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Low incidence of microplastics in coral reefs of Kāneʻohe Bay, Hawaiʻi, USA

Jeremy B. Axworthy [*,1], Katherine S. Lasdin, Jacqueline L. Padilla-Gamiño

School of Aquatic and Fishery Sciences, University of Washington, 1122 NE Boat St., Seattle, Washington 98105, USA

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ABSTRACT

This study investigated microplastic and other micro-debris pollution in sediment, seawater, sea cucumbers, and corals from fringing and patch reefs in Kāneʻohe Bay, Oʻahu, Hawaiʻi, USA. Microplastic pollution in Kāneʻohe Bay Bay was low compared to other tropical coral reefs. Microplastics were detected in sediments (29 %), sea cucumbers (9 %), and coral (0–2 %) samples but were not quantifiable. Seawater had quantifiable microplastic (< 0.5 mm) and macroplastic (> 0.5 mm) pollution, with mean concentrations ranging from 0.0061 to 0.081 particles m^{-3}. Most particles detected in seawater samples were larger, floating plastic debris consisting mostly of polyethylene, polypropylene fragments, and fibers. Across the other matrices, the most detected particles were polyester, polypropylene, and cotton fibers. These results provide baseline data for this important coral reef ecosystem, and further monitoring is recommended to understand the seasonal and long-term trends in microplastic pollution and its potential future impacts.

1. Introduction

Plastic pollution is wide-ranging and increasing rapidly, yet there is still much we do not know about the fate of plastics in marine environments. Millions of tons of plastic waste enter the oceans each year (Borrelle et al., 2020; Jambeck et al., 2015; Meijer et al., 2021), but unless countries drastically increase efforts to manage plastic waste, plastic emissions are predicted to increase substantially in the coming years (Borrelle et al., 2020). Microplastics, synthetic polymers <5 mm, are a consequence of plastic pollution (Barnes et al., 2009). They can enter the oceans as micro-sized particles (primary microplastics) or result from the breakdown of larger plastic debris (secondary microplastics) due to physical processes such as UV radiation and abrasion (Cole et al., 2011). Microplastic pollution has been documented in nearly every corner of the planet, from polar regions and sea ice to the tropics and coral reefs (Hall et al., 2015; Peeken et al., 2018). They are thought to accumulate on the sea surface, in seafloor and coastal sediments, in biogenic marine structures, and organisms (Kane and Clare, 2019; Lebreton et al., 2019; Reichert et al., 2022; Soares et al., 2023; Woodall et al., 2014). However, we still lack a comprehensive understanding of the fate of these persistent pollutants. Thus, monitoring these pollutants to better understand their fate in different ecosystems and their potential impacts on life on our planet is critical.

Coral reefs are essential ecosystems that support the livelihoods of millions of people, protect coastlines from erosion, and harbor incredible amounts of biodiversity (Moberg and Folke, 1999). Microplastics have been detected in coral reef environments, as well as in organisms living within these reefs, including corals themselves (Ding et al., 2019; Hall et al., 2015; Lei et al., 2021; Lim et al., 2022; Tang et al., 2021; Zhou et al., 2022). Chemical cues may drive microplastics ingestion by corals (Allen et al., 2017), or corals may inadvertently ingest them while feeding on zooplankton from the water (Axworthy and Padilla-Gamiño, 2019). Lab studies have demonstrated that exposure to microplastics can have deleterious effects on corals, including reduced growth and prey capture, impaired photosynthesis, compromised immunity, tissue necrosis, and bleaching (Chapron et al., 2018; Hankins et al., 2021; Lanctôt et al., 2020; Liao et al., 2021; Mendrik et al., 2021; Reichert et al., 2019, 2018; Tang et al., 2018). Moreover, corals and coral reefs can serve as long-term sinks for microplastics when they are incorporated into coral skeletons and other reef structures (Hierl et al., 2021; Reichert et al., 2022; Soares et al., 2023). In addition to microplastics, other micro-debris, such as semi-synthetic (e.g., rayon) and other anthropogenically altered (e.g., dyed cotton) micro-fibers may impact corals and other marine life (Kroon et al., 2018; Reichert et al., 2024). Improving the management of microplastic and other micro-debris, as well as gaining a deeper understanding of their ecological impacts, requires establishing robust baseline data and implementing consistent monitoring efforts.

Kāneʻohe Bay, Oʻahu, Hawaiʻi, supports a tropical coral reef

* Corresponding author at: 1122 NE Boat St., Seattle, WA 98105, USA.
E-mail address: jeremyax@uw.edu (J.B. Axworthy).
[1] Permanent address: 526 Belmont Ave E Apt 302, Seattle, WA 98102.

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ecosystem consisting of fringing reefs that line the shore and patch reefs distributed throughout the bay. The reefs are dominated by two coral species, rice coral, *Montipora capitata,* and finger coral, *Porites compressa*. Together, these two species comprise over 80 % of the coral assemblage in the bay (Jokiel, 1991). Past research has shown that *M. capitata* is reluctant to ingest microplastics ex situ (Axworthy and Padilla-Gamiño, 2019), but it is not known whether this species, or *P. compressa*, ingest them in the wild. Kāneʻohe Bay is also home to the hot dog sea cucumber, *Holothuria edulis*, which feeds on detritus in the sediment, potentially making it a good indicator of microplastic and other micro-debris pollution (Coc et al., 2021; Plee and Pomory, 2020). To our knowledge, microplastic pollution has not been assessed in Kāneʻohe Bay coral reefs despite the Hawaiian islands being a hotspot for plastic debris accumulation (Brignac et al., 2019). An assessment of microplastic contamination in Kāneʻohe Bay could establish an initial baseline and provide valuable insights into the distribution of microplastics within the reefs, identifying areas where they are most abundant.

Microplastic pollution varies considerably in tropical systems, with some reefs being heavily polluted and others being low to moderately polluted (Huang et al., 2021). Few studies, however, have conducted simultaneous sampling of environmental and biological samples (Ding et al., 2019; Lei et al., 2021; Lim et al., 2022; Tang et al., 2021; Zhou et al., 2022), which could provide a comprehensive understanding of the spatial extent of these contaminants and their risk to reef organisms. The goal of this study was to investigate microplastic contamination in Kāneʻohe Bay. Specifically, we aimed to determine the abundance of microplastics and other micro-debris in sediments, seawater, sea cucumbers, and corals from the bay. We hypothesized that fringing reefs, being closer to shore and sources of terrestrial runoff, are more contaminated with microplastics than patch reefs. We also hypothesized that microplastic contamination in corals correlates to seawater microplastic contamination since corals feed from the water column and that microplastic contamination in sea cucumbers correlates to sediment microplastic contamination because *H. edulis* feeds in the sediment.

2. Materials and methods

2.1. Sample collection

Environmental and biological samples were collected from two fringing reefs (K4 and K5) and two patch reefs (HIMB and P29) in Kāneʻohe Bay, Oʻahu, Hawaiʻi, in the summer of 2018 (Fig. 1). Environmental samples included surface water and sediment. Biological samples included sea cucumbers, *Holothuria edulis* and corals, *Montipora capitata,* and *Porites compressa* (DAR Special Activities Permit No. 2019–21; Table 1).

Sediments were collected on SCUBA from each reef between coral patches or at the bottom of the reef slope from depths between 2 and 8 m. Sediment was sampled from the top 10 cm of the seafloor using a stainless-steel spoon to fill a 0.47 L glass jar. Later, 200 g of wet sediment from each sample was double wrapped in pre-cleaned and weighed aluminum foil and dried overnight at 60 °C in a drying oven at the Hawaiʻi Institute of Marine Biology (HIMB). The samples, still wrapped in aluminum foil, were stored and shipped in plastic freezer bags to the University of Washington.

