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Macro implications of microplastics: a comparative study of microplastic distribution in Bahía Almirante, Bocas del Toro, Panama

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Sample collection on a mangrove island in Cayo Coral in the Bocas del Toro Archipelago, Panama

Abstract

Plastic is one of the most demanded materials in the modern world as it is durable and long lasting. However, that which makes it so commercially appealing also makes it environmentally degrading. Anthropogenic waste and specifically microplastics have been identified in natural habitats globally, with particular interest placed on marine ecosystems. This research aims to add to this pool by comparing microplastic concentrations in beach, mangrove, and shallow ocean sediment in Bahía Almirante, Bocas del Toro, Panama. Sediment samples were collected from each habitat type, processed, and then analyzed to obtain the number of microplastic particles per gram of dry weight. Ocean sediments were found to have significantly higher concentrations of microplastics than beach and mangrove sediments, however beach and mangrove sediments were not statistically different from each other. This implies that microplastics are more likely to be found in ocean sediment then in beach sediment, and that mangroves are not likely to act as filters for microplastics in coastal zones. Secondary microplastics and microfibers were the most prevalent type of microplastic found, which is consistent with previous research. It also points to plastic degradation rather than direct inputs as main sources of contamination. This study confirms the presence of microplastics in coastal zones in the Bocas del Toro Archipelago, concentrations of which will only stop increasing if plastic use and consumption are reduced.

Acknowledgements

I would first and foremost like to thank Eric Manzane for his willingness to invest his time and energy into my project. I would have been lost without his guidance, creative thinking, and ample advice. I could not have asked for a better advisor! I would also like to thank Philip Dustan for his help in figuring out my methodology in real time, without which I would have given up halfway through. A big thank you also needs to be given to the Smithsonian Tropical Research Institute, and more specifically to Plinio Góndola for practically saving my research through the use of work space, a drying oven, and a 0.25 mm sieve. Thank you to Ben Tan for helping me collect samples and to Lucy Murray for helping me collect my samples as well as my thoughts. Thank you also to Arcadio Castillo for putting me in contact with several useful resources, and to Alberto Machuca for transporting me to my research sites. Finally, a special thank you to Aly Dagang, Rhay Perez, and Yari Jimenez for creating a beautiful, exciting, and enriching program and for all of their support over the last three and a half months. With out all these people this project would not have been possible. Thank you!!!!

Contents

Often, when people throw away an item, they cease thinking about it. However, waste has a long journey ahead of it once it reaches a trash can, and if not handled properly, waste can end up in ecosystems and cause environmental and public health issues. One large source of waste is plastics – it is one of the most demanded materials today (Zabkov and Esiukova, 2016) and demand is increasing alongside population and consumption (Claessens et al., 2013). In 2015 alone, the United States generated 34.5 million tons of plastic, only 9.1% of which was recycled (U.S. Environmental Protection Agency, 2018). The majority of plastics produced are single use products that are thrown away a year or less after creation (Thompson et al., 2009).

Plastic's durability is both what makes it so appealing as a commercial material and what makes it so environmentally harmful. Plastics can take anywhere from years to centuries to break down (Thompson, 2015) – in fact, it is widely believed that every single piece of plastic ever created (that has not been incinerated) still exists on the planet today (Thompson et al., 2005). Never truly biodegrading, they simply fragment into smaller and smaller pieces until they are invisible to the naked eye (Kathiresan, 2003). These broken-down pieces are referred to as microplastics, generally defined as a piece of plastic that is 5 mm in size or smaller (Gross, 2015) and specifically defined in this research as plastic sized 5 mm – 0.25 mm.

Microplastics are mainly divided into four categories: microbeads, microfilms, microfibers, and microfragments. They are also classified into two types: primary microplastics and secondary microplastics (Cole et al., 2011; Startain et al., 2018). Primary microplastics are those manufactured to be smaller than 5 mm. These are often found in the form of packing materials or plastic pellets, called 'nibs' or 'nurdles,' which are resin granules used as raw materials for larger plastic products. Primary microplastics can also be found as exfoliants in face wash, in toothpaste, deodorant, or make up. As of July 2018, it is no longer legal to sell cosmetic products containing microbeads in the United States (Startain et al., 2018), however this will not prevent other sources of primary plastics from entering the environment. Secondary microplastics are those particles that have been degraded down from larger plastic sources due to physical, biological, and/or chemical processes (Cole et al., 2011). This can include processes such as wave action, ultraviolet radiation, heat, and pressure (Startain et al., 2018).

From Land to Marine Environments

This breaking down of material becomes an issue for organisms (including humans) when plastics enter the natural environment. Of the over 34 million tons of plastic produced in the U.S. in 2015, 75.4% was sent to landfills rather than recycled or reused (U.S. Environmental Protection Agency, 2018). However, a growing issue is that plastic waste often escapes waste processing streams and ends up in marine ecosystems. For example, synthetic clothing fibers may become detached during washing and enter waste water. These microfibers are unlikely to be adequately removed by treatment plants and therefore end up in marine ecosystems (Thompson, 2015). Most bulk and commercial plastics like polyethylene and polypropylene are buoyant and will float into the open ocean, often accumulating into oceanic gyres or garbage patches due to currents (Gross, 2015). Additionally, this statistic does not account for plastic litter that never makes it into waste processing systems in the first place.

