Microplastics Presence in *Rhizophora mangle* Roots throughout Fishponds and Open Coasts in Moloka'i, Hawaii

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Microplastics Presence in *Rhizophora mangle* roots throughout Fishponds and Open Coasts in Moloka‘i, Hawaii

By

Mia Hackett

An undergraduate honors thesis submitted in partial fulfillment of the requirements of the degree of Bachelor of Science in University Honors and Environmental Sciences

Thesis Advisor
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Portland State University
2023
Abstract
Microplastics (MP) are an emerging global contaminant that has drawn the attention of many researchers in the last few decades due to their growing environmental threats. Pieces of plastic smaller than 5 mm, they are specifically a high cause for concern in marine environments as their small size allows them to flow from inland into the ocean. Through this movement, MPs have been found in all marine ecosystems and ingested by hundreds of marine species often mistaking them for food. Labeled as one of the most threatened ecosystems, mangrove forests are already a large sink for a variety of contaminants now including MPs as they may be trapped within the sediment by mangroves’ unique aerial roots. As mangroves grow in coastal zones, these contaminants are often anthropogenic coming from both land and marine-based activities. Fishing activities have recently begun to be researched and associated with MP abundance, but one type of fishing, fishponds, has had no research done to understand MPs' presence. Fishponds are a tool used for a specific kind of seawater farming that take advantage of and utilizes the natural resources of the ocean's tidal movements to control the exchange of water and sediment build-up. This study focused on the fishponds spread throughout the island of Moloka’i Hawaii to evaluate the effect fishpond presence has on microplastics in Moloka’i Hawaii. The combination of mangrove roots' ability to trap sediment and fishponds' use of the ocean to control sediment flow may have an impact on the MP presence at these sites compared to open coasts. Therefore this study was conducted to evaluate how MPs found in the mangrove roots of Moloka’i, Hawaii are affected by the presence of fishponds versus along the open coast. Four to five hour digestions at 50 °C using Sodium hypochlorite were used to digest the organic matter of collected root samples, separated as coarse and fine roots. No statistical significance was observed for coarse or fine roots between open coasts and fishponds (p-values of 0.4723 and 0.7812).

1. Introduction
1.1 Microplastics in the Marine Environment
Since the mass production of plastic in the 1940s (Cole et al. 2011), the concern for plastic in the environment has risen. The durability of plastic makes it a favored material for many consumers. This same durability makes most plastics non-biodegradable (Tokiwa et al. 2009) leading to mass accumulations that negatively impact the environment. When exposed to ultraviolet light, these accumulated plastics fragment into smaller pieces, and eventually into microplastics (MP’s) (Auta et al. 2017). Defined as pieces of plastic less than 5mm in diameter (Arthur e al. 2008), MP’s have become an important emerging topic of research. They are categorized into two groups, primary and secondary. Primary MP’s are intentionally created plastic particles found in many daily used products such as facial cleansers, cosmetics, or dyes. Secondary MP’s result from plastic fragmentations (Maghaosian et al. 2022), through weathering.

Recently particular concern has been placed on understanding the effect MPs have on marine ecosystems. MP’s threaten marine ecosystems due to their microscopic size. With 80% of marine plastics believed to be derived from terrestrial origins, fragmented plastics can easily flow into marine ecosystems through the unidirectional flow of rivers (Mattson et al. 2015). Lebreton et al. (2017) found that at least a million tons of MP’s are entering the ocean from inland rivers annually. Once MP’s enter the marine environment they are mistaken for food (Jinadasa et al., 2022) by many organisms due to their shiny appearance or by filter-feeding
organisms; their ingestion may lead to bioaccumulation (Wayman & Niemann, 2021) and chemical leaching (Mattson et al. 2015).

The larger the quantity of MPs consumed by a species, the larger the harm to the species. Therefore, the larger the quantity of MPs consumed by a species, the larger the harm to the species. There are currently three main concerns for marine species ingesting MPs regularly. One, ingesting MPs can alter feeding habits as organisms feel full despite having consumed nothing beneficial to their health leading to reduced development. Two, the leaching of additives (e.g. flame retardants, antioxidants, plasticizers) from the plastics into the organism's tissues can cause adverse health effects resulting in death if concentrations are high enough. Three, through weathering MPs can absorb more harmful contaminants on top of the additives negatively impacting the marine species ingesting them (Issac & Kandasubramanian, 2021). Specifically, as endocrine disruptors, persistent organic pollutants (POPs) are large contaminants of concern affecting organisms' biological functions including reproduction (Johnson et al., 2013).

