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Microplastics absent from reef fish in the Marshall Islands: Multistage screening methods reduced false positives

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ABSTRACT

Island communities, like the Republic of the Marshall Islands (RMI), depend on marine resources for food and economics, so plastic ingestion by those resources is a concern. The gastrointestinal tracts of nine species of reef fish across five trophic groups (97 fish) were examined for plastics >1 mm. Over 2100 putative plastic particles from 72 fish were identified under light microscopy. Only 115 of these from 47 fish passed a plastic screening method using Fourier-transform infrared microspectroscopy (μFTIR) in reflectance mode. All of these were identified as natural materials in a final confirmatory analysis, attenuated total reflectance FTIR. The high false-positive rate of visual and μFTIR methods highlight the importance of using multiple polymer identification methods. Limited studies on ingested plastic in reef fish present challenging comparisons because of different methods used. No plastic >1 mm were found in the RMI reef fish, reassuring human consumers.

1. Introduction

Human activity has led to widespread plastic contamination throughout the world's oceans. Plastic marine debris is degraded and fractured as a result of exposure to ultraviolet light and physical abrasion from wind and waves (Andrady, 2011). As plastics degrade and become smaller microplastics (defined as < 5 mm, Arthur et al., 2009), they become available to be incidentally consumed by a diversity of marine organisms. As a result of growing plastic pollution, microplastics are ingested by many marine animals including reef and pelagic fishes, which are commonly consumed by humans (Baalkhuyur et al., 2018; Ory et al., 2018; Markic et al., 2019; Huang et al., 2023). In a recent meta-analysis, plastic ingestion was detected in 69.5 % of 555 marine and estuarine fish species examined worldwide (Savoca et al., 2021), which includes pelagic, demersal, and reef fishes across different feeding strategies and trophic levels. However, the detection method used (e.g., microscopy vs. spectroscopy) may bias results and lead to over or underestimation of plastic concentrations (Song et al., 2015; Hanvey et al., 2017). Polymer identification with Fourier transform infrared spectroscopy (FTIR) and Raman was performed in 49 % of the studies included in that meta-analysis. When microplastics are only identified visually and not verified with chemical polymer identification, up to 70 % of particles are shown to be misidentified as plastic (Lusher et al., 2017).

Ingested plastics can cause physical damage to GI tracts including abrasions, lesions, or gut blockage and can increase the risk of starvation when indigestible particles fill the stomach and reduce hunger (Jovanović, 2017). Ingestion can also cause direct mortality via gut obstruction and perforation, which has been documented in seabirds (Pierce et al., 2004), sea turtles (Orós et al., 2021), and marine mammals (Puig-Lozano et al., 2018). However, direct mortality caused by ingested plastic has not yet been documented in wild fish, and the physiological effects of plastic ingestion on wild organisms are not well known (Porcino et al., 2023). Sublethal physical impacts of microplastic

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ingestion in fish include decreased feeding performance, decreased mobility, reduced growth, poor body condition, neurotoxic effects, toxic effects on development, and increased mortality when co-exposed with viruses (de Sa et al., 2015; Critchell and Hoogenboom, 2018; Barboza et al., 2020; Ferrante et al., 2022; Contino et al., 2023; Seeley et al., 2023). In addition, some plastics contain toxic chemicals from additives added during manufacturing including plasticizers and flame retardants (Rani et al., 2015). Marine plastics also adsorb anthropogenic compounds present in the water including heavy metals, pesticides, fertilizers and other industrial chemicals including persistent organic pollutants (Rochman et al., 2014; Massos and Turner, 2017; Anbumani and Kakkar, 2018). These chemicals have a high capacity to desorb and transfer into the tissues of animals from ingested plastics (Hermabessiere et al., 2017), which can impact fish health and may pose food safety concerns for humans.

