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Chronological Accumulation of Microplastics in the Gulf of Mexico and Their Acute Effects on Coral Bleaching

by

Martina M. Plafcan

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Marine Science
with a concentration in Biological Oceanography
College of Marine Science
University of South Florida

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Isabel C. Romero, Ph.D.
Nicole D. Fogarty, Ph.D.

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Keywords: Ocean warming, Plastic pollution, Time trend, Zooxanthellae density, Marine debris

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Chapter 2 was made possible through the involvement of Mote Marine Laboratory and their collection of the corals used in this project. I thank Sharla Sugierski for her input in the study design for this work and Ian Maier for his help transporting the corals and assisting throughout the experiments.

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Abstract

Microplastics have accumulated in the environment since plastic production began. They have spread to all areas of the globe from marine trenches to mountains and they can be harmful to organisms. However, research on microplastics has only recently begun so it is unclear how they have changed over time in many regions of the ocean and how current oceanic concentrations might affect marine life. Additionally, organisms such as corals are under stress and have been declining due to climate change, so it is not yet known if microplastics exacerbate these threats. My thesis addressed these gaps in the literature by assessing the temporal and spatial dynamics of microplastics in an understudied basin as well as how current oceanic concentrations, in combination with thermal stress, affect the bleaching response of an important coral species. To assess how microplastics have changed over time and space in the Gulf of Mexico, I used agglutinated foraminifera from sediment cores that incorporated the pollutant into their tests. I found that microplastics were higher after plastic production began, which was an expected temporal trend. I also found a spatial pattern where microplastic concentrations were higher at deeper sites as well as those close to the Mississippi River. Then, I assessed how microplastics interacted with thermal stress in corals using a controlled laboratory experiment. I exposed fragments of the threatened Caribbean coral, *Acropora cervicornis*, to orthogonally crossed treatment levels of microplastic beads (0 and 11.8 particles L^{-1}) and water temperatures (ambient at 28°C and elevated at 32°C), then quantified zooxanthellae densities to measure the bleaching response. Regardless of microplastic treatment level, corals in the elevated temperature treatment were visibly bleached and necrotic (i.e., significant negative effect on zooxanthellae density) while those exposed to ambient temperatures remained healthy. However, there was not a significant microplastic effect at either individual (ambient temperature) or combined levels (elevated temperature). My thesis adds to the emerging literature on microplastics in the Gulf of Mexico and their effects on corals. Importantly, my study was the first to assess their temporal and spatial dynamics in deeper-water sediments in the GOM. Also, I used environmentally relevant microplastic concentrations to understand how current conditions affect corals. Additional work is needed to more fully identify the distribution of microplastics across the Gulf of Mexico to better understand how sensitive ecosystems are affected by this pollutant.

Introduction

Plastic pollution has been observed globally and its negative effects on the ocean are well known. Plastic debris can act as vectors to transport species to new locations which facilitates the spread of invasive species (Kühn et al. 2015). They can also adsorb chemicals and carry them throughout the ocean (Rochman 2015). Plastic ingestion or entanglement can lead to mortality of megafauna, such as birds and sea turtles (Sigler 2014, Kühn & van Franeker 2020). Recently, microplastics have become a major focal area in research on plastic pollution (Moore 2008). The small size (less than 5 mm) of the particles makes them easily ingestible by a wide variety of organisms across taxonomic levels. They are ubiquitous in the ocean, with an average of 11.8 microplastics L^{-1}, and have increased in concentration over time (Barrows et al. 2018). Further, they can be harmful to marine life because they can alter feeding behaviors, reproduction, and energy metabolism (Anbumani & Kakkar 2018). Microplastics enter the ocean directly, through discard from ships and coastal areas, as well as the breakdown of macroplastic pollution (Bondelind et al. 2020, Waldschlager et al. 2020). They can also enter the ocean indirectly through rivers, stormwater runoff, and wastewater treatment systems (Bondelind et al. 2020, Waldschlager et al. 2020). Their concentration at a particular location, therefore, depends on ocean hydrology, proximity to anthropogenic activities, and effectiveness of nearby wastewater management systems (Arthur et al. 2008). However, microplastics have accumulated in the environment since plastic first started to be produced yet their temporal dynamics remain unclear. Additionally, research on the effects of microplastic pollution on organisms is still in its infancy which leaves this topic relatively unknown. My thesis will address these important gaps in the literature to help us understand the temporal dynamics of microplastics and their effects on organisms.

Both macro- and microplastic pollution have increased due to the continuous increase in plastic production. Indeed, global plastic production increased from 1.5 to 230 million tons from 1950 to 2009 (PlasticsEurope 2010), with coinciding intensification of pollution. For example, 19 to 23 million tons of plastic entered the ocean in 2016 alone (Borrelle et al. 2020). Microplastics were first observed in zooplankton trawl samples in the early 1970s (Carpenter et al. 1972, Carpenter & Smith 1972) and their concentrations in the ocean have increased since the 1950s (Yao et al. 2019). Courtene-Jones et al. (2020) found decreased microplastics with increased age in sediment cores from Rockall Trough in the North Atlantic Ocean and Lin et al. (2021) found a similar trend in the East China Sea. Lately, efforts to determine historical concentrations of microplastics and understand their temporal dynamics have used sediment cores (Matsuguma et al. 2017, Dahl et al. 2021, Uddin et al. 2021). However, temporal dynamics of microplastics still remain unclear, as many studies do not employ the use of either reliable dating methods (e.g., ^{210}Pb) or comparisons to known markers (e.g., when plastic production began; Yao et al. 2019, Uddin et al. 2021). A recent study found microplastics can be incorporated into the tests of agglutinated benthic foraminifera (Birarda et al. 2021) which could be used to analyze this pollution in the ocean. However, no studies have identified temporal trends of microplastics in the Gulf of Mexico (GOM). This basin remains understudied for microplastics despite receiving freshwater discharge from the Mississippi River which is known to concentrate excess nutrients and pollutants (Rabotyagov et al. 2020, Scircle 2020).

Evidence has increased to suggest microplastics are harmful to marine life. Organisms can encounter microplastics passively through adhesion and actively through ingestion.

Adhesion occurs when microplastics stick to the surface of an organism which can have negative effects (Reichert et al. 2018). For example, microplastic adhesion decreased photosynthetic efficiency in marine algae, *Platymonas helgolandica* (Chen et al. 2020). Additionally, microplastic ingestion can cause harm such as through the release of toxic substances into organisms that were adsorbed onto the plastic (Verla et al. 2019), bioaccumulation (Maes et al. 2020), and introduction of infectious diseases (Fackelmann & Sommer 2019, Rotjan et al. 2019, Schlundt et al. 2020). Indeed, fish experienced changes in feeding patterns, damage to intestines, and alterations to their energy metabolism due to microplastic ingestion (Anbumani & Kakkar 2018). Coral reefs are ecologically and economically important (Brander et al. 2007, Woodhead et al. 2019), but microplastics can have adverse effects on them that could exacerbate current threats. General health effects such as decreased growth, bleaching, necrosis, and parasite infections have all been reported in response to microplastic exposure in corals (Reichert et al. 2018, Reichert et al. 2019, Syakti et al. 2019). However, studies that assessed the toxicological effects of microplastics on organisms commonly used unrealistically high concentrations. When experimental dosages were compared to environmental levels, only 17% of studies used environmentally relevant microplastic concentrations (Bucci et al. 2020). The use of unrealistically high concentrations can produce misleading results that are not applicable to current environmental conditions. Thus, it is still not clear how environmentally relevant concentrations affect the responses of corals to microplastics when combined with other stressors.

My thesis has addressed the gaps in the literature on the temporal dynamics of microplastics in the GOM and the combined effects of microplastics and additional stressors in corals. For my thesis, I investigated temporal dynamics of microplastics in the northern GOM to answer the study questions: (1) *Have microplastics in the GOM increased since plastic production began in the 1950s?* (2) *Do microplastics decline with distance from the Mississippi River?* (3) *How do microplastics vary with water depth?* I also assessed the potential interactive effects of microplastics and ocean warming on the bleaching response in a vulnerable coral species with ecologically relevant microplastic concentrations. Specifically, I answered the study question: *Does microplastic exposure interact with elevated water temperatures to exacerbate coral bleaching?*

Chapter 1: Temporal dynamics of microplastics in the Gulf of Mexico

Introduction

Microplastics are pollutants that have been observed in every global environment including the atmosphere, mountains, and marine trenches (Rios Mendoza et al. 2021). Their abundance has increased exponentially since the 1950s (Thompson et al. 2004, Uddin et al. 2021). Microplastics within sediments can be used to determine temporal dynamics of accumulation, but prior research has focused largely on surface layers and shallow environments. In comparison, shelf to slope depth sediments have been relatively understudied (Uddin et al. 2021). Additionally, microplastics can exhibit high spatial variability so it is essential to expand our studies across locations to understand the occurrence and distribution of this pollutant (Yao et al. 2019, Uddin et al. 2021). To address this gap, I assessed the temporal dynamics of microplastics from oceanic sediments in an understudied marine basin.

The primary method historically used to determine the temporal dynamics of plastics has been through analysis of specimen samples. For example, plankton samples revealed that macro- and microplastics in the North Atlantic Ocean and the North Sea have increased since the late 1950s, a trend which tracked the exponential increase of plastic production (Thompson et al. 2004, Ostle et al. 2019). More recently, researchers have used oceanic sediments to assess historical records of microplastics because they can act as sinks for various pollutants (Louvado et al. 2015, Rabotyagov et al. 2020, Scircle 2020). These sediments allow us to reconstruct microplastic presence and abundance over long timescales and have revealed increased concentrations through time (Yao et al. 2019). Indeed, microplastics in sediments have increased threefold in 16 years on Belgian beaches (Claessens et al. 2011) and from 1100 to 7000 microplastics kg^{-1} of dry sediment over 50 years in Japanese waterways (Matsuguma et al. 2017). Another way to assess the temporal dynamics of microplastics is to use agglutinated benthic foraminifera. These marine protists are found in sediments and glue the material around them to form their tests (Benito 2020). These organisms are often used to determine past environmental conditions (Edwards & Horton 2000), including the accumulation of microplastics over time (Birarda et al. 2021). Microplastic incorporation into foraminiferal tests occurs only when the organisms are in the sediment since they are frozen after collection which can reduce secondary contamination. Agglutinated foraminifera are found globally and can therefore be used to examine temporal changes in microplastics in previously unstudied locations.