1.2 Microplastics in Mangrove Forests

Mangrove forests grow in intertidal zones in tropical and subtropical latitudes acting as a buffer between the land and sea. While these forests only make up 5% of the world's coastal zone, (Alongi 2014), they contribute to the environment by protecting coastal zones from natural disasters; by being an estuary for many organisms, they are labeled as one of the most biodiverse ecosystems; and as a blue carbon storage source, they sequester about 24 million metric tons of carbon per year (Twilley & Rovai 2019).

Mangroves' unique aerial roots, roots that grow aboveground, allow the trees to live in unstable and anaerobic soil conditions at the edge of coastal zones (Nguyen et al. 2023). The aerial roots of mangroves also heavily contribute to increased water quality, sediment deposition, and reduce coastal erosion. The entanglement of the dense roots filters out contaminants (e.g. nitrates, phosphates) improving water quality as it flows out of the ecosystem. With aerial roots to slow down water flow, increased sediment deposition reduces coastal erosion (The Nature Conservancy 2020). Because of this movement, Duan et al. (2021) showed how increased accumulation through slowed water velocity also increased the buildup of MP's present. Their research suggests that mangroves may have comparatively high MP abundances. Furthermore, since mangroves act as a buffer between the land and sea they are subject to both terrestrial and marine activities that produce anthropogenic contaminants, including MPs (Weng et al. 2022). Therefore mangroves may be exposed to a greater number of MPs compared to other ecosystems as well as possessing a unique ability, through their roots, to trap those MPs into their environment.

The higher abundance of MPs present would result in a greater abundance of MPs ingested by the species living in mangrove forests. The ingestion of these MPs, and consequently the additional contaminants associated with them, can lead to drastic changes in feeding habits, reproduction, and general health of a species leading to death if contaminant toxins are high enough (Issac & Kandasubramanian, 2021). The loss of biodiversity that comes with species decline would negatively impact the mangrove's ability to function as it naturally does, altering the whole ecosystem. Furthermore, it would also lead to a decline in the ecosystem services mangroves provide (Masoud & Wild, 2004) including protecting coastal cities from natural disasters, storing blue carbon, and improving water quality by filtering out contaminants.
1.3 The Mangrove Forests of Molokaʻi Hawaii

Mangroves were introduced to the island of Molokaʻi, Hawaii in 1902 by the American Sugar Company to mitigate sediment transportation (Möhlenkamp et al. 2018). Seven taxa were reported as introduced, but now only two remain with the *Rhizophora mangle* (the red mangrove) being dominant. While not native, Hawaii’s favorable climate combined with the opportunistic nature of mangroves allowed them to spread to multiple islands, including all the main islands (Wester 1981). Through mangroves' widespread dispersal, they have grown on the coastlines where fishponds are located. (Fig. 1)

1.4 Molokaʻi Fishponds

Fishponds on Molokai are controlled reservoirs of seawater used for aquaculture that utilizes the natural resources already present in the ocean. A seawall of lava rock or coral forms a semicircle around the shore on a reef flat, allowing natural marine biota to remain. Movable gates (*makaha*) are built into these seawalls. The *makaha* is opened to allow fish to migrate in and then is closed when they try to migrate out, trapping them inside. (Fig 1)

At its peak, Hawaii had 488 fishponds spread throughout the islands (McDaniel 2018), producing almost 2 million pounds of fish per year (Tengan, 2023); however, the changes brought about by Westerners (e.g., a money economy and new diseases) in 1778 caused drastic decreases through disuse and disrepair (Costa-Pierce 1987). Prior to this disrepair, the island of Molokaʻi had the highest abundance of seawater ponds per area of land compared to the rest of Hawaii. In recent years there has been a push to restore the disrepaired fishponds and use them to serve the community once again. While fishpond abundance remains lower than it historically once was, as of 2017 thirteen fishponds have been restored statewide, six of which are in use, and of those six, three are located on Molokai (EPA, 2017).

