polyps, although approximately 90% of colonies were made up of completely aposymbiotic polyps. Colonies ranged in size from 0.516 cm^2 to 8.19 cm^2. Using dissecting forceps corals were hand fed different treatments of particles for 45-minute trials under a Stemi 508 Trino microscope with an ERc-5s Axiocam. An iPad with wireless Labscope software were used to record coral ingestion behavior (Fig. 5). Gooseneck lights were positioned in order to visualize coral behavior under scope. Two polyps were fed per colony during each trial and were fed as long as they would take particles from forceps during the 45-minute period. Particle treatments consisted of

either live *A. nauplii*, capsulated brine shrimp eggs, beach sand, benthic sand, fibers, large fragments (size 500-1000 µm), small fragments (size 250-500 µm), or fragments seasoned in ocean water for 3, 6, or 10 days. All particle types were imaged using a Stemi SV-11 stereo microscope equipped with an Olympus DPZ3 (17.2M) camera (Fig. 1). Plastics were placed on a polyp's tentacles using forceps. Particles were kept in 60 or 100 µm filter cups in dishes with 0.1 µm filtered seawater (FSW) before feeding to polyps in order to reduce chance of corals ingesting air bubbles attached to plastics.

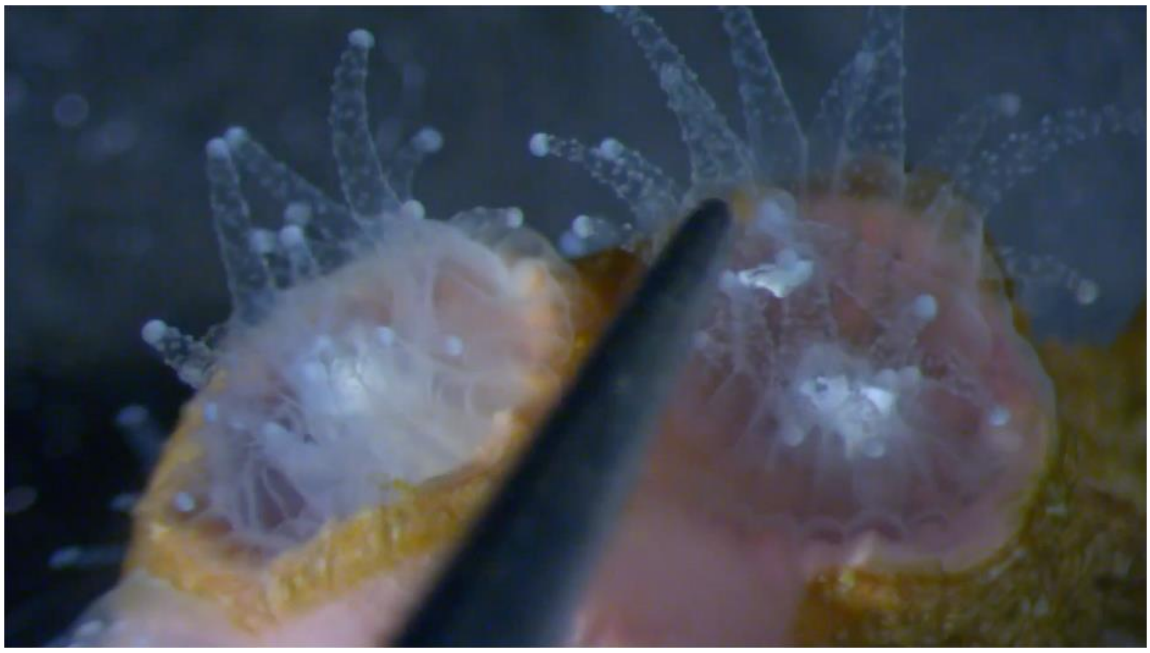


Figure 5. Image taken from fragment feeding video using Zeiss Stemi 508 Trino microscope with an ERc-5s Axiocam.

Seasoning Protocol and Sand Collection

Plastics were "seasoned" in Eel Pond next to the MBL campus in order to attempt to establish a biofilm on the plastics. Fragments sized 250-500µm were placed in falcon tubes with porous mesh on either side to let water constantly flow through. These falcon tubes were placed

in a mesh bag along with rocks to serve as weights, tied to the dock, and left in the water for 10 days. Plastics were collected at 3-, 6-, and 10-day intervals and were used in the feeding trials.

Two types of sand were collected from MBL's Garbage beach. "Beach sand" was from the surface, and the "benthic sand" was collected using SCUBA from 0.25 m^2 quadrats at a depth of 8 meters adjacent to live *Astrangia* colonies. Both sands were sieved for 250-500 μm particles. Beach sand was not washed, while benthic sand was washed in zinc chloride and rinsed in Deionized Water (DI).

Egestion Trials

During egestion trials, corals were fed five particles for each type of treatment. Treatments used for egestion trials were beach sand, small fragments (250-500um), large fragments (500-1000), and fibers. All polyps were monitored and recorded for egestion of particles using iPad Lab scope software and the ERc-5s Axiocam Zeiss attachment. Videos were time lapsed and set to take an image every 100 seconds (Fig. 12).

Calculations and Statistical Analysis

During each 45-minute feeding period, individual particles ingested by each polyp were counted. Feeding rates were calculated in units per hour, and polyp feeding rates were averaged per colony. For feeding rates, a Shapiro and Wilk's test was first used to test for normality. Because data was not normally distributed, a non-parametric one-way ANOVA was used to test for overall differences between groups (GraphPad Prism).

Based on egestion videos, the number of particles egested were counted every hour for each polyp. Polyps from the same colony were averaged. Total egestion time was determined by the number of hours elapsed between feeding and the time it took to egest all particles. A non-

parametric one-way ANOVA was performed for both egestion percentages and total egestion time.