The Gulf of Mexico (GOM) is a large marine basin affected by anthropogenic activities and receives freshwater discharge from the largest watershed in the United States, yet it remains understudied for microplastics. The GOM receives 60% of its river discharge from the Mississippi River which covers more than 16% of North America (Liu et al. 2013). This river system concentrates pollutants such as excess nutrients (Rabotyagov et al. 2020) and microplastics (Scircle 2020) and deposits them into the GOM. Sediments from shallow environments (i.e., beaches and estuaries) across this basin contained highly variable quantities of microplastics that ranged from 0 to 150 microplastics m^{-2} and 0 to 1940 microplastics kg^{-1} (Wessel et al. 2016, Beckwith & Fuentes 2018, Yu et al. 2018, Alvarez-Zeferino et al. 2020, Sanchez-Hernandez et al. 2021, Weitzel et al. 2021). Such high spatial variation was attributed to differences in local urbanization (Yu et al. 2018, Tunnell et al. 2020, Sanchez-Hernandez et al. 2021) and the physical characteristics of each site (i.e., wind, tides, currents; Wessel et al. 2016, Alvarez-Zeferino et al. 2020, Sanchez-Hernandez et al. 2021). Importantly, microplastics can be transported from shallow to deep water environments in bottom currents and can lead to accumulation in deeper-water sediments (Peng et al. 2018, Kane et al. 2020). However, these environments in the GOM are understudied which leaves the temporal dynamics of microplastics within this basin unknown. In response to this gap, I addressed the questions: (1) *Have microplastics in the GOM increased since plastic production began in the 1950s?* (2) *Do microplastics decline with distance from the Mississippi River?* (3) *How do microplastics vary with water depth?*

Methods

A team of researchers from the C-IMAGE Consortium collected sediment cores from the northern GOM aboard the R/V Weatherbird II from 2011 to 2013. They collected cores with an Ocean Instruments MC-800 multicorer (8 cores, 10 cm diameter, up to 70 cm in length) and then froze them (-20°C) until analysis in the laboratory. I used cores from six different locations from the studied region: three sites west of the Mississippi River discharge (MS4, MS5, MS6; referred to as Mississippi River cores) and three to the east on the West Florida Shelf (WFS1, WFS2, WFS3; referred to as West Florida Shelf cores; Figure 1.1). I chose these sites to examine microplastics across water depths and regions because they have been shown to be spatially variable (Uddin et al. 2021). Once in the laboratory, the samples were collected from each core with a calibrated, threaded-rod extrusion device at 2 and 5 mm intervals (Schwing et al. 2016). I chose samples that corresponded with time periods that dated before (WFS1, WFS2, WFS3, MS5, MS4: $n = 3$, MS6: $n = 1$) and after (WFS1: $n = 4$, WFS2: $n = 3$, WFS3: $n = 3$, MS5: $n = 4$, MS4: $n = 7$, MS6: $n = 8$) plastic production began (the 1950s). However, the samples were not from the same depths within the cores due to differences in sedimentation rates at each site. I washed the samples with a sodium hexametaphosphate solution to remove sediment and terrestrial particles from foraminiferal tests. Then I dried and stored the foraminifera at room temperature.

I stained the foraminifera with Nile Red to identify microplastics contained within the tests (Shim et al. 2016, Maes et al. 2017). I immersed the samples in Nile Red (5 mg L^{-1} acetone) for 30 minutes, then poured them over a glass fiber filter and dried them in an oven at 60°C for 12 hours (Shim et al. 2016, Maes et al. 2017). Next, I weighed the foraminifera and sorted them through a series of sieves into three size classes (63-<150 μm, 150-<300 μm, 300-5000 μm) (Schwing et al. 2018). I used a stereomicroscope (Amscope SF-2TRA, 10-30x magnification) with cyan (490 – 515 nm wavelength) LED lights, and an orange filter to count the stained microplastics within the agglutinated foraminifera tests. I only considered agglutinated foraminifera because they cement particles, including microplastics, together to form their tests (Bender & Hemleben 1988). In contrast, calcareous foraminifera precipitate their tests and cannot incorporate other materials as part of them (Erez 2003). I examined the entire sample, when possible, but if it was too large, I analyzed and weighed a portion of it. When I found foraminifera that contained microplastics, I separated them from the rest of the sample to be identified and photographed. I also counted fragments of foraminifera because some species, such as *Saccorhiza ramosa* and *Archimerismus subnodosus*, can be highly fragmentable. I determined the number of foraminifera that contained microplastics in each sample as well as the number of microplastics within each test. I then standardized the microplastic abundance data to the weight of the foraminifera in the sample analyzed (g). All microplastics I observed and counted were completely incorporated into the test. I identified the foraminifera to the genus or species level. Then, I took photographs of the foraminifera that contained microplastics with a

Canon EOS 2000D camera connected to a stereomicroscope (Nikon SMZ800N with Nikon LV-TV adapter) (Figure 1.2).

I determined the dates of each core with excess ^{210}Pb radioisotopes on Series HPGe (high-purity Germanium) Coaxial Planer Photon Detectors (Brooks et al. 2015, Schwing et al. 2017, Larson et al. 2018). I measured total ^{210}Pb (46.5 Kev), ^{214}Pb (295 Kev and 351 Kev), and ^{214}Bi (609 Kev) activities in disintegrations per minute per gram (dpm g^{-1}) (Brooks et al. 2015). I determined the supported ^{210}Pb *in situ*, with the averaged activity of ^{214}Pb (295 Kev and 351 Kev) and ^{214}Bi (609 Kev) as a proxy for ^{226}Ra (Brooks et al. 2015, Schwing et al. 2017). Then, I subtracted the supported ^{210}Pb from the total ^{210}Pb to give the excess ^{210}Pb which I used to date the last ~100 years of the core (Table 1.1; Brooks et al. 2015, Schwing et al. 2017). I used the Constant Initial Concentration (CIC) and Constant Rate of Supply (CRS) models based on the sediment accumulation rates for each core to assign ages to the layers (Appleby & Oldfieldz 1983, Binford 1990, Brooks et al. 2015).

I described the qualities of each site relative to anthropogenic activity. I used GIS to determine the distance of each site to the nearest coast, river, port, and tourist beach since they have been shown to be sources of microplastics to the ocean (Scircle 2020, Masia et al. 2021). WFS1 was the closest of the West Florida Shelf cores to the coast (115.8 km), nearest river (134.7 km), port (147.5 km), and beach (127.6 km), and WFS3 was the farthest (198 km, 220.3 km, 219.4 km, 213.2 km, respectively; Table 1.2). MS5 was the closest to the coast (54.1 km) and nearest river (91.9 km) of the Mississippi River cores, and MS6 was the farthest (91.1 km and 154.2 km, respectively; Table 1.2). Additionally, MS5 was the closest to the nearest port (77.7 km) and beach (81.3 km) and MS4 was the farthest of the Mississippi River cores (141.9 km and 137.6 km, respectively; Table 1.2). Importantly, all the Mississippi River cores were closer to the coast than the West Florida Shelf cores (Table 1.2). I reported diversity indices (Fisher's alpha, Equitability J, and Shannon's diversity index) that were previously calculated for WFS3, MS4, MS5, and MS6 to provide insight into the potential effects of microplastics on foraminifera communities (Romero et al. 2016, Schwing et al. 2018). All indices were higher with increasing distance from the nearest coast and river for the Mississippi River cores (Table 1.2). All indices for WFS3 were low compared to MS4 and MS6 (Table 1.2). I also received data for total foraminiferal species abundance within the surface layers of WFS3, MS4, MS5, and MS6 (Romero et al. 2016, Schwing et al. 2018). I did not analyze the foraminifera for microplastics for this dataset, so it only contained information on the total number of species and individuals. I determined the proportion of total foraminifera present of each species that contained microplastics within each depth of the cores. Although other species were found in the cores, I focused only on those that contained microplastics (i.e., total proportion). Then I calculated the proportion of the foraminifera species that contained microplastics (i.e., proportion with microplastics) to compare to the total proportions present. Data were not available for WFS1 and WFS2.

To examine the effects of water depth (fixed effect), time (fixed effect; pre-plastic production = 0; ≤ 1950, post-plastic production = 1; >1950), and region (fixed effect; Mississippi = MS, West Florida Shelf = FL) on microplastic abundance (response), I performed a generalized linear mixed model (GLMM) with core as a random effect to account for multiple measurements. I performed all analyses in R (R Development Core Team 2021), with package glmmTMB (Brooks et al. 2017) for the GLMM and the DHARMa package (Hartig & Hartig 2021) for residual diagnostics. I used Akaike information criterion (AIC) to determine the best model then tested for diagnostics to ensure the appropriateness of it. I determined the microplastic response data were zero-inflated so I assessed models that can handle large amounts of zeros (Zuur et al. 2009). The best was a zero-inflated negative binomial model with region and water depth as effects in the conditional model and time as an effect in the zero-inflation model (AIC = 288.3). I removed time as an effect in the conditional model after I determined its contribution was not significant ($p > 0.05$).

Results

I identified a total of 92 microplastics (Table 1.3) from 53 foraminifera tests (Table 1.4). Microplastics g^{-1} of sample were higher post-plastic production (the 1950s) throughout all cores (Figure 1.3). However, microplastics were found pre-plastic production in one core (WFS1), but these represented a very low proportion of the total microplastics observed across all cores (0.03). MS4 made up the largest proportion of microplastics g^{-1} of sample post-plastic production, followed by MS6, WFS3, WFS2, WFS1, then MS5 (Figure 1.4). Of the microplastics found, 78% were in foraminifera in the size range 300-5000 μm and 20% were in the 150-<300 μm size (Figure 1.5). Twelve foraminifera species and two fragments of tests that could not be identified contained microplastics. The most common species found within the cores that contained microplastics were *Archimerismus subnodosus* (proportion with microplastics = 0.28, total proportion = 0.41) and *Saccorhiza ramosa* (proportion with microplastics = 0.26, total proportion = 0.42; Figure 1.6).

The probability of obtaining a zero post-plastic production was lower than pre-plastic production ($z = -3.04$, $p < 0.01$, Table 1.5), which indicated there were more microplastics found after the mass production of plastic. Additionally, microplastics were greater in the Mississippi River cores ($z = 4.04$, $p < 0.01$, Figure 1.7) and increased with water depth ($z = 5.89$, $p < 0.01$, Figure 1.8).

Discussion

Using agglutinated foraminifera in sediment cores from the northern GOM, I found that microplastics increased following the mass production of plastic circa 1950. This study was the first to assess the temporal dynamics of microplastics in this basin. Microplastics also exhibited spatial variability due to differences between the regions studied and they were higher in deeper water depths. Although they are known to vary spatially in shallow environments in the GOM, microplastics have been previously unstudied in this basin's deeper-water sediments. My study was the first to report on microplastic distributions in shelf to slope depth sediments in the GOM.

Microplastics were higher post-plastic production in sediment cores collected from the northern GOM. This was expected because microplastic pollution has been shown to increase over time, similar to plastic production (Hale et al. 2020). Indeed, this has been well documented globally from coastal to offshore sediments (Claessens et al. 2011, Matsuguma et al. 2017, Yao et al. 2019, Courtene-Jones et al. 2020, Uddin et al. 2021). However, Matsuguma et al. (2017) found a core in Tokyo Bay where microplastics did not exhibit a temporal trend because of rapid sedimentation or sediment disturbance. Likewise, one core in my study exhibited a different trend than the others because the amount of microplastics found before and after plastic production were similar. In this core, the microplastics found at a depth that corresponded to time before plastic production began was likely due to foraminifera from shallow core depths being mixed deeper during extrusion, but this was not enough to affect its geochronology. Martin et al. (2022) reviewed literature on the temporal dynamics of microplastics in sediments and reported that several studies found the pollutant before plastic production began. This was attributed to either reworked sediments (e.g., bioturbation, pore water transport) or procedural contamination (Martin et al. 2022). Although it is important to minimize contamination, it can be difficult due to the small size of the microplastics and foraminifera. The contamination from the mixing of foraminifera from different sediment layers only occurred in one core but has revealed the need to take more precautions during the extrusion process.

The differences between the two focal regions highlight the spatial variability microplastics can exhibit. The GOM receives a large amount of riverine input (1100 km^3 yr^{-1}), of which roughly 655 km^3 yr^{-1} originates from the Mississippi River alone (Liu et al. 2013). Scircle (2020) estimated there were 87 to 129 trillion microplastics per day near the mouth of the Mississippi River. Although I only analyzed sediment, the Mississippi River is a large source of microplastics to the GOM and led to a greater amount of the pollutant to accumulate within the nearby Mississippi River cores. This distribution pattern has been reported in other systems. For example, Falahudin et al. (2020) also found microplastics in Indonesian bays were inversely proportional to distance from the mouth of a river. Additionally, the West Florida Shelf cores were located further from the coast which may have contributed to the differences between the studied regions. Indeed, several studies have reported microplastics decrease with increased distance from the coast and attributed this to being further from pollution sources (Graca et al. 2017, Zhang et al. 2019a, Zhang et al. 2019b, D'Hont et al. 2021). My study supports previous work that has found sites nearer to sources of pollution (e.g., rivers, coastlines) have greater abundances of microplastics (Yu et al. 2018, Tunnell et al. 2020, Sanchez-Hernandez et al. 2021).

