PH.D. DISSERTATION PROPOSAL

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This is to certify that this student's PhD Supervisory Committee had read and approved the Ph.D. Dissertation Proposal titled: THE IMPACTS OF CLIMATE CHANGE AND MICROPLASTICS POLLUTION ON CORALS AND CORAL REEFS

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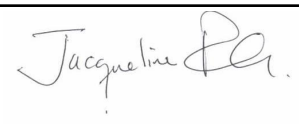
CHAIR	Jacqueline L. Padilla-Gamino		3/28/2-23
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THE IMPACTS OF CLIMATE CHANGE AND MICROPLASTICS POLLUTION ON CORALS AND CORAL REEFS

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A Dissertation Proposal

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Introduction

Coral reefs are critical ecosystems, both ecologically and economically, for over half a billion people worldwide (Moberg and Folke 1999). While occupying under one percent of the ocean floor, coral reefs are home to more than 25% of all marine species (Fisher et al. 2015). The immense biodiversity of coral reefs is due in part to the complex, diverse structures formed by scleractinian, or stony, corals. In addition to providing habitat for an extremely diverse group of taxa, these reef structures protect shorelines in tropical and sub-tropical regions from storms and coastal erosion (Moberg and Folke 1999). Coral reefs also support highly productive fisheries and tourism revenue for coastal inhabitants, especially in developing nations (McManus 1988). Despite the importance of coral reefs, their future remains uncertain due a rapid rise of anthropogenic stressors, on both global and local scales, over the past several decades (Hoegh-Guldberg et al. 2007).

The greatest threat to corals is global climate change caused largely by carbon dioxide emissions that lead to warming and acidification of the oceans (Hoegh-Guldberg et al. 2007). Scleractinian corals live in mutualistic, symbiotic relationship with dinoflagellate algae, where corals provide habitat for the algae which in turn provide photosynthetically derived carbon, or energy, to the coral (Muscatine and Porter 1977). However, this relationship breaks down with even slight (1 – 2 ºC) increases in seawater temperature and can lead to coral mortality. Since the effects of climate change are rapidly increasing, model projections predict that 75% of coral reefs will be subjected to annual severe bleaching before 2070 due to thermal stress alone (van Hooidonk et al. 2016). However, the fate of corals may be worsened in the face of additional stressors (Hughes and Connell 1999).

Local threats to corals include overfishing, disease, coastal development, and pollution, among others. These stressors are more easily managed than global climate change but can interact with its effects. In some cases, the interaction between local stressors and climate change can act additively or synergistically worsening the outlook for corals (Carilli et al. 2009; Ellis et al. 2019; Fisher et al. 2019). In other cases, however, interactions between local and global stressors can be antagonistic (Darling et al. 2010; Fisher et al. 2019). Many scientists agree that reducing local stressors on coral reefs can ameliorate effects of climate change (Hughes et al. 2003; Wooldridge and Done 2009; Donovan et al. 2021) which would buy time to implement coral reef conservation strategies until we reduce or reverse the impacts of global climate change.

Proteomics

Much is known about the effects of global and local stressors on corals at ecosystem, population, and organismal levels but we are only beginning to understand the cellular level impacts these stressors have on coral physiology. It is critical to understand how corals respond at the cellular level to provide conservation efforts with tools for evaluating corals' response to stress, such as molecular biomarkers. Analytical methods using "omics" technology are advancing rapidly and have elucidated much of the cellular components that corals have to respond to stress as well as cellular pathways that are activated under stressful conditions (Cziesielski et al. 2019). However, much of that research focuses on gene expression patterns which often does not paint an accurate

picture of what is occurring in cells at the time of interest (Gygi et al. 1999; Maier et al. 2009; Mayfield 2020). In contrast, few studies have investigated how proteins respond to stressors in corals, which paints a more accurate picture of cellular processes that are actively occurring at the time of sampling, and complements much of what is learned using genomics and transcriptomics (Pandey and Mann 2000; Tomanek 2014; Stuhr et al. 2018).

While there are fewer proteomics studies with corals than genomic or transcriptomic studies, the field is growing rapidly. Early proteomic investigations with corals relied on gel-based (e.g., 2-dimensional gel electrophoresis) methods to determine proteins of interest that can be then identified using mass spectrometry (e.g., Drake et al. 2013; Ramos-Silva et al. 2013; Mayfield et al. 2018). However, a new era of proteomics, e.g., "shotgun" proteomics, allows for the identification of up to thousands of proteins from considerably small samples by coupling liquid chromatography with tandem mass spectrometry (Wu and MacCoss 2002). This allows researchers to determine much of the proteome, or suite of proteins, present at the time of sampling. Downstream analysis of proteomics data can be used to elucidate active cellular and metabolic pathways when they are searched against annotation data bases such as Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG).

Microplastics

Figure 1: Microplastics (left) and zooplankton (right) are similar in size and shape making it difficult for marine organisms to distinguish them.