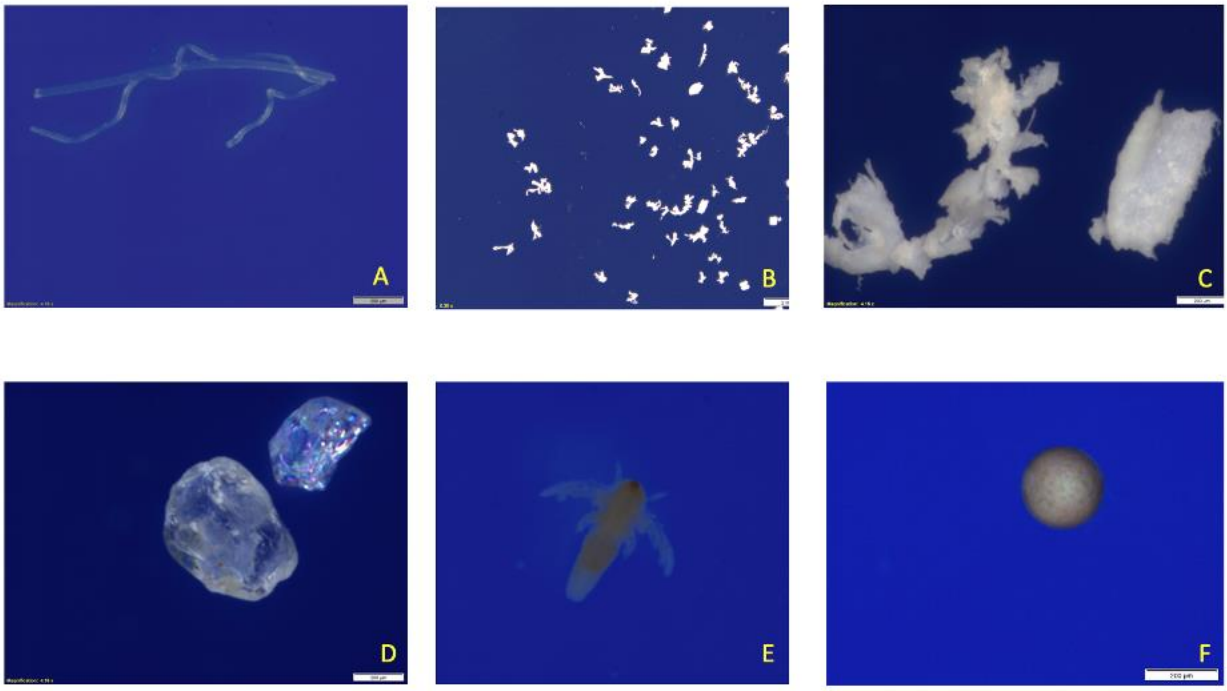


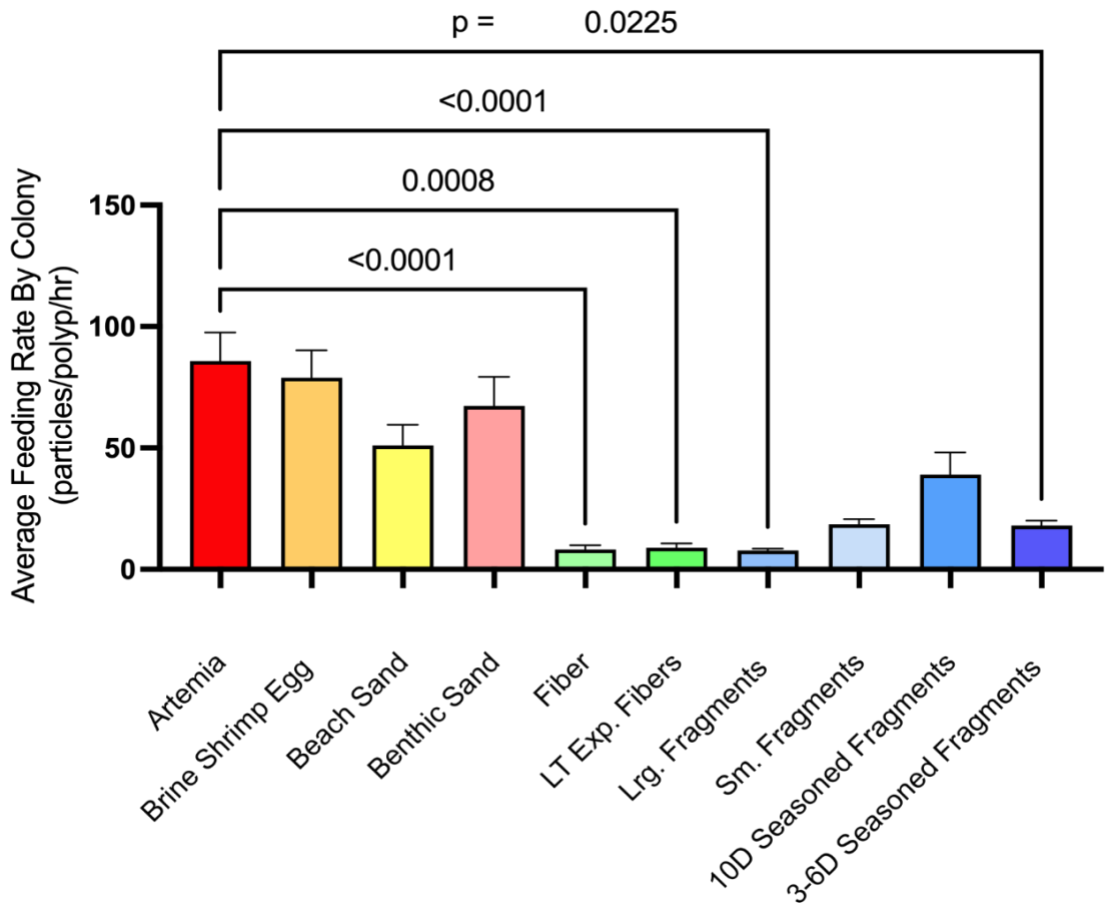
Figure 6. A. Fiber (6.6x magnification) B. 500-1000µm fragments (0.6 magnification) C. 250-500µm fragments (6.6x magnification) D. Beach Sand (6.6x magnification) E. *A. nauplii* (6.6x magnification) F. Brine Shrimp Egg (6.6x magnification)

Results

Based on hand-feeding trial data treatments were significantly different from each other (ANOVA, $F_{45} = 2.373$, $P < 0.05$). *Astrangia* polyps ingested the control treatment, *A. nauplii*, which is a natural prey, at a high average feeding rate of 104.5 ± 40.3 individual particle/ polyp/ hour^{-1} (N=13). Brine shrimp eggs were also fed on at a very high rate (86.5 ± 38.7, N=8

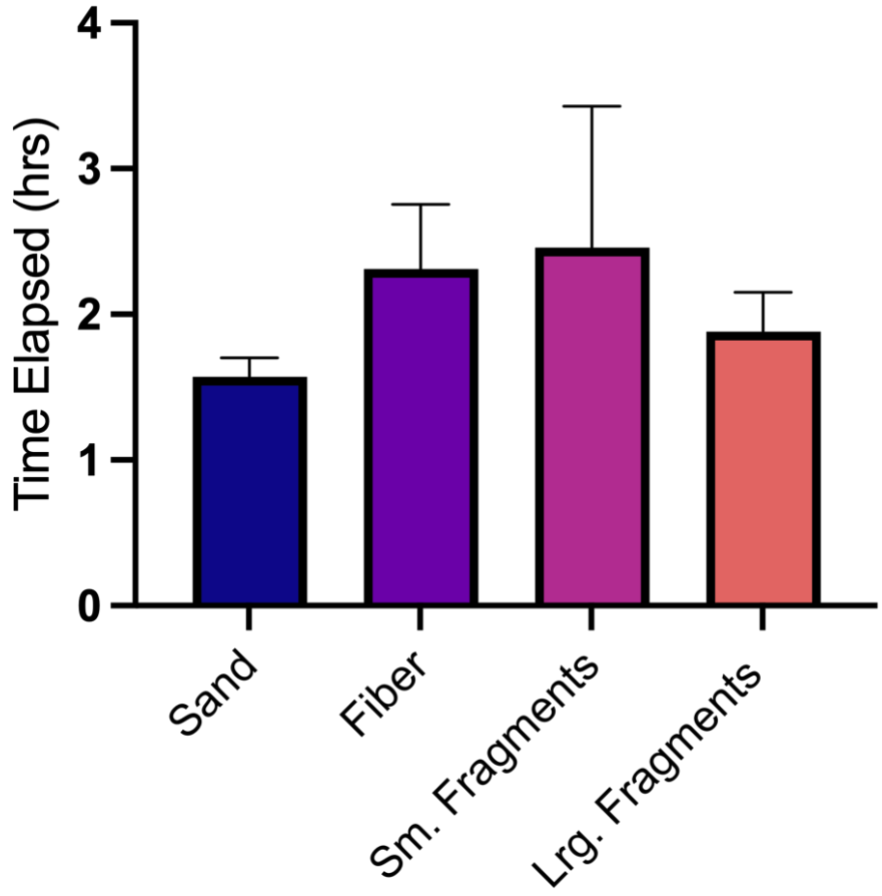
particle/poly/hr), along with the two different types of sand, beach sand (67.33 ± 26.7, N=6 particle/poly/hr) and benthic sand (51.1 ± 20.7, N=5 particle/poly/hr). The Artemia feeding rate is significantly higher than fibers (8.2 ± 6.0, N=12 particle/poly/hr), large fragments (51.1 ± 20.7, N=12 particle/poly/hr), and 3–6-day seasoned fragments (18 ± 5.75, N=8 particle/poly/hr). However, small fragments and 10-day seasoned fragments were not significantly different from the Artemia treatment. 10-day seasoned fragments were ingested at a rate of (39.0 ± 20.3 particle/polyp/hour N=5), and small fragments were ingested at a rate of (18.55 ± 5.30 particle/polyp/hour N=6) (Fig. 6).

Egestion time varied across treatments but was statistically similar across plastic types. Total egestion time showed no significant differences between sand and plastic treatments ($p = 0.6389$). Beach Sand (1.57 ± 0.296, N = 5 hrs), fiber (2.312 ± 0.9899, N = 5 hrs) sm. fragments (2.456 ± 2.380, N=6 hrs), lg. fragments (1.881± 0.6031 hrs) (ANOVA, $p > 0.05$) (Fig. 7).