Some island communities are exposed to a large amount of plastic marine debris due to their position relative to the so-called garbage patches (see Eriksen et al., 2014 for the locations of the accumulation zones). The Republic of the Marshall Islands (RMI) are located far away from the garbage patches (Fig. 1). The prevailing surface currents are towards the north and west (Mao and Yoshida, 1955) with little to no land mass upstream of RMI to provide floating plastic marine debris. Sources of marine plastic pollution in RMI are more likely from industrial fishing industries operating in the tropical Pacific Ocean (e.g. Escalle et al., 2022) and mismanaged waste from the islands themselves. These communities are highly dependent on marine resources for their food security and economy, putting them at greater potential risk of detrimental effects from plastic pollution. It is extremely time consuming to evaluate all plastic-associated effects (e.g., chemical additives in plastic, nanoplastics, etc.) but baseline data to evaluate the risk caused by plastic pollution is critical. This study aimed to establish the baseline exposure amount of plastics $>$ 1 mm ingested by Marshall Island reef fish. The authors hypothesize reef fish from nearshore areas on islands with a greater human population will have higher microplastic ingestion amounts than fish near sparsely inhabited islands. The lower cutoff size of 1 mm was chosen specifically as this is the smallest size of plastic that can be reliably handpicked with forceps. This baseline data can act as a starting point to determine potential impacts to fish from the ingestion of microplastics, and to humans from consuming those fish.

2. Methods

2.1. Sample collection

Samples were collected by the Marshall Islands Marine Resources Authority (MIMRA) between March and July of 2020 from six atolls: Majuro (developed, urbanized capital); Kwajalein (U.S. military base since World War II); Rongelap (few residents due to relocation following U.S. military nuclear bomb testing); Utirik (small remaining population following contamination from U.S. military nuclear bomb testing); Jaluit (previous military base and current conservation area); and Wotje (previous military base and small present population). The human population on these atolls ranges from $<$100 on Rongelap to $>$27,000 on Majuro, and they were selected by MIMRA because of their distinct historic and present land uses. Sampling at Kwajalein atoll included sample sites in three sub-areas that were of interest to local managers and community leaders: Ebadon, Mid-Corridor Oceanside, and South Kwajalein (Fig. 1).

At each of the six sites, sampling targeted three individuals of five species (15 total fish samples) representing multiple trophic levels. All samples were collected using spears or hook and line by MIMRA personnel, compliant with local regulations. Targeted species were selected by MIMRA based on the fish consumption preference of Marshallese. Reef fish are an important part of coastal fisheries in the RMI and therefore are an important food source locally (Pinca et al., 2009; Nalley et al., 2023). The priority targets included grazing herbivores (*Acanthurus triostegus* - kupãn/convict tang), browsing herbivores (*Naso lituratus* - bwilak/orangespine unicornfish), parrotfish (*Chlorurus sordidus* - merã/bullethead parrotfish), benthic invertivores (*Lutjanus gibbus* - jato/humpback snapper), and carnivores (*Epinephelus polyphekadion* - kũro/camouflage grouper). In cases where these taxa were not available, appropriate substitutions were made based on the most functionally similar species present (see Table 1 for sampled species at each site). The samples were wrapped in aluminum foil, labeled, placed in plastic bags, and stored frozen following collection. They were then shipped frozen on dry ice to the Hawai'i Institute of Marine Biology, where they continued to be stored frozen at −20 °C.

2.2. Quality control

Sample processing took place at the Center for Marine Debris Research in Waimānalo on the island of O'ahu in Hawai'i. To prevent contamination from airborne or surface microplastics, many quality