Surface water samples were collected using a manta net with an opening of 0.069 m^2 and a mesh size of 330 μm (Masura et al., 2015). At each reef, five ~500 m tows were performed from the windward side of

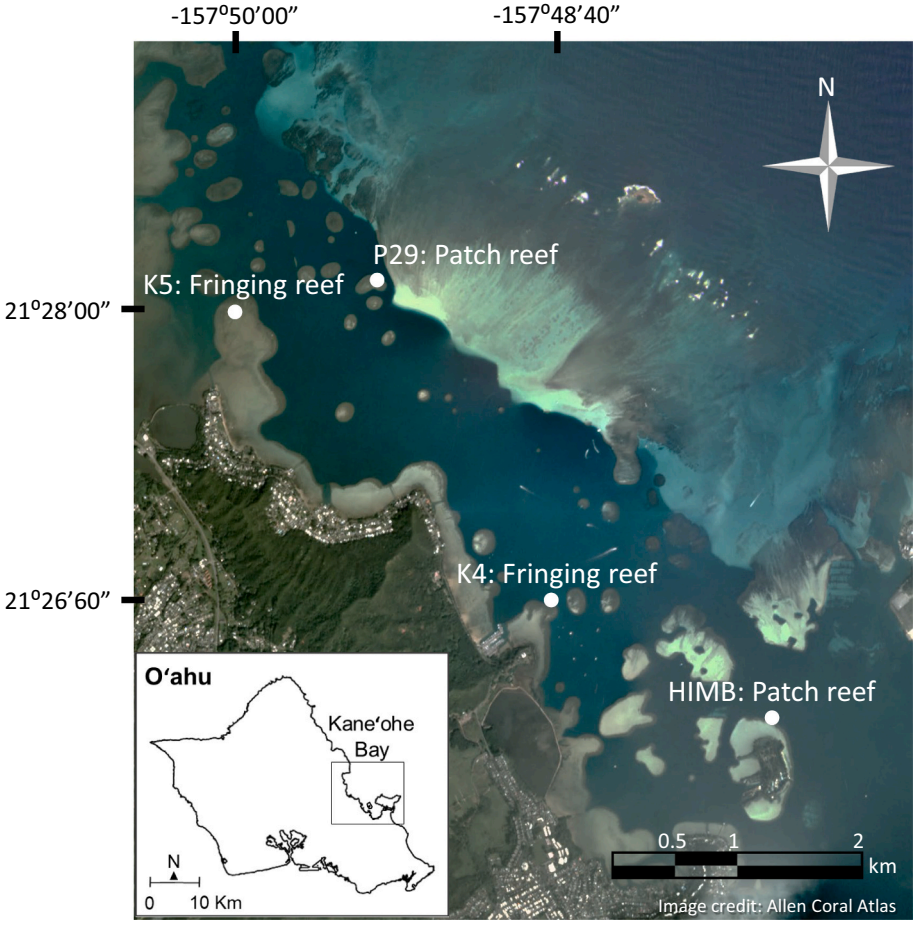

Fig. 1. Map of Kāneʻohe Bay and sample collection sites.

Table 1
Field site coordinates and sample size for each matrix.

Site	Latitude	Longitude	Sediment	Seawater	*H. edulis*	*M. capitata*	*P. compressa*
K4	21.26682	−157.48356	7	5	9	11	12
K5	21.27886	−157.49991	7	5	12	13	11
P29	21.28241	−157.49145	7	5	13	10	10
HIMB	21.26273	−157.47424	7	5	10	10	10

the vessel within 5 m of the reef crest above the reef slope. Distance was measured with a mechanical flowmeter (General Oceanics, USA) attached to the manta net, which was used to calculate the total volume of water filtered. At the end of each tow, the contents of the net were rinsed into the cod-end by pumping seawater along the outside of the net, then transferred to a pre-cleaned glass collection jar with equal amounts of seawater and 10 % formalin. Later, the samples were poured through a 250 μm stainless steel sieve and rinsed into a 200 mL plastic collection jar using Milli-Q water. An equal amount of 10 % formalin was added again to preserve the samples.

Organisms were collected at each reef on SCUBA. Coral fragments (~7 cm) from each site were collected using stainless steel toenail clippers (Revlon, USA) and placed in communal plastic freezer bags. Sea cucumbers were collected by hand and placed in plastic freezer bags. Upon surfacing, organisms were rinsed with copious amounts of filtered seawater (0.45 μm) to remove mucous and any adhered microplastics, then wrapped in pre-cleaned aluminum foil and frozen on ice. They were later stored at −80 °C at HIMB until they were shipped to the University of Washington, where they were stored at −20 °C until they were processed for microplastics.

2.2. Sample processing

Dried sediments were placed in a glass beaker and ground with a stainless-steel pestle. Large chunks of rubble were removed, and the samples were weighed to obtain dry weight. Density separations were performed by filling the beakers with 200 mL saturated sodium chloride (NaCl) solution and then stirring the solution vigorously for 1 min with a metal spatula (Thompson et al., 2004). The samples were allowed to settle for at least 24 h before they were decanted into clean glass jars. The process was repeated three times per sample, pooling the decanted solutions into single glass jars. The resulting solutions were vacuum filtered onto 5 μm polycarbonate filters, which were transferred into glass petri dishes and then wrapped in aluminum foil until they were analyzed for particles.

Preserved seawater samples were poured over a 250 μm stainless steel sieve and rinsed with filtered DI water to wash away the formalin. The contents on the sieve were visually inspected for micro- and macro debris, which were removed and stored in glass petri dishes for later analyses (Masura et al., 2015). The remaining contents on the sieve were backwashed into glass beakers. To digest biological material, 200 mL of 20 % potassium hydroxide (KOH) was added to the beakers, then they were covered with aluminum foil and placed in an incubator at 50 °C for five days (Lasdin et al., 2023). The beakers were swirled every 1–2 days to aid the digestion reaction. The contents of the beaker were vacuum filtered onto 5 μm polycarbonate filters, placed in glass petri dishes, then wrapped in aluminum foil until later analyses.

Sea cucumber samples were defrosted, then weighed, and measured lengthwise. The digestive tracts were dissected from the body cavities, laid on clean aluminum foil, and measured lengthwise. The digestive tract tissues were digested in glass beakers with 100 mL 20 % KOH solution at 50 °C for 3–4 days (Lasdin et al., 2023). The KOH solution was decanted into a clean glass jar, then vacuum filtered onto a 5 μm polycarbonate filter and stored as described above until further analysis. The sediments that remained following tissue digestion underwent density separation by adding 200 mL of saturated NaCl to the same beaker, stirring the solution vigorously for 1 min, and then allowing it to settle for at least 24 h (Thompson et al., 2004). The NaCl solution was decanted into a clean glass jar. Density separation was performed three times for each sample, and the decanted solution was pooled together in the same jar before undergoing vacuum filtration onto a 5 μm polycarbonate filter and stored as described above.

Coral fragments were thawed and rinsed heavily with filtered DI water before being placed into glass beakers for tissue digestion. 200 mL of 20 % KOH solution was added to the beakers, which were then placed in an incubator for five days at 50 °C (Lasdin et al., 2023). Following this step, coral skeletons were rinsed with filtered DI water into the same beaker to remove the remaining tissue. The digested tissue solutions were vacuum-filtered and stored until further analysis, as described previously.

Surface areas of the coral skeletons were obtained using 3D scanning (Reichert et al., 2016). In brief, coral skeletons were mounted on modeling clay, placed on a turntable, and rotated while scanning (Artec Spider) all sides at a 45 ° angle. The models were processed using Artec Studio (ver. 13). Individual frames with scans with an error value above 0.3 were discarded. “Crop surroundings” was used on each scan to remove auxiliary surfaces and random noise. “Global registration” was performed next to optimize meshes for further processing. To remove outliers, “eliminate noise” was used, and then the scans were fused into a single mesh using the “smooth fusion” function. Visible abnormalities were removed using “erase flaws,” and the resulting meshes were reduced using “simplify mesh.” Finally, the texture was applied to the meshes to show their surface color and textures, and the meshes were exported as .obj files. The meshes were imported into Meshlab (ver. 3.5.474) to obtain surface area measurements using the “compute geometric measures” function.

Coral skeletons were rinsed with filtered DI water to remove potential micro-debris contamination and then placed into glass beakers for skeleton dissolution. Skeletons were dissolved by adding ~250 mL of 10 % hydrochloric acid (HCl) solution and allowing the reaction to occur overnight (Oldenburg et al., 2021). If not fully dissolved by the next day, additional 50 mL aliquots of HCl were added as needed until no skeletal material remained. The resulting solution was vacuum-filtered and stored as described above.