It is estimated that about 10% of all produced plastics end up in oceanic ecosystems (Thompson, 2006), and about 80% of plastic waste present in these ecosystems comes from land (Andrady, 2011). Terrestrial inputs are not limited solely to coastal anthropogenic activities. Plastics can enter oceans through any freshwater system; these systems flow into oceans and thus are a main driver of oceanic plastic debris. Plastic pollution that does not come indirectly from land is deposited directly into oceans. Fishing gear is one of the main and most common types of marine sourced plastic (Cole et al., 2011). It is estimated that about 640,000 tons of fishing gear are discarded into oceans yearly, both from recreational and commercial fishing activities (Good et al., 2010).

Impacts of Microplastics on Coastal Zone Ecosystems

Primary and secondary plastics are found all over oceanic ecosystems, from shores to surface level to deep-ocean benthic zones (Startain et al., 2018). They are very hard to remove from the environment once present and because of this, they can easily work their way into food webs, contaminating both animals and human diets (Stolte, 2014). As of 2013, over 250 marine species worldwide were shown to have ingested microplastics, and this number has likely risen since then as concentrations worsen (Wright et al., 2013).

Anthropogenic waste such as food wrappers, cans, bottles and other single use items can pose both physical and chemical threats to marine wildlife. Physical harm can be caused when animals such as birds, fish, turtles, or other marine mammals become entangled in or consume waste. Entangled animals can drown or become injured or impaired, making them unable to escape predators. Similarly, plastics and trash mistaken as food and ingested can lead to suffocation or organ blockages that may then lead to death (Thompson, 2006). The degradation of plastics means that these particles can be ingested by animals as big as whales or as small as barnacles (Thompson et al., 2004).

This degradation can also create chemical hazards for marine wildlife. As ultraviolet radiation beaks down plastics, additives that make plastic more durable are caused to leach out into the environment (Chin Li, 2018). Paradoxically, plastics are an issue because they absorb pollutants as well. Particles concentrate hydrophobic contaminants that are already present in seawater from other sources onto their surfaces. Gut conditions may cause these contaminants to be released, transferring chemicals into the organism (Teuten et al., 2009). Once ingested and digested the organism would be exposed to much higher quantities of toxic materials since it is shown that these chemicals are multiple times more concentrated on plastic surfaces then in surrounding water (Thompson, 2015).

Microplastics can also alter the properties of sediments along shore lines. It has been shown that high concentrations can increase permeability as well as decrease heat absorption. Higher permeability can make organisms living in the sediment more likely to desiccate or dry out, and lower maximal temperatures can impact organisms such as sea turtles by affecting the sex-determination of eggs (Carson et al., 2011)

Global Concentrations of Microplastics and Waste

As global populations increase it becomes more relevant and important to study the spread and influence of microplastics, especially in oceanic ecosystems. Coastal zones are specific areas of interest to study because they are home to several different types of habitats – shore lines, coral reefs, seagrass, mangroves, lagoons, shallows, etc. – and they are important to separate from open ocean ecosystems because particles flow differently and are affected by different environmental factors (Chubarenko et al., 2018). So many types of organisms live in coastal zones, all of which are at risk to be harmed by the hydrophobic and heavy metal contaminants that microplastics carry, that it is necessary to locate hotspots for microplastic concentrations in order to further study their impacts (Chin Li, 2018).

Much research has already been done to identify such locations. One hotspot is the South Pacific Gyre. Oceanic plastics and other anthropogenic waste often accumulate in gyres because the majority of currents, drifts, and eddies lead into them (Martinez et al., 2009). Plastic zonation is also dependent on the density and size of plastic particles. Some sink, some are neutrally buoyant, and some float, thus distributing plastics to all parts of the water column (Cole et al., 2011). Additionally, biofouling or adherence to sediments can cause naturally buoyant plastic particles to sink (Zabkov and Esiukova, 2016).

Microplastic concentrations have been confirmed in the Atlantic Ocean and Caribbean Sea (Law et al., 2010), the Pacific Ocean (Moore et al., 2001), the Indian Ocean (Ng and Obbard, 2006), the Baltic Sea (Zabkov and Esiukova, 2016), the Gulf of Mexico (Wessel et al., 2016), and even the Arctic Sea (Obbard et al., 2014). However, the highest concentrations globally were found in the East Asian Sea. For example, in seas surrounding Japan, concentrations of 1.7 million pieces/km^2 were reported (Jambeck et al., 2015). This is in comparison to the North Atlantic Gyre with over 20,000 pieces/km^2 reported (Law et al., 2010) and the North Pacific Gyre with over 334,000 fragments/km^2 reported (Moore et al., 2001).

Plastics and plastic particles also concentrate along shore lines, which have waste inputs from both land and sea. Terrestrial sources are most prevalent in places with high human impacts and density, while oceanic sourced waste is deposited when caught in near-shore currents (Ryan et al., 2009). Studies have found microplastics on beaches in the United Kingdom (Browne et al., 2010; Thompson et al., 2004; Stolte et al., 2015), in the U.S. (Doyle et al., 2011; Carson et al., 2011), in South America (Ivar do Sul et al., 2009; Costa et al., 2010), Central America (Wessel et al., 2016), the Mediterranean (Turner and Holmes, 2011), Asia (Ng and Obbard, 2006; Fok and Cheung, 2015), and in Africa (Nel et al., 2017), to name a few places. While it is hard to compare concentrations due to a lack of standardization in reporting, some of the highest concentrations found were in Hong Kong with an average abundance of 5595 items/m^2 reported (Fok and Cheung, 2015), in Brazil with 200 items/0.01 m^2 reported (Costa et al., 2010), and in Germany with a maximum of 50,000 particles/kg reported (Liebezeit and Dubaish, 2012).