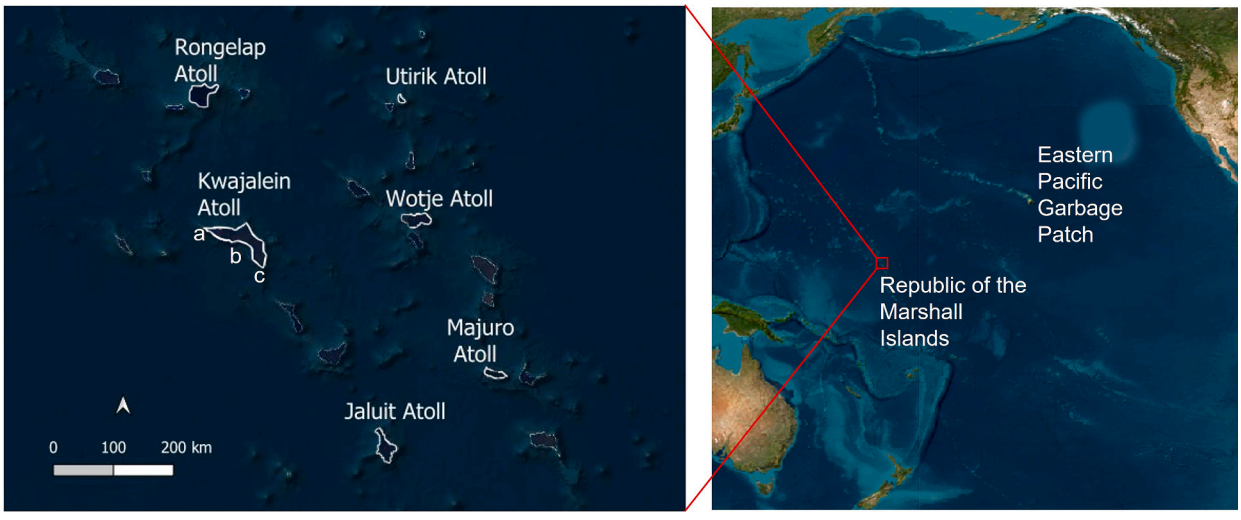

Fig. 1. Map of sampling sites within the Republic of the Marshall Islands. Three sites were sampled on Kwajalein Atoll: a) Ebadon, b) Mid-Corridor Oceanside, c) South Kwajalein.

Table 1
Metadata for the 97 fish samples examined, including Atoll (and location), trophic group, species, individuals per species per location (n), fish total length (cm; mean +/− SD), and weight (grams; mean +/-SD).

ATOLL	TROPHIC GROUP	SPECIES	n	LENGTH (CM) [mean (SD)]	WEIGHT (G) [mean (SD)]
Kwajalein: South	Scraper-Parrotfish (microphage)	*Chlorurus sordidus*	3	26.60 (4.85)	398.37 (218.84)
	Herbivore (browser)	*Naso lituratus*	3	30.80 (7.45)	388.20 (158.00)
	Benthic Invertivore	*Lutjanus gibbus*	3	33.60 (4.18)	466.37 (178.56)
	Carnivore	*Epinephelus polyphekadion*	3	31.37 (4.21)	486.73 (144.30)
Kwajalein: Ebadon	Scraper–Parrotfish (microphage)	*Chlorurus microrhinos*	1	39.7	939.1
	Scraper–Parrotfish (microphage)	*Chlorurus sordidus*	2	57.10 (4.38)	2092.85 (308.65)
	Herbivore (browser)	*Naso lituratus*	3	29.13 (6.06)	555.20 (303.96)
	Benthic Invertivore	*Lutjanus gibbus*	3	32.67 (1.80)	529.87 (150.22)
	Carnivore	*Lutjanus bohar*	1	66.7	5000
	Carnivore	*Plectropomus laevis*	1	69.8	4800
	Carnivore	*Variola louti*	2	46.05 (1.34)	936.10 (11.60)
Kwajalein: Mid-Channel	Benthic Invertivore	*Lutjanus gibbus*	3	31.10 (1.01)	382.43 (11.72)
	Carnivore	*Epinephelus polyphekadion*	3	33.93 (2.41)	865.83 (366.50)
Jaluit	Herbivore (browser)	*Naso lituratus*	3	36.07 (2.00)	357.43 (115.10)
	Benthic Invertivore	*Lutjanus gibbus*	3	30.20 (0.95)	392.60 (23.58)
	Carnivore	*Epinephelus polyphekadion*	3	41.30 (2.61)	929.27 (578.49)
Majuro	Scraper-Parrotfish (microphage)	*Chlorurus sordidus*	3	28.77 (6.53)	358.93 (31.25)
	Herbivore (grazer)	*Acanthurus triostegus*	3	17.40 (0.79)	131.20 (14.77)
	Herbivore (browser)	*Naso lituratus*	3	33.67 (7.99)	442.57 (109.87)
	Benthic Invertivore	*Lutjanus gibbus*	3	25.73 (0.75)	228.57 (3.67)
	Carnivore	*Epinephelus polyphekadion*	3	27.57 (1.18)	315.90 (69.50)
Rongelap	Scraper-Parrotfish (microphage)	*Chlorurus sordidus*	3	34.60 (0.53)	798.80 (45.04)
	Herbivore (grazer)	*Acanthurus triostegus*	3	14.73 (0.71)	89.27 (11.87)
	Herbivore (browser)	*Naso lituratus*	3	35.80 (2.08)	448.57 (14.39)
	Benthic Invertivore	*Lutjanus gibbus*	3	26.37 (8.26)	302.17 (245.71)
	Carnivore	*Variola louti*	1	43	750.3
Utirik	Scraper-Parrotfish (microphage)	*Chlorurus sordidus*	3	30.17 (9.05)	585.60 (501.79)
	Herbivore (grazer)	*Acanthurus triostegus*	3	16.90 (0.80)	132.83 (17.73)
	Herbivore (browser)	*Naso lituratus*	3	36.60 (0.87)	457.73 (20.50)
	Benthic Invertivore	*Lutjanus gibbus*	2	20.40 (1.56)	125.40 (29.27)
	Carnivore	*Epinephelus polyphekadion*	3	35.73 (1.02)	667.27 (95.87)
Wotje	Scraper-Parrotfish (microphage)	*Chlorurus sordidus*	3	32.93 (5.76)	665.17 (315.06)
	Herbivore (grazer)	*Acanthurus triostegus*	3	16.60 (1.35)	114.73 (29.50)
	Herbivore (browser)	*Naso lituratus*	3	35.83 (5.51)	464.87 (185.71)
	Benthic Invertivore	*Lutjanus gibbus*	3	24.93 (0.81)	206.80 (17.10)
	Carnivore	*Epinephelus polyphekadion*	3	30.80 (4.55)	692.23 (567.59)