Microplastics pollution has recently emerged as a contaminant of growing concern. They are defined as synthetic, or partially synthetic, particles with a size range of 1µm to 5mm (Frias and Nash 2019). Primary microplastics enter the environment at an already small size and originate from sources such as cosmetics and industry. Secondary microplastics result from the degradation of larger plastics already present in the environment (Cole et al. 2011). Microplastics are impacting organisms and ecosystems across the entire planet, from deep-sea trenches to Arctic sea ice (reviewed in Auta et al. 2017). In the marine environment, they are particularly problematic for planktivorous organisms because of their similarity in size and shape to

zooplankton (Fig 1). Effects of ingesting microplastics by marine organisms include decreased growth and fecundity, increased immune response, changes in behavior and mortality (reviewed in Auta et al. 2017).

Reef building corals are affected by microplastics in various ways. Hall et al. (2015) was the first study to show that corals can ingest microplastics. Since that article, research have shown that exposure to microplastics by corals can cause decreased growth, necrotic lesions, tissue and skeletal overgrowth, increased immune response, and bleaching (reviewed in Huang et al. 2021). Microplastics can also be incorporated into a coral's skeleton (Hierl et al. 2021; Reichert et al. 2022) with yet unknown consequences. Furthermore, plastics can act as vectors for disease when infectious microbes colonize the plastic's surface and are transferred to corals. In a study spanning 159 reefs across Southeast Asia and Australia, corals that were in contact with plastic were up to 80 times more likely to be diseased than those not in contact with plastic (Lamb et al. 2018).

Although research efforts are increasing, we still have much to learn about spatial and temporal patterns of microplastics abundance in coral reefs. Depending on their density and other physical and biological properties, microplastics can float on the ocean's surface, remain in the water column or sink to the seafloor (Zhang 2017). Ocean currents and terrestrial inputs also affect the spatial distribution of microplastics (Jambeck et al. 2015; Zhang 2017; Yu et al. 2018). However, most microplastics that enter the marine environment will eventually settle on the seafloor because of changes to their physical properties that make them less buoyant, such as degradation and/or colonization of biota (Kowalski et al. 2016; Kooi et al. 2017). In addition to sampling seawater and sediment, a useful tool for assessing microplastics accumulation and distribution in marine environments is to sample them from benthic organisms like corals (Rotjan et al. 2019) and sea cucumbers (Mohsen et al. 2019). Corals, being suspension feeders, remove microplastics from the water column, whereas deposit-feeding sea cucumbers ingest microplastics from the sediment.

Exposure to climate change stressors and microplastics pollution has potential to impact corals. Increased seawater temperatures due to climate change can cause corals to bleach resulting in decreased energy for the coral (Hoegh-Guldberg and Smith 1989). Some coral species can mitigate nutritional deficits caused by bleaching through increased feeding on zooplankton during recovery from bleaching, a phenomenon known as heterotrophic plasticity (Grottoli et al. 2006). However, corals have been shown to ingest microplastics while feeding on zooplankton prey, thus increased heterotrophy may come with undesirable side effect of increased ingestion of microplastics (Axworthy and Padilla-Gamiño 2019). It has also been suggested that some corals may preferentially ingest microplastics over zooplankton prey (Allen et al. 2017; Rotjan et al. 2019). Ingesting microplastics while in a bleached state may increase the energy deficit already faced by corals since ingesting, and egesting, microplastics is considered an energetically costly behavior (Hall et al. 2015; Reichert et al. 2018; Chapron et al. 2018). In my first chapter of my dissertation (Axworthy and Padilla-Gamiño 2019), we were the first study to ask the question: Do thermal stress induced bleached corals ingest more microplastics than non-bleached corals? A key finding in that study showed that bleached corals ingested less microplastics than

non-bleached corals. This was a surprising result, since previous research showed that bleach corals feed more heavily than non-bleached corals (Grottoli et al. 2006; Palardy et al. 2008). Other key results include: 1) that the coral species tested had different microplastic ingestion rates, 2) one species only ingested microplastics when prey was also present, and 3) ingesting microplastics did not affect the corals' feeding rate on prey.

Corals may be a significant sink for microplastics pollution via active and passive removal mechanisms. Corals actively remove microplastics from seawater when they ingest them and passively remove them when microplastics adhere to the coral's surface or mucus. Studies on four coral species from the Red Sea showed that microplastics adhesion rates were 40 times greater than ingestion rates suggesting that adhesion is a more important removal mechanism than ingestion (Martin et al. 2019; Corona et al. 2020). However, it is not known how microplastics type, water velocity and coral morphology influence these processes. Different plastics vary in chemical composition, and morphologically, which may affect chemical or physical sensory processes that corals use to detect and feed on prey (Allen et al. 2017). Colony morphology and hydrodynamic properties, such as water velocity, may affect how corals and microplastics interact, affecting both ingestion and adhesion processes (Helmuth and Sebens 1993; Sebens et al. 1997; de Smit et al. 2021)

It is important to understand how both global and local stressors stand to impact coral reefs to be better prepared to conserve these delicate ecosystem engineers as our world changes and these pressures increase. My PhD dissertation will address the problems of coral bleaching due to climate change and microplastics pollution on corals and coral reefs. Specifically, the goals of my PhD dissertation include:

1. **Determine how thermal stress affects microplastics ingestion by corals.**
2. **Explore changes in protein expression and corresponding metabolic pathways in bleached corals compared to non-bleached corals.**
3. **Characterize spatial patterns of microplastics pollution in Kāneʻohe Bay, Hawaiʻi.**
4. **Determine the effects of different water velocities, microplastic types and coral morphologies on microplastics ingestion and adhesion rates in corals.**

Chapter 1: Microplastics ingestion and heterotrophy in thermally stressed corals

In this study, I performed experiments in Hawaiʻi to test the hypothesis that bleached corals ingest more microplastics than non-bleached corals. The hypothesis was based on previous research showing that some corals increase heterotrophy during recovery from bleaching as a way to increase energy supply when their symbiotic algae are decreased (Grottoli et al. 2006; Palardy et al. 2008). Full details of that study, which is published in *Scientific Reports*, can be found here: https://www.nature.com/articles/s41598-019-54698-7.

Chapter 2: Shotgun proteomics identifies active metabolic pathways in bleached coral tissue and intraskeletal compartments

In this study, we performed a coral bleaching experiment in Hawai‘i, followed by proteomics and bioinformatics analyses of those corals at the University of Washington. Our main goal was to determine changes in protein expression and associated metabolic pathways between bleached and non-bleached corals. Full details of that study, which is part of a special issue titled “The Cellular Stress Response and Physiological Adaptations of Corals Subjected to Environmental Stressors and Pollutants” in *Frontiers in Marine Science*, can be found here: https://www.frontiersin.org/articles/10.3389/fmars.2022.797517/full.

Chapter 3: Microplastics pollution in seawater, sediment, corals, and sea cucumbers in Kāne‘ohe Bay, Hawai‘i

In this study, I will characterize spatial patterns of microplastics abundance in Kāne‘ohe Bay, Hawai‘i. This bay is located on the windward side of Oahu and supports a highly studied sub-tropical coral reef ecosystem. Historically, there have been many studies examining coral reef ecology, pollution, eutrophication, coral disease, and corals’ physiological responses to stressors such as bleaching. However, to date no studies have addressed the scale and impact of microplastics pollution in the area. My proposed research will begin to address this knowledge gap by sampling seawater, sediment, corals, and sea cucumbers from Kāne‘ohe Bay for microplastics.

Kāne‘ohe Bay’s reef system includes both fringing and patch reefs that are heavily populated with the coral species, *Montipora capitata* and *Porites compressa*. Fringing reefs line most of the coastline within the bay while reef patches are distributed away from shore throughout the bay, separated by silty benthic sediments. Since fringing reefs are adjacent to the shore, where there are multiple sources of terrestrial input, such as rivers and fishponds, I hypothesize that we will detect higher abundance of microplastics from fringing reefs than from patch reefs.

Methods

Sample collection

Samples were collected from two fringing reefs, K4 (21.26610º N, 157.48358º W) and K5 (21.28028º N, 157.49981º W), and two patch reefs, HIMB (21.26159º N, 157.47489º W) and P29 (21.25903º N, 157.47265º W) (Fig 2), for sediment, seawater, corals and sea cucumbers in summer 2018 (Fig 2).

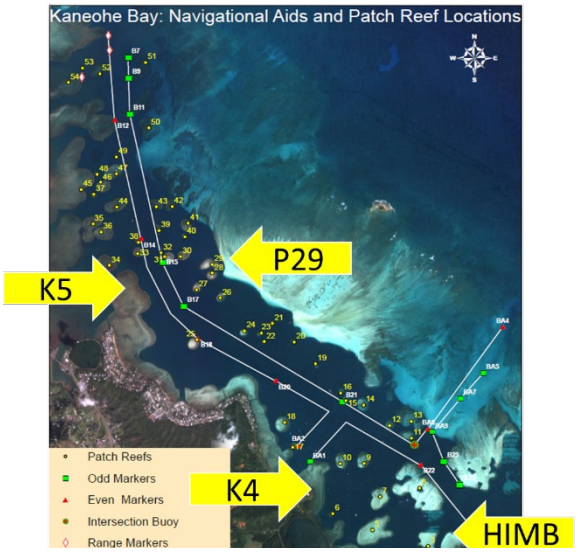


Figure 2: Sampling locations for seawater, sediment, corals and sea cucumbers in Kāne‘ohe Bay, Hawai’i

Seawater (n = 5 per site) was collected using a manta net with an opening of 0.069 m^2 and a mesh size of 330 µm. The manta net was towed for ~500 m over the fore reef at a distance <5 m from the reef crest. To prevent plastic contamination in

the manta net, it was towed using a natural fiber rope from the windward side of the vessel. At the end of each tow, contents in the net were rinsed with seawater to the cod end from the outside of the mesh using a manual pump. The cod end was emptied into pre-cleaned glass containers and mixed with an equal volume of formalin to preserve the samples. Back on land, the seawater samples were poured onto a 250 µm stainless steel sieve and then backwashed into 0.24 L pre-cleaned plastic jars with filtered (1 µm) water and mixed with an equal volume of formalin. The sample containers were wrapped with parafilm to secure them, then shipped to the University of Washington (UW).