The model found that microplastics were both higher and more variable in deeper waters in the GOM. Microplastics may be higher in deeper waters because of oceanographic processes and features such as bottom currents, erosion, or grain size (Kane et al. 2020, Lechthaler et al. 2021, Sun et al. 2021). Importantly, these results contrast with other research that found microplastics decrease with water depth due to increased distance from the coast and thus pollution sources (D'Hont et al. 2021, Manbohi et al. 2021, Uddin et al. 2021). However, I found that microplastics were lower at sites that were further from the coast, but they were higher in deeper waters. This indicates another process, such as bottom currents, in the GOM transported microplastics to these deeper-water depths (Hamilton & Lugo-Fernandez 2001). However, due to the high uncertainty associated with my results, it is still unclear how microplastics vary with water depth in the GOM. Additional research is needed to better understand microplastic distribution in sediments and how oceanographic processes affect it.

Little is known about microplastic incorporation into foraminifera tests; however, my study contributes new information on this topic. Based on my results, larger foraminifera are found to integrate microplastics into their tests, but they do not selectively incorporate them. Microplastics were mostly found in the larger foraminifera, specifically the 300–5000 μm and 150-<300 μm sizes. This could be because the larger the foraminifera, the more likely it is to encounter microplastics to incorporate due to a greater surface area, whereas the smaller individuals would encounter less of the pollutant. However, this hypothesis has not been fully studied, to my knowledge. Additionally, the most common species, *Saccorhiza ramosa* and *Archimerismus subnodosus,* that were found to contain microplastics were also common within the cores. This suggests foraminifera may not be seeking out microplastics to incorporate into their tests over other materials. Although, more research is needed to determine whether some species preferentially incorporate the pollutant into their tests and how this could affect them.

The diversity indices lend some information about the condition of the benthic foraminiferal communities and their response to anthropogenic activity. There were higher values for the diversity indices for the Mississippi River cores with increased distance from the coast and nearest river. This indicates foraminiferal communities closer to the coast and river were less diverse and may have been affected by their proximity to anthropogenic activities and plastic pollution inputs. Indeed, one study found that microplastics can leech chemicals into foraminifera, weaken their tests, and induce oxidative stress (Birarda et al. 2021). However, WFS3 was the farthest site from the coast, and it had low values for the diversity indices compared to the Mississippi River cores. The foraminiferal community at this site could have been affected by other factors that were outside the scope of this study, such as oxygen concentration, grain size, and concentration of total organic carbon (Bouchet et al. 2012).

Microplastics are a global, ubiquitous pollutant and this was the first study to examine them in deeper-water sediments of the GOM, but it highlights the need for further research. My study found that microplastics were higher post-plastic production and closer to the Mississippi River. Additional locations across the GOM should be studied since microplastics exhibited spatial variability. Further work can also examine the relative contributions of other rivers that are sources of microplastics to the GOM. While uncertainty was high, I also found that microplastics were higher in deeper water depths, but more work is needed to understand mechanisms that may drive this distribution, since others have found the opposite relationship (D'Hont et al. 2021, Manbohi et al. 2021). Finally, microplastics were found in larger foraminifera but did not appear to be selectively incorporated into their tests. However, there is little research on this topic so more work is needed to assess the interactions between agglutinated foraminifera and microplastics.

Appendix A: Tables and Figures for Chapter 1[1.1]

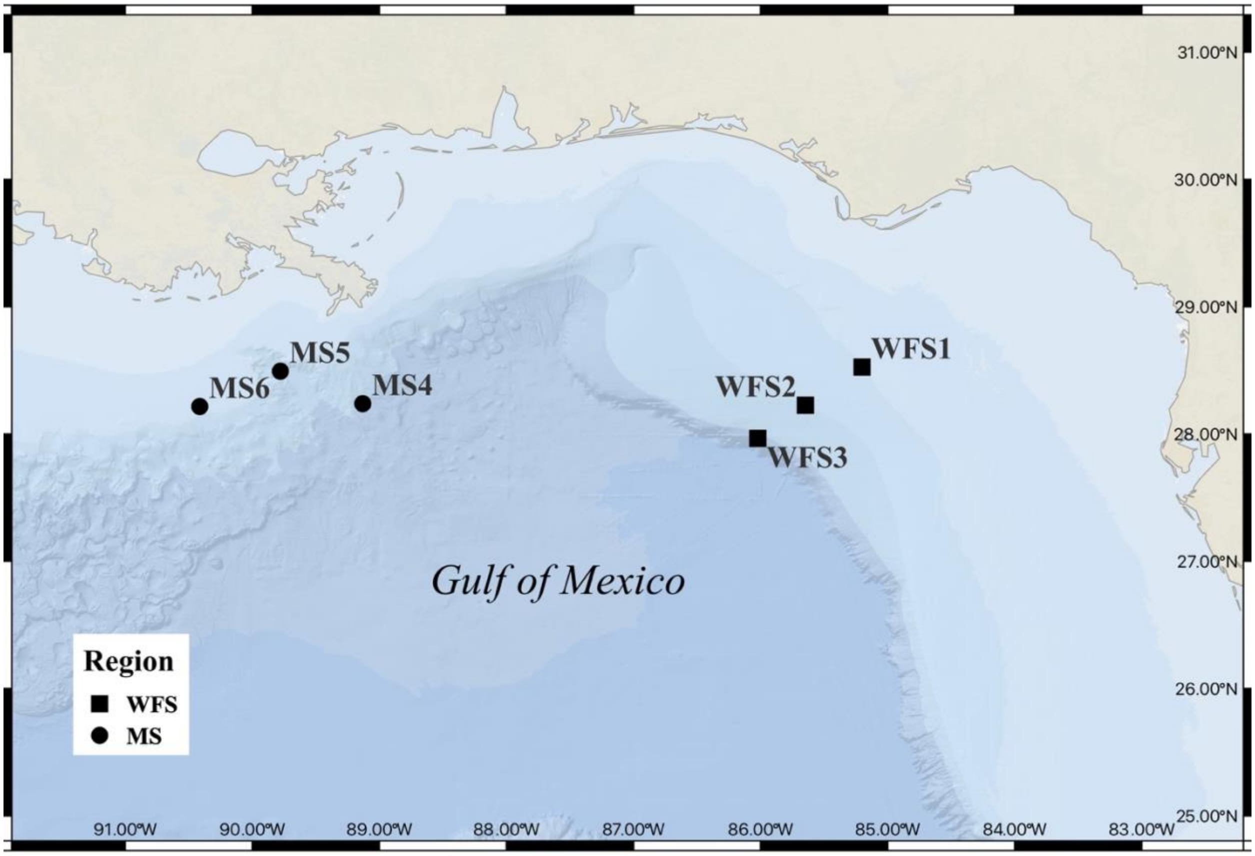

Figure 1.1. Map of locations where sediment cores were sampled. Sediment cores are indicated by the squares and circles. Circles are the Mississippi River cores and squares are the West Florida Shelf cores.

[1.1] The tables and figures appear as they were ordered in the chapter.

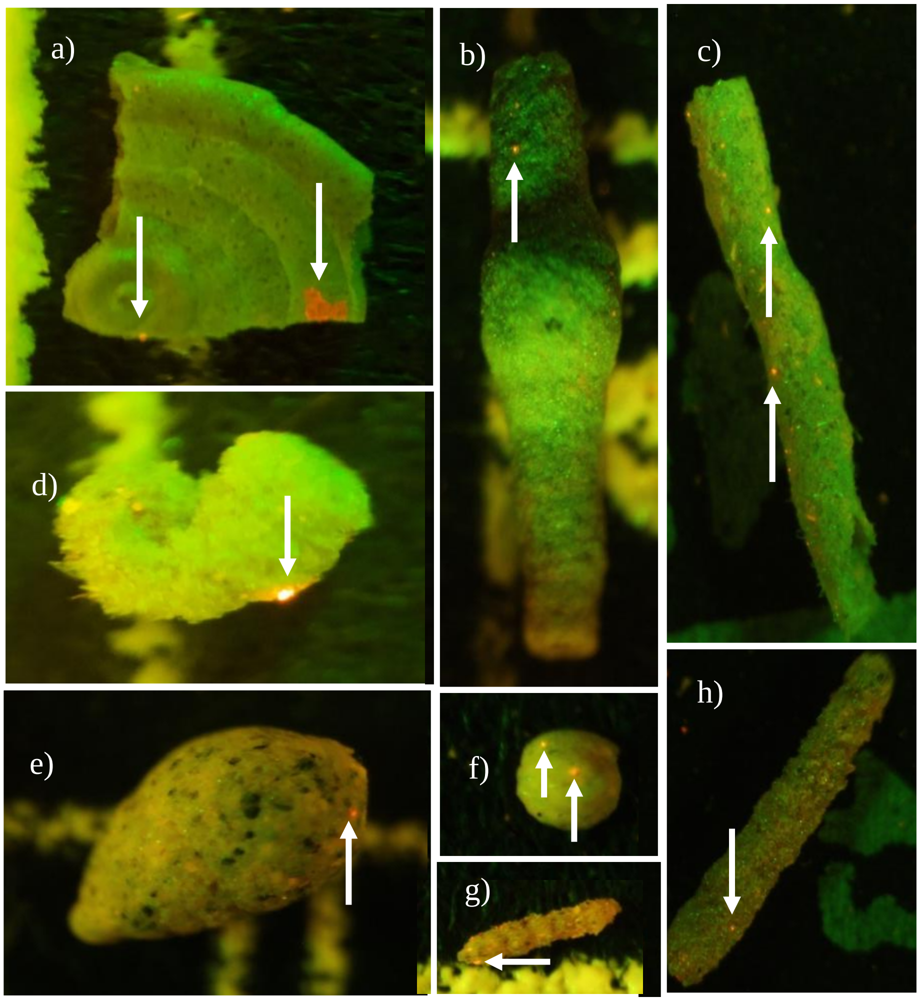

Figure 1.2. Images of fluorescing microplastics incorporated into foraminifera: a) *Ammodiscus tenuis*, b) *Archimerismus subnodosus*, c) *Saccorhiza ramosa*, d) *Ammobaculites* spp., e) *Hippocrepina* spp., f) *Trochammina squamata*, g) *Bigenerina nodosaria*, and h) *Hyperammina friabilis*.

Table 1.1. Year and uncertainty assigned to each depth interval within each core.

Core	Depth interval (mm)	Year	Uncertainty
MS4	0-2	2012	1.47
	2-4	2012	1.47
	4-6	2011	1.47
	6-8	2010	1.48
	8-10	2010	1.48
	100-105	1966	1.94
	105-110	1962	2.00
	130-135	1946	2.40
	190-195	<1900	NA
	195-200	<1900	NA
MS5	0-2	2013	1.28
	50-55	2008	1.28
	95-100	2003	1.30
	215-220	1965	1.86
	280-285	1943	2.48
	310-315	1933	2.76
	395-400	1907	3.53
MS6	0-2	2014	2.22
	2-4	2013	2.22
	4-6	2013	2.22
	6-8	2013	2.22
	8-10	2013	2.22
	90-95	1998	2.40
	95-100	1997	2.42
	220-225	1967	3.14
	390-400	1901	4.13
WFS3	0-2	2011	2.22
	2-4	2010	2.23
	10-12	2005	2.31
	100-105	1938	4.62
	110-115	1932	4.79
	190-195	<1900	NA
WFS2	0-2	2011	2.91
	4-6	2009	2.94
	16-18	1998	3.24
	60-65	1922	17.64
	105-110	<1900	NA
	190-195	<1900	NA
WFS1	0-2	2011	2.83
	2-4	2011	2.84
	14-16	2006	2.87
	105-110	1955	3.17
	115-120	1943	3.23
	130-135	1904	3.32
	190-195	<1900	NA

Table 1.2. Descriptions of the sites and diversity indices for each core. NA indicates data were unavailable.