ANOVA, $F_{45} = 2.373$, $P<0.05$

Figure 7. Feeding rates by treatment. Error bars represent S.E.M. Pairwise comparisons between control and significantly different p values were determined using Dunns comparison of means.



ANOVA, $F_{20} = 0.4$, $P>0.05$

Figure 8. Average total egestion time by treatment per individual polyp.

Discussion

This study examined *Astrangia poculata* ingestion and egestion on microplastics, building on former studies examining *A. poculata* preferential feeding on biofouled plastics, specific size-classes, and shape (Rotjan et al. 2019; Allen et al. 2017; Hankins et al 2021). This work specifically examined coral microplastic feeding in a novel method of microscopic video recording of both ingestion and egestion behavior during feeding trials.

These results suggest that *A. poculata* may be feeding more on biofouled plastics compared to non biofouled plastics, which conflicts with some previous work that reported non bio fouled plastics are ingested at higher rates because of possible phagostimulants in naive plastics (Allen et al. 2017) (Fig. 7) In the mushroom coral *Danafungia scruposa* it was found that biofouled plastics were more likely to be ingested and retained by this species (Corona et al. 2020). It was hypothesized that a biofilm may make it harder to differentiate between normal food items and alien items like plastics (Corona et al. 2020). This could have been the case in our treatments, although 3–6-day seasoned plastics had a similar feeding rate to non-seasoned plastics of the same size class (250-500μm). It could be that 3-10 days was not enough time to develop a significant biofilm. Given that we did not check for a biofilm on the seasoned fragments, we cannot know for sure that biofilms had developed, and what kind of biofilms were present if they had. Further, these plastics were “seasoned” not biofilmed. Biofilmed plastics are potentially hazardous to coral health and pose a threat of disease, as shown by Rotjan et al., (2019) who found that *Astrangia* can co-ingest *Escherichia coli* with plastic.

We observed that *A. poculata* ingested both types of sand at a high rate, suggesting something about the sand may have been difficult to differentiate from normal food. This is

different from what Allen et al. (2017) found after hand-feeding *A. poculata* organic-free sand, in which the sand particles elicited little to no feeding response. It is possible that the sand we used still contained organic matter or biofilms and was of more interest to the corals. (Fig. 7) More studies should examine *A. poculata* egestion of non-food items compared to plastic.

We observed that small fragments (250-500 μm) were generally ingested at a higher rate compared to the large fragment size class (500-1000 μm), although corals that were interested in plastic would still consume high levels of 500-1000 μm fragments. This difference may be due to polyp satiation, as large fragments could be observed filling the mesenterial cavity of polyps. However, despite this, during the 45 minutes most polyps would continue to feed once they had ingested a fragment. Variation could also be observed from different polyps, and polyp size is most likely a contributing factor in ability to ingest more or less fragments. While *A. poculata* has not been studied in the context of different microplastic size classes, Hankins et al. (2021) found that the tropical coral species, *Pseudodiploria clivosa,* ingested microspheres of the size classes 425-500 μm and 850-1000 μm at similar rates, while *A. cervicornis* had a strong preference for the 425-500μm size class over 850-1000 μm during an exposure experiment (Hankins et al. 2021).

Feeding and egestion responses of corals to microfibers have been less studied compared to spheres or fragments, most likely due to their difficulty to prepare and quantify. However, fibers are the most abundant type of plastic found in field corals (Rotjan et al. 2019). Fibers in this study ranged greatly in size, from 200 μm - 2 mm, however due to the ease of grasping fibers with forceps it is most likely that larger fibers were used in hand feedings. To our knowledge this is the first experiment hand-feeding fibers to *A. poculata*. For corals that were interested in

feeding, fibers almost always elicited a feeding response, despite their drastically different shape in comparison to fragments.

Plastic egestion across types has not been well studied. This study examined egestion of fibers, small fragments (250-500 μm), large fragments (500-1000 μm), and sand. There was no significant difference between egestion of the different size classes or the sand particles. Fragments were usually egested particle by particle within a few hours but could also be seen condensing in the polyp so that multiple particles were egested by the polyp at once. In a comparison of particle number and egestion time, no significant difference was observed, (Fig. 8). suggesting that polyps are in fact able to condense fragments and egest them together, despite feeding on them individually. Further, the data presented from this experiment illuminates the diverse array of feeding preferences in the context of microplastics by *Astrangia poculata.*

Chapter III: Effects of Microplastic Exposure on *Astrangia poculata* Growth and Asexual Reproduction

Introduction

Recently, it has been reported that scleractinian corals can incorporate microplastics into their skeleton after five months of continuous plastic exposure (Reichert et al., 2021). Investigators have suggested that corals may even act as long-term sinks for microplastic deposition, accumulating permanently in their skeleton as they grow (Reichert et al., 2021; Hierl et al., 2021). It is important to understand how short-and long-term plastic ingestion and accumulation may affect long-term coral health and physiology. To my knowledge, previous studies have focused on calcification rates and growth, measured as tissue surface area, of corals

ingesting microplastics at least 65 µm in size (Huang et al., 2021; Soares et al., 2021). A previous study that examined the effects of microplastics on the tropical coral species *Pseudodiploria clivosa* and *Acropora cervicornis,* found significantly reduced calcification and tissue surface area in plastic treatments after a 12-week exposure (Hankins et al., 2021). Another study found significantly reduced growth rates in the cold-water coral *Lophelia pertusa,* after a 69-day incubation with both micro and macro–plastics (Chapron et al., 2018). Finally, significantly reduced calcification, surface area, and volume parameters were measured in two out of four tropical coral species tested after a six-month exposure (Reichert et al., 2019).

To study the effects, if any, of microplastic exposure and ingestion on *A. poculata* growth rates, I exposed colonies to microplastics with different shapes over a six-week period. I separately exposed colonies of *A. poculata* in individual aquaria to three differently shaped polystyrene particles sized 9-15 µm since, to our knowledge, this size class has not been tested with *A. poculata* feeding. I chose polystyrene because it is one of the most common types of plastics found in the ocean and is one of the five major plastic pollutant types (Browne et al., 2015; Andrady et al 2009). I compared different shapes of microplastics because of the major presence of irregularly shaped plastics in the marine environment, and the lack of microplastic and marine invertebrate studies using irregular versus spherical microplastics (Baroja et al., 2021). Finally, I chose these plastics because they are on the small size scale compared to other plastic studies, and to our knowledge, this size class has not been tested with *A. poculata* feeding.

Methods

Animal maintenance

Colonies of *Astrangia poculata* were collected off the coast of Dumpling Island, Groton, CT (41.2879° N, 72.0189° W) via SCUBA. Fragments were kept in a wet lab with flow through seawater until use in experiments. Colonies were cut to approximately 0.1-0.3 cm^2 fragments and allowed to recover for 48 hours. Individual colony fragments were then haphazardly placed onto glass chambers and exposed to one of five different exposure treatments (three different microplastics, pollen particles, and one control). A total of six replicates were done per treatment. Colonies were loosely covered with saran wrap and maintained in an incubator at 15℃, with a 10:14 L:D cycle. Chamber water was changed 2x a week, and colonies were fed approximately 1 mg of live *Artemia sp. nauplii* 3x week and libitum.

Microplastic exposure trials

Colonies were exposed to one of four particle treatments for a total of six weeks. Treatments consisted of Polystyrene Latex particles (Magsphere Inc.) in the shape of a "snowman" (12.5x15.3µm, cat #: PNT015UM)", "pear" (9x10.5µm, cat #: PNT010UM), and "sphere" (10µm, cat #: PM010UM). A treatment of Ragweed pollen particles (Polysciences, 19-20µm, cat #: 07673-1) was also included to account for particle interference and was used as an experimental control. A control treatment of colonies not exposed to microplastics, or other particles was also used. A 1L particle stock solution of 1 µm FSW was prepared prior to experiment start and stored in the same temperature-controlled incubator as the treatments. Microplastics were distributed by pipetting aliquots from homogenized stock solutions a few centimeters away from each colony at a targeted concentration of 5,000 particles/L^{-1}. Colonies were exposed to the treatments 3x per week. Water from each exposure was quantified throughout the experiment to spot-check whether microplastics were being ingested by the colonies. To determine ingestion, three sub-samples were collected from the water of each

treatment after colonies had fed on a weekly basis. Water samples were processed with a BD Accuri C6 Plus flow cytometer to determine particle counts.