Sediment samples (n = 8 per site) were collected using pre-cleaned, 0.47 L mason jars while SCUBA diving from depths between 3 – 8 m. The mason jars remained sealed until immediately before each sample was collected. Sediment from the top 10 cm of the seafloor was scooped into the jars using a stainless-steel spoon and immediately covered with a lid when the jar was full of sediment. Back on land, 200 g (wet weight) of each sediment sample were dried in pre-cleaned aluminum foil boats at 80º C for 12 h then packaged carefully in Zip-lock bags for shipping to UW.

Corals, *Montipora capitata* (n = 15 per site) and *Porites compressa* (n = 15 per site), were collected while SCUBA diving from depths between 3 – 8 m. Fragments approximately 15 cm long were cut from coral colonies that were at least 5 m away from each other using stainless-steel toenail clippers (Revlon, USA). All samples were stored underwater in a plastic Ziplock bag. Upon surfacing, each coral fragment was rinsed with copious amounts of filtered seawater (1 µm) to remove potential plastic contaminants and mucous, then wrapped in pre-cleaned aluminum foil and stored on ice. Back on land, the coral samples were stored at -80º C until they were shipped on dry ice to UW.

Sea cucumbers, *Holorthuria edulis* (n = 15 per site, except for K4: n = 9), were collected by hand while SCUBA diving from depths between 3 – 8 m. At each site, all sea cucumbers were stored in a plastic Ziplock bags until surfacing. At the surface, each sea cucumber was rinsed with copious amounts of filtered seawater (1 µm) to remove potential plastic contaminants and then wrapped in pre-cleaned aluminum foil and stored on ice. Back on land, sea cucumber samples were stored at -80º C until they were shipped on dry ice to UW.

Microplastics extraction

To extract microplastics from each matrix type (e.g., seawater, sediment, coral, sea cucumber), different methods, chosen for their cost-effectiveness and low environmental impact, will be used (Kavya et al. 2020).

Microplastics will be extracted from sediment in 2 steps: 1) density separation and 2) vacuum filtration. For each sample, sediments will be transferred from the aluminum foil boat through a stainless-steel funnel into a pre-cleaned 1000 ml glass beaker and the dry weight of the sample will be recorded. Density separation will be performed three times for each sample using saturated a sodium chloride solution (NaCl, density = 1.2 g ml^{-1} at 25º C). Each beaker with sediment will be filled to 300 ml with NaCl, stirred for one min with a stainless-steel stirrer and allowed to settle for a minimum of 22 h. The supernatant will be decanted into pre-cleaned glass

mason jars and sealed with the lid upside down to avoid contamination from the lid's rubber gasket. The supernatant from each sample will be vacuum filtered onto 5 µm polycarbonate filters and stored in glass petri dishes.

Microplastics from seawater will be extracted in 3 steps: 1) sieving, 2) digestion and 3) vacuum filtration. The seawater sample with formalin solution will be poured onto a 250 µm stainless-steel sieve. Large debris will be rinsed with filtered DI water onto the sieve and then removed and saved to potentially examine later. The remaining material on the sieve will be backwashed into a pre-cleaned 500 ml glass beaker with filtered DI water. Digestion of biological material in the sample will be performed by adding 200 ml of 20% potassium hydroxide (KOH) solution to each beaker followed by incubation at 50º C for 72 h. The solution will then be vacuum filtered onto 5 µm polycarbonate filters and stored in glass petri dishes.

Microplastics from both coral species will extracted in 3 steps: 1) tissue digestion, 2) skeleton dissolution and 3) vacuum filtration. Surface area of each coral measurement will be determined using 3D scanning. Coral fragments will be placed on a turntable, scanned two times from different angles using a 3D scanner (Artec spider), processed using 3D scanner software (Artec Studio 13) to create a 3D model. The 3D model will be exported to MeshLab (ref) software where surface area will be determined using the "Compute Geometric Measures" function. Following surface area measurements, each coral fragment will be transferred to a pre-cleaned 500 ml glass beaker and 200 ml of 20% KOH solution will be added, followed by incubation at 50º C for 7 days or until the tissue is fully digested. The KOH solution will be saved in a pre-cleaned mason jar until vacuum filtration. The remaining coral skeleton will be dissolved in filtered Cal-Ex II, a solution containing formalin, ethanol and formic acid, until complete dissolution of the skeleton is achieved. The remaining Cal-Ex II and KOH solutions will be vacuum filtered onto 5 µm polycarbonate filters and stored in glass petri dishes.