While comparatively much less research has been done on these ecosystems, for the purposes of this research it is important to note that microplastics have also been found in mangrove forests. Average concentrations of 9.2 particles/250 g were reported in Singapore (Nor and Obbard, 2017), 418 particles were found in a mangrove forest in Malaysia (Barasarathi et al., 2014), and mangroves in Ecuador were found to have higher microplastic concentrations in surrounding water than in intertidal and bed sediment (Domínguez et al., 2017). Mangroves can serve as a dumping ground, accumulating waste and debris that tides bring in (Kathiresan, 2003) from fish farms, coastal development, or recreational areas (Nor and Obbard, 2017). It has been shown that more microplastics are found in mangrove sediment where there is higher adjacent human activity (Nor and Obbard, 2017), however, microplastics have also been found in mangroves isolated from human activity (Barasarathi et al., 2014).

Research Objectives

Clearly a substantial amount of research has been done on microplastic concentrations in beach sediments and in the oceanic water column both separately and comparatively (as cited above), however there is much less research on mangrove plastic concentrations and little to no research systematically comparing all three habitat types. Mangroves are important ecosystems within coastal zones as they provide habitats, nurseries, and feeding grounds for countless organisms. They also act as a buffer between land and sea, protecting communities from natural disasters and erosion, and preventing pollutants and waste from entering waterways (Barbier, 2016). Because of this, the flow of debris between these ecosystems is fundamental to study in order to form a baseline of information for future studies on how microplastics affect coastal zone organisms.

This research attempts to compare all three habitats by collecting sediment samples from two different sites within each ecosystem type and analyzing them to find out what, if any, is the difference between microplastic concentrations in beach, mangrove, and shallow ocean sediments in Bahía Almirante, Bocas del Toro, Panama. This was done with the goal of discovering if there is a difference in the amount of microplastics present in these ecosystems to then identify if microplastics get trapped in a specific habitat within larger coastal zones.

Methods

Ethics

Care was taken at each step of the methodology to reduce the use of plastic and the creation of waste while reusing as many materials as possible. While this was difficult due to lack of resources it was still a primary concern. Additionally, all sample collection sites were disturbed as minimally as possible by taking care during collection to leave no trace. Only 6 L of sediment were collectively removed from each beach and mangrove site and any organisms accidently collected were returned to nature. The traps used to collect oceanic sediment were attached to thin pieces of rebar that were placed in sandy sediment as often as possible to reduce their cumulative impact on organisms such as seagrass or coral. It was not found that any of the sediment collection processes impacted the research sites past the time of collection.

Site Description

Figure 1: Map of sample collection sites in the Bocas del Toro Archipelago. Beach sites are marked in yellow, mangrove sites in green, and ocean sites in purple.

The Bocas del Toro province of Panama is located in the most southwestern corner of the country along the Caribbean coast. To the northwest lies Costa Rica and to the east is the province of Veraguas. Bahía Almirante and the Laguna de Chiriquí combine to create the largest estuary on the Caribbean coast of Central America. This estuary contains the six major islands of the Bocas del Toro Archipelago, several smaller islands, numerous mangrove cays, and a narrow continental shelf, making it home to many important habitat and organisms (Meylan et al., 2013). Bahía Almirante is relatively isolated from oceanic influences as it is enclosed by the mainland, islands, and mangroves, and contains no significant passageways into the Caribbean Sea or the Laguna de Chiriquí (Kaufmann and Thompson, 2005). This is an interesting place to study microplastics not only because of this separation and the unique ecosystems and biodiversity, but also because of the complex interactions between humans and the environment here. There is a recently instated plastic bag ban and a current movement to ban plastic bottles, however there is also a high presence of consumption and therefore waste due to the large tourism industry. Additionally, many studies have shown that there is not necessarily a direct correlation between higher human populations and higher plastic concentrations (Alomar et al., 2016; Laglbauer et al., 2014; Reisser et al., 2013). Based on this, even areas within the Bocas Archipelago with low human impacts are still likely to have plastic contamination.

Beach Sediment Sampling

The beach sediment samples were collected at La Playita and Punto Carenero. Both are located along the Isla Carenero Trail on Isla Carenero. La Playita is a small beach 45 meters long and 2 meters deep on the eastern side of Carenero about 500 meters from the Vista Azul Resort. The sediment was course sand and the beach was covered with mossy green and white algae. At the time of sample collecting, the water was calm with few waves and only 1-3 pieces of trash were noticed. Punto Carenero is also located on the eastern side of Carenero and is 60 meters long and 4 meters deep. The sediment was fine sand and during sample collection the water was rough. Countless pieces of trash were noted at the top of the beach along the Isla Carenero Trail and on surrounding beaches.

Figure 2: Transect line along the wrack line at Punto Carenero during the first day of sample collection

At each beach, three samples were taken on two separate days for a total of six samples per site. Specific sampling locations for each beach were chosen by running a transect along the entire length of the beach and dividing it into equal segments. On the first day of sample collecting, the beach was divided into four equal parts, with samples taken at the first, second, and third quarter markings. The second day of collection the beach was divided into six parts, with samples taken from the first, third, and fifth markings. This was done to eliminate the possibility of sampling the same exact spot on the beach twice. A sample area of 25 cm x 10 cm x 4 cm (1 L) was taken from the wrack line, as this is the highest point of high tide and where microplastics should be most dense (Startain et al., 2018).