2.3. Particle analysis

Filters were visually inspected under a microscope (10–40× magnification) for suspected microplastics and other anthropogenic microdebris. All particles that did not appear to have a cellular structure, did not crumble when probed, and did not make a scraping sound when prodded were analyzed (Lusher et al., 2020). Photographs were taken of each particle, and their length, color, and type (morphology) were recorded. For length measurements, the longest dimension of each particle was recorded, i.e., length of fibers or ferret diameter of fragments. Particles were divided into size classes of 0–500, 501–1000, 1001–2000, 2001–3000, 3001–4000, 4001–5000, and >5000 μm. Color observations included primary and secondary colors: black, brown, clear, and white. Particle types included fibers, fiber bundles, films, fragments, and beads (Lusher et al., 2020). The particles were rinsed in filtered 70 % ethanol and transferred to gold-plated slides for micro-Fourier transformed infrared (μ-FTIR) analysis.

Particles were identified using a Nicolet iN10 μ-FTIR (Thermo Fisher Scientific), equipped with a liquid nitrogen cooled, mercury cadmium

telluride array detector. Scans were taken with an aperture of 150 μm × 150 μm in attenuated total reflectance (ATR) mode using a germanium crystal, with a resolution of 8 cm $^{-1}$, using Omnic Picta software (ver. 1.8.240). Spectra were detected in the spectral range of 650–4000 cm^{-1}. Up to three attempts were made to identify particles: attempt 1) included 16 added scans and ATR pressure of 15, attempt 2) included 64 added scans and ATR pressure of 15, attempt 3) included 64 added scans and ATR pressure of 2. Spectra were compared with an in-house compilation of spectral libraries (Supplementary materials). Particles were considered identified if the best spectral match score was ≥70 (Lasdin et al., 2023). Representative FTIR spectra and images of particles detected in this study are presented in Fig. 2.

Particle identifications were used to classify particles into four groups based on their origin: plastic, anthropogenic, natural, and unknown (Miller et al., 2021; Lasdin et al., 2023). Plastic refers to synthetic polymers, such as polypropylene or polyester. Anthropogenic refers to cellulosic materials, including cellophane, cellulose, and rayon, and includes dyed (i.e., black or blue) cotton. The anthropogenic category was used because the spectral similarity of these materials makes it difficult to distinctly identify them and because, although cellulosic materials originate from natural compounds (i.e., cellulose), the resulting products are not natural, sensu stricto, and may contain anthropogenic chemicals that could have ecological implications. Natural particles refer to non-dyed (i.e., white or clear) cotton and wool, fur, wood, etc. Unknown particles refer to particles that did not meet the spectral match threshold of 70 or were lost during μ-FTIR analysis.

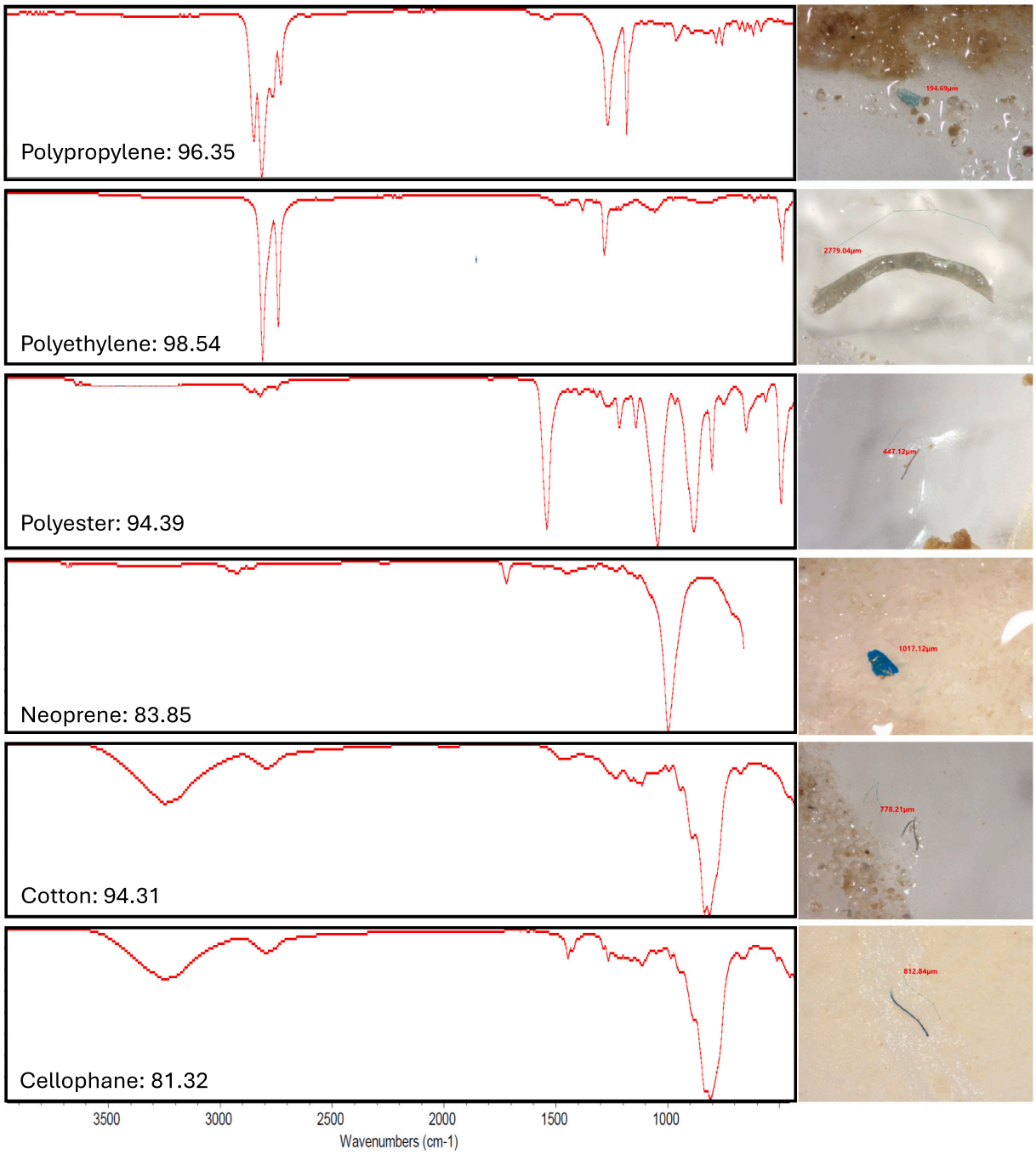

Fig. 2. FTIR spectra, match scores, and microscope images of particles representative of the most abundant polymers and anthropogenic materials detected in this study. Note the spectral similarity between cotton and cellophane, which complicates distinguishing between the two materials.

2.4. Quality assurance/quality control

Multiple steps were taken to reduce microplastic and other microdebris contamination (Brander et al., 2020). Materials and equipment used for field work were rinsed with filtered DI water or filtered seawater before and in between each use. In the lab, all personnel were encouraged to wear clothing made of non-plastic material (i.e., cotton and wool) and were required to use a lint roller and wear 100 % cotton lab coats. Two HEPA filters were run simultaneously while working in the lab. Lab benches and equipment were cleaned with filtered 70 % ethanol each day work was performed. Lab work was performed efficiently to reduce the time for contamination to occur. Procedures that did not involve harmful chemicals were performed in a laminar flow hood. All reagents and water were filtered to 1.2 μm. The use of plastic labware and field equipment was avoided whenever possible. All glassware used in the lab was baked in a muffle furnace at 500 °C for 4 h before first use and was washed, then rinsed 3× with filtered DI water, then rinsed again with filtered 70 % ethanol between each use. Airborne controls and procedural blanks were used for lab procedures to account for contamination. Procedural blanks were not used in the field (discussed later).

