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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: Comparing Fishponds and Open Coasts in Moloka'i, Hawaii

By

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Abstract
Due to their growing environmental threat, microplastics (MP) are an emerging global contaminant that have drawn the attention of many researchers in the last few decades. Pieces of plastic smaller than 5 mm, they are 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 sampled and ingested by hundreds of marine species that often mistake 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. These contaminants may be trapped within the sediment by mangroves' unique aerial roots. As mangroves grow in coastal zones, anthropogenic contaminants are often 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 MP presence. Fishponds are controlled reservoirs of seawater used for aquaculture that utilize 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 that fishpond presence has on microplastics. 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. MP counts were similar between coarse and fine roots between open coasts and fishponds ($p=0.4723$ for coarse roots and $p=0.7812$ for fine roots).

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 (MPs) (Auta et al. 2017). Defined as pieces of plastic less than 5mm in diameter (Arthur e al. 2008), MPs have become an important emerging topic of research. They are categorized into two groups, primary and secondary. Primary MPs are intentionally created plastic particles found in many daily used products such as facial cleansers, cosmetics, or dyes. Secondary MPs 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. MPs 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 MPs are entering the ocean from inland rivers annually. Once MPs enter the marine environment they are often mistaken for food (Jinadasa et al. 2022) due to their shiny appearance.

The larger the quantity of MPs consumed, the more likely it will cause harm to a species (Horton et al. 2018). There are currently four main concerns for marine species ingesting MPs regularly. First, ingesting MPs can alter feeding habits through falso satiation as organisms feel

full despite having consumed nothing beneficial to their health leading to reduced development (Sorrentino & Senna 2021). Second, 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 (Botterell et al. 2019). Thirdly, 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). Finally, MPs can bioaccumulate in the organism, gradually accumulating the abundance of MPs and their chemicals present in the organism (Wayman & Niemann 2021).

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 reduced coastal erosion. The entanglement of these dense roots filter out contaminants (e.g. nitrates, phosphates) improving water quality as the water 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 MPs present. Their research suggests that mangrove ecosystems 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 (Wang 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 would result in a higher abundance of MPs being 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 the general health of a species (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 Moloka'i Hawaii

Mangroves were introduced to the island of Moloka'i, 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 Moloka'i Fishponds

Fishponds on Moloka'i, are controlled reservoirs of seawater used for aquaculture that utilize 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 Moloka'i 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 Moloka'i (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) 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 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 MPs 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 MP presence in the mangrove forests throughout Moloka'i, Hawaii.

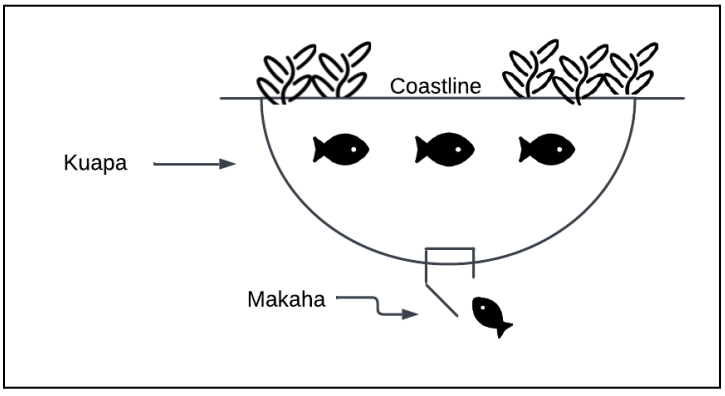

Fig 1. A diagram representing the structure of the fishponds on Moloka'i with mangroves present. The *kuapa* (seawall) forms a semi-circle around the coastline while the *makaha* (gate) opens and closes allowing the fish to swim in and then trap them. The back line of plants represents where the mangroves 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 locations 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 were 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. Sites, Coastline Habitat, and Transect types for every sample location from West to East.