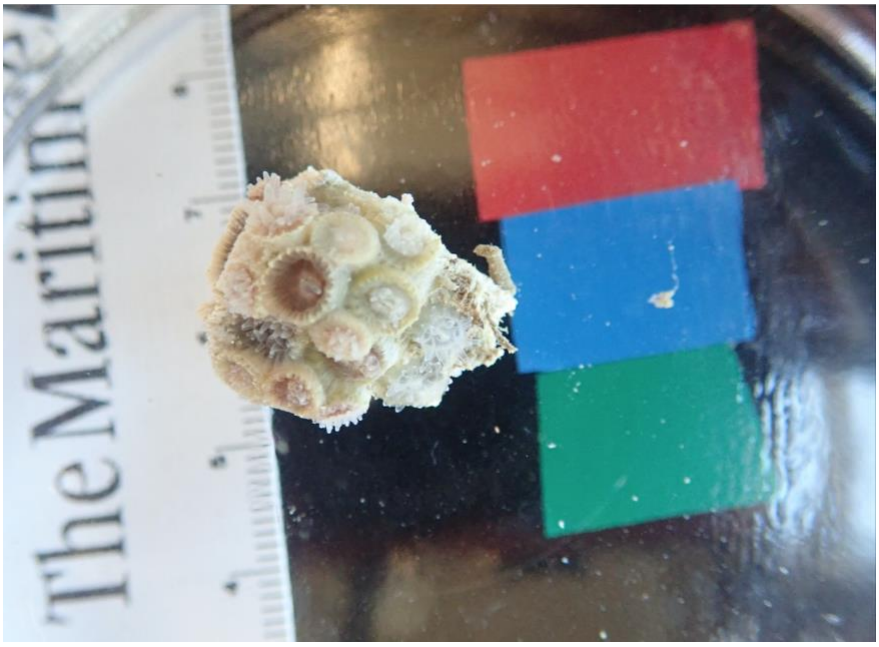


Figure 9. Image used for surface area analysis in Image J software.

Data analysis

Colonies were imaged at the beginning, middle, and end of the exposure period to determine growth and asexual reproduction. An Olympus digital camera was mounted on a tripod and balanced (Fig 9). Growth was measured by determining the surface area (cm^2) using Image J® software (NIH). This was done by setting the scale to a reference scale on the image,

and tracing around the perimeter of the coral skeleton. Asexual reproduction was determined by counting polyp number based on imaging at each time point.

Statistical Analysis.

Change in surface area and polyp number were calculated by finding the difference between initial measurements and final measurements for each treatment. For the surface area data, individual measurements were normalized to polyp number for each corresponding time point. A Shapiro and Wilks' test was used to test each data set for normality, and a One-Way ANOVA was performed for surface area and polyp data. Tests were performed using GraphPad Prism version 9.3.0 for macOS, GraphPad Software, San Diego, California USA, www.graphpad.com.

Results

Growth data

Colonies in all the treatments grew, measured as change in area, during the duration of the experiment. No significant differences were found for change in colony area between any of the treatments (Fig 2; ANOVA, df = 25, F = 2.245 $p > 0.05$). Generally, colonies exposed to the "snowman" and "pear" polystyrene particles had the most growth ($0.082 \pm 0.049 cm^2$), ($0.052 \pm 0.046\ cm^2$) followed by cultures exposed to the control treatment ($0.04 \pm 0.018 cm^2$), the pollen treatment, ($0.031 \pm 0.014\ cm^2$) and the spheres ($0.025\ cm2 \pm 0.041 cm^2$). Daily changes in surface area ranged from $6.00 \times 10^{-4} \pm 9.90 \times 10^{-4}\ cm^2$ for the sphere treatment to an increase of $0.082 \pm 0.001 cm^2$ for the pear microplastic treatment (Table 1) (Fig. 10).

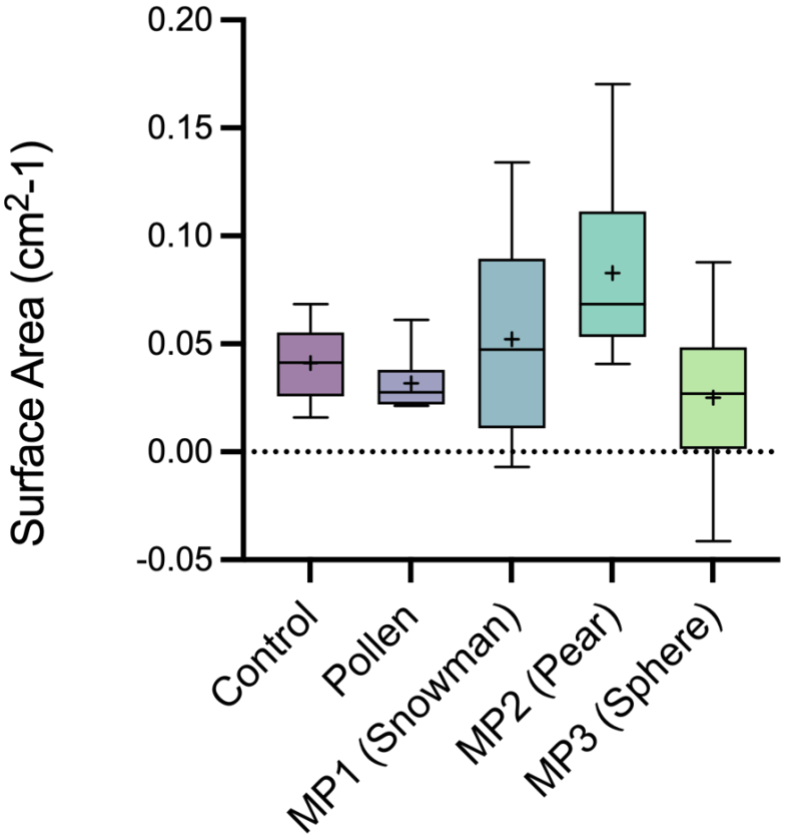


Figure 10. Differences in surface area in cm^2 over the six-week experiment period by treatment. Bars represent median, whiskers represent maximum and minimum value. + Symbol represents the mean of each treatment.

Asexual reproduction

All colonies exhibited asexual reproduction or "budding", quantified by changes in polyp number. No significant difference in polyp number was found between treatments (Fig 3; ANOVA, df = 25, F = 2.223 $p > 0.05$). The control treatment exhibited the most change in polyp number (2.2 ± 3.114, N = 5), followed by the pollen treatment (1.33 ± 2.581, N = 6). Microplastic treatments had the lowest rates of asexual reproduction, beginning with the "pear"

shape (0.33 ± 2.804, N = 6), followed by negative values (resorption of polyps) by the "snowman" shape treatment (-0.167 ± 2.34, N = 6), and "sphere" treatment (-1.67 ± 2.338, N = 6). Daily polyp rates ranged from a decrease of -0.004 ± 0.082 for the "snowman" treatment and an increase of 0.052 ± 0.070 for the control treatment (Table 1) (Fig. 11).