As Hawaii continues to repair and use their fishponds it is important to evaluate the impact MPs may have on them. The mangroves present at the fishponds make this situation unique as Dual et al. (2021) study suggested that the mangrove's aerial roots slow down the velocity of water allowing MPs to build up in the trapped sediment (Duan et al. 2021). The MPs caught in the mangrove's sediment and roots may be mistaken for food (Jinadasa et al., 2022) by the fish caught in the fishponds. Fishponds are being repaired with the intent to help lower food scarcity throughout the islands (Tengan, 2023); however, the ingestion of MP’s by the captured fish may lead to negative health consequences for people ingesting them (Jinadasa et al., 2022). Therefore this study, as the first of its kind, aims to help understand how the natural landscape (coastlines) and traditional fishing practices (fishponds) affect MPs present in the mangrove forests through Molokaʻi, Hawaii.

**Fig 1.** A diagram representing the structure of the fishponds on Molokaʻi with mangroves present. The *kuapa* (seawall) forms a semi-circle around the coastline while the *makaha* (gate) opens and closes allowing the fish to swim in and then trap them. The back line of plants represents where the mangroves are on the coastline in relation to the fishpond.
2. Materials and Methods

2.1 Study Area

All mangrove root samples were collected from the island of Moloka‘i, located in the center of Hawaii’s eight islands. Three root clumps were collected by hand per transect at 1-3 transects per site during the collection of samples in Hawaii in March of 2022 from a total of eleven sites; five sites were from locations with fishponds and seven from locations along the open coast (Table 1; Fig. 2). Fishponds are semi-enclosed or fully enclosed aquaculture areas in various states of use/disrepair and open coast sites are locations not protected from the sea by any structures. Once collected, the root samples were placed in aluminum foil, labeled, and frozen for later MP extraction and analysis.

**Table 1.** Location names for sites and transects where roots were collected from fishponds locations and open coast.

<table>
<thead>
<tr>
<th>Fishpond Site</th>
<th>Pualoa</th>
<th>Keawanui</th>
<th>Ali‘i</th>
<th>MS/KE</th>
<th>Kupeke</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transect</td>
<td>Sea</td>
<td>Culvert</td>
<td>Sea</td>
<td>Sea</td>
<td>Sea</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Open Coast Site</th>
<th>Newport</th>
<th>Newport</th>
<th>Niaupala</th>
<th>Mile 9</th>
<th>Mile 9</th>
<th>Coconut Grove Farms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transect</td>
<td>Land</td>
<td>Sea</td>
<td>Sea</td>
<td>Land</td>
<td>Sea</td>
<td>Sea</td>
</tr>
</tbody>
</table>

**Fig 2.** Sample locations for fishponds (x) and open coasts (circles) throughout Moloka‘i, HI.
2.2 Microplastics Safety and Quality Control Protocols - Field and Laboratory

To avoid the introduction of microplastics (MP) to the samples post-collection, specific QA/QC measurements were taken in the field and in the laboratory. In the laboratory, cotton clothing was worn under pink lab coats as pink is a less environmentally observed MP color (Hidalgo-Ruz et al., 2012) so any pink MP’s found in the samples were labeled as procedural contamination.

Glass was used in place of plastic, when possible. All equipment was triple-rinsed with DI water through a 20-micron sieve. For the purpose of this paper, when equipment is stated as rinsed, that indicates that it was triple rinsed by DI water that had been passed through a 20-micron sieve. Once rinsed, equipment was immediately placed in the fume hood and covered, limiting the number introduced. When the samples were open in the fume hood, the hood was placed on emergency purge.

Airfalls and operational controls were used to quantify any accidental MP introduction. For the field, air falls of sieved DI water were placed out during the whole sample collection process at each location. Both control types consist of DI water placed in glass beakers covered with rinsed aluminum foil. Air falls were open any time the samples were opened and exposed; it accounted for any MP’s that fell onto/into the samples from the surrounding environment. Operational controls were mock samples that went through the same procedures as the samples without the roots present. They accounted for MPs that may be added to the samples through the processing and digesting of samples.