Sites	Coastline Habitat	Transect Types
Farms	Open Coast	Sea
Coconut Grove	Open Coast	Sea
Newport	Open Coast	Land, Sea
MS/KE	Fishpond	Sea
Ali'i	Fishpond	Sea
Mile 9	Open Coast	Land, Sea
Keawanui	Fishpond	Culvert
Pualoa	Fishpond	Sea
Niaupala	Open Coast	Sea
Kupeke	Fishpond	Sea

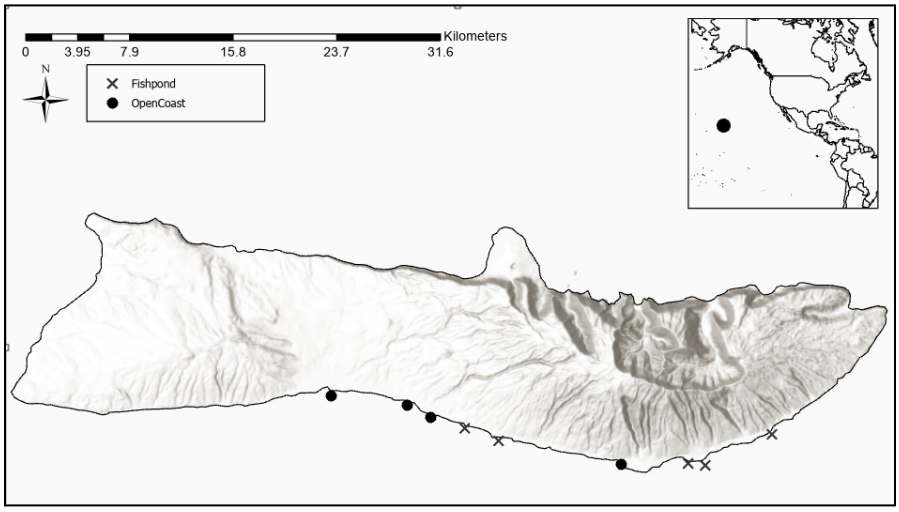

Fig 2. Sample locations for fishponds (x) and open coasts (circles) throughout Moloka'i, HI.

2.2 Microplastics Safety and Quality Control Protocols - Field and Laboratory

To avoid the introduction of MPs 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 MPs 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 potential for MP introduction. When the samples were open in the fume hood, the hood was placed on emergency purge.

Airfalls and procedural controls were used to quantify any accidental MP introduction. Both control types consist of DI water placed in glass beakers covered with rinsed aluminum foil. For the field, air falls of sieved DI water were placed out during the whole sample collection process at each location. For the lab, air falls were open any time the samples were opened and exposed; it accounted for any MPs that fell onto/into the samples from the surrounding environment. Procedural 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 processing, digesting, and microscopic analysis.

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 μm) were placed on top of each other to catch coarse roots (CR) and fine roots (FR). Each root ball was placed on top of the sieves and rinsed with DI water to remove excess organic materials. 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 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 fine roots were separated from each other. A glass pipette was used to rinse off both root types and remove any surface MPs 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 °F 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 MPs 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 fine (diameter ≤1 mm) and coarse (>1 mm diameter, up to a maximum of 5 mm) roots. 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 a 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 (Pfeiffer & Fischer 2020). 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 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.

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 MPs 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 MPs later on. The same process of filtration was applied for all the air falls and procedural controls from the past procedures.

2.7 Microscope

MPs were counted using 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 included: Is the potential MP unequal in thickness throughout the whole fiber? Is the potential MP displaying heterogeneous color variation? Does the potential MP posses visible cellular structure? If the answer is yes to any of these three questions, it is not counted as an MP. Other guidelines for identifying MPs included 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 conduct 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

3.1 MPs in procedural and airfall controls

10 MPs were found in the sample processing, digestions, and filtering AFs, their lengths ranged from 0.0343µm to 0.7241µm with a mean length of 0.1741µm (Table 2). For the procedural controls of these processes, 4 MPs were found ranging from 0.2207µm to 0.5265 µm with a mean length of 0.2844 µm (Table 2). Averaging out to 0.2051 MP/slide, 21 MPs were found in the AFs from microscopic analysis ranging from 0.0125 to 0.3574 with a mean length of 0.1086 (Table 2).

Table 2. The average abundance and length (µm) of MP of all airfalls per day (n=19) and procedural controls (n=12) during sample processing, digestions, and filtering and of all airfalls (n=33) during microscope analysis.

Type	Abundance	Length (µm)
Airfall	0.68	0.17
Procedural Control	0.78	0.28
Microscope Airfalls	0.21 mp/slide	0.11

3.2 MPs in root samples: numbers, colors, and sizes

The majority of observed MPs 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 in a root from an open coast site (2.86%) (Fig.3).