Microplastics from sea cucumbers will be extracted in 4 steps: 1) dissection of the gastrointestinal tract (GT), 2) digestion of the GT, 3) density separation and 4) vacuum filtration. Sea cucumbers will be dissected in a pre-cleaned glass baking dish. The GT will be excised by making an incision from the mouth to the anus to open the body cavity and then severing the connection of the GT from the ring canal (near the mouth) and the cloacal muscles (near the anus). During the dissection step, total sea cucumber length and circumference, wet weight, GT length, and if it is possible to determine, sex will be recorded. The excised GT will be placed in a pre-cleaned 250 ml glass beaker and digested in 50 ml 20% KOH solution, incubated at 50º C for 72 h. After digestion, the KOH solution will be decanted into a pre-cleaned glass mason jar for storage until vacuum filtration. Density separation will be performed three times using saturated NaCl. The NaCl solution will be decanted into pre-cleaned glass mason jars, followed by vacuum filtration as described previously.

Microplastics quantification and identification

Suspected microplastics will be quantified visually using a microscope and their chemical composition will be determined using micro-Fourier transformed infrared spectroscopy (µ-FTIR). Polycarbonate filters will be examined under a stereo microscope at 10 – 40X

magnification. Suspected microplastics will be counted and their shape (bead/pellet, fragment, film or fiber), size (µm) and color (red, yellow, blue, green, orange, purple, white, black and clear) will be recorded. A subset of particles (≥20%) will be analyzed using µ-FTIR (Nicolet iN10, Thermo Fisher Scientific). Analysis will be performed in attenuated total reflection mode using a germanium crystal. The spectra produced will be searched against the Thermo Fisher Aldrich™ Polymers FT-IR Spectral Library to identify particle composition. Particle identification will be accepted at a match score ≥70 (Ding et al. 2019).

QA/QC

To prevent possible contamination of plastic particles from the environment, reagents and procedures, several steps will be taken (Brander et al. 2020). The laboratory where all analyses will occur will be cleaned regularly throughout the experiments and two HEPA filters will be run constantly. All lab personnel will be required to wear clothing with at least 70% natural fiber (100% if possible), use a lint roller upon entrance to the lab and wear 100% cotton lab coats. All procedures that do not require the use of dangerous chemicals (i.e., formalin, KOH and Cal-Ex II) will be performed in a laminar flow hood to prevent contamination. All labware will be rinsed three times with filtered DI water and wiped with Kimwipes wetted with filtered 70% ethanol before each use. All reagents will be filtered to 1.2 µm using GF/F filters. One procedural blank will be processed alongside each batch of samples at each step in the analyses to account for contamination from reagents and procedures. Airborne control filters, 90 mm wetted glass fiber filters in a glass petri dish, will be used to quantify microplastics that fall out of the air near each procedure. The quantity of microplastics determined in the procedural blanks and airborne controls will be subtracted from the sample data to account for contamination.

This study is currently underway. In Summer and Fall 2021, I troubleshooted the extraction methods for each matrix and learned how to use the µ-FTIR. In Fall 2021, I practiced using a 3D scanner to retrieve surface area measurements from coral fragments. Currently, microplastics extractions have been preformed on all sediment samples and one third of the sea cucumber. I will begin extractions from corals and seawater in Summer 2022. I expect to finish extractions from sea cucumbers by the end of Spring 2022 and corals and seawater by the end of Summer 2022. Filters from sediment samples are currently being analyzed for suspected microplastics. I have processed about one third of them to date. I expect to have all filters from all matrices processed by Fall 2022.

Significance

This study will be the first to characterize microplastics pollution in an ecologically and economically important area of a sub-tropical coral reef environment in Hawai‘i. I aim to determine if the concentration of microplastics in water and sediment correlate to the concentration of microplastics in corals and sea cucumbers, respectively, since corals are suspension feeders and sea cucumbers are deposit feeders. This could help monitoring efforts in determining the risk of microplastics pollution for organisms with different feeding modes and/or identify potential bioindicators of microplastics pollution. Additionally, by comparing microplastic abundances at reefs that are affected differently by terrestrial input, this research

could help management efforts in prioritizing which types of coral reefs (fringing or patch) are at greater risk of microplastics pollution thus enabling them to implement effective conservation strategies.

Chapter 4: Microplastics removal from seawater by reef building corals under different water velocities

In this study, I will investigate the effects of different plastic types, water velocity and coral morphology on microplastics ingestion and adhesion by corals. Different plastics are characterized by a suite of different properties, from chemical composition (e.g., polycarbonate, acrylic, polyester, etc.) to morphological features (e.g., pellet, fiber, etc.). The chemical composition, including the polymer type as well as additives that give plastics different properties, can potentially influence how an organism senses it or is able to interact with it (Allen et al. 2017). Morphological features of microplastics, such as shape and size, can affect how likely they are to be ingested by organism (Hankins et al. 2018; Corona et al. 2020). Water velocity may affect how likely corals and particles, such as microplastics, are to interact (Helmuth and Sebens 1993; Sebens et al. 1997) by exerting influence on the boundary layer at the corals surface. Higher water velocity reduces the boundary layer and vice versa, thus affecting transport and/or interception of plastic particles in seawater. Finally, a coral's morphology has the potential to affect hydrodynamic properties, such as the boundary layer and presumably plays a role in the likelihood of coral and particle interactions (Helmuth and Sebens 1993; Sebens et al. 1997). I will test the hypotheses that microplastics type, coral morphology, and water velocity affect microplastics ingestion and adhesion in a controlled laboratory experiment (Fig 3).