Each sample was then sifted through a stacked arrangement of 5 mm and 0.25 mm metal sieves, using water to ensure all solids were transferred through. Any solid larger than 5 mm and smaller than 0.25 mm was discarded while the remaining materials were placed in an aluminum tray and dried in a 90^{o}C drying oven for 17 hours. Once dried, each sample was weighed and then transferred into a covered container (Masura et al., 2015).

A flotation method to remove sediments was applied by adding a 300 g/L saline solution (Masura et al., 2015) to each sample to create a 1:2 ratio of sample to solution (Ng & Obbard, 2006). The sample was agitated using a metal spoon for two minutes and then left to settle for 6 hours (Ng & Obbard, 2006). As many of the floating solids as possible were removed and saved, while the remaining sediments and solution were placed in a pot. The materials in the pot were stirred and allowed to settle for one hour, and any remaining floating materials were removed, saved as a 13th sample, and all non-floating solids were discarded. This was done as a precaution to make sure that no floating materials were missed, as it was difficult to remove floating solids from each sample while in their containers.

Once all floating solids were collected, 20 mL of one molar NaOH solution and 20 mL of 5% acetic acid solution were added to each sample to hydrolyze any remaining organic and carbonate materials. Each sample was checked after 4 hours. If natural materials were still visible, an additional 20 mL of 2-molar NaOH solution was added and the samples were left to sit overnight for 14 hours. If natural materials were still present after 14 hours, water was added to these samples to equalize their volume and pure NaOH was added to bring the concentration to 2.4 g/20 mL to create a 3-molar solution. These samples were left to sit for a final 2 hours.

Next, a second flotation was implemented in order to float all microplastics to the top of the sample. To increase the salinity, 6 g of salt per 20 mL volume was added to each sample. The samples were stirred to dissolve the salt and then allowed to sit for 24 hours. After 24 hours, all floating materials were removed using a metal spoon and tweezers as needed and drained through a coffee filter. Each filter was placed on a petri dish and allowed to air dry overnight, loosely covered in aluminum foil (Masura et al., 2015).

Each sample was examined under a light microscope at 40x magnification to identify any microplastics present. Particles were identified as plastic based on the following criteria (Nor & Obbard, 2014):

1. Size is between 5 mm – 0.25 mm
2. No visible cellular or organic structures
3. Fibers are equally thick throughout their entire length and are not tapered or frayed at ends
4. Particles are homogenously colored
5. Particles are not segmented, or appear as twisted flat ribbons

Potential microplastics were visually verified under 100x and 400x as needed. The number of microplastics as well as their length (0.25 – 1 mm, 1 – 3 mm, or 3 – 5 mm), color, and type (primary plastic, microfiber, or microfragment) were recorded.

Mangrove Sediment Sampling

Mangrove sediment samples were collected on two separate mangrove islands in Cayo Coral, located southwest of Isla Bastimentos. Both were islands comprised of *Rhizomorpha mangle*, the first about 150 m x 500 m in size, and the second about 75 m x 200 m. The first site was not muddy, had ankle deep water, and abundant new growth. Only four pieces of trash were noted, and leaf litter covered most of the ground making it easy to walk on. The second site had knee deep water and was slightly muddier than the first. In the center of the island was a patch of grass about 7 m in diameter. There was also a dock built into the island, however it did not seem to be in use. This

Figure 3: (Left) Mangrove sediment sample collection process; (Right) A mangrove sample during the first flotation process to remove sediments

second site exhibited less new growth than the first, however more trash was noticed, especially on the second day of sample collecting.

In each mangrove location, three samples were taken each day over two days for a total of six samples per site. Specific sampling locations were chosen by walking away from the drop off point in a randomly chosen cardinal direction for 10 m, then 10 m in another randomly selected direction, and repeated one more time. A sample area of 25 cm x 10 cm x 4 cm (1 L) was taken from each point using a knife to cut through any roots and a spoon to scoop up any water above the hole.

Each sample was then drained through a stacked system of 5 mm and 0.25 mm sieves. Water was used to remove any sediment trapped in the roots and to transfer all solids through the sieves. All solids larger than 5 mm and smaller than 0.25 mm were discarded. The remaining solids were placed in an aluminum tray and dried for 17 hours in a 90^{o}C drying oven. Once dried, the samples were transferred into a covered container and weighed.

After this, the same series of sediment removal, hydrolyzation, microplastic flotation, and analyzation as applied to the beach sediment samples was applied to the mangrove sediment samples.

Oceanic Sediment Sampling

Passive sediment traps, rather than active manual sediment sampling, was used to collect shallow oceanic sediments. Each sediment trap consisted of a 40 cm x 2 in PVC tube with a cap on the bottom end tied to a 1 yd long rebar. The first set of sediment traps was placed at Punto Hospitál, located on the northwestern tip of Isla Solarte, about 200 m off shore. The ocean habitat and floor consisted mainly of dead coral, with a few large brain corals and a reef on the northwestern end. The southeastern end was shallower and sandier. On both the sediment trap placement and collection days, there were strong currents and large swells with low visibility of about 2-4 m. The second site was La Playita, located on the eastern side of Isla Carenero. The sediment traps were placed about 200 m off shore in between two seagrass meadows on sandy sediment. On the day the traps were placed, the current was light and there was high visibility. On the collection day there was low visibility of about 1 – 2 m.