Core	Water depth (m)	Distance to coast (km)	Name of nearest river	Distance to nearest river (km)	Name of nearest port	Distance to nearest port (km)	Name of nearest tourist beach	Distance to nearest tourist beach (km)	Fisher's alpha	Equitability J	Shannon
WFS1	150	115.77	Apalachicola	134.7	Port St. Joe	147.5	St. George Island	127.63	NA	NA	NA
WFS2	400	159.15	Apalachicola	179.55	Port St. Joe	181.35	St. George Island	174.4	NA	NA	NA
WFS3	1200	197.98	Apalachicola	220.26	Port St. Joe	219.41	St. Joseph Peninsula	213.2	6.50	0.54	1.81
MS4	1187	77.12	Mississippi	102.8	Port Fourchon	141.89	Grand Isle Beach	137.62	11.21	0.84	3.00
MS5	550	54.12	Mississippi	91.87	Port Fourchon	77.69	Grand Isle Beach	81.32	6.64	0.76	2.46
MS6	72	91.13	Mississippi	154.2	Port Fourchon	100.1	Grand Isle Beach	114.43	16.35	0.91	3.55

Table 1.3. The number of microplastics contained within each species from all cores.

Microplastics per species

Species	MS6	MS4	MS5	WFS3	WFS2	WFS1	Total
Ammobaculites spp.	0	1	0	0	0	0	1
Ammodiscus tenuis	0	14	0	0	0	0	14
Archimerismus subnodosus	11	6	1	8	0	0	26
Bigenerina nodosaria	3	0	0	0	0	0	3
Botellina labryinthica	0	0	0	0	0	1	1
Hippocrepina spp.	0	0	0	0	0	1	1
Hormosinella distans	0	0	0	0	1	0	1
Hyperammina friabilis	1	7	0	1	0	0	9
Lagenammina difflugiformis	3	0	0	0	0	0	3
Martinotiella occidentalis	2	0	0	0	0	0	2
Saccorhiza ramosa	0	2	0	10	15	0	27
Trochammina squamata	0	0	0	0	0	2	2
Unknown sp. A	0	0	0	0	0	1	1
Unknown sp. B	0	0	0	0	0	1	1
Total	20	30	1	19	16	6	92

Table 1.4. The number of each species that contained microplastics from each core.

Species with microplastics

Species	MS6	MS4	MS5	WFS3	WFS2	WFS1	Total
Ammobaculites spp.	0	1	0	0	0	0	1
Ammodiscus tenuis	0	5	0	0	0	0	5
Archimerismus subnodosus	7	5	1	3	0	0	16
Bigenerina nodosaria	1	0	0	0	0	0	1
Botellina labryinthica	0	0	0	0	0	1	1
Hippocrepina spp.	0	0	0	0	0	1	1
Hormosinella distans	0	0	0	0	1	0	1
Hyperammina friabilis	1	5	0	1	0	0	7
Lagenammina difflugiformis	2	0	0	0	0	0	2
Martinotiella occidentalis	1	0	0	0	0	0	1
Saccorhiza ramosa	0	2	0	7	5	0	14
Trochammina squamata	0	0	0	0	0	1	1
Unknown sp. A	0	0	0	0	0	1	1
Unknown sp. B	0	0	0	0	0	1	1
Total	12	18	1	11	6	5	53

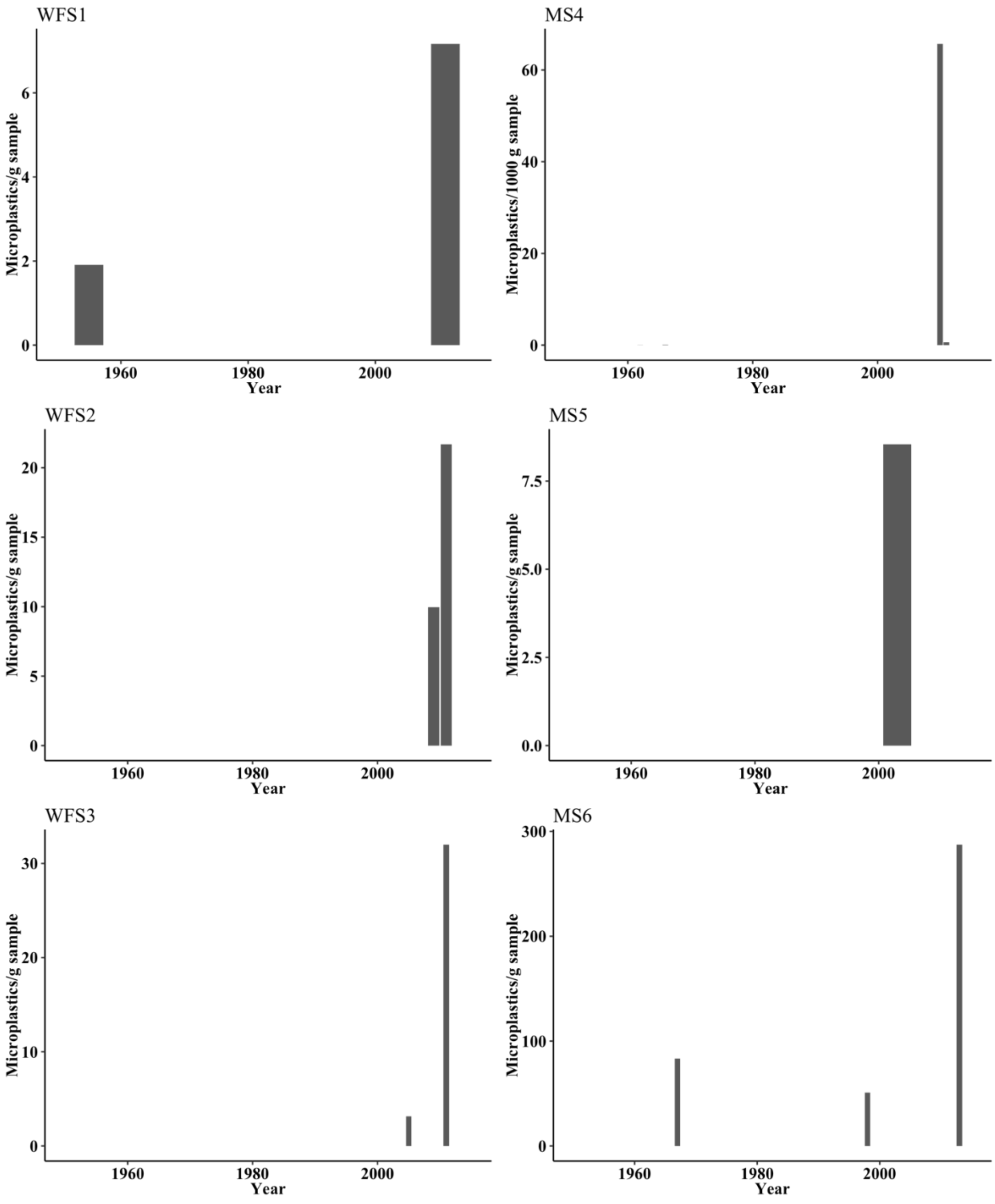

Figure 1.3. Total number of microplastics g^{-1} of sample per year with a focus on time periods with microplastics (>1950).

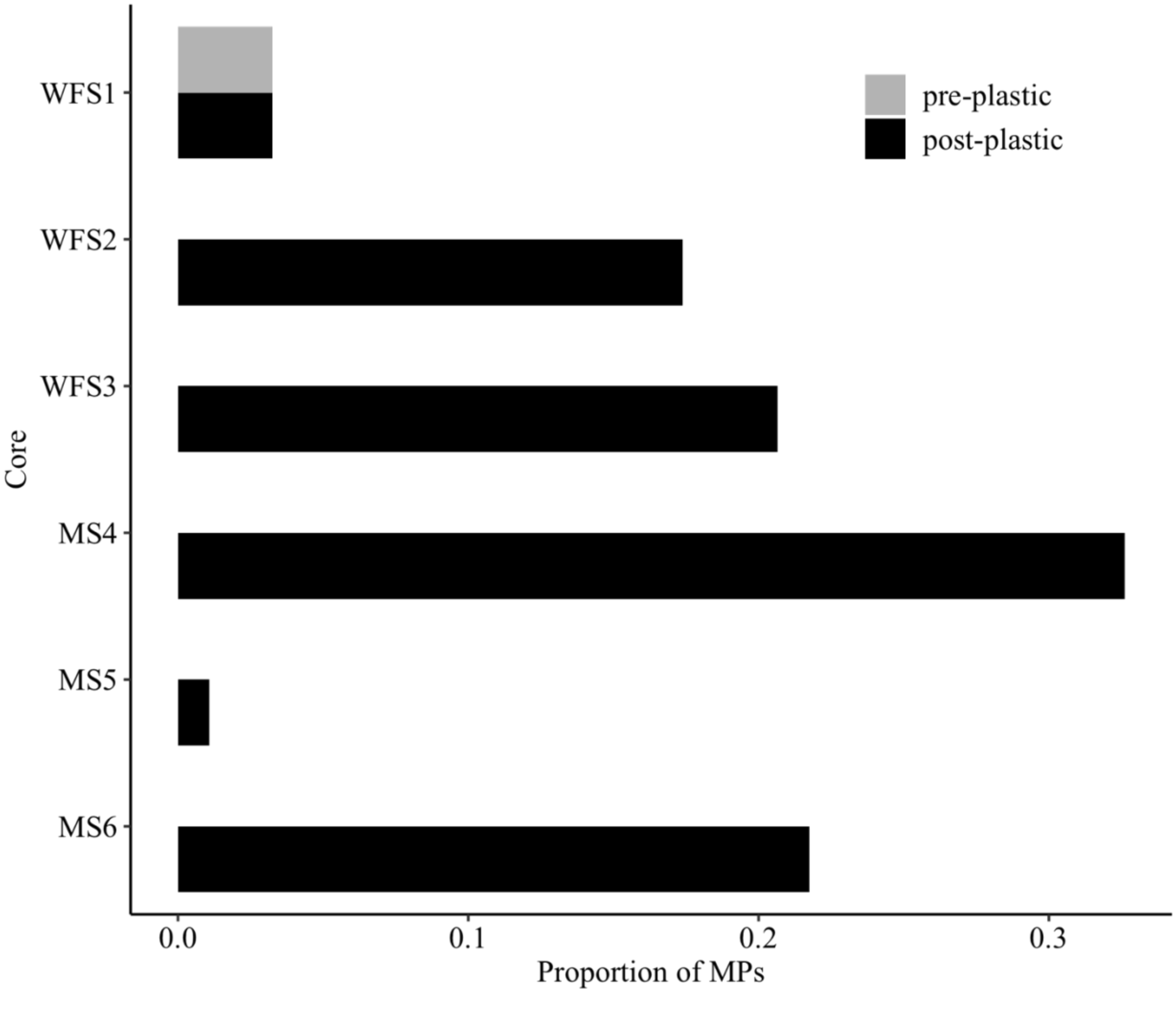

Figure 1.4. Proportion of microplastics within each core relative to the total observed across all cores. Time periods are separated between pre-plastic production (gray) and post-plastic production (black). Pre-plastic is ≤1950 and post-plastic is >1950.

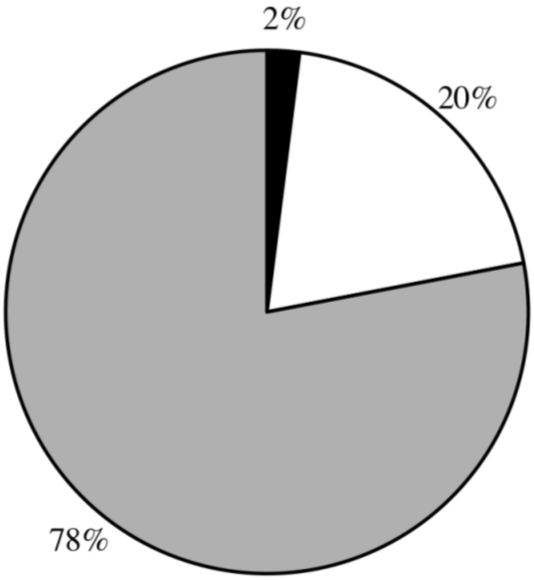

Figure 1.5. Size classes of foraminifera that had microplastics incorporated into their tests for all cores. 63-<150 μm (black), 150-<300 μm (white), 300-5000 μm (gray).