2.5. Limit of detection/limit of quantification

Samples were processed in batches of four. For each batch, one airborne control and one procedural blank were used. Airborne controls consisted of 90 mm glass fiber filters stamped with a grid and placed in a glass petri dish. The petri dish was uncovered whenever samples were exposed to the environment to account for particles that fell out of the air. Procedural blanks were empty vessels treated the same way as the field-collected samples to account for contamination from lab equipment and reagents. Particles on the airborne control and blank filters were analyzed as described above and used to calculate the limits of detection (LOD) and limits of quantification (LOQ) (Dawson et al., 2023). Particle data from one procedural blank, used for a batch of sediment, was omitted from analyses because it contained an unusually high number of particles (> 50) that were mostly cellulosic, which we suspect came from using a Kimwipe when cleaning the petri dish. To calculate LOD and LOQ, particle counts from the airborne controls and blanks were summed for each batch, and then the mean and standard deviation of the particle count for each matrix (i.e., sediment, seawater, sea cucumbers, and coral) and particle origin (i.e., plastic, anthropogenic, natural, unknown), were determined. LOD for each matrix and particle origin was calculated as the mean respective particle count plus three times the standard deviation. LOQ for each matrix and particle origin was calculated as the mean plus ten times the standard deviation. The number of samples that had particle counts above LOD and LOQ were reported and samples that had particle counts above LOQ were analyzed further to determine differences between collection sites.

2.6. Data analysis

All data analyses were performed using R Studio (ver. 2023.12.1). Natural particles were not included in the analyses. For samples with particle counts above LOQ, the LOQ was subtracted from the particle count (Dawson et al., 2023), and the resulting values were normalized to matrix-specific parameters as follows: sediment dry weight (particles kg^{-1}), seawater volume (particles m^{-3}), sea cucumber body length (particles cm^{-1}), coral surface area (particles cm^{-2}). Samples with particle counts below LOQ were converted to zero. Since none of the data were normally distributed, Kruskal-Wallis tests were used to test for differences in particle concentrations between sites for each particle origin class within each matrix. Differences were considered significant when $P < 0.05$. Particle characteristics (identification, type, size, and color) are reported for samples with particle counts above LOD. Pearson's correlation was used to test the relationships between the concentration of microplastics between seawater and corals and between sediment and sea cucumbers at different sites.

3. Results

Across all matrices, we extracted a total of 1474 particles. All suspected microplastic particles were analyzed via μ-FTIR spectroscopy. Of those, 15 % were plastic ($n = 224$), 55 % were of anthropogenic origin ($n = 814$), 5 % were natural materials ($n = 80$), and 24 % were not identified ($n = 356$). Over 88 % of particles were fibers ($n = 1309$) or fiber bundles (n = 2), 8 % were fragments ($n = 118$), 2.7 % were films ($n = 41$), and the remaining particles were beads (n = 2) and foams (n = 2).

3.1. Sediment

Microplastic particles were detected in 7 out of 28 (25 %) sediment samples across all sites ($n = 7$ per site; Table 2, Fig. 3A). Three samples from each site K4 and HIMB and one sample from each site K5 and P29 had microplastic counts above LOD (Fig. 3A). No sediment samples had microplastic counts above LOQ (Table 2). Polypropylene was the most abundant polymer type in sediments, followed by polyester, olefin, polyethylene, acrylic, neoprene, and vinyl ester (Fig. 4A). Fibers were the most common type of microplastic particles observed, followed by fragments and one bead (Fig. 4B). The majority of microplastic particles were <500 μm (Fig. 4C). Most microplastic particles were blue, and the rest were clear, black, white, green, and red (Fig. 4D).

No sediment samples contained anthropogenic particles above the LOD or LOQ (Table 2, Fig. 3A).

Particles of unknown origin were detected in 7 out of 28 (25 %) sediment samples across all sites (Table 2, Fig. 3A). Two samples from site HIMB and one sample each from sites K4 and P29 had unknown particle counts above LOD (Fig. 3A). No sediment samples had unknown particle counts above the LOQ (Table 2).

3.2. Seawater

Microplastic particles were detected in 18 out of 20 (90 %) surface seawater samples across all sites ($n = 5$ per site; Table 2, Fig. 3C). Five samples from both sites K4 and HIMB, as well as four samples from both sites K5 and P29, had microplastic counts above the LOD (Fig. 3C). The most abundant polymer type of microplastics in seawater was polyethylene, followed by polypropylene, polyester, neoprene, olefin, polyamide, and acrylonitrile butadiene styrene (Fig. 4A). Fragments were the most abundant microplastic type, followed by films and fibers (Fig. 4B). Most microplastic particles in seawater were between 500 and 2000 μm (Fig. 4C). Blue was the most common microplastic particle color in seawater, followed by black, clear, white, green, orange, red, and yellow (Fig. 4D).

Microplastics were quantifiable in 12 (60 %) seawater samples across all sites (Table 2). Five samples from site K4, three samples from both sites K5 and HIMB, and one sample from site P29 had microplastic counts above the LOQ. Based on the samples with quantifiable microplastic counts, fringing reefs, K4 and K5, had microplastic particle concentrations of 0.081 ± 0.017 (mean ± 1 SE particles m^{-3}) and 0.030 ± 0.017, respectively (Fig. 6). Patch reefs, P29 and HIMB, had microplastic particle concentrations of 0.050 ± 0.050 and 0.024 ± 0.012, respectively (Fig. 6). There was no difference in microplastic concentration between sites (Kruskal-Wallis, chi-squared = 5.63, df = 3, p-value = 0.13). An observation worth noting is that the plastic fibers found in the seawater samples were generally larger and more weathered than fibers observed in other sample matrices.

No micro-sized particles of anthropogenic origin were detected in seawater samples (Table 2, Fig. 3C).

Four micro-sized particles of unknown origin were detected in seawater samples across all sites (Table 2, Fig. 3C). Three samples from K5 and one sample from K4 had unknown micro-sized particle counts

Table 2
Limit of detection (LOD) and limit of quantification (LOQ) and the number of samples with particle counts above them for each matrix and each particle origin. Particles were pooled from airborne controls and procedural blanks. The LOD is equal to the mean number of particles plus three times the standard deviation. The LOQ is equal to the mean number of particles plus ten times the standard deviation (Dawson et al., 2023).

Matrix	Origin	LOD (# particles)	LOQ (# particles)	# samples above LOD	# samples above LOQ
Sediment	Plastic	4.58	13.19	7	0
$n = 28$	Anthropogenic	25.47	69.00	0	0
	Unknown	8.22	22.90	7	0
Seawater (micro)	Plastic	0.39	1.17	18	12
$n = 20$	Anthropogenic	3.11	8.51	0	0
	Unknown	3.06	8.46	4	1
Seawater (macro)	Plastic	0.39	1.17	15	10
n = 20	Anthropogenic	3.11	8.51	0	0
	Unknown	3.06	8.46	0	0
Holothuria edulis	Plastic	1.60	4.89	4	0
$n = 44$	Anthropogenic	13.23	35.74	3	0
	Unknown	4.93	14.15	3	0
Montipora capitata	Plastic	3.01	9.06	1	0
n = 44	Anthropogenic	17.34	44.66	2	0
	Unknown	4.74	13.90	2	0
Porites compressa	Plastic	3.01	9.06	0	0
$n = 43$	Anthropogenic	17.34	44.66	0	0
	Unknown	4.74	13.90	1	0

above LOD (Fig. 3C). One seawater sample from site K4 had an unknown micro-sized particle count above LOQ (Table 2, Fig. 3C).

Seawater was the only matrix in which macro-sized particles (> 5000 μm) were detected. Macroplastic particles were detected in 15 out of 20 (75 %) surface seawater samples across all sites (Table 2, Fig. 3E). Five samples from site HIMB, four samples from site K5, and three samples from each site K4 and P29 had macroplastic particle counts above LOD (Fig. 3E). The most abundant polymer type was polyethylene, followed by polypropylene, acrylonitrile butadiene styrene, polyamide, and polystyrene (Fig. 4A). The most abundant particle type was fibers, followed by films, foams, and a fragment (Fig. 4B). Blue was the most common macroplastic particle color, followed by black, clear, green, brown, orange, purple, red, and white (Fig. 4D).

Macroplastics were quantifiable in 10 samples across all sites (Table 2). Four samples from HIMB, three samples from K5, two samples from K4, and one sample from P29 had macroplastic counts above LOQ. Based on the samples with quantifiable macroplastic counts, fringing reefs, K4 and K5, had macroplastic particle concentrations of 0.011 ± 0.0069 (mean ± 1 SE particles m^{-3}) and 0.032 ± 0.014, respectively (Fig. 6). Patch reefs, P29 and HIMB, had macroplastic particle concentrations of 0.0061 ± 0.0061 and 0.077 ± 0.029, respectively (Fig. 6). There was no difference in macroplastic concentration between sites (Kruskal-Wallis chi-squared = 6.83, df = 3, p-value = 0.077).