2.3 Sample Processing

Once ready for analysis, samples were removed from the freezer. A collection of metal sieves were used to separate the roots from the mud balls they were collected in. Two sieves, 250 and 125 microns, were placed on top of each other to catch the roots. The first-micron size collected coarse roots (CR) and fine roots (FR), and the second-micron size collected ultra-fine roots. Each root ball was placed on top of the sieves and rinsed with DI water to remove excess organic materials. Once the coarse and regular fine roots were fully rinsed off, the first sieve was taken off to separate the ultra-fine roots from small organic material. When all the roots were fully rinsed they were placed in metal weigh boats, wrapped in aluminum foil, properly labeled, and placed back in the freezer.

This procedure took place outside of the fume hood, as the consistent flow of sieved DI water and a place for the rinsed-off excess material were not available within it. An air fall was set up near the sink where rinsing took place and the time was recorded to account for how long each sample was exposed to the air. To prevent contamination between sites, the sieves were rinsed off between every sample. They were rinsed until no organic material from the root balls was left, then gently scrubbed with a cotton dish scrub, and then rinsed once more to remove any added cotton fibers.

2.4 Drying Separated Roots

Once placed in the fume hood, coarse and normal fine roots were separated from each other. A glass pipette was used to rinse off both root types and remove any surface MP’s that may have been added from the open exposure of the root rinsing procedure. Once fully rinsed, the separated roots were placed back in metal weigh boats and loosely wrapped in labeled foil. Each foil-wrapped root sample was placed in a drying oven for 24 hours at 100 degrees Celsius or until fully dried. A blank control with a weigh boat also wrapped in aluminum foil was placed
in the oven as well to account for any MP that might be added during this drying process outside the fume hood.

2.5 Digestion and Filtration

When the roots were fully dried they were removed from the oven to set up for digestion. Each sample was separated by coarse and fine roots. Fine roots are pieces thinner than a strand of hair while coarse roots accounted for the rest. The weight for each sample was taken and once weighed, each sample was placed in a rinsed 500 ml glass beaker covered with aluminum foil. 100 ml of household Clorox bleach was double-filtered through the 20-micron sieve and added to the beaker with the roots. Sodium hypochlorite (NaClO), the main ingredient of bleach, was responsible for digesting organic material. Lastly, a glass stir bar was added to the beaker helping with the breakdown of organic material. The beakers were placed on a five-spot hot plate for four to five hours at a temperature of 50 °C. The length of digestion depended on the speed at which the sample was digested. All samples were checked at four hours and if the root material was fully digested they were removed from the hot plate. If there was still material to digest, they were left on the hot plate for another hour.

Again a blank control beaker was set up, which received the same treatments and procedures as the samples but did not receive any sample matter. This control accounted for the introduction of MP from the digestion equipment and bleach solution.

A glass filtration apparatus was used to separate the bleach solution from the leftover undigested material. The apparatus was rinsed and then rinsed filter paper (MilliporeSigma, MF-Millipore, Membrane Filter) five microns in size was placed on it. Once the total solution had passed through the filter paper, it was placed in a rinsed petri slide. Depending on how well the solution filtered through, the sample was split between one to three slides for ease of microscopic visualization of MP’s later on. The same process of filtration was applied for all the air falls and operational controls from the past procedures.

2.7 Microscope

MP’s were detected through the use of a compound microscope. A snorkel hood angled above the microscope platform helped control the airflow around the microscope, preventing the introduction of new MPs. A blank microscope slide was laid open near the microscope to act as the control quantifying any MP in the air as the sample was open and exposed.

Under the microscope, the slides were read from left to right. If a potential MP was observed, light pressure was applied with a dental scaler. If it did not break and met all the other MP requirements, it was recorded as a MP. Additional questions asked to identify MPs include: Is the potential MP unequal in thickness thick throughout the whole fiber? Is the potential MP displaying heterogeneous color variation? If the answer is yes to any of these three questions, it is not counted as an MP. Other guidelines for identifying MP’s included a lack of visible cellular structure, a smooth appearance, and fibers having uniformity through their length, allowing for some fraying at the ends. However, there were exceptions to every one of these rules depending on the situation.