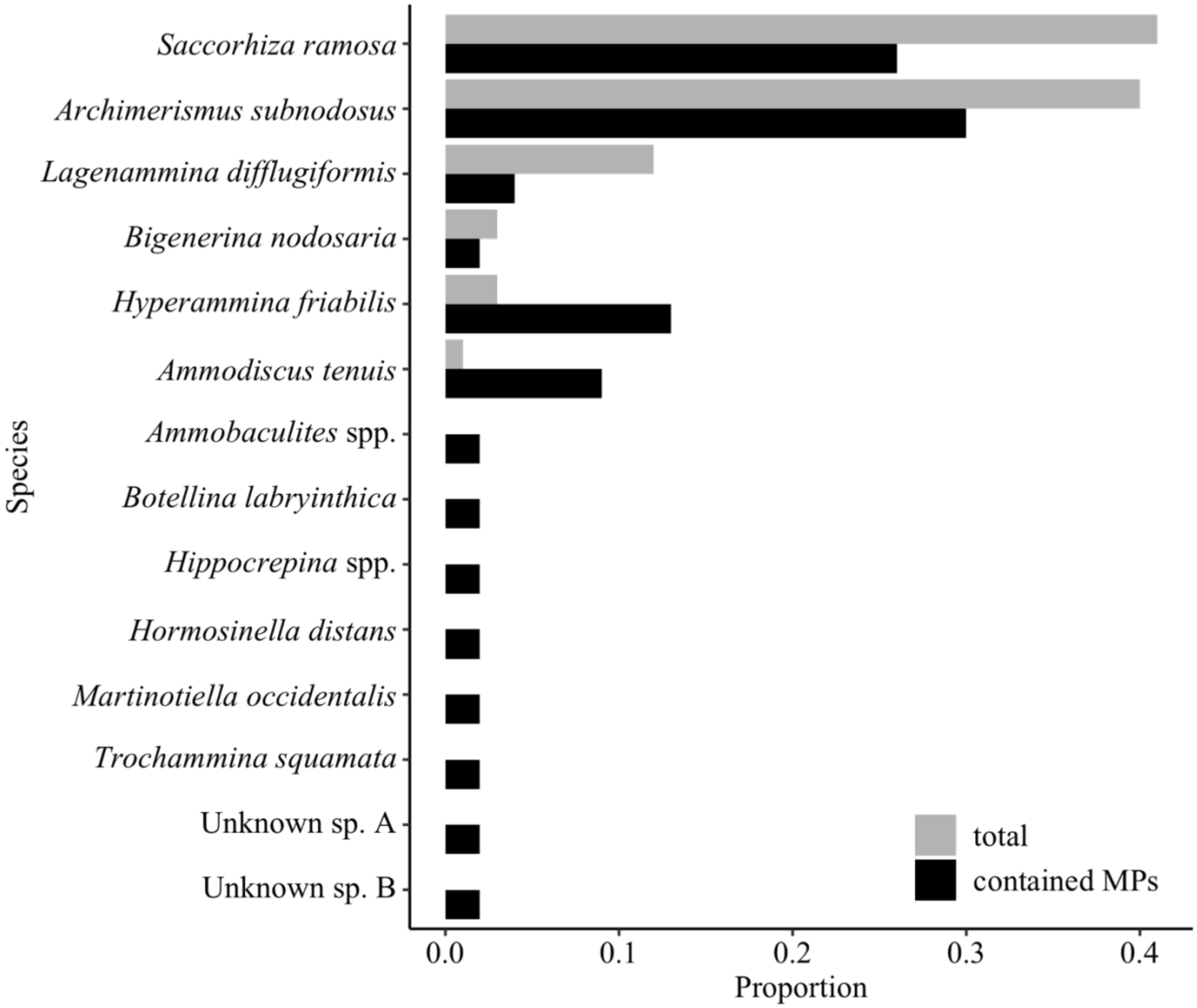

Figure 1.6. Proportion of each species that were found to contain microplastics in their test from all cores (black) and the total proportion of those same species that were found within the cores (gray; except WFS1 and WFS2).

Table 1.5. Output of GLMM to evaluate the effects of region (Mississippi = regionMS), time (post-plastic production = group1), and water depth on microplastics g^{-1} of sample incorporated in agglutinated foraminifera tests. The West Florida Shelf region and pre-plastic production time were used as model reference. ($\alpha = 0.05$, **p < 0.05**)

Conditional model

	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	0.396	0.648	0.612	0.541
regionMS	3.454	0.856	4.035	**0.000**
water depth	0.004	0.001	5.889	**0.000**

Zero-inflation model

	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	2.483	1.055	2.354	**0.019**
group1	-3.689	1.212	-3.043	**0.002**

Random effects

	Variance	Std. Dev.
Core	7.188e-09	8.478e-05

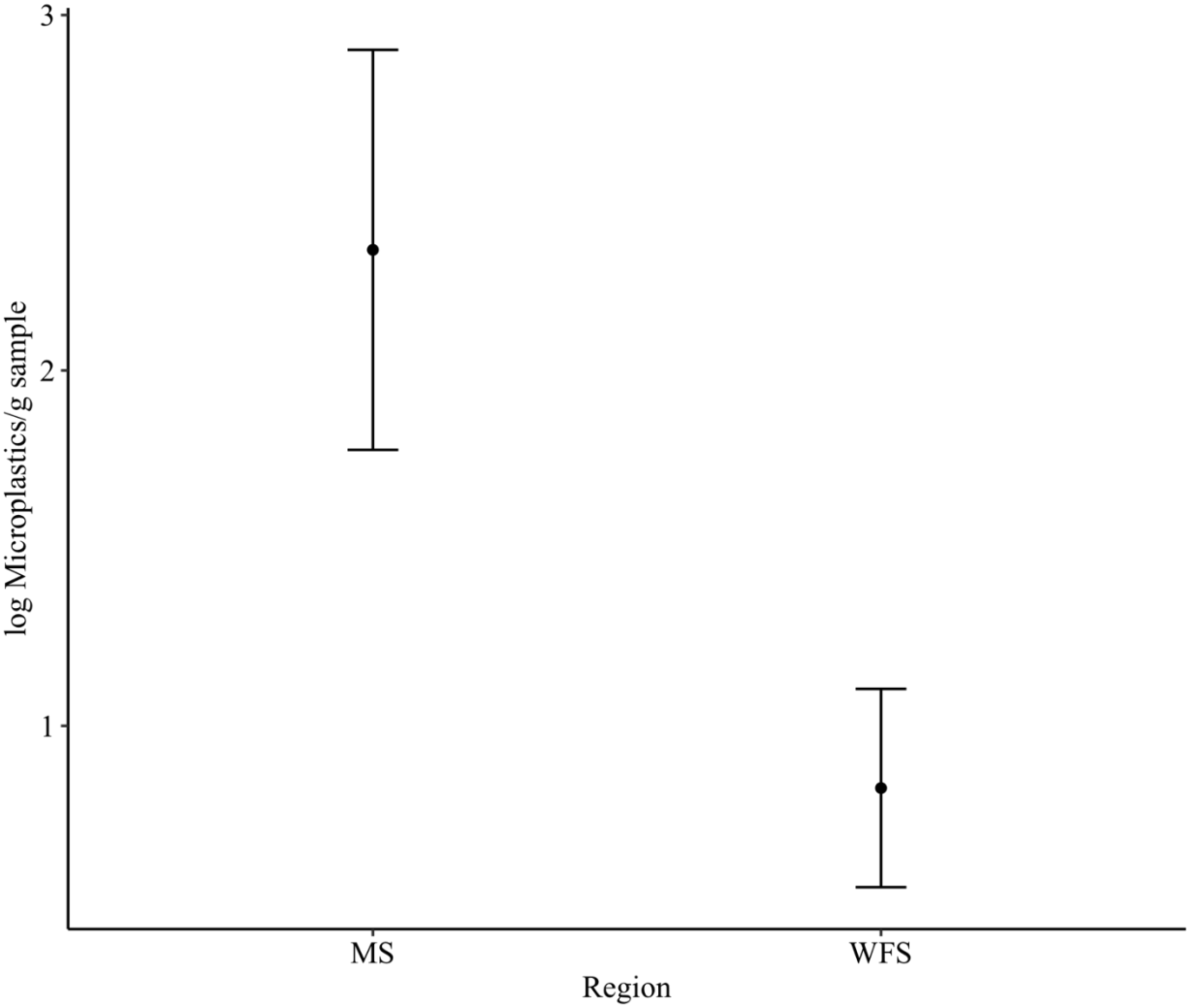

Figure 1.7. Log transformed microplastics g^{-1} of sample for each region. MS is the Mississippi River region and WFS is the West Florida Shelf region. Circles are the mean and whiskers are one standard error.

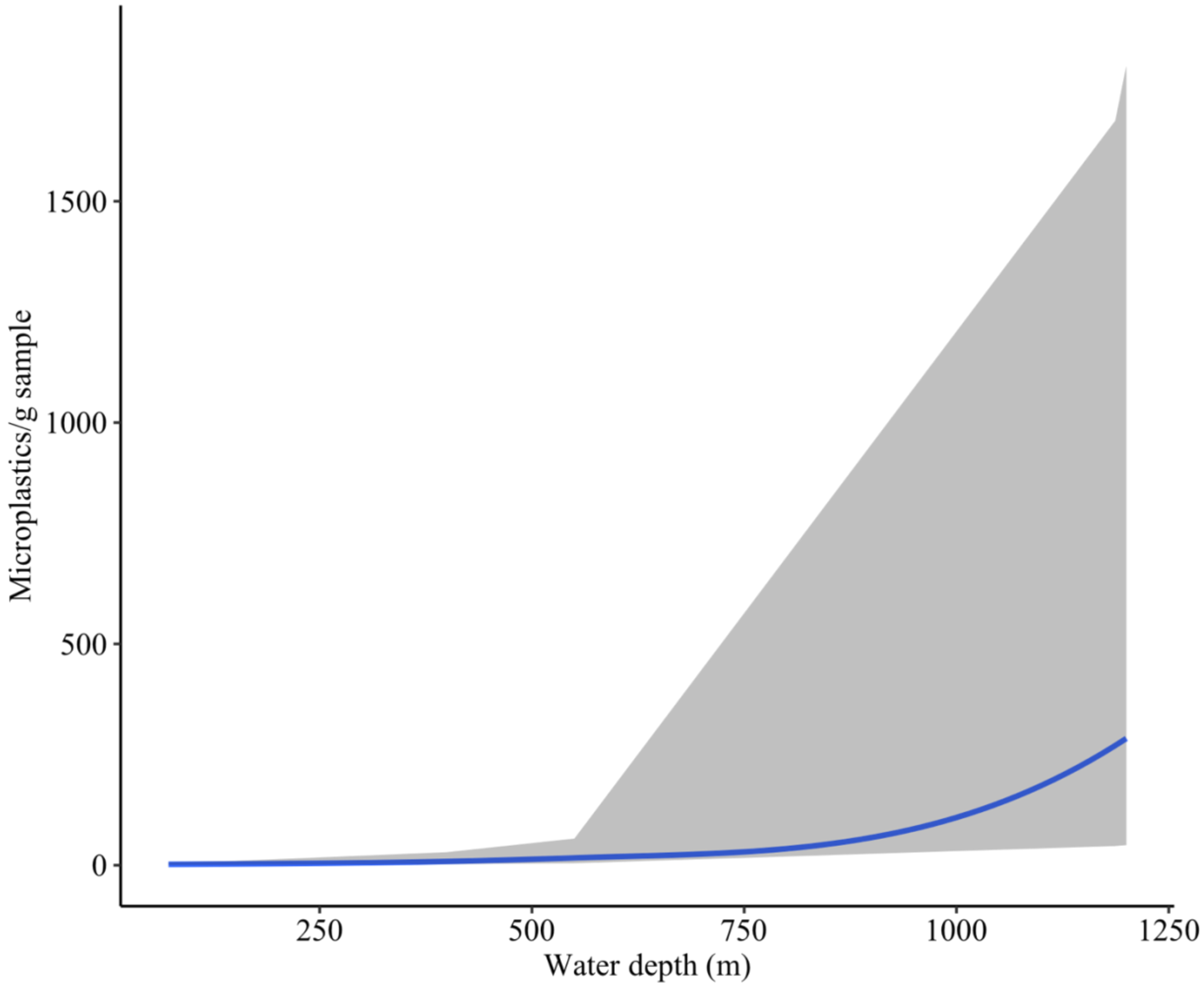

Figure 1.8. Modelled effect of water depth on microplastics g^{-1} of sample. The gray envelope represents 95% confidence intervals.