No macro-sized particles of anthropogenic or unknown origin were detected in seawater samples (Table 2, Fig. 3).

3.3. Sea cucumber

Microplastic particles were detected in 4 out of 44 (9 %) *H. edulis* samples across all sites ($n = 9$–12; Table 2, Fig. 3B). Two samples from P29 ($n = 13$) and one sample from each site K4 (n = 9) and HIMB ($n = 10$) had microplastic counts above LOD (Fig. 3B). No *H. edulis* samples contained microplastics above LOQ (Table 2). The most abundant polymer type was polyester, followed by polypropylene, olefin, and polyamide (Fig. 4A). All detected microplastics in *H. edulis* were fibers (Fig. 4B). Most microplastic particles in *H. edulis* were <500 μm (Fig. 4C). Black was the most common plastic particle color, followed by blue and orange (Fig. 4D).

Anthropogenic particles were detected in 3 out of 44 (7 %) *H. edulis* samples (Table 2, Fig. 3B). Two samples from K4 and one sample from P29 had anthropogenic particle counts above LOD (Table 2). No *H. edulis* samples contained anthropogenic particles above LOQ (Table 2). The most abundant material type was cotton, followed by cellophane, cellulose, and rayon (Fig. 5A). All detected anthropogenic particles in *H. edulis* were fibers (Fig. 5B). The most abundant size class of anthropogenic particles in *H. edulis* was 0–500 μm (Fig. 5C). Black was the most common fiber color, followed by blue, red, and clear (Fig. 5D).

Particles of unknown origin were detected in 3 out of 44 (7 %) of *H. edulis* samples, all from site K4.

3.4. Coral

Microplastic particles were detected in 1 out of 44 (2 %) *M. capitata* samples across all sites (n = 10–13; Table 2, Fig. 3D). The sample was collected from site K5 (Fig. 3D). Of the microplastics recovered from that sample, three were purple and black polyester fibers, and one was a red acrylic fiber, all between 1000 and 3000 μm (Fig. 4A – D). No *M. capitata* samples had microplastics above LOQ (Table 2).

Anthropogenic particles were detected in 2 out of 44 (5 %) *M. capitata* samples across all sites (Table 2, Fig. 3D). One sample from site K4 and one sample from site P29 had anthropogenic particle counts above LOD (Fig. 3D). None of the *M. capitata* samples had anthropogenic particles above LOQ (Table 2). The most abundant material type was cotton, followed by cellophane, rayon, and cellulose (Fig. 5A). All detected anthropogenic particles in *M. capitata* were fibers (Fig. 5B). The most abundant size class of anthropogenic particles in *M. capitata* was 1000–2000 μm (Fig. 5C). Black was the most common fiber color, followed by blue, red, clear, and orange (Fig. 5D).

Particles of unknown origin were detected in two *M. capitata* samples (Table 2, Fig. 3D). One sample from HIMB and one sample from P29 had unknown particle counts above LOD (Fig. 3D). No *M. capitata* samples had unknown particle counts above LOQ (Table 2).

No microplastics or anthropogenic particles were detected in *P. compressa* (Table 2, Fig. 3F). Particles of unknown origin were detected in one *P. compressa* sample from site P29 (Table 2, Fig. 3). That sample did not contain a particle count above LOQ (Table 2).

3.5. Correlation tests

Correlation tests between corals and seawater or sea cucumbers and sediment could not be performed because no coral or sea cucumber samples had quantifiable particle concentrations.

4. Discussion

Here we present, to the best of our knowledge, the first assessment of microplastic, and other micro-debris, pollution in the coral reef ecosystem in Kāneʻohe Bay, Oʻahu, Hawaiʻi. Overall, there was very low

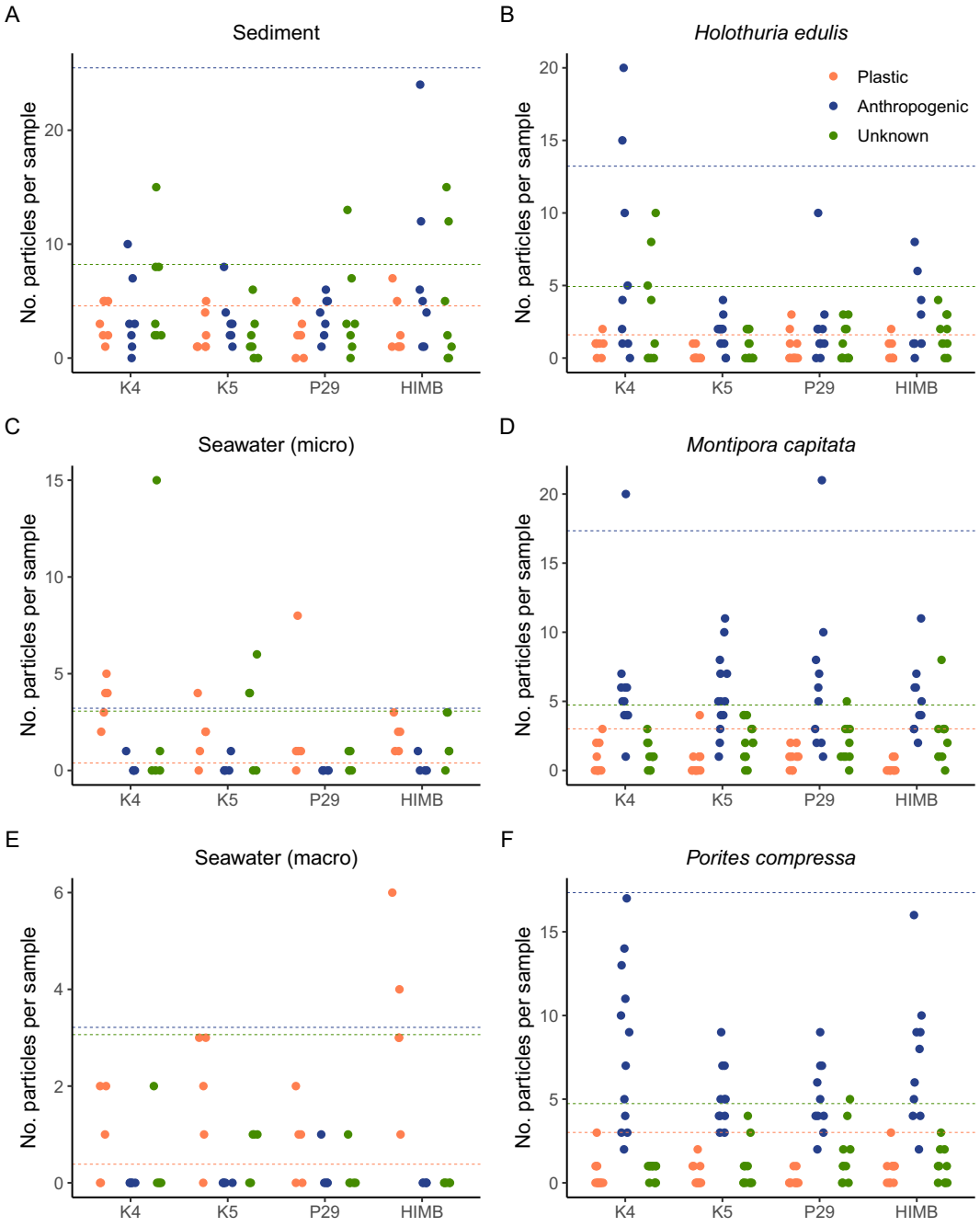

Fig. 3. Number of particles by site for each matrix from samples collected from Kāneʻohe Bay, Oʻahu, Hawaiʻi, and the limits of detection (dotted lines). Particle origins (plastic, anthropogenic, and unknown) were determined by μ-FTIR spectroscopy. Plastic particles include synthetic polymers. Anthropogenic particles include cotton, rayon, cellulose, and cellophane, which are derived from natural materials but have been modified anthropogenically (e.g., dyed, etc.). Unknown particles did not meet the criteria for a good spectral match (≥ 70) or were lost during analysis. Dotted lines represent respective limits of detection.

microplastic contamination in the matrices studied, with seawater having the only quantifiable microplastic contamination. Given these low contamination levels, we could not support our hypotheses that fringing reefs in the bay are more contaminated than patch reefs or that microplastic contamination in corals and sea cucumbers correlates to microplastic concentrations in seawater and sediment, respectively. Our data, based on a comprehensive sampling regime that covered a range of matrices known to be contaminated with microplastics, suggests that microplastic pollution is likely not a serious threat to Kāneʻohe Bay's coral reefs.