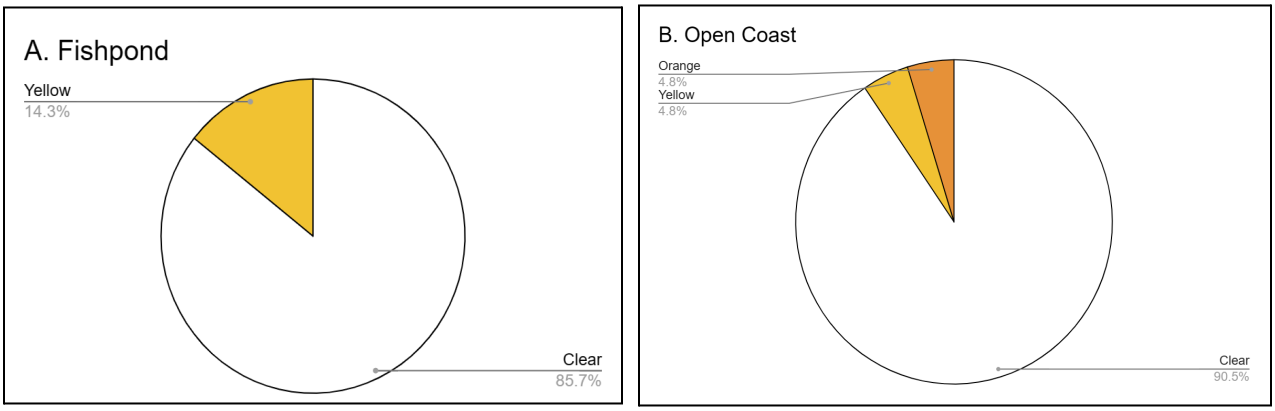

Fig 3. Observed MP colors between transects with fishponds (A) versus open coast (B). Coarse and fine roots were combined for a total of 14 MPs at fishpond transects and 21 MPs at open coast transects. The three colors found were clear, yellow, and orange.

In total 35 MPs were observed across all 12 transects combining both fine and coarse roots with 14 MPs at fishponds transects (n=5) and 21 at open coasts transects (n=7). Per transect coarse roots had a range of 0-7 MPs averaging 1.75 MPs; fine roots were similar ranging from 0-6 averaging 1.17 MPs per transect. At only fishponds, coarse roots had an average MP of 2.74, and fine roots had an average of 2.35; comparatively at open coasts coarse roots had an average MP abundance of 4.52 and fine roots had an average of 3.01 (Table 3).

Coarse roots had a slightly higher abundance of MP than fine roots at fishponds and open coasts. For both fine and coarse roots 3-NPT-Land had the highest abundance of observed MPs for open coasts transects and 10-PLA-Sea for fishponds transects. No MPs were observed at 12-KPK-Sea in fine or coarse roots. There were no MPs found in coarse roots at 4-MSK-Sea,

6-M9-Sea, 11-NIP-Sea, and in fine roots at 1-FMS-Sea, 3-NPT-Sea, 5-ALL-Sea, and 8-KWU-CVT. (Fig. 2)

P-values greater than 0.05 indicate no significant difference in MP abundance or length between fishponds and open coasts for both coarse (abundance: p=0.4723; length: p=0.3916) and fine (abundance: p=0.78125; length: p=0.8181) roots (Table 2 &3).

Table 3. N, mean, standard error, 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.

Coarse Roots	Fishpond	Open Coast
Mean	2.7414	4.5239
SE	1.1266	1.8303
P-Value	0.47228	

Fine Roots	Fishpond	Open Coast
Mean	2.34885	3.0071
SE	1.8969	1.4085
P-Value	0.78125	

The average length of MP in coarse roots samples was 0.15µm (range 0.03µm to 0.67µm) and 0.25µm (range 0.05µm to 0.84µm) for fine roots. At only fishponds, coarse roots had an average MP length of 0.1656µm and fine roots had an average of 0.16µm; comparatively open coasts coarse roots had an average MP length of 0.19µm and fine roots had an average of 0.26µm (Table 4).

Table 4. The mean, standard error, and p-value for MP length (µm) between locations with fishponds (n=5) vs the open coast (n=7) for both coarse and fine roots.