control steps were taken (Hermsen et al., 2018). All protocol steps for microplastic processing (e.g., fish dissection, GI tract dissection, sieving, and microscopic evaluation) were performed in a HEPA-filtered laminar flow hood (AirClean 600 PCR Workstation). Glassware and metal dissection utensils were scrubbed, sonicated in soapy water for 20 min, rinsed three times with tap water and three times with Millipore high-purity deionized water (resistivity = 18.2 MΩ cm^{-1}; 0.2 μm filtered; hereinafter referred to as high-purity water), then baked in a muffle furnace (Thermo Scientific Thermolyne) at 450 °C for 4 h. Utensils that could not be baked (ceramic knives and forceps) or needed to be used again on the same day were sonicated in soapy water, rinsed three times with tap water and three times with high-purity water, then rinsed once with trace analysis grade methanol (Honeywell International) followed by rinsing three times with high-purity GC grade hexane (Honeywell International) from a Teflon squirt bottle. Cleaned utensils were wrapped in baked foil until use. All foil used throughout the project was baked at 450 °C for 4 h. Three blanks were created using the same materials as the fish dissection. A piece of foil was rinsed with high-purity deionized water, capturing approximately 10 mL of water in a pocket of the foil. In the laminar flow hood, all utensils used in a dissection (stainless steel scissors, ceramic forceps, ceramic knife) were dipped into the water pocket. The water was transferred to a new piece of foil, and new utensils were dipped in the water. The water was then folded into a pouch, wrapped in another piece of foil, placed in a Ziplock bag, frozen at −20 °C, then transferred to −80 °C, in the same protocol as the dissected GI tracts. The blanks were processed according to the methods described below for assessment of >1 mm sized microplastics.

2.3. Sample processing

Fish samples were allowed to thaw to room temperature, measured for length and mass (OHAUS Scout Pro SP4001 with a 0.1 g resolution), and photographed. Held with forceps hooked through the gills or mouth, the fish was rinsed with high-purity water from just behind the gills to the tail before processing to remove potential microplastics from the outside of the fish just before the incision into the body cavity was made. The fish was dissected on a piece of pre-cleaned aluminum foil in a laminar flow hood (Fig. 2).

Great care was taken to avoid touching the fish with gloves, and all handling of the fish was done with pre-cleaned aluminum foil, ceramic knives and forceps, and stainless-steel scissors as needed. The fish was sliced from the cloaca towards the head to remove the gastrointestinal (GI) tract from the esophagus to the anus, and the sex was recorded when possible. GI tracts were separately stored in pre-cleaned foil at −80 °C until further processing for microplastics could occur. The remainder of the fish (i.e., the whole fish minus the GI tract) was rewrapped in precleaned foil, labeled, and stored at −80 °C for organic and inorganic contaminant analyses (Nalley et al., 2023).