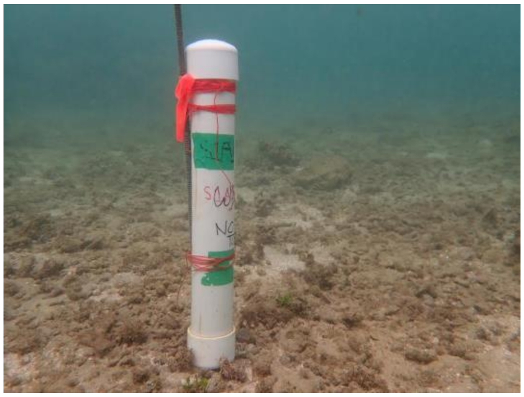


Figure 4: A closed, full sediment trap moments before removal

At each site, six sediment traps were hammered into the ocean floor at an average depth of 2 m. The traps were placed 10 m apart from each other, parallel to shore to reduce depth variability. The traps were collected three days after placement. Each trap was capped underwater before removal to keep any sediment kicked up during the removal process from entering the trap.

The contents of each trap were then poured through a stacked system of 5 mm and 0.25 mm sieves. Water was used to rinse out each trap to ensure no sediments were left and to transfer all solids through the sieves. Anything larger than 5 mm and smaller than 0.25 mm was discarded, and the remaining solids were put into an aluminum tray and dried at 90^{o}C in a drying oven for 17 hours. The dried solids were then transferred into a covered petri dish and weighed.

The same processes of hydrolyzation, microplastic flotation, and analyzation as applied to the beach and mangrove sediment samples were then applied to the oceanic sediment samples.

Results

Of the beach sediment samples and ocean sediment samples 100% contained microplastics, while 91.67% of mangrove sediment samples contained microplastics. The single mangrove sample in which no plastics were found contained very high levels of organic material that were unsuccessfully hydrolyzed during processing, thus making it possible that plastics were present but not able to be found.

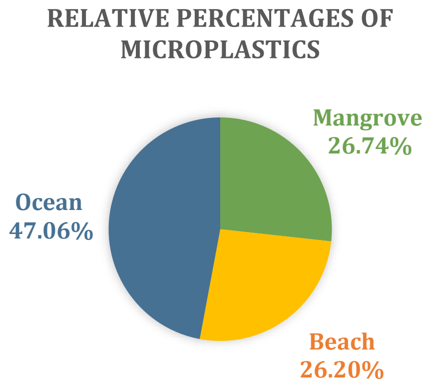
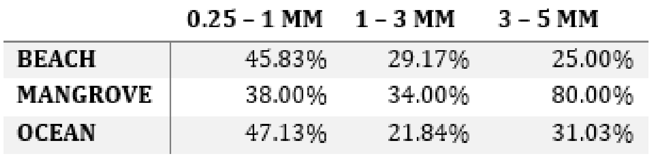


Figure 5: Percent of microplastics per habitat of the total number of microplastics found

In total, 187 microplastic particles were identified across all three habitat types. Out of the 187, 26.2% were found in beach sediment, 26.74% were from mangrove sediment, and 47.06% were from oceanic sediment.

Secondary microfibers were the most prevalent type of microplastic found. In the beach sediment, 65.31% of microplastics were fibers, while 80% of mangrove microplastics and 63.64% of ocean microplastics were fibers. In total, 68.45% of all microplastics identified were microfibers and 31.55% were microfragments. No primary microplastics were found.

Overall, microplastics sized between 0.25 – 1 mm were the most common, however this was only specifically true for the beach and ocean sediments, which contained 45.83% and 47.13% microplastics within this range, respectively. Mangrove sediment mainly contained microplastics in the category of 3 – 5 mm, with 80% of identified plastics falling within this size range.



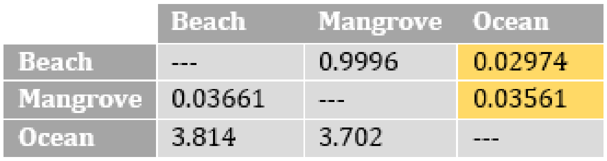
	0.25 – 1 MM	1 – 3 MM	3 – 5 MM
BEACH	45.83%	29.17%	25.00%
MANGROVE	38.00%	34.00%	80.00%
OCEAN	47.13%	21.84%	31.03%

Figure 6: Table showing the percentages of microplastics found per habitat type in each of three size range categories

The average number of microplastic particles per gram of dry weight was also calculated per habitat type. Beach sediment samples contained an average of 0.0054 ± 0.0063 particles/g, mangrove sediment samples contained an average of 0.69 ± 1.22 particles/g, and ocean sediment contained an average of 72.71 ± 114.97 particles/g.

Statistical Analysis

A one-way analysis of variance (ANOVA) was run to compare the microplastic concentrations between beach, mangrove, and ocean sediment to determine overall significance; a p-value of 0.018 was reported for α = 0.05.