4.1. Microplastics in environmental samples

Surface seawater had the highest detected levels of microplastics (0.049–0.11 particles m^{-3}) in our study compared to the sub-surface matrices. Compared to studies that used similar collection methods (i.e., surface water plankton trawl and mesh size: 80–333 μm, and FTIR or Raman spectroscopy), the sea surface microplastic concentrations detected in Kāneʻohe Bay were on the same order of magnitude as the Maldives (0.02–0.65 particles m^{-3}; Saliu et al., 2018, 2019), the South China sea (0.148 to 0.842 particles m^{-3}; Wang et al., 2019), Nansha Reef, China (0.056 particles m^{-3}; Tan et al., 2020), and Sri Lanka (0.28–0.52 particles $m^{-3;}$ Sevwandi Dharmadasa et al., 2021).

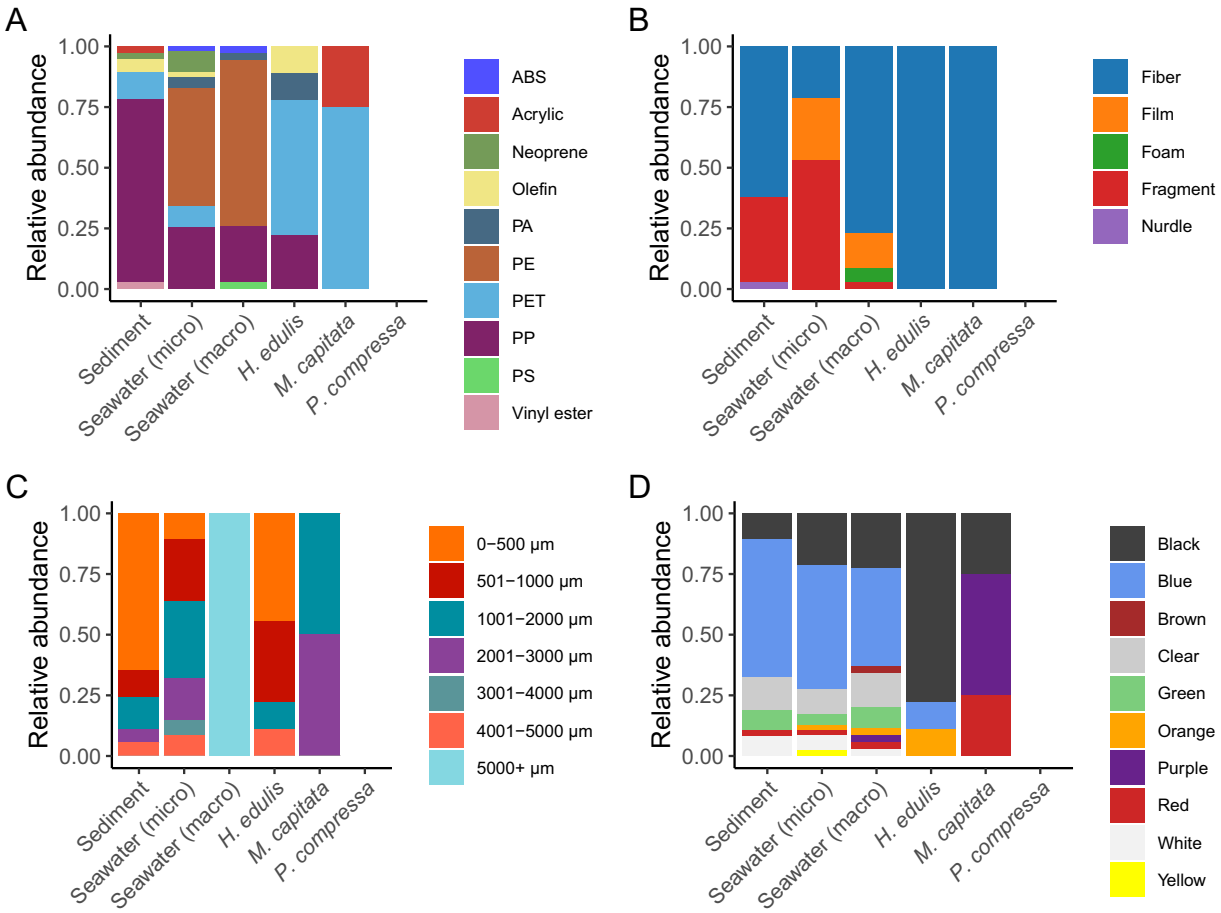

Fig. 4. Characteristics of plastic particles detected in different matrices. A) plastic polymer identified by µ-FTIR, B) particle type, C) particle size (maximum dimension), and D) particle color.

Conversely, sea surface microplastic concentration in Kāneʻohe Bay was much lower than in the Gulf of Mannar, India (6000–126,000 particles m^{-3}; Patterson et al., 2020, 2022), and Rameswaram Island, India (24000–96,000 particles m^{-3}; Jeyasanta et al., 2020). Differences in sampling methods make it difficult to compare our data to other studies, highlighting the need for harmonization between studies. However, many recent studies employ bulk sampling to obtain seawater samples (Huang et al., 2021). Unlike plankton tows, which are limited by mesh size, bulk sampling methods can capture smaller particles than plankton nets. The trade-off is that plankton nets allow more efficient sampling of greater water volumes. Thus, our sampling technique likely underestimated the amount of smaller-sized microplastics and other microdebris, but we can more confidently infer particle concentration over a larger spatial scale.

Microplastic contamination levels in the sediments, sea cucumbers, and corals from Kāneʻohe Bay were considerably lower compared to those found in surface water. This indicates that a significant portion of the plastic debris polluting the bay consists of positively buoyant, floating particles. Floating plastics may sink over time when they degrade into smaller particles and become fouled (Chubarenko et al., 2016), which in turn could enter the sediment or be ingested by biota. However, it is likely that much of the floating plastic debris will end up on the shorelines of the islands. Driven by large-scale circulation patterns and climatic variations, a substantial amount of floating marine debris that enters the North Pacific Ocean makes its way onto Hawaiʻi's coastlines (Brignac et al., 2019; Howell et al., 2012; Ribic et al., 2012). Coastlines on the windward side of the islands, such as Kāneʻohe Bay, are more heavily impacted as onshore winds help carry debris to the shore (Brignac et al., 2019). In alignment with the findings of Brignac et al. (2019), the dominant plastics detected in sea surface waters in this study were polyethylene and polypropylene, which have low densities, making them resistant to sinking. Thus, a potential explanation for the relatively low detection of microplastics in subsurface samples could be that floating plastic debris entering Kāneʻohe Bay via ocean currents and wind-driven processes are rapidly transported to the shore before becoming negatively buoyant and sinking to the reefs.

Other potential reasons for the low abundance of microplastics detected in subsurface samples could be associated with the characteristics of sediment and our extraction method. The upper layer of sediment in the reef slopes of Kāneʻohe Bay is fine silt, which is very soft and loose. This could result in particles sinking deeper into the sediment beyond the depth of our sampling, which was limited to the top 10 cm. Therefore, we may not have sampled deep enough to find many particles. This could also explain why we detected only a few microplastics in sea cucumbers, which feed on detritus in the upper sediment layers. Additionally, we may not have detected much microplastic contamination in sediments and sea cucumbers due to the density separation method used. Saturated NaCl has a density of approximately 1.2 mg mL^{-1}, limiting its ability to float certain denser types of plastic, such as polyester and polystyrene. Other solutions with higher densities are more efficient at separating more types of plastic, such as zinc bromide or sodium iodide (Quinn et al., 2017), but these chemicals are not as cost-effective or environmentally friendly as NaCl. We acknowledge that by using the methods employed in this study, we might not have detected particles with higher densities, which have been detected in Hawaiian seafloor habitats (Brignac et al., 2019).