Chapter 2: Microplastics do not affect bleaching of *Acropora cervicornis* at ambient or elevated temperatures

Note to reader

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Introduction

Coral reefs provide recreational, commercial, and ecological services, which makes them a valuable marine habitat (Woodhead et al. 2019). Despite their importance, coral reefs are threatened by a suite of global and local stressors. Globally, climate change is affecting ocean temperatures which are expected to increase by 2.6–4.8°C at the surface by 2100 (Rogelj et al. 2012, Pachauri et al. 2014), and can result in coral bleaching. The global effects of ocean warming on coral reefs are evidenced by the significant degradation and collapse of reef ecosystems since bleaching can lead to coral mortality (Pratchett et al. 2018). Due to the continual rise in ocean temperatures, there has been an increase in the frequency and intensity of coral bleaching events (Riegl et al. 2009, Hughes et al. 2018). The Florida Keys and Caribbean are among the most degraded reefs, with 63% continuous decline in coral cover between 2007 and 2016 (Jones et al. 2020), however reef degradation began decades before the recent changes (Schutte et al. 2010). In addition to rising water temperatures, there is a growing concern about the effects of microplastics on coral-reef systems. Although some early studies have demonstrated that microplastics can negatively harm corals (Hankins et al. 2018, Reichert et al. 2018, Tang et al. 2018, Tang et al. 2021), the responses have been equivocal among species examined (Reichert et al. 2018, Reichert et al. 2019). We therefore lack an understanding of how microplastics may interact with elevated ocean temperatures, and how this emerging stressor may affect bleaching in most coral species. Addressing this research gap will help us to broaden our understanding of the generalities of the individual and combined effects of these two anthropogenic stressors on sensitive coral-reef ecosystems.

Exposure to microplastics in corals has been demonstrated to cause a variety of negative effects. Adhesion of microplastics to a coral's surface can cause localized bleaching and tissue necrosis (Reichert et al. 2018), but further harm can occur when corals ingest them. There is some evidence to suggest corals accidentally ingest microplastics when they try to capture food (Axworthy & Padilla-Gamino 2019). This prevents corals from obtaining real food due to time spent handling the plastic (Savinelli et al. 2020) and imparts satiation by filling their gastrovascular cavity (Rotjan et al. 2019). These responses can have important implications on their energy budgets because the movements involved with capturing, ingesting, and egesting microplastics are energetically costly (Reichert et al. 2019). In addition, a reduction in food consumption to replenish energy lost when handling the microplastics could ultimately cause an energy deficit (Savinelli et al. 2020). This may have profound repercussions when corals are stressed, such as in ocean warming conditions, since they need energy to cope with these stressors. However, only three studies to date have examined how microplastics and ocean warming interact in corals. Reichert et al. (2021) found equivocal effects of microplastics on five species of coral. Although microplastics exacerbated the effects of temperature on bleaching in one species, it did not affect bleaching in three species, and even reduced it in one (Reichert et al. 2021). Increased photosynthetic efficiency, upregulation of heat shock proteins, or increased heterotrophic feeding were potential explanations for why *Montipora digitata* bleached less when thermally stressed (Reichert et al. 2021). However, Axworthy and Padilla-Gamino (2019) found corals reduced feeding on *Artemia* but not on microplastics following thermal stress and suggested this could cause an energy deficit. Additionally, Mendrik et al. (2021) observed reduced photosynthetic activity in *Acropora* spp. exposed to microplastic fibers at ambient temperature likely due to an increase in reactive oxygen signaling species, an indicator of stress, but this effect was not found at high temperatures. The authors suggested the corals acclimated to thermal stress by producing oxidative enzymes which also protected them from the microplastic stress (Mendrik et al. 2021). Ultimately, the stress and energy deficits caused by microplastics combined with stress from elevated temperatures could interact to produce either an additive or synergistic effect on coral bleaching, but further work is needed to examine this.

Acropora cervicornis is an important reef-building species in the tropical western Atlantic region that provides ecosystem services such as habitat for organisms and storm protection of shorelines (Moberg & Folke 1999, Woodhead et al. 2019). This species is particularly susceptible to bleaching and other stressors and has been declining in abundance over time (Langdon et al. 2018). In fact, *A. cervicornis* has been listed as critically endangered by the International Union for Conservation of Nature (Aronson et al. 2008), and some estimates have suggested it may not survive past 2035 due to its susceptibility to bleaching (Langdon et al. 2018). Its recent decline in abundance, combined with fast growth rates, reliance on asexual propagation, and ecological importance, have made it a focal species for restoration efforts in the Caribbean (Johnson et al. 2011, Young et al. 2012). *A. cervicornis* has been shown to ingest microplastics (Hankins et al. 2021) but the effects of doing so remain unclear. Given the prevalence of microplastics in Caribbean waters (Rose & Webber 2019, Garces-Ordonez et al. 2021), the warming trend in the region (Chollett et al. 2012, Kuffner et al. 2014), and the drastic declines of *A. cervicornis* (Aronson et al. 2008), it is imperative to assess the effects of the combined stressors (microplastics and elevated temperatures) on this sensitive coral. To address this knowledge gap, we asked: *Does microplastic exposure interact with elevated water temperatures to exacerbate bleaching in A. cervicornis?* To test this study question, we performed controlled laboratory experiments where we manipulated temperature and microplastic concentrations and quantified the amount of bleaching or tissue loss.

Materials & Methods

We conducted experiments in the University of South Florida's College of Marine Science (CMS) aquarium facility. Mote Marine Laboratory (Summerland Key, Florida, USA) donated *A. cervicornis* fragments comprising two genotypes from several colonies each; the genotypes had moderate to high tolerance to heat stress (Muller et al. 2021). Corals were obtained from Mote Marine Laboratory under National Marine Sanctuary Permit FKNMS-2015-163-A3. We performed the experiments on coral fragments of the moderately heat-tolerant genotype in November 2020 and the high heat-tolerant genotype May - June 2021. We glued coral fragments to ceramic tiles upon arrival at the CMS and fed the corals 2.5 g per 100 gallons of a dried zooplankton mix per manufacturer recommendations (Reef-roids, PolypLab). We stored the fragments in a 190 L acclimation tank at the CMS for two weeks at 28°C prior to the experiments. Lighting consisted of T5 High Output fluorescent lights (two 440nm wavelength and two 15000K bulbs in each fixture in each tank) with an 8:16 hour (light:dark) photoperiod. We used this photoperiod due to mortality associated with longer light periods in preliminary experiments, but this photoperiod is consistent with previous studies on corals maintained in the laboratory (Schutter et al. 2011). We used two submersible pumps (Model 3, Danner) to maintain circulation throughout the tank and a titanium heater to maintain temperature (Titanium 800+, Finnex) and controller (Apex Lite, Neptune Apex Systems). We made seawater with Reef Crystals Reef Salt (Instant Ocean) mixed with deionized water to a salinity of 35.

We used a fully orthogonal design to test the effects of temperature and microplastic exposure on coral bleaching. Specifically, we crossed two temperatures, 28°C and 32°C, with two microplastic concentrations, 0 microplastics L^{-1} and 11.8 microplastics L^{-1}. We choose 28°C to match the ambient water temperature at time of collection since Mote raised the corals in an offshore nursery. The higher temperature (32°C) was within the predicted range for the tropical western Atlantic region by year 2100 (Rogelj et al. 2012, Pachauri et al. 2014). The microplastic concentration reflected the global average of 11.8 microplastics L^{-1} (Barrows et al. 2018). We placed 2-3 coral fragments in each of the eight 8.26 L experimental tanks per treatment combination. We kept experimental tanks within a water bath to keep their temperature stable. Freytes-Ortiz and Stallings (2018) developed this system to examine the effects of ocean warming on marine organisms. We placed the heater in the water bath with pumps on opposite ends to circulate the water. Each experimental tank contained a wave maker (JVP-110 528 gallons hr^{-1}, Sunsun) to generate flow and an airstone. We performed water changes of approximately one-third the tank volume every other day and measured water quality for eight parameters: temperature, calcium, alkalinity, nitrite, salinity, pH, nitrate, and ammonia. We randomly selected two tanks from each treatment four times throughout the experiment to test the water, and all tanks were ultimately examined. Water quality throughout each experiment was within an acceptable range except on the last day of the experiment for the moderately heat-tolerant genotype (Fig. 2.1). Two tanks had high levels of ammonia, nitrite, and nitrate caused by the tissue necrosis and mortality of the coral fragments in those tanks due to the elevated water temperature.

For the high temperature treatment, we increased the water temperature 0.5°C each day until it reached 30°C. We held the temperature at 30°C for four days, then increased by 0.5°C per day until it reached 32°C where it remained constant for six days. This rate of temperature increase mitigated any effects of thermal shock. When the temperature was held at 32°C, the tanks were maintained at 28 ± 0.02°C (mean ± SE) and 32 ± 0.02°C (mean ± SE). We added fluorescent green low-density polyethylene microbeads with a diameter range of 212-250 µm (1.025 g cc^{-1}) and 300-355 µm (1.010 g cc^{-1}) directly to the tanks at a concentration of 11.8 microplastics L^{-1} (5.9 particles L^{-1} of each size) (Barrows et al. 2018). We chose these microplastic sizes based on what the small-polyp *A. cervicornis* (1.26mm-2.03mm) can ingest. Prior to the experiments, we kept the microplastics in saltwater for at least one week to accumulate a biofilm. We added the microplastics to both the elevated and ambient temperature treatments after the first temperature increase along with food to initiate a feeding response. After the microplastics were added, they mostly floated on the surface on the first day and then were suspended in the water column for the remainder of the experiment. During water changes, we separated the microplastics and added them back to the tank to ensure consistent microplastic concentrations throughout the study duration. As a result of microplastic exposure, ingestion was an assumed response due to evidence by Hankins et al. (2021) and video we collected (Fig. 2.2).

We used a protocol to minimize contamination (Brander et al. 2020, Cowger et al. 2020), that we modified for corals. We separated the tanks from the rest of the room with a heavy-duty tarp to limit airborne contamination. We wore 100% cotton clothing to limit fiber shedding, thoroughly rinsed hardware (e.g., containers, glassware) with deionized water before use, and covered them in aluminum foil if not used immediately. We also rinsed our arms thoroughly with deionized water up to the elbows and wiped down all other surfaces with paper towels and deionized water.