	Beach	Mangrove	Ocean
Beach	---	0.9996	0.02974
Mangrove	0.03661	---	0.03561
Ocean	3.814	3.702	---

Figure 7: Results from post hoc Tukey HSD test with significant comparisons noted in yellow

When the ANOVA results showed significant difference, a post hoc Tukey HSD test was calculated to compare all possible pairs of habitats to reveal specific points of statistically significant differences, the results of which can be found in Figure 7.

Discussion

Interpretation of Statistical Analysis
The ANOVA showed that there was a significant difference between microplastic concentrations at the $p < 0.05$ level for the three conditions [F(2, 30) = 4.604, p = 0.018]. Since this significance was found, the post hoc Tukey HSD test was run to determine which concentrations were significantly different. The Tukey test showed that the mean concentrations in beach (M = 0.0054, SD = 0.0063) and mangrove (M = 0.69, SD = 1.22) sediments were significantly different from water sediment (M = 72.71, SD = 114.97). However, there was no significant difference between the beach and mangrove microplastic concentrations. Taken together, these results suggest that one would expect to find higher concentrations of microplastics in oceanic sediment than in beach or mangrove sediment in Bahía Almirante.

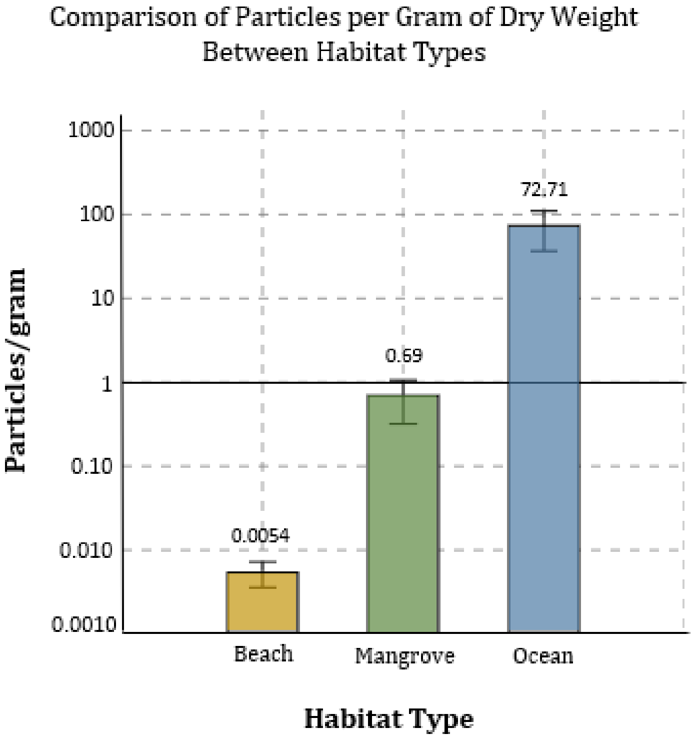


Figure 8: Comparison of microplastic particles per gram of dry weight between the three habitat types. A log scale was used to prevent the graph from being skewed towards outlying large values

Larger Implications
One of the main objectives of this research was to identify if one or more habitat exists within coastal zones in Bahía Almirante and the Bocas del Toro Archipelago that microplastics get trapped in. Based on the statistical analysis, it seems as though microplastics centralize in the ocean rather than on land since oceanic sediment had significantly more particles/g than beach sediment. Additionally, mangrove islands do not appear to act as a filter for microparticles, for if they did it would be expected that mangrove concentrations would be higher or at least equal to that of surrounding water.

It is also interesting to note that despite direct anthropogenic inputs of trash and plastic on land, beaches did not have significantly more microplastics than mangrove islands. This may imply that the main sources of plastic contamination on beaches are larger or whole, undegraded plastics rather than microplastics. Observations of several pieces of trash at La Playita and large amounts of trash at Punto Carenero support this theory. Trash included plastic bottles, plastic and metal lids, cans, and food wrappers. Similarly, no noted oceanic trash (apart from one floating diaper) was noticed at either sediment trap site, which, coupled with the significant difference in plastic particle concentrations between beaches and ocean sediment, may imply that microplastics are the main source of plastic debris in the ocean.

However, looking only at microplastics, the statistic that 100% of particles identified were secondary microplastics shows that the dominant source of microplastics across all habitat types is due to plastic degradation rather than direct deposits. This is consistent with previous research in the Western Tropical Atlantic Ocean and in the Gulf of Mexico where the majority of microplastics identified in beach samples were secondary (Ivar do Sul et al., 2014), and 100% of microplastics in beach sediment were secondary (Wessel et al., 2016), respectively. This prevalence is common in tropical areas as plastics and other waste are exposed to higher temperatures and more extreme degradation conditions (Andrady, 2011).

Figure 9: (From left to right) A blue microfiber; a pink microfragment; and a clear, biofouled microfragment attached to a piece of organic material

The findings of this research are congruent with reports from similar parts of the world in terms of types of microplastics found, however, when comparing concentrations found in beach samples from these studies, the concentrations in Bahía Almirante are much lower. In the Equatorial Western Atlantic Ocean, 194 plastic particles were found across 13,708 g of beach samples, leading to a concentration of 99 particles/g, over 18,000 times higher than those found on the Bocas del Toro beaches (Ivar do Sul et al., 2009). In the Gulf of Mexico, sampled beaches along the ocean contained 50.6 ± 9.96 particles/m^2 (Wessel et al., 2016). Our data is reported in particles/g, however Wessel et al. took sample areas of 25 cm x 25 cm x 3-6 cm, while our sample areas were 25 cm x 10 cm x 4 cm. Wessel et al. disregarded depth and reported in m^2, so doing the same for our data (since they are approximately the same depth) we found 150.78 ± 88.17 particles/m^2. This is about 3 times larger than concentrations found in the Gulf of Mexico.