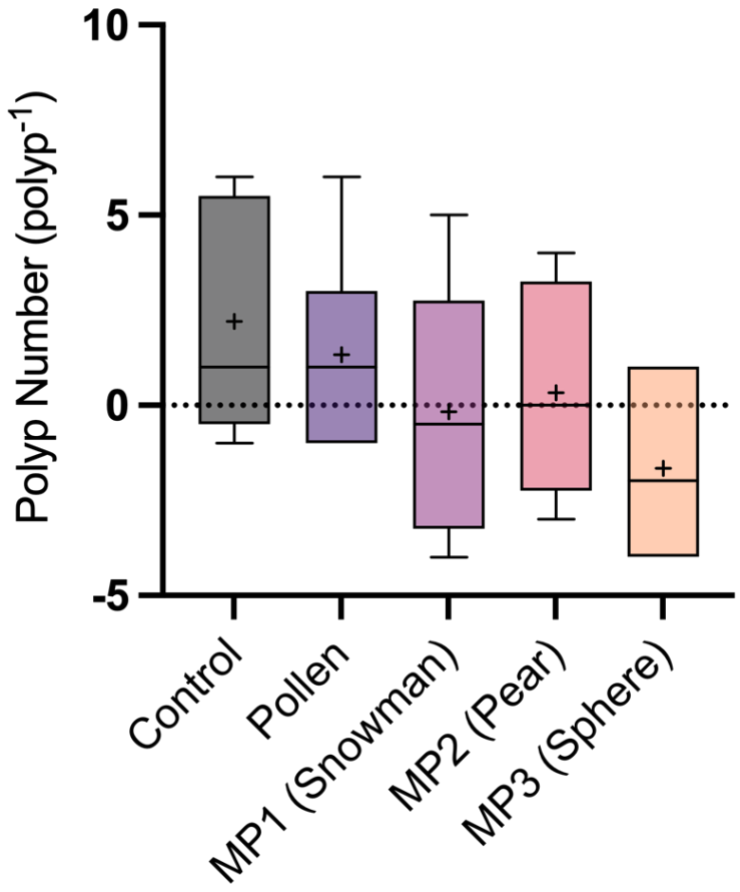


Figure 11. Differences in polyp number over the six-week experiment period by treatment. Bars represent median, whiskers represent maximum and minimum value. + Symbol represents the mean of each treatment.

Table 1. Changes in daily growth rate and polyp number for colonies exposed to each treatment. Data shown as mean ± SD

	Change in Polyp Number per day	**Change in Surface Area (cm^2) per day**
Control	0.052 ± 0.070	$0.001 \text{ x} \pm 4.44 \text{ x } 10^{-4}$
Pollen	0.032 ± 0.061	$7.54\text{x } 10^{-4} \pm 3.53\text{x } 10^{-4}$
MP1 (Snowman)	-0.004 ± 0.082	0.052 ± 0.002
MP2 (Pear)	0.008 ± 0.068	0.082 ± 0.001
MP3 (Sphere)	-0.004 ± 0.056	$6.00 \text{ x } 10^{-4} \pm 9.90 \text{ x } 10^{-4}$

Discussion

In this study I investigated whether exposing *Astrangia poculata* colonies to irregular shaped microplastics over a six-week period would affect coral growth and reproduction, by measuring surface area and polyp number. No significant differences were found between treatments over the six-week period for surface area or polyp number. However, surface area had the most

variability in colonies exposed to microplastic treatments, and polyp number showed decrease in colonies exposed to the microplastic treatments.

Growth rates of *A. poculata* have been investigated previously by S.P Grace, in which colony polyp numbers were counted seasonally on field corals in Narragansett Bay, Rhode Island. He reported that during the months of September to December, temperature decreased from 20 °C to 7 °C, and colonies increased on average 5 - 10 polyps over the four-month period (Grace 2017). This increase is equivalent to 0.04 - 0.09 polyps per day, which is similar to the range in polyp number change recorded for the control and pollen fed treatments, which was an average of 0.052 and 0.032 polyps per day (Grace 2017) (Table 1.). Over the coldest winter months from December to March when temperatures were as low as 5°C, Grace observed that intertidal colonies lost polyps equivalent to a rate of 0.09 polyps per day, and in this study, it was found that the sphere-shaped microplastic treatment colonies reduce polyp number by an average of 0.004 polyps per day (Grace 2017) (Table 1).

Dimond and Carrington (2007) also measured change in polyp number of *A. poculata* seasonally in Narragansett Bay, RI, and found that symbiotic and aposymbiotic colonies showed significant decreases in polyp number in the winter months, but significant increase in the warmer months (Dimond ad Carrington 2007). The study temperature in this experiment (15°C) is most comparable to the field data temperatures in Narragansett Bay from May to July, in which our control and pollen treatments increased in polyp number at a lower rate (0.052 and 0.032), within range of Dimond and Carrington's study, which was between 0 and 0.1 polyps per day (Dimond ad Carrington 2007) (Table 1).

A comparison of these laboratory results to these field data suggests that microplastic exposure could cause a similar stress response in coral asexual reproduction as cold temperature. Generally, reduction in polyp number happens when coral is experiencing tissue loss from

extreme stress, which can then lead to coenosarc reduction, and skeletal loss (Dimond and Carrington 2007). Something else to note is that we found differing results for surface area and polyp number. For example, while the "pear" shaped plastic treatment showed the most increase in surface area, there was no change in polyp number, suggesting there was skeletal growth but no asexual reproduction. Colonies in the "sphere" shaped microplastic treatment also had the second highest growth rate but decreased in polyp number. This finding suggests that exposures to microplastics in the smaller size range (9-15 μm) may have more of an effect on asexual reproduction than skeletal growth rates of *A. poculata*.

These data indicate that while smaller microplastics did not significantly affect colonies over six weeks, it is possible that feeding on plastics did have some effect on growth and asexual reproduction (measured by surface area and polyp number, respectively). Similarly, Hankins et al. (2018) found no difference in calcification after a 48-hour exposure of PE microbeads (90–106, 425–500, and 850–1000 μm) at a concentration of 30 mg/L. Another study from Berry et al., (2019) found limited effects of PE and PP microbead exposure on coral embryo development, fertilization, and early life stages. In addition, Mendrik et al., (2021) found species specific effects of microplastic exposure, with no significant changes in photochemical efficiency and respiration for the species *S. hysterix*, but significant stress response in *Acropora sp.* Whether the results we found were due to the length of exposure or the type of microplastics used needs further study. It is also possible that given more time than six weeks, this type of exposure may have produced more of an effect. Other growth studies have measured coral growth after a longer exposure period, in the past at six months, five months, or two months, (Reichert et al., 2019; Chapron et al. 2018; Mouchi et al., 2019).

The concentrations of plastic used in this study (5000 particles/L^{-1}) are generally higher than concentrations found in the environment but are similar to the concentration used in other

marine invertebrate microplastic studies (Baroja et al., 2021; Soares et al., 2021; Huang et al., 2021). I used a higher microplastic concentration than is normally found in the environment but is still within the range of other microplastic studies. In 10,000 km North-South transects of the Atlantic Ocean, Pabortsava and Lampett (2020) measured high concentrations of Polyethylene, Polypropylene, and Polystyrene at 10m, 50-170m, and 100-270m. The highest concentrations were commonly at 10-meter depth and were measured as high as 2000-4000 particles m^3 (Pabortsava and Lampett 2020). Given that those temperate corals are often found closer to shore in the 10-meter range, these high levels are concerning, and are close in concentration to what I used in this experiment. A higher concentration of microplastics may have a significant impact on coral physiology but would not be environmentally significant.

It is possible that microplastics on the smaller size scale, do not have as great or immediate of an effect on growth. Most studies testing microplastic effects on corals have used plastic at least 100 μm in size (Huang et al., 2021; Soares et al., 2021). To my knowledge, no other studies have exposed the species *A. poculata* to plastics less than 100 μm in size (Huang et al., 2021; Soares et al., 2021). Many studies have also not compared the effects of different shapes and types of plastics on coral growth as we tried to investigate in this study. This work confirmed ingestion of particles in the size class 9-15 μm sized by *A. poculata.* This confirms a wider range of particles are ingested than previously reported and highlights the need for more information is needed on the feeding preferences and ingestion capabilities of *A. poculata*, in relation to microplastic size and type. Further, it begs the question if any effects of microplastics consumption on growth is due to gut fullness (retention time) or interference with feeding particles (mechanical).