Compared to other studies, very little microplastic pollution was detected in Kāneʻohe Bay corals (see review by Huang et al., 2021). For

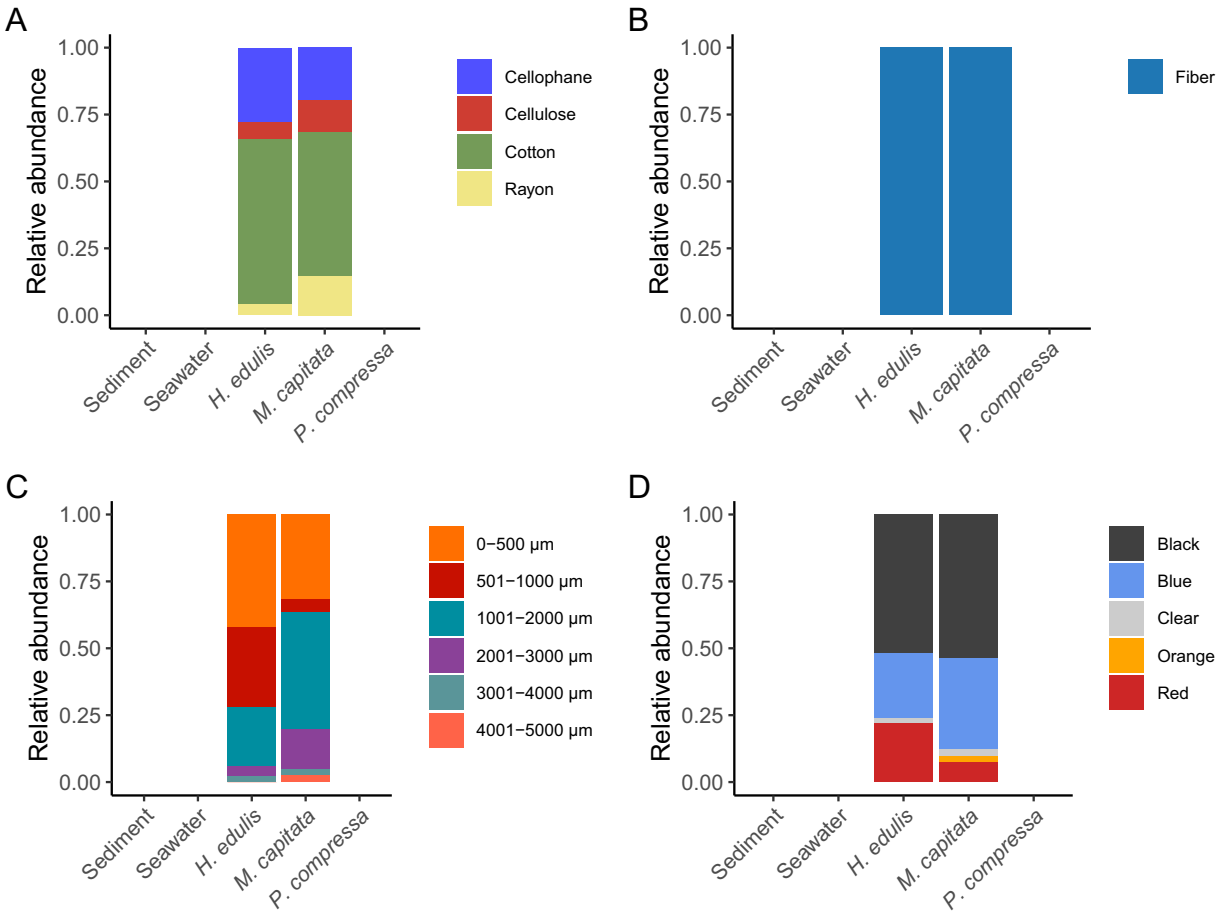

Fig. 5. Characteristics of anthropogenic particles detected in different matrices. A) material identified by µ-FTIR, B) particle type, C) particle size (maximum dimension), and D) particle color.

example, microplastic contamination in corals from atolls of Xisha Islands, China, ranged from 0.9 to 2.5 particles cm^{-2} (Zhou et al., 2022), and corals from Hainan Island, China, had microplastic levels of nearly five particles cm^{-2} (Tang et al., 2021). Since we rinsed coral fragments to remove potential contaminants before processing, we were only able to detect microplastics that were ingested by the corals or microplastics that had been incorporated internally by corals prior to sampling. For *M. capitata*, this was not surprising since our past research has indicated that it does not readily ingest microplastics (Axworthy and Padilla-Gamiño, 2019). Given that, and the fact that we used the identical extraction method for *P. compressa*, it is reasonable to conclude that neither of these species ingest many microplastics. However, adhesion to surface tissue appears to be a more important mechanism for coral-microplastic interactions. Microplastic adhesion rates may be up to 40 times higher than ingestion rates for some corals (Corona et al., 2019; Martin et al., 2019). The adhesion of microplastics to coral surfaces can lead to tissue necrosis, bleaching, and overgrowth, ultimately leading to their deposition into the skeleton (Hierl et al., 2021; Reichert et al., 2018). However, corals may be able to remove adhered microplastics using the same mechanisms for removing sediments (Stafford-Smith and Ormond, 1992; Martin et al., 2019; Bejarano et al., 2022; Axworthy et al., *in review*).

We acknowledge that we may have underestimated the abundance of microplastics associated with corals, specifically those adhered to their outer tissue layers, which could explain the low levels of microplastic contamination detected. To our knowledge, only one field study has quantified both ingested and adhered microplastics in wild corals (Rani-Borges et al., 2023). However, determining the ratio of adhesion to ingestion rates is challenging because microplastic abundance was reported in different units. Experimental studies have reported ratios of microplastic adhesion to ingestion in corals ranging from 40:1 (Corona et al., 2019; Martin et al., 2019), 3–4:1 (Axworthy et al., *in review*), and 2:1 (Isa et al., 2024). These differences are likely due to variations in experimental design (e.g., species, microplastic concentrations, flow conditions, and quantification techniques), making it difficult to extrapolate the number of potentially adhered microplastics in this field study. However, the low levels of microplastics detected or quantified in the sediments and seawater surrounding the corals suggest that they were not heavily polluted. For future studies, it is important to use consistent units and sampling protocols that account for adhered microplastics and distinguish between ingested and adhered microplastics in corals to gain a more comprehensive understanding of the extent of these pollutants.

4.2. Method justifications and limitations

Recently, there has been a call for more transparency when reporting the use of experimental controls in environmental microplastic studies (Dawson et al., 2023; Lao and Wong, 2023; Munno et al., 2023). We chose to use the LOD/LOQ method suggested in Dawson et al. (2023) because it was reported to differentiate microplastic contamination between samples and controls with over 95 % accuracy. The method was easy to employ; however, the detection and quantification limits (LOD and LOQ) depended on the standard deviation of particles in control samples. Given the variability in particle numbers on control filters, this likely increased our detection limit, potentially explaining the lower pollution levels observed compared to other studies. Interestingly, LODs and LOQs for anthropogenic particles (dyed cotton, cellulose,

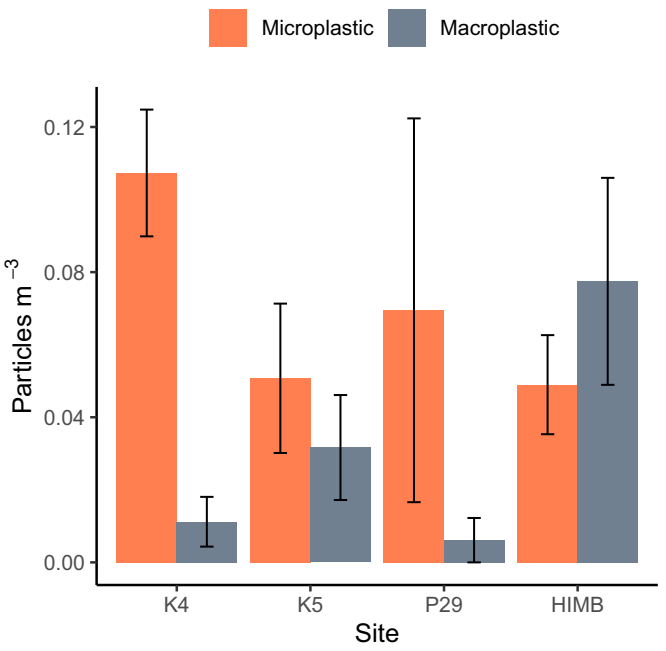

Fig. 6. Mean microplastic and macroplastic concentration in seawater by collection site. The data represent quantifiable particle concentrations in samples with particle counts above the limit of quantification based on control data. Microplastics were not quantifiable for sediment, sea cucumbers, or corals. Error bars represent one standard error.

cellophane, and rayon) were considerably higher than the ones for plastic and unknown particles. These anthropogenic particles were almost entirely small fibers, indicating fibers shed from clothing (Carney Almroth et al., 2018). While we made a great effort to reduce contamination in the lab, there was still considerable and variable contamination. Given this uncertainty in contamination levels, the use of stringent LOD/LOQ methods for accounting for our control data was warranted, and we encourage other labs to follow the same method.