The GI tracts were thawed at room temperature inside the precleaned foil. While inside of the laminar flow hood, the GI tracts were removed from the foil and placed into a glass baking dish using a metal spatula. The foil was rinsed three times with high-purity water over the glass

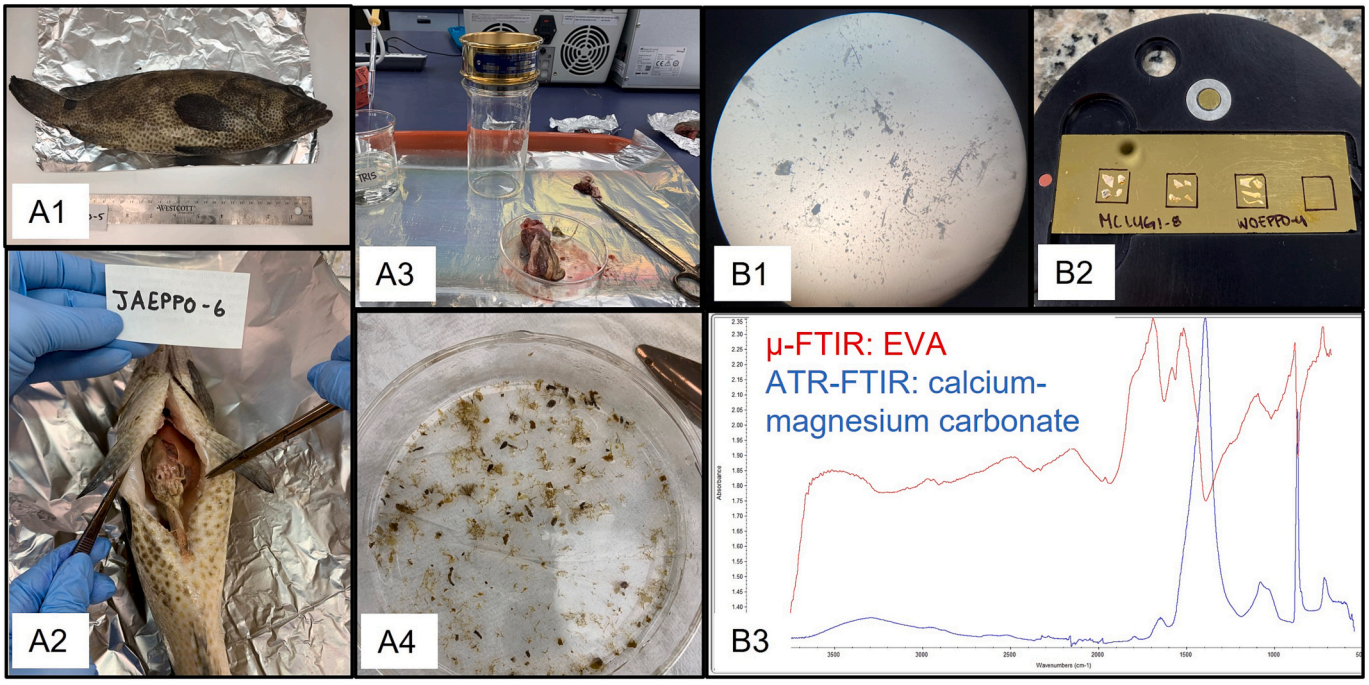

Fig. 2. Overview of the methods used in fish dissection and plastics >1 mm identification. A1: Measurements of the whole fish. A2: Dissection of the fish to remove GI tract. A3: GI tract removed from the fish and placed in a petri dish to be cut open. The contents of the GI tract were put in the 1 mm sieve (in the background of the photo). A4: Contents of GI tract >1 mm after sieving. B1: Visual identification of potential plastic particles at 10× magnification using a dissecting microscope. B2: Potential plastic pieces on gold slide for analysis on the μFTIR. B3: Spectra of a particle identified on the μFTIR as EVA (red) but shown to be a natural material when analyzed by the ATR-FTIR (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

baking dish to retain any materials from the GI tract. All GI contents were scraped out using a metal spatula into the glass baking dish. GI tracts were then rinsed three times with high-purity water over a 1 mm sieve that was placed over a glass beaker, where all liquids and filtrate were collected. This ensured all remaining particles from the GI tract were captured. Any large identifiable prey items were recorded and preserved for future secondary microplastic ingestion analysis. The contents from the sieve were then carefully scooped into a petri dish for visual analysis described below.

2.4. Particle analysis and microplastic identification

All suspected microplastic particles went through a three-stage screening process with strict quality assurance criteria to ensure all particles were correctly identified. Particles were first subject to visual inspection (1: Visual Analysis), then analyzed using Fourier-transform infrared microspectroscopy (μFTIR) in reflectance mode (2: Screening Analysis) and, finally, attenuated total reflectance FTIR (ATR-FTIR) (3: Confirmatory Analysis). The details of each of the three analyses are described next.