Results from this study represent an acute exposure to microplastic ingestion, as animals were directly delivered each type of several times a week. Most studies, on the other hand,

constantly exposed corals to microplastics by keeping plastics in suspension (Huang et al., 2021). It is possible a combination of exposure mode and particle size allowed for the colonies to continue feeding, explaining the higher growth rates observed. These differences in methodology can account for some of the differences observed. By contrast, in this study we fed plastics to corals three times a week, and while plastics could not be visually detected, uneaten plastics presumably floated to the bottom of the dish over time.

In conclusion, I did not find significant effects of microplastic ingestion on *A. poculata* growth as other studies using similar concentrations did on tropical coral species. Our findings, however, indicate other potential effects of smaller microplastics on colony growth and suggest future studies should investigate this topic further. Plastics smaller than 100 μm in diameter should be tested more in coral microplastic growth studies.

Chapter IV: Effects of Microplastic Exposure on *Astrangia poculata* Respiration Rates

Introduction

Respiration rates can provide valuable insight into invertebrate organism health and functioning. Increased respiration rates have been associated with stress responses in corals and anemones (Edmunds, 2005; Leggat et al., 2011; Nii and Muscatine, 1997; Mendrik et al. 2021). Further, I hypothesized that since microplastics have been shown to have physiological effects on factors like growth and photosynthesis (Mendrik et al. 2021; Hankins et al. 2018), respiration

would also be affected. However, effects of microplastic on coral respiration have so far been inconclusive, with studies finding conflicting results, that are often species specific (Mendrik et al. 2021). Boodraj and Glassom (2022) recently investigated respiration rates after microplastic exposure over a 4-week period and found no significant effects on the tropical species *Anomastraea irregularis* and *Pocillopora verrucosa*. Mendrik et al. (2021), on the other hand, found heightened respiratory function in *Acropora* sp. colonies after a 12-day exposure period, but no significant changes in other species tested.

In the past, *Astrangia poculata* respiration rates have been measured to compare respiration rates and thermal tolerance in colonies from Rhode Island and Virginia (Aichelmann et al. 2019). However, respiration rates of *A. poculata* have not been investigated in the context of microplastic exposure. I hypothesize that continuous microplastics exposure and furthermore, ingestion, might be correlated with higher respiration rates in corals. I also hypothesize that different plastic size classes may present varying levels of effects, due to different levels of disturbance and selective ingestion based on size. This experiment aims to investigate how continuous microplastic exposure may or may not influence *A. poculata* respiration rates over a total five-week exposure period.

Methods

Coral Collection and Maintenance

Corals were collected by SCUBA off the Marine Biological Laboratory dock and were maintained in the MBL Marine Resources Center in a flow-through, filtered sea water system. Astrangia colonies were shipped to Connecticut College overnight, after which they were maintained in 1 μm filtered seawater with aeration. During the experiment, coral colonies were

individually fed 1mL of live brine shrimp 3x a week. Each coral colony used was approximately 1 cm^2 in size. 100% water changes were conducted once a week using seawater, and the same concentrations of plastic stock solutions. Salinity was maintained at 29-33 ppt and ambient light was maintained at 12-hour light/dark cycles. The temperature in experimental jars averaged 22 °C, recorded using a HOBO temperature and light logger.

Plastic Treatments

A total of four treatments were used, with six replicates each, consisting of 425-500 μm polyethylene spheres (Cospheric Inc), 125-250 μm polyethylene spheres (Cospheric Inc), "Snowman" (12.5x15.3μm, cat #: PNT015UM) polystyrene particles, and a control of 1μm filtered seawater (no microplastics present).

Experimental Design

Corals were maintained in individual 10 oz glass jars filled with 300 mL 1μm filtered seawater and lightly covered to prevent evaporation, in plastic exposures at a targeted concentration of 2000 particles L^{-1}. Plastic particles were kept in circulation by bubbling air into each jar using individual airline tubing connected to air pumps (Tetra 77850 Whisper Air). After five weeks of plastic exposure, corals were transferred to 300 mL glass dishes with filtered seawater with no plastic exposure.

Respirometry Measurements

Respirometry measurements of colonies were done using the Q-Box AQUA Aquatic Respirometry Package**.** Corals were exposed to plastics for a total of 5 weeks, during which respiration was measured at week 3, week 5, and week 8. Respirometry time points were conducted over a three-day period, and treatments were measured in the same order every time. Respiration was measured using a Logger Pro respiration q-aqua box system. A 120 mL chamber was kept in a water bath filled with 1 μm filtered seawater. The total volume of the system in use

was measured at 190 mL. The respiration of three colonies from each treatment were measured for each timepoint. Blanks were conducted every time new water was used. For each experimental respiration measurement, colony respiration was measured for 1 hour in the light (30 minutes to allow for recovery from handling time), and 30 minutes in the dark to adjust for photosynthetic symbionts that could contribute separate respiration signals.

Imaging

Each colony was imaged for surface area and polyp number analysis at experimental start, middle, and end. Corals were imaged using a MEIJI dissecting scope and smartphone snap zoom attachment, with a millimeter ruler next to each colony for calibration.

Statistical analysis and Calculations

Respirometry calculations were done by subtracting each experimental value from the blank value. Total oxygen consumption for each coral colony was calculated by multiplying measured dissolved oxygen slopes by the respirometer volume (L) subtracted from the coral volume (mL), and multiplying by time measured (s), over individual coral mass (kg).

A Two-Way ANOVA was performed on both light and dark respiration data sets, comparing weeks three and five. A One-Way ANOVA was performed to compare light and dark treatment measurements, as well as differences between treatments for each time point (GraphPad Prism).

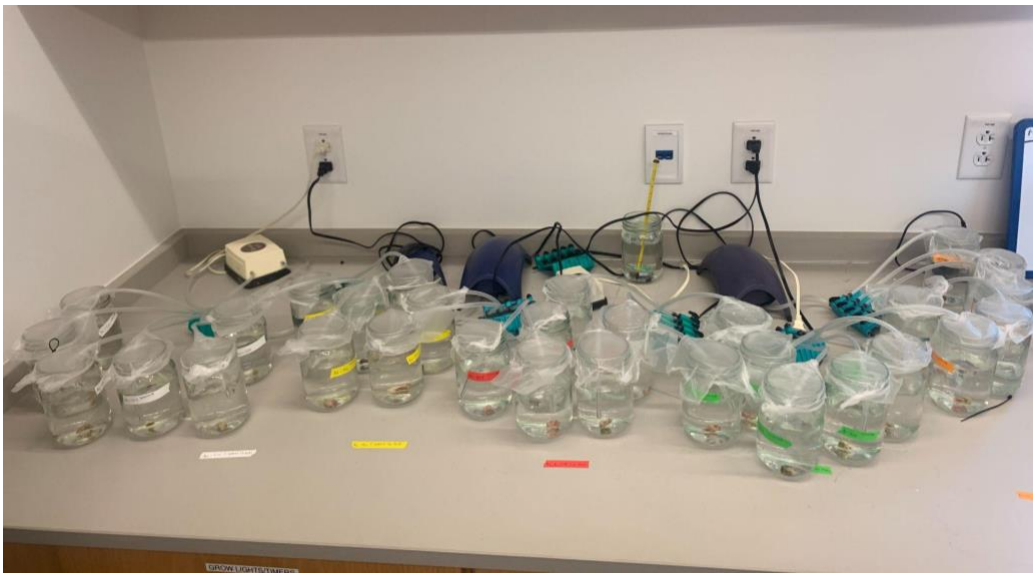


Figure 11. Exposure set up with separate labeled microplastic treatments.