Coarse Roots	Fishpond	Open Coast
Mean	0.1656	0.1942
SE	0.0674	0.0760
P-Value	0.3916	

Fine Roots	Fishpond	Open Coast
Mean	0.1639	0.2640
SE	0.0595	0.1479
P-Value	0.8181	

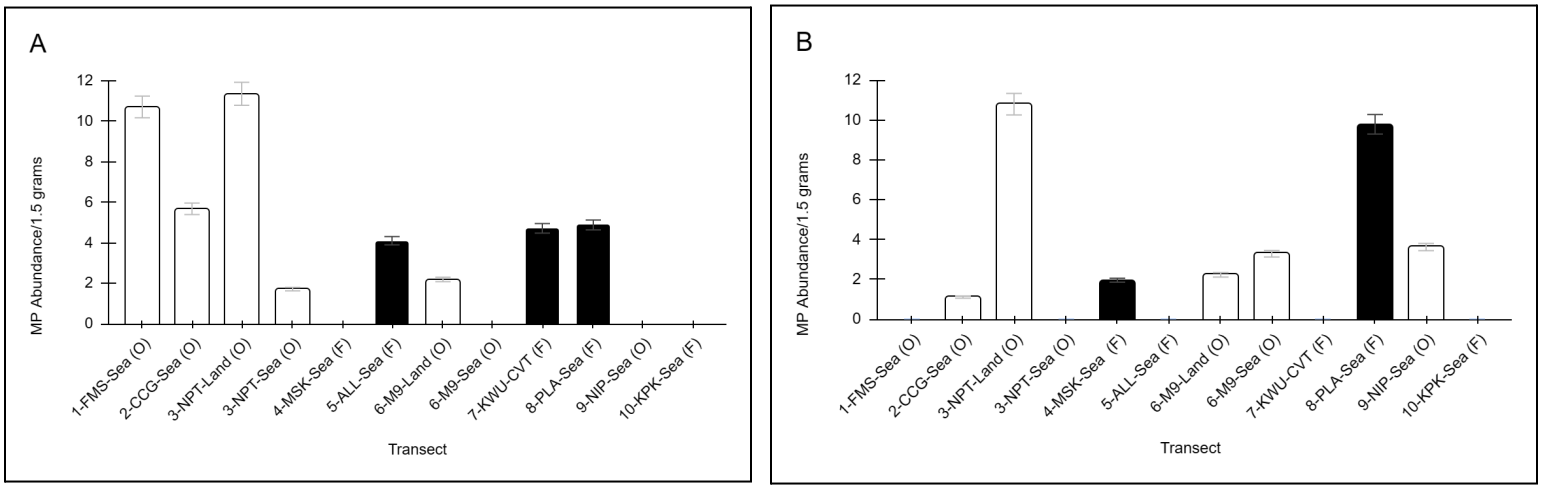

Fig 4. Observed mean MPs abundance by transect (n=3) and standard errors for coarse roots (A) and fine roots (B) after values were standardized to 1.5 grams per transect between sites with fishponds (F) (solid bars) present versus the open coast (O) (open bars) with SE set at 0.05. Each transect accounts for a collection of three root samples. Transect sampling locations run west to east along the horizontal axis.

4. Discussion

MPs were found in fine and coarse roots at fishponds and open coasts. Overall, there was a low abundance observed with little differences between the variables examined for fishponds and open coasts, and root sizes. The variables examined were MP length, color, and abundance. The presence of potential MP contamination in the lab was also noted and examined.

4.1 Air Falls and Procedural Controls

The mean length of MPs for AFs and PCs was lower than those found in the samples. Microscopic analysis AFs had the shortest lengths averaging 0.1086 µm and then AF and OC means from sample processing, digesting, and filtering averaged 0.6842 µm and 0.7778 µm (Table 4). Comparatively, the MP mean for coarse roots was 0.1656 µm for fishponds and 0.1942 µm for open coasts, and for fine roots it was 0.1639 µm for fishponds and 0.2640 µm 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 sample processing, digestions, filtering, or microscopic analysis.

4.2 Microplastic Abundance, Length Color, and Abundance,

4.2.1 Microplastic Length

There was no difference in MP length between those found in fishponds or along the open coasts for either root sizes. The lack of difference might suggest that the MPs from both fishponds and open coasts are deriving from the same source.

Though the mean MP lengths between coarse and fine roots for open coast transects were similar, at fishponds, coarse roots had a lower mean length (0.1656 µm) compared to fine roots (0.1942 µm). The difference in length might suggest that, similar to how mangrove roots have the ability to filter out contaminants such as nitrates or phosphates (The Nature Conservancy 2020), perhaps their roots also have the ability to break down these microscopic plastics.

While no research has been performed specifically looking at the impact mangrove roots may have on breaking down MPs, Liu et al. (2022) found the presence of mangrove forests do have positive impacts on the breakdown of MPs. The research showed that once MPs entered mangrove forests they were impacted by many factors including sunlight, wind erosion, and seafloor erosion that gradually broke the MP particles down (Liu et al. 2022). Due to the impact mangrove forests have on MP size, further research focusing on the impact of their roots is necessary to understanding their potential influence on MP size and abundance.