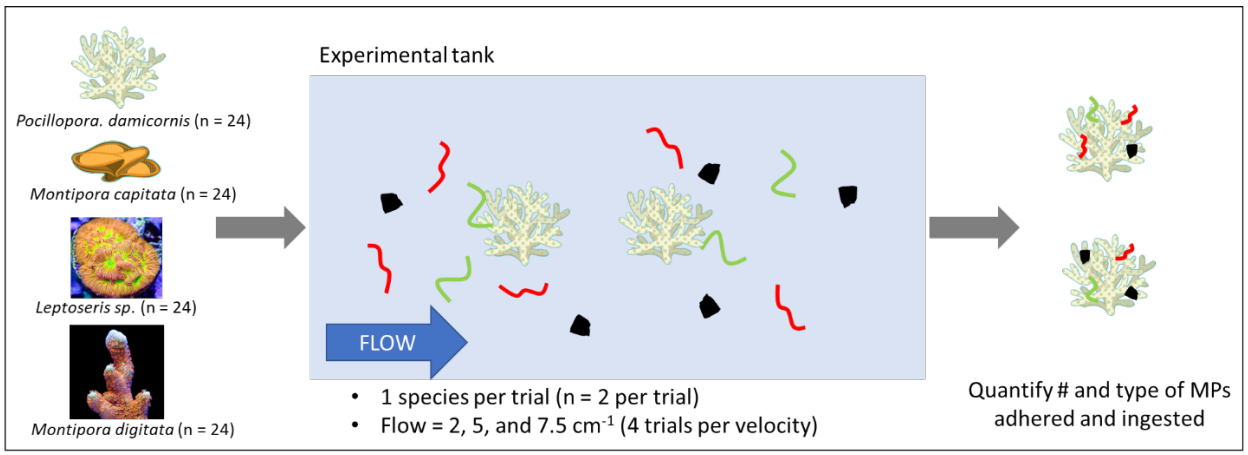


Figure 3: Experimental design for chapter 4, Microplastics removal from seawater by reef building corals under different water velocities. The left side of the figure shows the four coral species. The middle of the figure shows the orientation of the coral fragments on the experimental stage under the microplastics treatment. The right side of the figure shows microplastics adhered to the surface of the coral fragments that will be quantified under a microscope. Ingestion will be quantified by digesting coral tissue followed by vacuum filtration and counting particles under a microscope dissection under a microscope.

Methods

Coral species

Fragments of *Montipora capitata*, *Pocillopora damicornis*, *Montipora digitata* and *Leptoseris sp*. will represent four distinct coral morphologies in this study. *M. capitata* and *P. damicornis* were collected from the wild in Hawai‘i in summer 2018 and maintained in our aquatic facility until the present. Both species have small polyps, ca. 0.8 -1 mm in diameter. *M. capitata* is morphologically plastic and grows in simple branching and plate-like morphologies or it can display both morphologies in the same colony. Specimens of *M. capitata* used in this study display a mix of these morphologies. *P. damicornis* grows in a complex branching morphology. My previous research shows that both of these species ingest microplastics experimentally, however, at vastly different rates given the same concentration of microplastics (see figure 2 in Axworthy and Padilla-Gamiño, 2019). Additionally, we showed that *P. damicornis* only ingests microplastics when prey is also available. Fragments of *M. digitata* and *Leptoseris sp*. were purchased from a local aquarist. *M. digitata* has small polyps, ca. 1 mm in diameter and grows in a simple branching morphology. *Leptoseris sp*. has larger polyps (ca. 5 mm in diameter) than the other species used in this study and displays an encrusting morphology. To my knowledge, no studies have examined microplastics interactions with either *M. digitata* or *Leptoseris sp*.

Microplastics treatments

Microplastics to be used in the experimental treatments were obtained through a collaboration with the Sofield lab at Western Washington University (WWU). Three types of weathered microplastics will be used in this study. Red acrylic fibers, green polyester fibers and black polystyrene fragments were weathered in Bellingham Bay as part of a toxicology study by the Sofield lab at WWU. Briefly, microplastics were generated from textiles (fibers) or plastic forks (fragments) in a blender with Nanopure™ water (Johnson 2021). The resulting particles were sieved resulting in a range of particle sizes from 63 – 1000 µm. Microplastic fibers and fragments were deployed in Bellingham Bay, 0.3 – 0.6 m above the bottom sediment for 69 days in Autumn 2018. Upon collection from the field, all particle types were dried and stored at -20º C.