Using a dissecting microscope (10× magnification) with a white background (and then repeated under a black background), all contents in the glass baking dish from the GI tract were carefully inspected for potential microplastics (approximately 5 mL at a time into a 9.5 cm diameter glass petri dish). Suspect plastics were first identified visually by their color, shape, and texture, placed on an aluminum pan, covered with foil, and allowed to dry at room temperature for particle analysis. After the petri dish was fully inspected and suspect plastics were removed, the contents were poured through the 1 mm sieve that was over a 400 mL glass beaker. Once all material was removed from the baking dish, it was rinsed three times with high-purity water to capture any remaining particles and a final inspection process was completed. No particles >1 mm were collected from the three blank samples.

Potential microplastics were first placed on a gold slide (Thermo Fisher Scientific, Madison, WI, USA) and analyzed using μFTIR (iN10 MX Thermo Fisher Scientific, Madison, WI, USA) with a 15× objective. Using the Thermo Fisher Scientific OMNIC Picta software, FTIR spectra and length/width dimensions of each particle were collected using the Particle Wizard. Mosaics were captured with 150 μm × 150 μm aperture and 100 μm × 100 μm step size. Spectra were collected in reflectance mode with 16 scans and 8 cm^{-1} resolution from 675 cm^{-1} to 4000 cm^{-1}. All spectra were searched through the in-house reflectance FTIR spectral library containing 31 natural materials and 94 items consisting of 19 polymers, and the best match from Pearson correlation with a match quality index of ≥70 % was recorded. The μFTIR is used in this step because its automation allows for larger numbers of small particles to be analyzed in a relatively short amount of time.

Particles identified as potentially synthetic plastic polymers by μFTIR were set aside for further confirmatory analysis. Each of these particles were weighed using a Sartorius microanalytical balance to 0.001 mg and then analyzed on a Thermo Fisher Scientific iS5 ATR-FTIR (Madison, WI USA). No additional sample preparation was done and the entire particle was used for polymer identification. Spectra were collected from 4000 cm^{-1} to 550 cm^{-1} with a data interval of 0.482 cm^{-1}. Resolution was set to 4 cm^{-1} with 16 scans. The ATR diamond crystal was cleaned with 70 % (volume fraction) isopropanol and a background scan taken before running each sample. All spectra were searched through spectral libraries provided with the OMNIC software (1000 + spectra), as well as the in-house spectral library of 31 natural materials and 25 synthetic polymers of 122 items. Final determination of each particle's material was made using >70 % match scores from the ATR-FTIR. ATR-FTIR spectra provide greater certainty because they rely on multiple thorough commercial spectral libraries, which do not currently exist for most polymers using reflectance mode with the μFTIR. In addition, ATR-FTIR spectra are inherently more resolved with clear peaks, while reflectance spectra often have poorly defined peaks (De Frond et al., 2023).

In the narrative review for this paper, a literature review was conducted using the web-based search engine Google Scholar with the following keywords/phrases: “plastic ingestion by reef fish” OR “plastic ingestion by fish” between 2010 and September 2023. The publications

were screened for studies related to reef fish in the Pacific Ocean.

3. Results

A total of 97 GI tracts from nine species of reef fishes were inspected for plastic particles >1 mm (Table 1). On average, 10 GI tract samples were examined per species (range: 1 to 23), with an average total fish length of 31.5 cm (range: 14.1 to 69.8 cm) and an average fish weight of 490 g (range: 79.3 g to 5 kg). Information for individual fish can be found in Table S1.

Through visual analysis, a total of 2147 suspected plastics >1 mm were found in the GI tracts in 72 of the 97 fish and warranted further analysis. The μFTIR (2: Screening Analysis) was used to screen all 2147 suspect plastic particles and only 115 of the microparticles from 47 fish were identified putatively as synthetic polymers by μFTIR, and most of these were unusual because they are not commonly found in the environment (Table S2). For example, 25 particles matched with polyurethane (PU), and 29 particles matched with polyether block amide (PEBAX). These identifications were suspect because these polymers are not commonly found in Pacific island marine environments (Brignac et al., 2019) and PEBAX is a specialty polymer in low production quantities. Using the ATR-FTIR (3: Confirmatory Analysis), these 115 suspect particles were all determined to be natural materials, most frequently calcium carbonate, sand, algae, or keratin. Collected spectra were searched through an in-house library of 31 natural materials to confirm their identification. The ATR-FTIR results confirmed that none of the suspected plastic particles were synthetic polymers (Table 2, Table S2). Therefore, this analysis shows that of the nine reef fish species with a diversity of foraging guilds in the Marshall Islands, the total incidence rates of ingesting plastics >1 mm was 0 % (0 of 97 fish).