The microscope software Zeiss labscope was used to visualize the slides through a computer and record MP features. The software was used to take pictures of confirmed MPs. MP length, color, shape, and position were also recorded.
After each sample was inspected, the air fall microscope slide was looked through to account for any addition of MPs to the sample slide from the atmosphere as it was open. If a MP was observed, length, color, shape, and position were recorded for it as well.

2.8 Statistical Analysis

The dried root weights were standardized to 1.5 grams to run statistical analysis. Two single-factor ANOVA tests were used to compare 1) MP abundance between fishponds and open coasts and 2) MP length between fishponds and open coasts. The analysis was applied to both fine and coarse roots to compare how root size may affect MP abundance and length.

3. Results

High p-values indicate no statistical significance for MP abundance between fishponds and open coasts for both coarse (0.4723) and fine (0.7182) roots (Table 2). This is the same for MP length, there was no statistical significance observed between fishponds and open coasts for both coarse (p-value of 0.3916) and fine (p-value of 0.818) (Table 3).

Coarse roots had a higher abundance of MP than fine roots. For both fine and coarse roots 3-NPT-Land had the highest abundance of observed MP for open coasts transects and 10-PLA-Sea for fishponds transects. No MP’s were observed at 12-KPK-Sea for fine or coarse roots. For coarse roots alone no MPs were 4-MSK-Sea, 6-M9-Sea, 11-NIP-Sea, and for fine roots alone no MP’s were observed at 1-FMS-Sea, 3-NPT-Sea, 5-ALL-Sea, and 8-KWU-CVT. (Fig. 2)

The majority of observed MP’s in the transects were clear (88.56%), however, there was also a limited amount of yellow for fishponds and open coast (8.57%) and one orange for the open coast (2.86%) (Fig.4).

The mean MP length for AFs and OCs was lower than both fine and coarse roots for fishponds and open coasts. The length of microscopic analysis AFs was the lowest at 186.608 (Table 5), and AF and OC averages from sample processing, digesting, and filtering were at 327.611 and 358.314 (Table 4). Comparatively, MP mean for coarse roots was 564.759 for fishponds and 838.043 for open coasts, and for fine roots it was 973.294 for fishponds and 854.849 for open coasts (Table 3).

Table 2. N, mean, standard deviation, and p-value for MP abundance between locations with fishponds (n=5) vs the open coast (n=7) for both coarse and fine roots; all values were standardized to 1.5 grams per transect.

<table>
<thead>
<tr>
<th>Coarse Roots</th>
<th>Fishpond</th>
<th>Open Coast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>2.74137</td>
<td>4.52386</td>
</tr>
<tr>
<td>StDev</td>
<td>2.51922</td>
<td>4.84256</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.47228</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fine Roots</th>
<th>Fishpond</th>
<th>Open Coast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>2.34885</td>
<td>3.00711</td>
</tr>
<tr>
<td>StDev</td>
<td>4.24152</td>
<td>3.72652</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.78125</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Coarse Roots</th>
<th>Fishpond</th>
<th>Open Coast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>564.7591</td>
<td>838.0432</td>
</tr>
<tr>
<td>StDev</td>
<td>521.0284</td>
<td>776.823</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.3916</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fine Roots</th>
<th>Fishpond</th>
<th>Open Coast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>973.2946</td>
<td>854.8485</td>
</tr>
<tr>
<td>StDev</td>
<td>757.9568</td>
<td>1040.1347</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.8181</td>
<td></td>
</tr>
</tbody>
</table>
Fig 2. Observed MP abundance in coarse roots (A) and fine roots (B) after values were standardized to 1.5 grams per transect between sites with fishponds (solid bars) present versus the open coast (open bars) with SE set at 0.05. Transect sampling locations run west to east along the horizontal axis.

Fig 4. Observed MP colors between transects with fishponds (A) versus open coast (B). Coarse and fine roots were combined for a total of 14 MPs at fishpond transects and 21 MPs at open coast transects. The three colors found were clear, yellow, and orange.