For the proposed experiment, the microplastic treatment will be a concoction of all particle types (herein referred to as “microplastics” for simplicity) described in the previous paragraph. Because our coral inventory (n = 24 of each species) is not large enough to run experiments with each plastics type individually, a concoction of all microplastic types will be used for each experimental trial. Equal parts of each type of microplastics will be added to a solution of filtered seawater and coral food (Benereef, Benepets) prepared on the day of each trial. The total concentration of microplastics treatments for the experimental trials will be 1.5 microplastics ml^{-1}. This concentration is higher than what is commonly observed on coral reefs, but it will allow for a robust statistical comparison of adhesion and ingestion rates of each plastic type.

Experimental system and trials

Microplastics exposure trials will be performed in a custom experimental race-track style fiber reinforced plastic tank (Fig 4). The ovular tank has an outer diameter of 0.91 m x 0.61 m and an inner diameter of 0.61 m x 0.30 m. The total volume of the tank is ~95 L. It is equipped with a variable speed pump (Nero 3, Aqua Illumination) and a 20 cm x 20 cm clear polycarbonate viewing window positioned in front of the stage where the corals will be placed for the trials. During the experimental trials, a high-speed video camera will be used to observe coral and microplastic interactions as well as to track particle movements to determine hydrodynamic processes that affect the interactions. Particle tracking will also be used to determine water velocity before and during the experimental trials. Three water velocities will be tested, 2, 5 and 7.5 cm^{-1}, encompassing a range of velocities observed on coral reefs. The tank will be filled with UV sterilized seawater and heated with a submersible 250 W aquarium heater. It will be fitted with a laminar flow apparatus to reduce the water flow to our target velocities. Aeration will be used to maintain suspension of the microplastics particles.

Experimental trials will occur after sunset (adjusted to 1500 in the lab) when corals typically display more active feeding. Two fragments of a coral species will be arranged on the stage at 1000 to allow them to acclimate to the experimental setting. At 1500, corals will be given a concoction of weathered microplastics and food (described above) and the exposure will last for 2 h. Based on preliminary trials and previous research (Axworthy and Padilla-Gamiño 2019) two hours will be sufficient to measure both adhesion and ingestion of microplastics. At the end of the trial, corals will be immediately removed from the system, photographed and each type of microplastics adhered to the coral surface will be quantified under a microscope, aided by UV light. The coral fragments will then be stored at -20º C until ingestion is quantified.

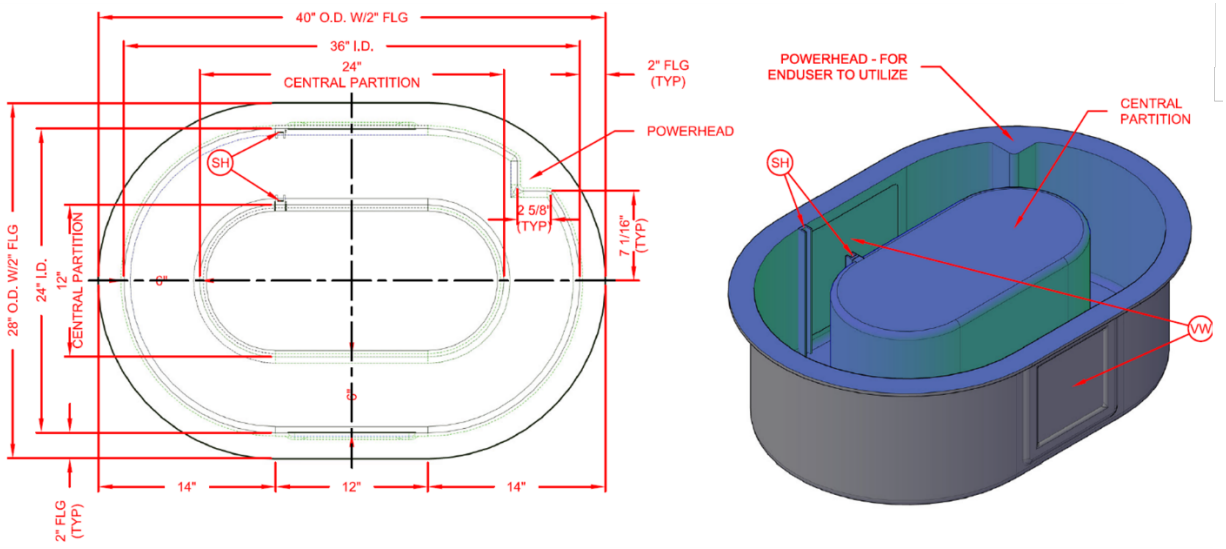


Figure 4: Experimental RFP tank design with dimensions (in imperial units). VW = viewing window; SH = screen holder. The laminar flow apparatus will be installed in the SH.