In addition to synthetic polymers (i.e., microplastics), it has been suggested that other anthropogenic micro-debris should also be considered in environmental samples (Cesa et al., 2017; Macieira et al., 2021; Miller et al., 2021; Wright et al., 2013). In line with this, we classified the observed particles by their origin based on the material categories proposed by Miller et al. (2021), which include plastic, anthropogenic (cellulosic materials altered by human activity), natural, and unknown. This classification offers a more comprehensive view of micro-debris pollution in our samples. The distinction between plastic and anthropogenic micro-debris is valuable as it contributes to a deeper understanding of the effects of anthropogenic debris on marine life (Kroon et al., 2018; Reichert et al., 2024), beyond just microplastics. Moreover, this allowed us to identify potential sources of micro-debris contamination in the lab, especially when combined with distinct LODs and LOQs for each category. This approach can help guide future lab work aimed at reducing contamination during the analysis of environmental samples.

In hindsight, our LOD and LOQ might have been higher if we had used procedural blanks in the field, which we acknowledge we overlooked. However, we are confident that there was little chance for contamination during most aspects of field collections. Upon surfacing, the organisms collected underwater were immediately rinsed with filtered (1.2 μm) seawater for contamination and concealed in pre-cleaned foil; then, they were rinsed again before processing in the lab. Sediment collected underwater was immediately stored in closed glass mason jars. A potential source of contamination in those samples could have come from the rubber gaskets in the lids, but we did not detect rubber in any samples. Seawater samples could have been contaminated by formalin or when the collection jars were open to the air. However, contamination would have likely consisted of small particles or fibers, while the plastics we observed in seawater samples tended to be larger, mostly macroscopic particles.

4.3. Implications

Sub-surface habitats in Kāneʻohe Bay show minor contamination with microplastics, to the extent that microplastics were detected but not quantifiable. This may sound reassuring for the corals and other organisms that live there, but it should be noted that our sampling occurred over one summer, and as such, our data represents just one point in time. While it rains on most days in the Hawaiian Islands, most rainfall occurs during the rainy season from November to March. During the rainy season, it is possible that more microplastics are transported to the bay via river discharge and other sources of runoff (Lebreton et al., 2017). Seasonal fluctuations in microplastic contamination in coastal waters have been documented in other places, with increases typically associated with monsoon seasons (James et al., 2021; Jong et al., 2022; Nakano et al., 2024). Moreover, storm surges and wind could result in the resuspension of microplastics from the seabed sediments (Zhang, 2017). Consistent monitoring over multiple seasons should be implemented to better understand temporal trends in microplastic pollution in Kāneʻohe Bay. The results of this study can serve as critical baseline data for future studies.

The results of this study indicate that surface waters of Kāneʻohe Bay were more polluted by microplastics than the subsurface environments. Based on the season when we collected samples and the geography of the study area, it is most likely that the observed plastic pollution entered the bay from the North Pacific Ocean, likely originating from so-called “garbage patches” (Brignac et al., 2019). The most likely fate for these floating plastic debris is the bay's shorelines, where they can accumulate. This could have implications for coastal organisms, where microplastic debris could alter the composition of shoreline sediments, and for tourism, where plastic debris could render the waterfront less attractive for tourists. Hawaiʻi has multiple programs that address marine debris, both governmental (Hawaii Department of Land and Natural Resources, n.d.; Hawaii Sea Grant, n.d.; NOAA Marine Debris Program, n.d.) and non-governmental (Hawaiʻi Wildlife Fund, n.d.; Ocean Defenders Alliance, n.d.; Sustainable Coastlines Hawaiʻi, n.d., etc.). These programs will be critical for managing shoreline plastic pollution. However, it is essential to implement global efforts to reduce plastic waste in the North Pacific Ocean and worldwide. This approach ensures that states are not solely responsible for cleaning up waste that they did not generate.

Coral reefs are under increasing pressure from a suite of stressors, but is all the attention given to microplastics pollution on reefs warranted? Reef-building corals are the keystone sentinels of tropical reefs, and laboratory experiments have demonstrated a range of negative physiological effects in corals exposed to microplastics (Chapron et al., 2018; Hankins et al., 2021; Lanctôt et al., 2020; Liao et al., 2021; Mendrik et al., 2021; Reichert et al., 2019, 2018; Tang et al., 2018). However, many studies acknowledge that microplastic treatment concentrations in experiments exceed what corals experience in the field or use particle types (e.g., microspheres) that are not commonly observed on reefs (Axworthy and Padilla-Gamiño, 2019; Bove et al., 2023; Chen et al., 2022; Hankins et al., 2021, 2018; Lanctôt et al., 2020; Liao et al., 2021; Montalbetti et al., 2022; Rotjan et al., 2019; Tang et al., 2018). Some studies have attempted to investigate the effects of environmentally relevant microplastic treatments on corals (Bejarano et al., 2022; Berry et al., 2019; Boodraj and Glassom, 2022; Mendrik et al., 2021; Plafcan and Stallings, 2022; Reichert et al., 2022), and those studies often conclude that there are minimal or uncertain effects on certain corals (Bejarano et al., 2022; Berry et al., 2019; Boodraj and Glassom, 2022; Plafcan and Stallings, 2022). Moreover, there is little evidence that corals in the field are suffering significantly from microplastics (Lim et al., 2022; Tang et al., 2021; Zhou et al., 2023). The effects of climate

change, most notably coral bleaching and mortality due to rising temperatures, are impacting corals at enormous and rapid scales (Eakin et al., 2019; Hoegh-Guldberg, 1999; Hughes et al., 2018). Localized issues, such as nutrification, disease, overfishing, and even macroplastic pollution could also contribute to substantial coral declines (D'Angelo and Wiedenmann, 2014; Green and Bruckner, 2000; Lamb et al., 2018; Roberts, 1995). Reducing plastic pollution (macro and micro) can alleviate additional stress on already vulnerable organisms, potentially avoiding additive or synergistic effects that can worsen their condition. Given the undeniable trend of increasing plastic pollution, proactive measures to curb its impact are crucial for the long-term health of marine ecosystems like coral reefs. We recommend ongoing monitoring of plastic pollution in reefs and efforts to deepen our understanding of its implications. However, without decisive action to reduce global emissions, mitigate the effects of climate change, and address imminent local stressors in tropical systems, we risk losing our corals before fully understanding the extent of plastic pollution in them.

5. Conclusion

In the summer of 2018, microplastic contamination in Kāneʻohe Bay was relatively low compared to other tropical coral reefs, providing valuable baseline data regarding these persistent pollutants. Most plastic pollution was concentrated in surface waters, consisting of floating plastic debris that will likely end up on the bay's shorelines rather than in the reefs below the water's surface. Further monitoring and laboratory testing are recommended to better understand seasonal and long-term trends in microplastic pollution and the potential future impacts of microplastic pollution in Kāneʻohe Bay. However, microplastics are likely a relatively minor form of stress to coral reefs compared to climate change's more significant impacts.

CRediT authorship contribution statement

Jeremy B. Axworthy: Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Katherine S. Lasdin:** Writing – review & editing, Methodology, Formal analysis. **Jacqueline L. Padilla-Gamiño:** Writing – review & editing, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The datasets generated during and analysed during the current study are available in the Figshare repository, https://doi.org/10.6084/m9.figshare.26097718

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2024.116996.

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