4.2.2 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. Similar color patterns between all transects were also observed by Martí et al. (2020) from a global collection of oceanic MPs in which they found the top three most abundant colors were white and clear (47%), yellow and brown (26%), and blue-like (9%) (Martí et al., 2020). Martí 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 reason for the high amount of clear MPs observed in all transects is the use of sodium hypochlorite (NaClO), to digest the samples' organic matter as bleach can strip the color

of MPs. However, Monteiro et al. (2022) found no effect of the use of NaClO digestion on visual or surface structure changes to MP exposed to NaClO for 60 hours at 50 °C (Monteiro et al., 2022). Similarly, Pfeiffer and Fisher (2020) found a marginally significant 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 minimal degradation of the sampled MPs,

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

4.2.3 Microplastic Abundance

The abundance of MPs 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 across 12 samples for both fine and coarse roots with 14 MPs at fishponds transects (n=5) and 21 at open coasts transects (n=7). There were no differences for either root size in MP abundance between fishpond and open coasts samples, this may indicate the MPs in mangrove roots are derived from inland rather than oceanic sources. This parallels the statement that 80% of marine plastic is from terrestrial origin (Mattson et al. 2015).

Coarse roots had a slightly higher abundance of MP than fine roots (Fig. 2), with MPs present at 8 of the 12 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 easternmost transect, 10-KPK-Sea, a fishpond transect far from town; and both root size classes had the most MP present at 3-NPT-Land, an open coast transect the site closest to the town center. Along the western transects, coarse roots had the highest 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 rather than oceanic origin. As mangroves grow in the intertidal zone and are subject to both marine and terrestrial contaminates (Wang et al. 2022), follow-up research focusing on the derivation of MP in mangroves would be beneficial in preventing their future introduction to the ecosystem.

4.3 The Importance of Identifying Microplastics in Fishponds

As the last decade has brought up a resurgence of repairing the ancient method of aquaculture farming, fishponds, it is important to understand any possible concerns for MP contamination. Recently the non-profit Hui Hoʻoleimaluo has been repairing fishponds across all islands to create a more sustainable food production and help lower food scarcity. Therefore, as these fish are being caught to feed the community, it is important that there was a lack of abundance of MPs at both open coasts and fishponds, as well as a lack of significance between open coasts and fishponds. The lack of significant difference indicates the fish are not exposed to a greater abundance of MP by being trapped in the fishponds. The lack of abundance suggests a lower threat of ingestion for the fish, leaving a lower threat to the nearby communities relying on the fish as a food source.

A learning center at one of the repaired and working fishponds on Moloka'i, Keawanui, is providing the local, state, and research communities 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 to both the health of the mangrove ecosystem as well as the health of the community relying on this food source

5. Acknowledgments

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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. Transects run west to east (1-10).

Transect Name	**Fishpond or Open Coast**	**Fine Roots**			**Coarse Roots**			**Transect Total**	
		Weight (g)	*MP Abundance*	*Total MP per 1.5 g*	*Weight (g)*	*MP Abundance*	*Total MP per 1.5 g*	*MP Abundance*	*Total MP per 1.5 g*
1-FMS-Sea	Open Coast	0.31	0	0.0000	0.28	2	10.7143	2	10.7143
2-CCG-Sea	Open Coast	1.345	1	1.1152	1.32	5	5.6818	6	6.7971
3-NPT-Land	Open Coast	0.417	3	10.7914	0.396	3	11.3636	6	22.1550
3-NPT-Sea	Open Coast	1.7	0	0.0000	1.75	2	1.7143	2	1.7143
4-MSK-Sea	Fishpond	2.294	3	1.9616	0.732	0	0.0000	3	1.9616
5-ALL-Sea	Fishpond	4.836	0	0.0000	1.096	3	4.7170	3	4.7170
6-M9-Land	Open Coast	1.345	2	2.2305	0.684	1	2.1930	3	4.4235
6-M9-Sea	Open Coast	0.456	1	3.2895	0.097	0	0.0000	1	3.2895

7-KWU-CVT	Fishpond	1.345	0	0.0000	0.9375	1	4.7170	1	4.7170
8-PLA-Sea	Fishpond	0.46	3	9.7826	1.2285	4	4.8840	7	14.6666
9-NIP-Sea	Open Coast	0.414	1	3.6232	0.576	0	0.0000	1	3.6232
10-KPK-Sea	Fishpond	1.3565	0	0.0000	0.9375	0	0.0000	0	0.0000

Table 7: Every labeled root samples for both fishponds (n= 15) and open coasts (n=21) with unaverged MP abundance and weight for fine and coarse roots. M represents instances where a transect did not have all three samples present at the time of lab work. X represents instances where samples were missing weights or lengths. Transects run west to east (1-10).