Table 4. The average abundance and length of MP for all airfalls (n=19) and operational controls (n=12) during sample processing, digestions, and filtering.

<table>
<thead>
<tr>
<th>Airfall</th>
<th>Average</th>
<th>Operational Control</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abundance</td>
<td>0.68421053</td>
<td>Abundance</td>
<td>0.77777778</td>
</tr>
<tr>
<td>Length</td>
<td>327.61131579</td>
<td>Length</td>
<td>358.31391667</td>
</tr>
</tbody>
</table>
4. Discussion

**Microplastic Abundance**

The abundance of MP present per transect was low for both fishponds (ranging from 0-7) and open coasts (ranging from 0-6) (Appendix). In total 35 MPs were observed for both fine and coarse roots with 14 at fishponds and 21 at open coasts. The high p-values comparing the fishpond and opencoast values, 0.4723 for coarse roots and 0.7812 for fine roots, indicate no statistical significance between the two for either roots. The lack of statistical significance between sites may indicate the MPs in mangrove roots are derived from inland rather than oceanic. This parallels the statement that 80% of marine plastic is from terrestrial origin (Mattson et al. 2015).

Coarse roots had a higher abundance of MP than fine roots which is reflected in the MP per transect (Fig. 2). Coarse roots had MPs present at 8 of the 12 of the transects for a total of 21 MPs, while fine roots had them present at 7 of the 12 transects with a total of 14 MPs. Both roots had no MP present at the eastern most transect, 10-KPK-Sea, a fishpond transect; and both roots had the most MP present at 3-NPT-Land, an open coast transect on the western side. Along the western transects, coarse roots had the most MP abundance at 1-FMS-Sea, 2-CCG-Sea, and 3-NPT-Land all near the island's largest town, Kaunakakai. The higher abundance near this large town suggests a possible connection between population size and MP presence in mangrove roots connecting to the possibility that a larger portion of the MP abundance is coming from terrestrial origin rather than oceanic. As mangroves grow in the intertidal zone and are subject to both marine and terrestrial contaminates (Weng et al. 2022), follow-up research focusing on the derivation of MP in mangroves would be beneficial in preventing their introduction to the ecosystem from the source.

**Microplastic Length**

High p-values for MP length between fishponds and open coasts for both coarse (0.3916) and fine (0.8181) roots indicate no statistical significance between them (Table 3). This lack of difference might suggest that the MPs from both fishponds and open coasts are deriving from the same source.

The mean MP lengths between coarse and fine roots for Open Coast transects were similar, with only a 16.805 difference. Comparatively at fishponds coarse roots had a lower mean length (564.759) compared to fine roots (973.295). This may indicate that as mangroves uptake MPs at the ends of their roots, the finest parts, there might be something in the movement to up the coarse roots that breaks down the size of the MPs. As mangrove roots have the ability to help filter out contaminants such as nitrates or phosphates (The Nature Conservancy 2020), perhaps their roots have the ability to help break down these microscopic plastics. Further researching the difference between the mangroves fine and coarse for MP length would indicate the possibility of using these plants to help break down the MPs throughout coastlines.

<table>
<thead>
<tr>
<th>Airfall</th>
<th>Abundance</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>0.2051282 mp/slide</td>
<td>186.6079232</td>
</tr>
</tbody>
</table>

**Table 5.** The average abundance and length of MP for all airfalls (n=33) during microscope analysis.
**Microplastic Color**