To visually compare treatments throughout the experiments, we measured the response to thermal stress daily based on severity of coral bleaching and a visual estimate of percent surface area affected by tissue loss (i.e., necrosis). Coral bleaching occurs when the tissue loses its color due to the expulsion of zooxanthellae which makes the coral appear white, whereas tissue necrosis is the loss of tissue (Hoegh-Guldberg & Smith 1989, Rodolfo-Metalpa et al. 2005). The ordinal bleaching scale we used was none (0), low (>0-25%), partial (25-50%), high (50-75%), and total (75-100%). Immediately following the conclusion of the experimental trials, we placed all corals in a -20°C freezer for at least one hour, then removed them one at a time, and sprayed them with artificial seawater to remove the tissue (Johannes & Wiebe 1970). We preserved collected tissue in 2% formalin. Next, we recorded the total homogenate volume (i.e., the volume of the zooxanthellae, seawater, and formalin), homogenized it, and counted zooxanthellae on 10 grids of a Neubauer-improved hemocytometer under a light microscope. To obtain the total zooxanthellae count for each fragment, we divided the average cell count per grid by the volume of the hemocytometer chamber, then multiplied by the total homogenate volume. We used the aluminum foil method from Marsh Jr (1970) to calculate the surface area of each fragment. To do this, we completely and snugly covered each coral skeleton in aluminum foil with no overlap, and then weighed the foil. Then we weighed five 100 cm^2 foil sheets and calculated their mean mass as a reference. Next, we calculated the coral surface area by multiplying the reference foil surface area and coral foil weight then dividing by the reference foil weight. Finally, we quantified zooxanthellae density by dividing the zooxanthellae count of each coral fragment by its surface area.

To examine the additive and synergistic effects of temperature (fixed effect) and microplastics (fixed effect) on zooxanthellae density (response), we performed a generalized linear mixed model (GLMM) with tank included as a random effect. We determined the zooxanthellae response data were zero-inflated, and therefore examined several models that are capable of handling a large number of zeros (Zuur et al. 2009). We performed all analyses in R (R Development Core Team 2021) using glmmTMB (Brooks et al. 2017) for the GLMM and DHARMa (Hartig & Hartig 2021) for residual diagnostics. We used Akaike information criterion (AIC) to determine the best model then tested for diagnostics. We also determined that genotype did not affect zooxanthellae density ($p = 0.55$), and because we were not interested in its effects, per se, we pooled the data across genotypes. Our final model, that was deemed the best, was a zero-inflated, negative binomial model that examined the main effects of temperature and microplastic as well as an interaction between the two (AIC = 3893.1).

Results

Bleaching did not occur in the ambient temperature (28°C) treatment but was extensive in the elevated one (32°C). Indeed, 97.5% of corals in the high temperature treatment were visibly bleached and 75.3% experienced tissue necrosis (Fig. 2.3). These observations held regardless of microplastic presence. Further, zooxanthellae density was strongly affected by elevated temperature ($z = -8.15$ $p < 0.001$, Table 2.1). However, zooxanthellae density was not affected by either microplastics alone ($z = 1.07$, $p = 0.29$) or in combination with elevated temperature ($z = 1.04$, $p = 0.30$). Neither elevated temperature ($z = 0.01$, $p = 0.99$) nor microplastic presence ($z = 0.17$, $p = 0.87$) contributed to excess zeros in the zero-inflated model.

Discussion

Using a short-term laboratory experiment, we have shown that the presence of microplastics, when combined with thermal stress, did not alter the bleaching response of *A. cervicornis*. Importantly, these experiments were conducted using environmentally relevant microplastic concentrations. Research focused on the potential effects of microplastics on corals is an emerging field, and this study was one of the first to examine the orthogonal effects of microplastics with thermal stress (Axworthy & Padilla-Gamino 2019, Reichert et al. 2021). As expected, elevated temperature reduced the zooxanthellae densities of the coral, but we found no individual or interactive effects of the microplastics.

The literature to date has been equivocal regarding the effects of microplastics on coral bleaching. The results from our study are consistent with previous research on *Porites lutea* and *Heliopora coerulea* at ambient temperature (Reichert et al. 2018, Reichert et al. 2019), but in contrast with studies that have found microplastic exposure can cause bleaching and tissue necrosis in *A. muricata* and *Pocillopora verrucosa* (Reichert et al. 2018, Reichert et al. 2019, Syakti et al. 2019). Similar to our study design, Reichert et al. (2021) examined the combined effects of microplastics and climate-change induced ocean warming, and found more severe bleaching in microplastic-treated fragments of *Pocillopora verrucosa* at elevated temperature. However, consistent with our results, Reichert et al. (2021) did not find an additive or synergistic effect of microplastics at elevated temperatures in *A. muricata, Porites cylindrica,* and *Stylophora pistillata.* The contrasting results among species highlights the species-specific responses corals have to microplastics.

Previous studies have attributed the different responses to microplastics among coral species to variation in their reliance on heterotrophic feeding (Reichert et al. 2019, Tang et al.

2021). Corals typically rely on photosynthesis to meet their energy demands but can supplement this with heterotrophic feeding (Grottoli et al. 2006), which makes them vulnerable to microplastics through ingestion. Microplastics have been shown to be stressful to corals (Tang et al. 2018), which can deplete their energy (Hankins et al. 2021). In response to reduced energy, corals may increase heterotrophic feeding which leads to increased interactions with microplastics, additional stress, and energy depletion, subsequently causing bleaching (Reichert et al. 2019). Some coral species rely more on heterotrophic feeding than others, thus they are more vulnerable to microplastics while species that do not rely as much on heterotrophic feeding limit their interactions with microplastics and suffer less bleaching (Reichert et al. 2018, Reichert et al. 2019). This is especially concerning at elevated temperatures where corals can have heterotrophic plasticity in response to thermal stress (Grottoli et al. 2006), however we did not see an effect at either ambient or elevated temperatures. Microplastics were not stressful to *A. cervicornis*, possibly because they have small polyps that ingest less microplastics than large-polyp corals (Hankins et al. 2018, Hankins et al. 2021). Despite a reliance on heterotrophic feeding (Towle et al. 2015), the smaller polyp size could have led to lower rates of microplastic ingestion which limited the interactions *A. cervicornis* had with the microplastics. Therefore, the stress and energy consumption associated with microplastic exposure was limited which prevented bleaching. However, it is unclear how many microplastics these corals ingested since the goal of this study was to assess the effects of microplastic exposure on coral bleaching rather than to specifically measure ingestion. Microplastic ingestion has been observed in this coral species, so we assumed it occurred throughout the experiments.

Experimental conditions may have also played a role in the lack of a microplastic effect in our study. For example, the response of corals to this pollutant has been shown to be dependent on microplastic concentration (Syakti et al. 2019, Reichert et al. 2021). The choice of concentration(s) to use in experimental studies can be complicated since they are dynamic both spatially (Barrows et al. 2018) and temporally (Courtene-Jones et al. 2020). Microplastic concentrations range from 0 to 220 particles L^{-1} in the global ocean (Barrows et al. 2018), $3x10^{-5}$ to 14 particles L^{-1} in the tropical western Atlantic Ocean, and approximately 6 particles L^{-1} in the Caribbean (Ivar do Sul et al. 2014, Barrows et al. 2018). Due to the large range of microplastic concentrations found in the global ocean, we used the global oceanic average to make it applicable to a broader range of locations. Our results align with previous work that did not find an effect of microplastics at concentrations reflective of current oceanic conditions (Syakti et al. 2019, Bucci et al. 2020, Reichert et al. 2021), whereas studies that have found stronger effects on zooxanthellae densities used 17 times, and higher, the concentration we used (Reichert et al. 2018, Reichert et al. 2019). For example, Reichert et al. (2021) found lower photosynthetic efficiency, mortality, and bleaching in two coral species when exposed to 2500 microplastics L^{-1} at ambient and elevated temperatures but not at lower concentrations (2.5, 25, and 250 microplastics L^{-1}). Our finding is important because it indicates that bleaching in *A. cervicornis* is not exacerbated by realistic microplastic concentrations observed on average in the global ocean, and ocean warming remains a larger threat. It is important to consider our experiments took place in a controlled laboratory setting and used a single, static microplastic concentration. However, corals can be exposed to temporally variable microplastic levels due to ocean dynamics which could result in a different response locally compared to a controlled laboratory setting. Microplastic size can also play an important role in the effects on organisms. For example, Syakti et al. (2019) found smaller microplastics had a stronger effect on bleaching compared to larger ones. Indeed, studies that assessed the effects of microplastics on corals have used a range of microplastic sizes from 1-500 µm, which could lend to the varying results. In this study, we used a mixture of two different microplastic sizes (212-250 and 300-355 µm) to simultaneously expose the corals to different sizes of plastic which is more representative of actual ocean conditions. In addition to microplastic concentration and size, the particle shape could have played a role in the lack of a response to the microplastics (Bucci et al. 2020, Mendrik et al. 2021). Photosynthesis in two coral species were altered in different directions (increase and decrease) by different microplastic shapes (fibers and spheres; Mendrik et al. 2021). Additionally, it remains unclear whether polymer type could affect responses to microplastics (Bucci et al. 2020). Indeed, most studies on corals, including ours, have used polyethylene microplastics (Hankins et al. 2018, Axworthy & Padilla-Gamino 2019, Lanctôt et al. 2020, Hankins et al. 2021). In contrast, few have used other polymer types (e.g., polystyrene, polypropylene) (Tang et al. 2018, Corona et al. 2020, Mendrik et al. 2021), so it is difficult to determine the role it may have on how corals respond to microplastics.

Conclusions

In our study, we orthogonally crossed temperature and microplastics to assess the effects of these combined stressors on bleaching in *A. cervicornis*. We found that microplastics had no effect on the bleaching response of *A. cervicornis* at ambient and elevated temperatures. Based on the minimal effect of microplastics observed in this study, *A. cervicornis* could be more tolerant to microplastics; however, further research will need to be conducted on this species to discern this. Also, our experiment assessed the short-term effects of microplastics combined with thermal stress on corals. Long-term experiments are needed to determine how organisms may respond to prolonged exposure to microplastics. While rising ocean temperatures remain a known major threat to corals, microplastic research on corals is still in its infancy. Future work should continue to test for the combined effects of microplastics and other stressors (e.g., ocean acidification, disease) in other coral species to understand how microplastics interact with previously identified stressors in coral-reef ecosystems. Additionally, studies should focus on using realistic microplastic concentrations to make their studies relevant to current and near future conditions but could also use a range of concentrations to identify whether response thresholds exist. Indeed, such efforts could be important since microplastic concentrations will likely continue to increase in the ocean as plastic production continues to grow. Such an effort would also add to the well-studied and often modeled effects of two other major anthropogenic stressors, global warming and ocean acidification.