A study of environmental matrices in mangrove ecosystems in Ecuador found similar microparticle distributions to this study. Their research concluded that 76.9% of microplastic contamination found was from water, versus 11.2% from intertidal sediment and 11.9% from bed sediment (Domínguez et al., 2017). This aligns with our findings that water in coastal zones contains significantly more microplastics than near-by mangrove bed sediment.

Alternatively, compared to previous research, mangrove concentrations were much higher than those found in mangrove sediment in Singapore where concentrations of 9.2 ± 5.9 particles/250 g dry weight were reported (Nor and Obbard, 2013). Converting the data found in Bocas del Toro mangrove islands in Cayo Coral to particles/250 g shows concentrations of 171.49 ± 305.34 particles/250 g; this is about 19 times larger than in Singapore. This is even more interesting when it is considered that the Singaporean study looked at a much larger size range – from 0.04 - 5 mm versus the 0.25 - 5 mm range in our study – and that 58% of microplastics found were smaller than 0.04 mm. On the other hand, there is a point of congruity with this research in the types of microplastics. In Cayo Coral, 80% of microplastics found were microfibers, and in Singapore, 72% were microfibers, both representing the majority of particles identified.

Comparing the concentrations in ocean sediment to past studies is a bit more difficult, as most of the research found analyzes plankton net drags and water concentrations instead of sediment concentrations, and thus reports findings in particles/km^2 instead of by mass. While the beach sediment samples could be converted to m^2 and then if needed, km^2, the same cannot be done with the ocean sediment samples, as they were collected in 1 L sediment traps, only small portions of which were sediment that was analyzed. However, one study with congruent units was found analyzing bottom sediments in the Baltic Sea. There, concentrations of 34 ± 10 items/kg were reported, which is over 2,000 times smaller than Bahía Almirante concentrations when converted from particles/g to per kg. However, microfibers were the most prominent type of microplastic found in both locations. The researchers in the Baltic Sea explain that this dominance may be due to the physical properties of microfibers in comparison to different types of microplastics (such as fragments or films) and the way in which these properties interact with currents and sedimentation. In the Baltic Sea, more microfibers than microfragments were found closer to shore. This is potentially because higher current velocities, like those found in deeper water, are required to transport microfragments, versus shallow water's slightly slower velocities required to transport microfibers (Zabkov and Esiukova, 2016).

Additionally, it should be noted that concentrations of 1414 ± 112 pieces/km^2 were found in a study that compiled 22 years' worth of data from ship tows in the Caribbean Sea. This was similar to concentrations in other areas close to land, such as the Gulf of Maine (1534 ± 200 pieces/km^2), whereas concentrations farther from land were higher, like at 30^{o}N (along the same latitude as Florida) where 20,328 ± 2324 pieces/km^2 were found (Law et al., 2010). If this trend is consistent, it could be concluded that microplastic concentrations in Bahía Almirante are generally lower in comparison to other open ocean locations, as the bay is largely enclosed. This again highlights the need for standardized reporting in the world of microplastic research in order to facilitate meaningful and accurate comparisons and conclusions.

Overall, when comparing this research's findings to reports from similar parts of the world or similar habitats, it seems as though the types of microplastics most prominent (secondary and microfibers) are comparable, whereas the actual concentrations of microplastics per habitat differ, sometimes with large orders of magnitude. These differences can potentially be explained by the geography of Bahía Almirante. The bay is bounded by a coastal swamp and mangroves to the northwest, the Panama mainland to the southwest, and the Bocas del Toro Archipelago on the north and southwestern sides. Because of this, there is restricted watershed and limited circulation from the Caribbean Sea or even with the adjacent Laguna de Chiriquí (Kaufmann and Thompson, 2005). Overall, these factors create a relatively closed system with low oceanic influence, potentially altering the flow of microplastics and thus making concentrations within the bay different from concentrations in similar areas and habitats, be in on beaches, in mangroves, or in water.

Possible Sources of Error

Due to the nature of ecology, the natural world, and science in general, there was absolutely room for both environmental and human error. On both beach sample collection days it was raining, which not only could have altered the contents of the samples but made it difficult to standardize the sample sizes. Additionally, a few samples were lost to the ocean – one sediment trap quite literally disappeared in the days between placement and removal, while another spilled in transport – and another mangrove sample's container shattered during processing. This could have affected concentration averages as some data was not able to be collected.

Human error was present during the research process as well. Due to a lack of equipment and lab access, it was difficult to follow previously tested and confirmed methodologies (i.e. NOAA's *Laboratory Methods for the Analysis of Microplastics in the Marine Environment*). This meant that methods had to be adjusted in real time because it was impossible to anticipate before hand what materials would be available, meaning that some steps of sample processing – mainly organic material hydrolyzation and microplastic flotation – may not have been executed as precisely or worked as well as they could have with unlimited resources.