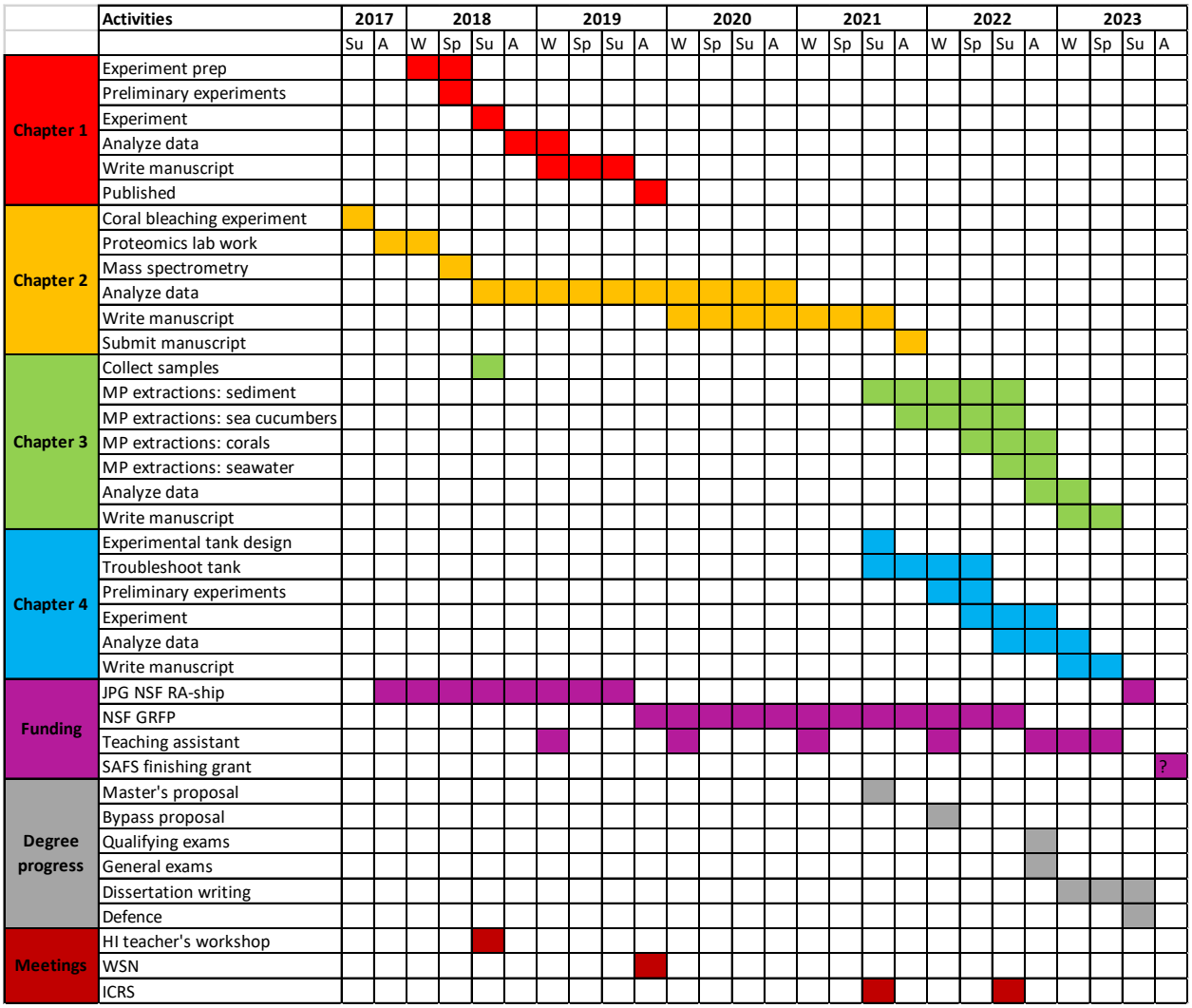
Analysis

Microplastics adhesion and ingestion rates will be quantified visually and via digestion and filtration, respectively. Immediately following the experimental trials, the number of each type of microplastics adhered to the coral surface will be counted using a stereo microscope (10 – 45X magnification) an UV light. Microplastics adhered to the up- and down- stream sides of the coral fragments will be counted separately which will allow me to compare microplastics and coral interactions with regards to hydrodynamic processes observed using particle tracking data. Microplastics ingested by corals will be quantified by digesting the coral tissue in 20% potassium hydroxide solution at 50º C for 72 h followed by vacuum filtration onto glass fiber filters. Microplastics on the filters will be counted under a microscope with the aid of UV light. The remaining coral skeleton will be scanned using a 3D scanner (as described in chapter 3) to determine the surface area. Microplastics adhesion and ingestion rates will be reported as the number of each microplastics type per polyp and per cm^2 of coral surface. A three-way analysis of variance will be used to determine the effects of microplastics type, coral species, and water velocity on microplastics adhesion and ingestion rates for coral.

Significance

This study will help determine the risk of microplastics exposure to coral reefs and help guide management strategies in conserving these important ecosystems. Determining what types of microplastics are more likely to interact with corals (via adhesion or ingestion) will provide information about which microplastics mitigation strategies to prioritize. Knowing how a coral's morphology influences microplastics and coral interactions can tell us which coral species are at more risk of microplastics exposure. Finally, learning how water velocity affects coral and microplastics interactions can tell us about which environments or reef zones stand to be more impacted by microplastics pollution.

Project Timeline



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