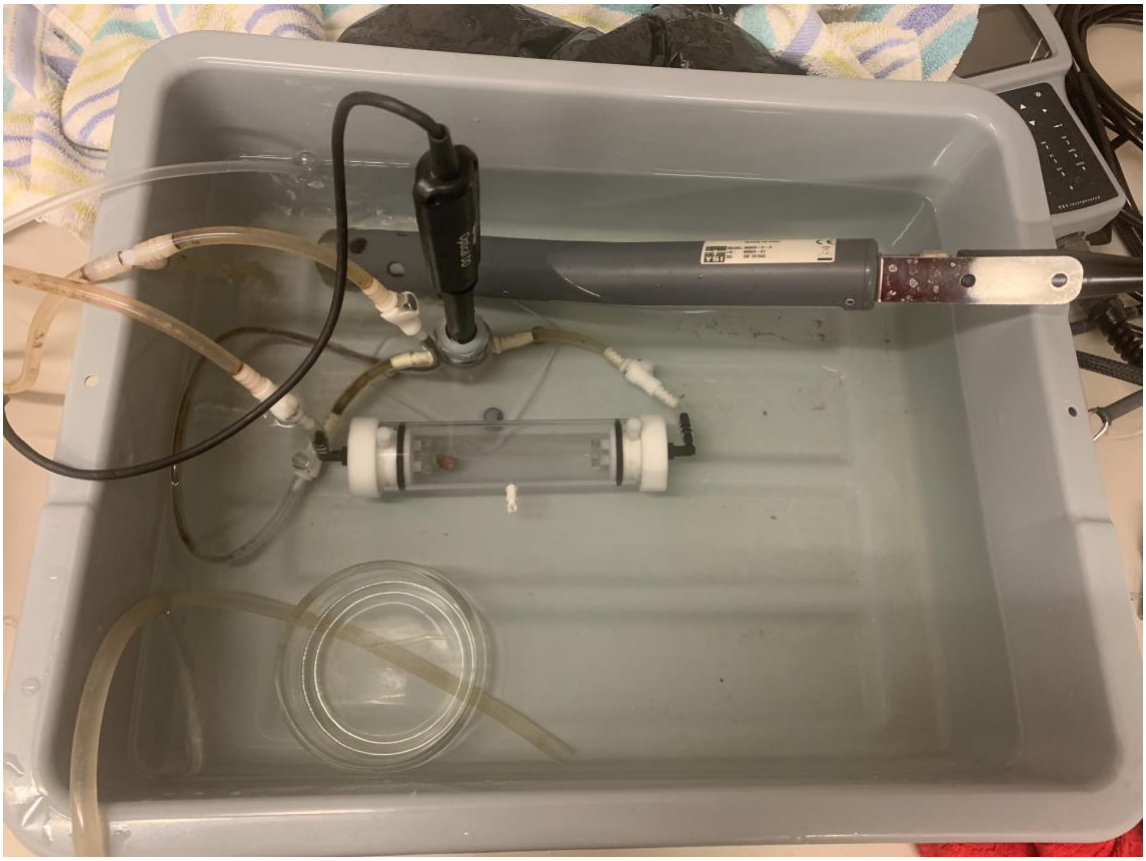


Figure 12. Respirometry set up with a colony in a chamber and water bath. YSI system is also pictured in the water bath, used for monitoring salinity and temperature.

Results

There was no significant difference between all four treatments from weeks three and five. Light respiration data from week 0 to week 3 trended to decrease in respiration rates on average except for the 500 µm treatment. (Fig 13; Two-Way ANOVA, df = 3, F = 2.579 p > 0.05) The 500 µm treatment increased from (39.34 ± 14.08 to 73.00 ± 22.05 mg O_2/kg/hr). The 10 µm treatment decreased from (41.33 ± 40.87 to 19.51 ± 25. 64 mg O2/kg/hr), the 200 µm treatment decreased from (41.60 ± 82.06 mg O_2/kg/hr), to (19.51 ± 25.64 mg O_2/kg/hr), and the control treatment decreased from (43.80 ± 11.50 to -7.65 ± 15.32 mg O_2/kg/hr). It was predicted that plastic treatments would be different from the control treatment, however in this case there was not statistically significant difference.

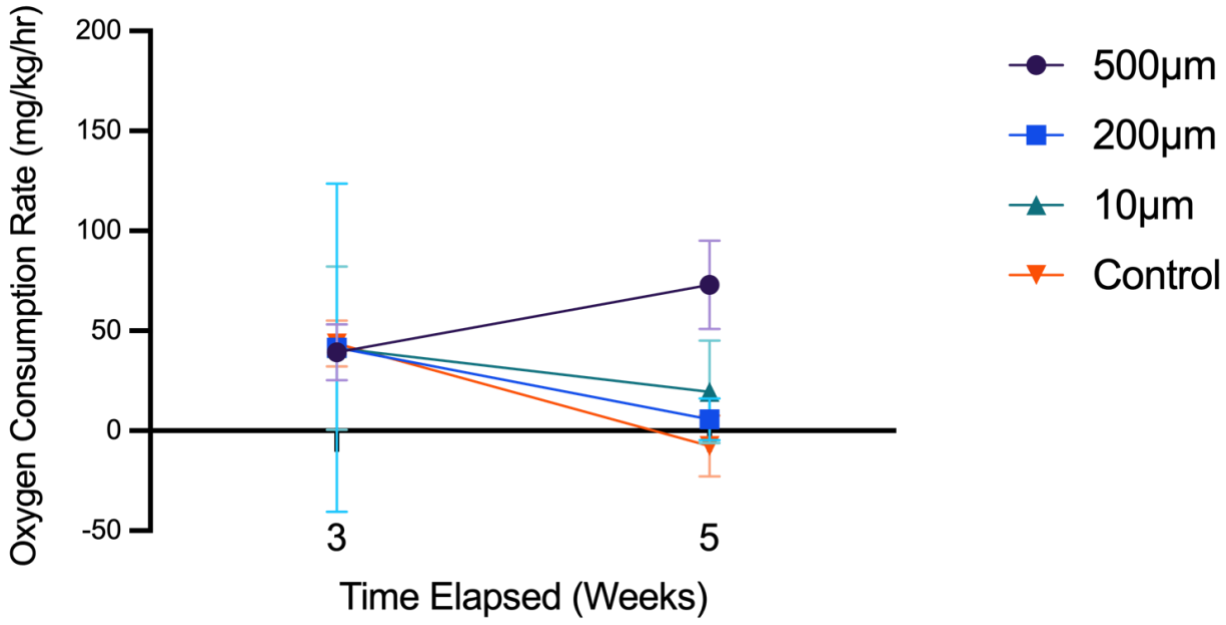


Figure 13. Average light respiration (mg O_2/kg/hr) by treatment and time point over a three-week exposure period with standard deviation bars.

As with the light treatments, there was no significant difference between week three and five treatments. (Fig 14; Two-Way ANOVA, df = 3, F = 1.429 p > 0.05) However, the 500 µm treatment still increased from an average of (39.774 ± 14.876 to 49.783 ± 9.31 mg O_2/kg/hr). The 10 µm treatment decreased from (24.00 ± 14.92.87 to -23.64 ± 29.00 mg O_2/kg/hr), the 200 µm treatment decreased from (74.42 ± 80.00, to 6.16 ± 13.62 mg O_2/kg/hr), and the control treatment decreased from (56.34 ± 5.40 to -4.54 ± 15.00 mg O_2/kg/hr).