Clear was the most common color observed in these MPs. 85.7% of the MP found at fishpond transects were clear while the remaining 14.3% were yellow. Similarly at open coast transects, 90.5% were clear. 4.8% were yellow and 4.8% were orange (Fig. 4). The color palettes observed for this study were insignificant in their differences between fishponds and open coasts. However, similar color patterns between all transects were also observed by Elisa Marti et al. (2020) who from a global collection of oceanic MPs found the top three most abundant colors to be white and clear (47%), yellow and brown (26%), and blue-like (9%) (Marti et al., 2020). Marti et al. (2020) also observed a smaller difference between colors with white and clear MPs at 47%, while the results of this study found a notably higher amount of clear plastics at 85.7% and 90.5%. A possible concern for the high amount of clear MPs observed in all transects is the use of sodium hypochlorite (NaClO), to digest the sample's organic matter. The use of bleach may have aided in stripping the color of the observed MPs. However, Monteiro et al. (2022) found no effect of the use of NaClO for digestion and found visual or surface structure changes to MP exposed to NaClO for 60 hours at 50 °C (Monteiro et al., 2022). These results also collaborate with Pfeiffer and Fisher (2020) who found a slightly insignificant effect on MP sample weight when digesting at 40-50 °C (Pfeiffer and Fisher, 2020). These studies demonstrate that following the methodology of keeping the time exposure in NaClO at four to five hours with a temperature of 50 °C should result in insignificant changes to the sampled MPs.

Rather than discoloration from the digestion process, Issac and Kandasubramanian (2017) observed yellow discoloration from oxidation during the weather of the plastic. This discoloration may be attributed to the presence of stabilizers used in the production of the plastic.

**Microplastics in Fishponds**

The lack of significant difference between MP presence in fishponds and the open coasts suggests no higher abundance of MP observed at fishponds. Organizations, like the non-profit Hui Ho‘oleimalu‘o, are dedicated to repairing these fishponds across all islands for a more sustainable food production and helping lower food scarcity (Kauanoe, 2023). Therefore as the last decade has brought up a resurgence of repairing this ancient method of aquaculture farming, it is important to understand any possible concerns for MP contamination. A learning center at one the repaired and working fishponds on Moloka‘i, Keawanui, is providing the local, and state, and research community the opportunity to study and experience a working fishpond (EPA, 2017). Utilizing this resource to further understand possible contamination from MPs to the caught fish before serving them to the community would be beneficial both the health of the mangrove ecosystem as well as the health of the community relying on this food source.

**Air Falls and Operational Controls**

The mean length of MPs for AFs and OCs was lower than those found in the samples. Microscopic analysis AF had the lowest length value of 186.608, and then AF and OC means from sample processing, digesting, and filtering were at 327.611 and 358.314 (Table 4). Comparatively the MP mean for coarse roots was 564.759 for fishponds and 838.043 for open coasts, and for fine roots it was 973.294 for fishponds and 854.849 for open coasts (Table 3). The difference in these lengths suggests a limited amount of the AF and OC MPs got introduced to the samples through any of the sample processing, digestions, filtering, or microscopic analysis. in in the samples.
5. Acknowledgements

Many thanks to Dr. Elise Granek for welcoming me into the Applied Coastal Ecology Lab and taking me in as her advisee throughout this long process. I am also extremely grateful to Geoffrey Szafranski for trusting me with his project and helping guide me through the process of creating, performing, and answering a scientific question. Lastly, I’d like to acknowledge and thank Elise Yu for all her help in the lab, and Sophia Hackett and Sylvia Pozzoni for helping with the edits of this thesis.

References


**Appendix**

**Table 6**: Every labeled transect for both fishpond (n=5) and open coasts (n=7) with the MP abundance for fine and coarse roots, their MP abundance transect total, and MP abundance per 1.5 grams.

<table>
<thead>
<tr>
<th>Transect Name</th>
<th>Fishpond or Open Coast</th>
<th>Fine Roots</th>
<th>Coarse Roots</th>
<th>MP Transect Total</th>
<th>Total MPs per 1.5 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-FMS-Sea</td>
<td>Open Coast</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2-CCG-Sea</td>
<td>Open Coast</td>
<td>1</td>
<td>5</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>3-NPT-Land</td>
<td>Open Coast</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>3-NPT-Sea</td>
<td>Open Coast</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>4-MSK-Sea</td>
<td>Fishpond</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>5-ALL-Sea</td>
<td>Fishpond</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>6-M9-Land</td>
<td>Open Coast</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>6-M9-Sea</td>
<td>Open Coast</td>
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<td>0</td>
<td>1</td>
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</tr>
<tr>
<td>7-KWU-CVT</td>
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<tr>
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<td>4</td>
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</tr>
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<td>0</td>
</tr>
<tr>
<td>10-KPK-Sea</td>
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</tbody>
</table>