Similarly, a lack of experience with microplastics could have led to the misidentification of particles as microplastics or, alternatively, some particles that were plastic could have been overlooked. The methodologies being followed relied on visual inspection of floating solids to ensure that nothing was missed, however, microplastics are microscopic and therefore it is possible that not all plastics were removed from the original samples. Some plastic particles may not have even floated to the surface in the first place if they were excessively biofouled or if they were attached to more dense sediment.

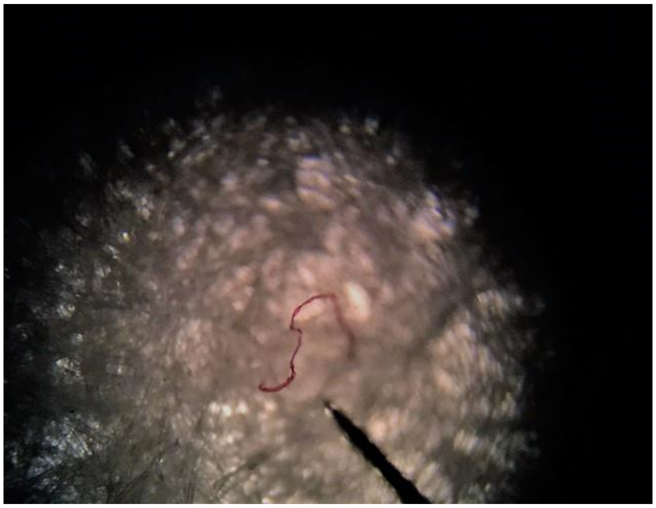


Figure 10: An example of a pink microfiber that was most likely a piece of contamination from a shedding hand towel

Finally, contamination was an issue across all habitats' samples. The culprit: a pink hand towel, bought in a time of need, now infamous for shedding its brightly colored fibers everywhere. Samples were covered for as much of the processing time as possible, however this was clearly not enough, as 69.32% of samples contained microfibers that most likely were sourced from said towel. Overall, 50 of these pink fibers were found, and while it was impossible to confirm which came from the towel and which were in the samples originally, anything that resembled a towel fiber (in comparison to a fiber taken directly from the towel and viewed under the microscope) was not counted towards the particles/g average per habitat. Other, less certain sources of contamination could have come from the plastic containers and petri dishes that had to be used since glass was not available, and from other microparticles floating in the air or from human contact.

Recommendations and Future Research

The main recommendation on which to improve this research would be to conduct it while having access to a laboratory and to unlimited use of equipment. Plastic containers and other plastic products had to be used at several points during the sample collecting and processing, which may have resulted in contamination, because there was not access to the correct quantity or size of glass containers. Additionally, a better hydrolyzing agent would have been useful (such as 30% hydrogen peroxide, as recommended by NOAA), as the hydrolyzation methods that were created on site did not fully remove all organic and carbonite materials.

Future research should include a confirmation of this study with more ideal conditions. Along with that, these findings will hopefully lead to further direct comparisons within coastal zone and between larger marine habitats to continue to identify specific ecosystems in which microplastics are most prevalent. More research should particularly be conducted in mangrove ecosystems, as there is currently very little information on microplastic concentrations within them and as they are important ecological hotspots; microplastics in these habitats have the potential to have high ecological impacts.

Finally, as most answers do, the findings of this research only lead to further questions. What are the main sources of microplastics on land? In oceans? In mangroves? Are there differences in microplastic concentrations between mangrove islands and land-locked mangroves? Are there differences in microplastic concentration in oceanic sediment versus the water itself? How are microplastic concentrations in Bocas del Toro and in the Caribbean changing over time? How are these concentrations affecting marine organisms of all types and sizes? How are individual habitat types within larger marine ecosystems connected to each other and to ecosystems around the world? Finding the answers to these questions will not be easy but will be integral to the continual study of microplastics and their greater impacts over time and space.

Conclusion

This research undoubtably proves the existence of microplastics in beach, mangrove, and ocean sediments in Bahía Almirante and in the Bocas del Toro Archipelago, Panama. When comparing microplastic concentrations between habitat types, ocean sediments contain significantly more microplastics than the beach or mangrove sediments, whereas beaches and mangrove islands contain statistically similar concentrations. Secondary microplastics and microfibers are the most prevalent types of microplastics found, which is consistent with previous research on beach and mangrove microplastic concentrations. The prevalence of secondary microplastics points to plastic degradation as a main source of contamination, rather than direct plastic inputs. Differences in microplastic concentrations in habitat types in Bahía Almirante when compared to past studies are potentially explained by the bay's geography as a relatively closed system, separated from large oceanic influences.

These findings are important as they emphasize that marine organisms, especially those living directly in water, are at risk of being harmed by the mal-effects microplastics can cause. These include, and are not limited to, organ blockages by ingestion, entanglement leading to injury or death, and illness or death from ingesting toxins either present in plastics or that have been concentrated on plastic surfaces from surrounding water. Due to the small size of microplastics, they are essentially impossible to effectively eliminate from any habitat type. While some larger pieces of plastic and trash can be removed to prevent them from degrading into micro sizes, the impacts of contamination will still exist. It would be a cop out to say that the take away from this research is that removal methods must me improved upon. Instead, it is vital to reduce plastic use and consumption and to find biodegradable but still commercially viable alternatives to plastic. This anthropogenic creation should not exist in natural habitats, but it is too late to reverse this action. The next best option is to halt the increase of marine microplastics and waste contamination by emphasizing reduce and reuse over recycle and refuse, and by minimizing the creation of waste in all aspects of life.

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