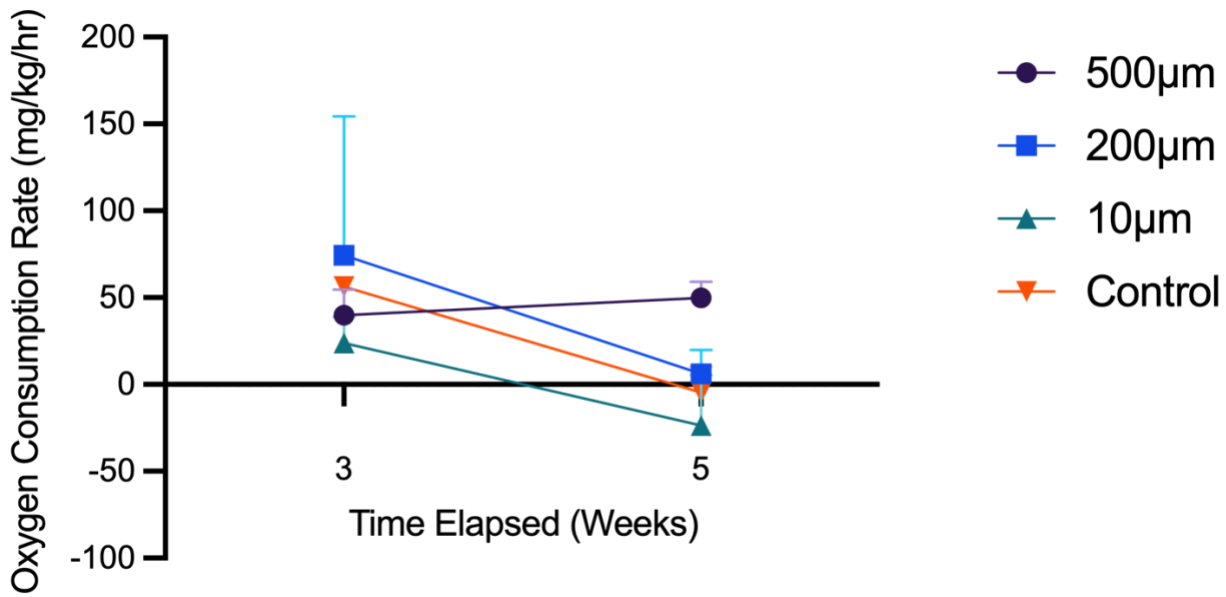


Figure 14. Average dark respiration (mg O_2/kg/hr) by treatment and time point over a three-week exposure period with standard deviation bars.

There was no statistical difference found between light and dark measurements between all treatment measurements from week three and five measurements (Welch t-test, $t(18.19) = 0.4119$, $p > 0.05$; $t(17.74) = 0.7950$, $p > 0.05$) However, there were significant differences between treatments for dark measurements from week three, and light and dark measurements from week five (ANOVA, $F_{10} = 8.695$, $P < 0.05$; $F_{11} = 12.96$, $P<0.05$; $F_{11} = 5.755$, $P < 0.05$). The only set of measurements without significant difference was light measurements from week three (ANOVA, $F_{11} = 0.004$, $P > 0.05$).

Discussion

Differences between treatments were not significant overall, however preliminary data suggests some patterns in respiration rates over three weeks between treatments (Figs. 13 and 14). Given that data could be trending towards significant difference in week 8, It is useful to note that respiration rates increased for the 500 μm plastic treatments, but overall decreased for all other treatments over a three-week exposure period. Dark and light measurements followed a similar pattern, with more variation in the dark respiration rate measurements. Since there was no significant difference between light and dark measurements, this pattern suggests that light and dark did not have a significant effect on respiration measurements, and that symbiotic algae respiration rates were not significant. However, since there was significant difference between treatments for both light and dark measurements in week five, this suggests that treatments significantly diverged from each other after week three and were no longer in a statistically similar range like they were based on week three light measurement data (Fig. 13).

While there were not statistically significant differences between timepoints, the preliminary data I presented here suggests that 500 μm plastics may have had a stress effect on

the corals given that they exhibited higher respiration rates compared to other treatments. Previously, *Astrangia poculata* respiration rates have been measured in the context of high temperature stress (Aichelman et al. 2019; Dimond et al. 2012). It is possible that large plastic particles could also cause a stress effect. Exposure to large particles in constant suspension could cause a similar effect to sedimentation stress, which has been found to significantly increase respiration rates in tropical species like *Merulina ampliata* (Browne et al., 2014). In addition, the data I have presented here are only from weeks 3 and 5 of exposure. Week 8 data will exhibit respiration rates after 3 weeks of no exposure, which could be significantly different from exposure respiration rates.

During the winter months, *A. poculata* will begin a state of torpor, and exhibit slower respirations rates and tissue thinning (Dimond et al. 2012). Given that temperature remained relatively constant in the exposure beakers, this response would not make sense. Generally, *A. poculata* can maintain stable respiration rates when it is experiencing temperatures between 11.5 and 23 ℃ (Dimond et al. 2012). Given that the control treatments decreased in respiration rates as well, slowed respiration may have been an artifact of the jar ecosystems. However, given that differences not significant, this is also probably within the range of normal fluctuations.

Given the variance in the data, however, it is also possible that the plastic did not have any significant effect on *A. poculata* respiration rates. Lack of microplastic respiration effects were also observed by Boodraj and Glassom (2022), and mixed species effects were observed by Mendrik et al. (2019). Other factors and limitations to consider are the varying plastic size classes used in this experiment. Generally, the 200 μm size class did not circulate as the 500 μm in the jars, so exposure to this size class may have been more limited. It is also possible that Astrangia, being a more resilient coral, is less affected by microplastics than more sensitive, tropical corals.

Overall, respiration rates I present here agree with the previous data suggesting that microplastic effects are inconclusive; However, with all the data analyzed from this experiment, further results with significant findings could be obtained.

Conclusions

This project aimed to address a multitude of questions related to how microplastics affect the physiology and behavior of a local temperate coral in New England, *Astrangia poculata.* The behavioral findings show that *A. poculata* feeds on a variety of plastic types and other organic material, however it will egest all particles within a relatively short period, agreeing with prior data that most plastics are egested within six hours (Allen et al. 2017; Rotjan et al. 2019). In addition, *A. poculata* feeds on spherical plastic and food items at a statistically similar rate – conflicting with previous findings from a 50/50 choices assay (Rotjan et al. 2019) Based on these data, they clearly prefer food and organic particles over plastic, however they will still feed on plastics at an alarmingly high rate. In addition, this study shed light on how different types of plastics like fibers and fragments are still egested within a relatively similar time frame. The question of why corals feed on plastic at all remains to be fully investigated, however this data supports the idea that corals prefer biofilmed plastics. It could be that organic matter like biofilms adhered to the plastic make it desirable to ingest as this work suggests, or it could be that there are chemosensory qualities in the plastic itself that may make it palatable as previous research has hypothesized (Allen et al. 2017). While this research indicates there is still more to be understood about *A. poculata* ingestion and egestion behavior in response to microplastics, it has further developed the selectivity and retention times of *A. poculata* in response to different plastic types.

Data from the physiology-based experiments did not show any significant effects over a five- and six-week period, however there was a huge amount of variability – contributing to a need for further understanding of potential long term stress effects on this species in the literature. Colonies measured for respiration and growth exhibited what could be initial stress responses based on a five- and six-week period. However, it is also possible that microplastics do not have a significant physiological effect on this species, as other authors have found with certain tropical species (Boodraj and Glossam 2022; Mendrik et al. 2021)

Future research for microplastics and coral studies should focus on physiological effects over a longer time period, with more environmentally relevant concentrations. Long-term effects of chronic ingestion and egestion are still relatively unknown. In addition, a variety of plastic types should be used more frequently in experimentation, like fibers and fragments, as well as other irregular shapes, instead of the standard spherical particle. Nanoplastics, plastics ranging from 1-1000 nm, should also be further investigated in the context of coral health, as they have been understudied (Li et al 2020).

Another important piece of the puzzle is to quantify plastic concentrations inside field corals, and around coral habitats in a diversity of locations. Further mapping tropical coral plastic concentrations could inform the severity of the issue. In addition, *A. poculata* could provide a useful system for testing differences in plastic feeding in aposymbiotic and symbiotic colonies. This has already been initially tested in *Aiptasia* colonies (Romanó de Orte et al. 2018), however may still provide useful insights for *A. poculata and* other photosynthetic anthozoans. Future research should also investigate ingestion and egestion together further, along with physiological and possible energetic impacts that chronic egestion may have. Understanding coral and other invertebrate filter feeder responses to different types of microplastics is paramount. Microplastic exposure could also have the potential to have epigenetic effects, and if plastic is present in

skeletons, it is possible that plastic will be present in larvae. Sexual reproduction and microplastics has been severely understudied.

Overall, these data contribute significant findings to the literature – and provide more questions to ask surrounding the effects of microplastics on corals, and benthic marine invertebrates. For now, plastic is not going away– but neither is *Astrangia poculata.*

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