Sample Site	**Fishpond or Open Coast**	**Fine Roots**				**Coarse Roots**			
		Weight (g)	*MP Abundance*	*MP Length (μm)*	*MP Color*	*Weight (g)*	*MP Abundance*	*MP Length (μm)*	*MP Color*
1-FMS-Sea#1	Open Coast	0.26	0	-	-	0.16	1	0.09	clear
1-FMS-Sea#2	Open Coast	0.05	0	-	-	X	0	-	-
1-FMS-Sea#3	Open Coast	X	0	-	-	0.08	1	X	clear
2-CCG-Sea#1	Open Coast	O	1	0.15	clear	O	4	X, 0.12, 0.42, 0.09	clear, clear, clear, clear
2-CCG-Sea#2	Open Coast	X	0	-	-	X	0	-	-
2-CCG-Sea#3	Open Coast	0.66	0	-	-	X	1	0.67	yellow
3-NPT-Land#1	Open Coast	O	1	X	clear	O	0	-	-
3-NPT-Land#2	Open Coast	O	2	0.09, 0.11	clear, clear	O	2	X, 0.05	clear, clear
3-NPT-Land#3	Open Coast	0.139	0	-	-	0.132	1	X	clear
3-NPT-Sea#1	Open Coast	0.56	0	-	-	0.16	1	0.47	clear
3-NPT-Sea#2	Open Coast	0.98	0	-	-	0.08	1	0.38	clear
3-NPT-Sea#3	Open Coast	0.16	0	-	-	1.51	0	-	-
4-MSK-Sea#1	Fishpond	0.241	1	0.38	clear	0.211	0	-	-
4-MSK-Sea#2	Fishpond	0.978	1	0.48	clear	0.416	0	-	-
4-MSK-Sea#3	Fishpond	1.074	1	0.45	clear	0.105	0	-	-
5-ALL-Sea#1a	Fishpond	0.312	0	-	-	0.18	2	0.07, 0.12	clear, clear
5-ALL-Sea#2a	Fishpond	0.624	1	0.35	clear	0.173	0	-	-
5-ALL-Sea#3a	Fishpond	0.414	0	-	-	0.127	0	-	-
5-ALL-Sea#1b	Fishpond	1.45	0	-	-	0.186	0	-	-
5-ALL-Sea#2b	Fishpond	0.94	0	-	-	0.28	0	-	-
5-ALL-Sea#3b	Fishpond	1.096	0	-	-	0.15	0	-	-
6-M9-Land#1	Open Coast	X	0	-	-	0.02	0	-	-
6-M9-Land#2	Open Coast	O	2	0.84, 0.04	clear, clear	O	1	0.08	clear
6-M9-Land#3	Open Coast	X	0	-	-	0.256	0	-	-
6-M9-Sea#1	Open Coast	0.031	0	-	-	0.032	0	-	-
6-M9-Sea#2	Open Coast	0.051	1	0.41	clear	0.034	0	-	-

6-M9-Sea#3	Open Coast	0.374	0	-	-	0.031	0	-	-
7-KWU-CVT#1	Fishpond	O	0	-	-	O	1	0.04	yellow
7-KWU-CVT#2	Fishpond	X	0	-	-	0.156	0	-	-
7-KWU-CVT#3	Fishpond	X	0	-	-	0.056	0	-	-
8-PLA-Sea#1	Fishpond	X	0	-	-	O	2	0.03, 0.18	clear, clear
8-PLA-Sea#2	Fishpond	O	1	X	clear	0.621	1	0.36	clear
8-PLA-Sea#3	Fishpond	0.23	3	0.41, 0.07, 0.08	clear, clear, yellow	0.198	0	-	-
9-NIP-Sea#1	Open Coast	0.138	1	0.05	orange	X	0		
9-NIP-Sea#2	Open Coast	O	0	-	-	O	0	-	-
9-NIP-Sea#3	Open Coast	0.138	0	-	-	0.288	0	-	-
10-KPK-Sea#1	Fishpond	X	0	-	-	0.35	0	-	-
10-KPK-Sea#2	Fishpond	X	0	-	-	0.275	0	-	-
10-KPK-Sea#3	Fishpond	X	0	-	-	O	0	-	-