Appendix B: Tables and Figures for Chapter 2[2.1]

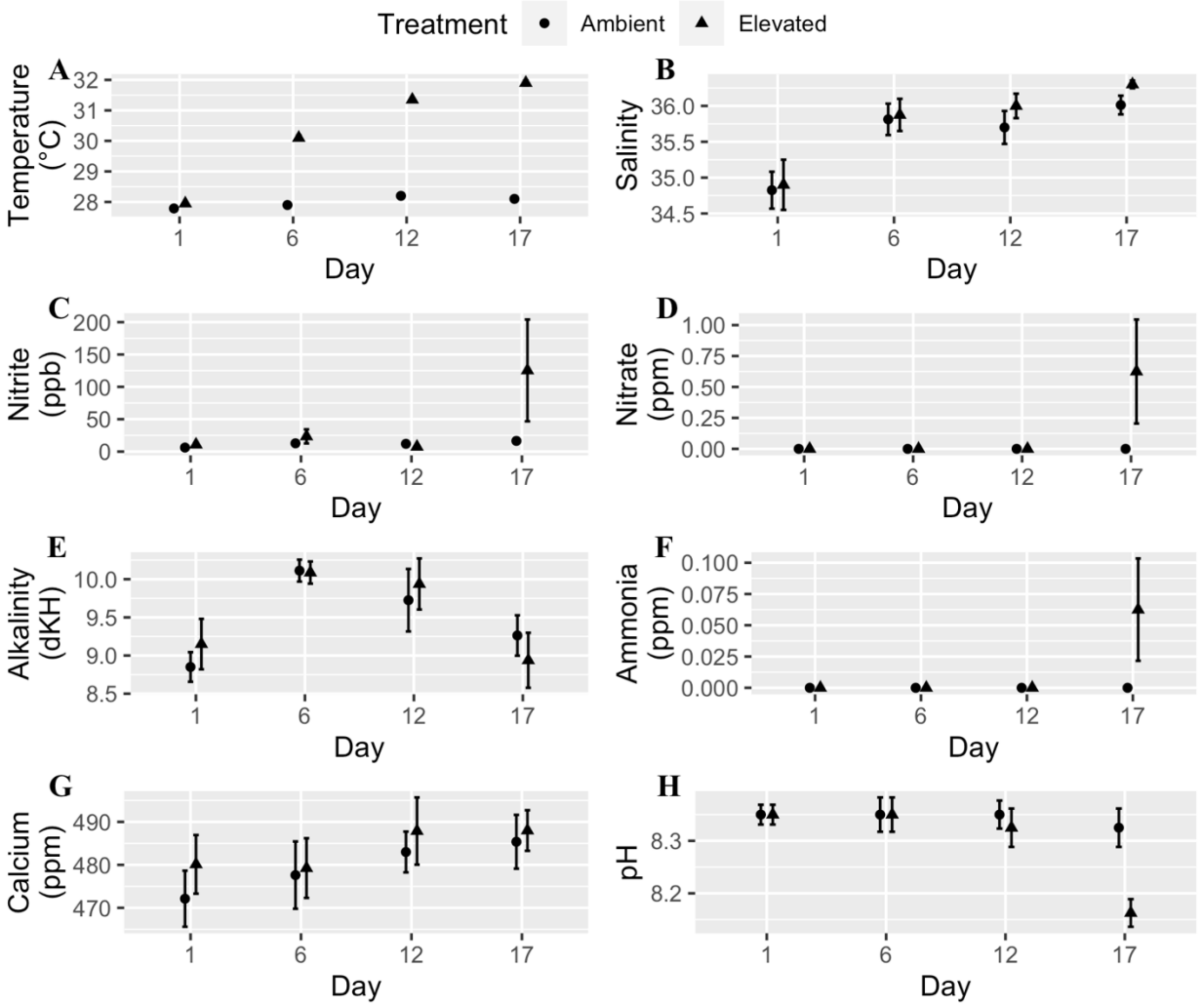


Figure 2.1. Water quality parameters (mean + SE) of tanks for each day measured throughout the experimental trials. Circles are the ambient temperature tanks and triangles are the elevated temperature tanks. (A) Temperature, (B) Salinity, (C) Nitrite, (D) Nitrate, (E) Alkalinity, (F) Ammonia, (G) Calcium, (H) pH. Two tanks had high levels of ammonia, nitrite, and nitrate caused by tissue necrosis and mortality of the coral fragments in those tanks due to the elevated water temperature which raised those values for the last day of the experiment.

[2.1] The tables and figures appear as they were ordered in the chapter.

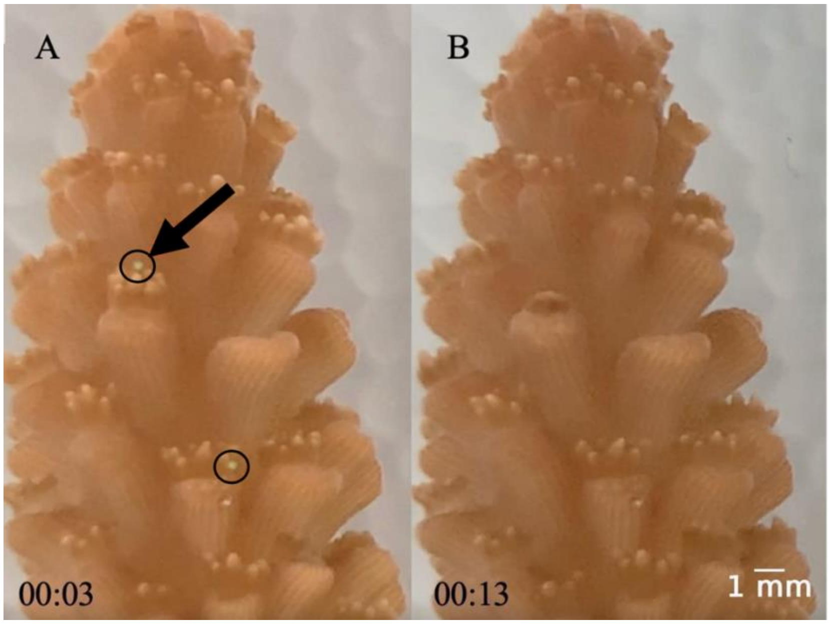


Figure 2.2. Images of coral capturing a microplastic. The black arrow points to the microplastic that was captured in panel B. Time stamps for each picture are at the bottom (note: these images were taken from a video which is sped up 20x). Note that there is a second microplastic visible in panel A, but it was not captured by the coral during this recording. Both microplastics are circled in black in panel A.

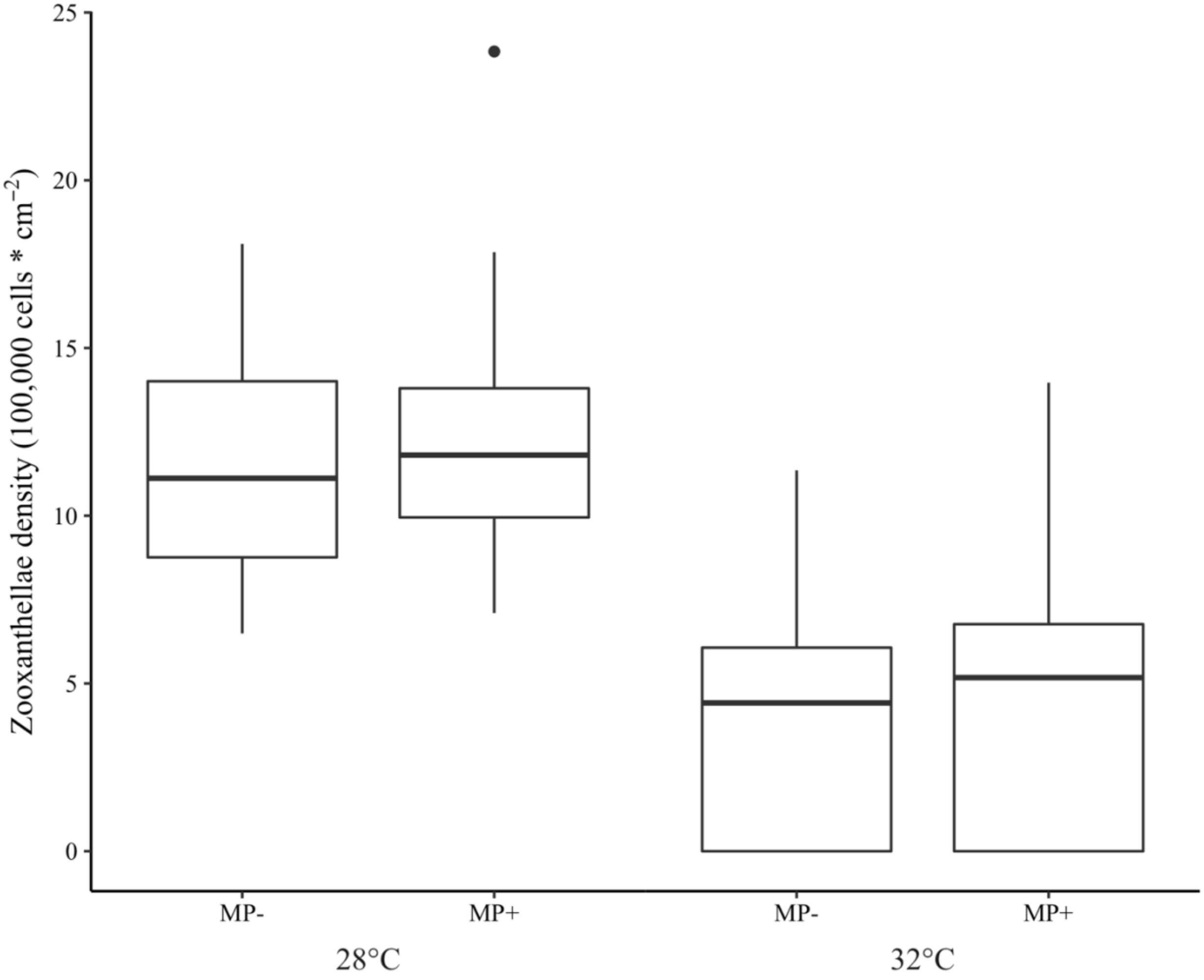


Figure 2.3. Boxplot of zooxanthellae densities (100,000 cells * cm^{-2}) for each treatment. Presence of microplastics is indicated with MP- (absent) and MP+ (present). Temperature treatments, 28°C and 32°C, are indicated below the microplastic treatments. The boxes represent the median (horizontal line inside box), the first and third quartiles (lower and upper lines of the box, respectively) which shows the interquartile range, and the lower and upper whiskers represent the range within 1.5 * interquartile range. The additional point represents an outlier.

Table 2.1. Output of GLMM to evaluate the effects of temperature and microplastic exposure on the zooxanthellae density. Ambient temperature (28°C) and MP- (absent) were used as model reference ($\alpha = 0.05$, **$p < 0.05$**).

Conditional model

	Coefficient	Std. Error	z value	Pr(>|z|)
Intercept	13.944	0.057	245.27	**<2e-16**
Temp32	-0.713	0.088	-8.15	**3.69e-16**
MP2	0.085	0.080	1.07	0.287
Temp32:MP2	0.128	0.123	1.04	0.298

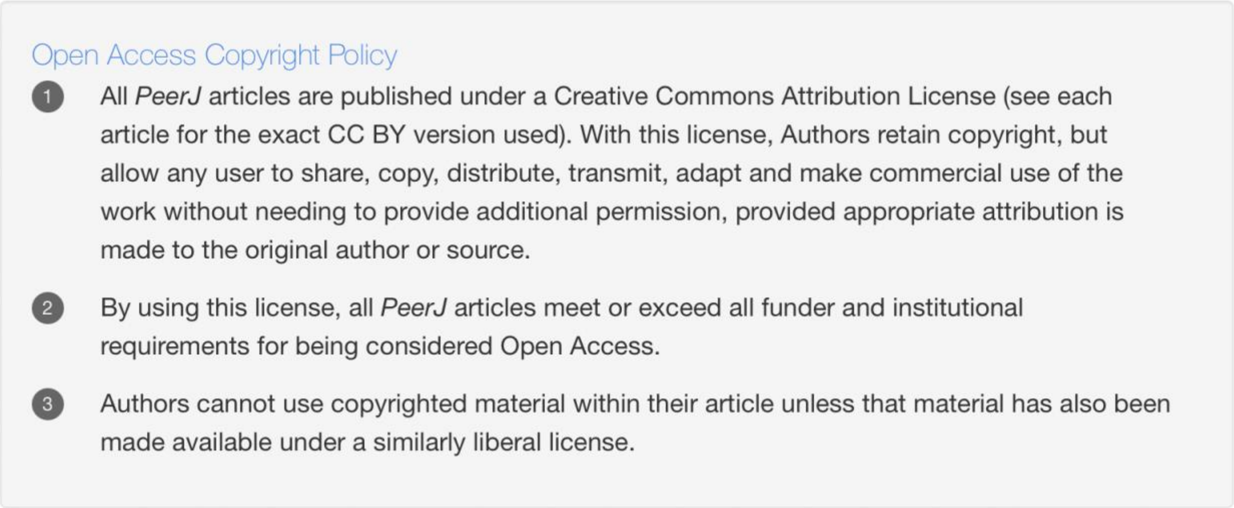
Zero-inflation model

	Coefficient	Std. Error	z value	Pr(>|z|)
Intercept	-19.698	2086.822	-0.009	0.992
Temp32	18.850	2086.822	0.009	0.993
MP2	0.080	0.481	0.166	0.868

Random effects

	Variance	Std. Dev.
Tank	0.011	0.105

Appendix C: Permissions for Chapter 2



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