The fish species with the most putative plastic fragments before μFTIR screening were *C. sordidus* which are corallivores (Table 2). Parrotfish GI tracts contained large quantities of white fragments that resembled white microplastic fragments commonly found in Hawaii (Figs. 2 part B2 and S1). These were all found to be calcium carbonate, which comprises coral skeletons.

There was one exception where one fish was confirmed to have a synthetic polymer in its GI tract, but this item was a nylon fishing line that was attached to a metal fishing hook that had pierced the mouth and esophagus of a humpback red snapper (*Lutjanus gibbus*). It was determined to be the fishing line and hook used to capture the specimen and not an ingested piece of marine debris.

Our literature search revealed five studies of plastic ingestion in reef fish in the Pacific Ocean (Forrest and Hindell, 2018; Markic et al., 2018; Garnier et al., 2019; Nie et al., 2019; Huang et al., 2023). Among the 42 fish species studied in the Pacific Ocean, the frequency of occurrence for plastic ingestion was extremely variable, from 0 % in this study to 100 % in parrotfish from the South China Sea (Table 3). The mean ingestion quantities rarely exceeded one particle per fish with the maximum being nine microfibers per parrotfish from the South China Sea. The data are limited and the methodological differences among studies are substantial, making species and geographical comparisons difficult. Species and location differences could not be statistically tested because all fish had no ingested plastics; therefore no further data analysis was warranted.

4. Discussion

A three-stage screening process was used to thoroughly investigate plastic ingestion by reef fish in the Marshall Islands. The first stage, visual analysis, has been mentioned as an appropriate method for particles >50 μm (De Frond et al., 2023). However, visual inspection is insufficient at differentiating microplastics from natural materials. In one report, up to 70 % of particles handpicked from a sample that resembled microplastics were shown not to be made of plastic (Lusher et al., 2017). The difficulty in visual identification can be seen in this study, with all 2100+ particles picked from samples as potential microplastics later being verified as natural materials. Over 50 % of the particles picked from samples (1123 particles) came from 17 parrotfish (*C. sordidus*; Table 2 & S1). Parrotfish are corallivores, and ingest large amounts of calcium carbonate fragments that can easily be mistaken for white microplastics. The second stage, reflectance μFTIR, is an automated screening technique useful for analyzing large numbers of particles; however, because collecting spectra of microplastics in reflectance mode is relatively unexplored, thorough commercial spectral libraries in this mode are not yet available. The absence of specific materials in spectral libraries can make it difficult, if not impossible, to identify particles. For example, many natural materials like animal fur or wool are not in libraries, limiting the usefulness of spectra matching (De Frond et al., 2023). This was not the case in our study; natural materials including sand, keratin, and algae were present in our in-house libraries. The poor matches are due to poor focusing on the particles, a surface coating of organic material, or the inherent properties of reflectance mode spectra that make them less resolved and therefore less specific (Fig. S2). In reflectance FTIR, both diffuse and specular components contribute to the spectrum making it more complex and not always directly comparable to spectra collected in ATR or transmission modes (Picollo et al., 2014). Of the three modes of FTIR, attenuated total reflection (ATR), reflectance, and transmission, ATR is the most accurate (De Frond et al.,

Table 2
Incidence rate of microplastics in fish gastrointestinal tracts examined in this study as determined at each step of the 3-step screening process: (1) Visual assessment, (2) μFTIR Screening Analysis, (3) ATR-FTIR Confirmatory analysis.

Species	Trophic Group	Length (cm) mean ± SD (range)	Number of Fish Examined	Number of Fish with Suspected Plastics ID by Visual Assessment (# of particles)	Number of Fish with Suspected Plastics ID by μFTIR (# of particles)	Number of Fish with Ingested Plastic Confirmed with ATR-FTIR
Acanthurus triostegus	Grazer	16.4 ± 1.3 (14.1–18.3)	12	9 (170)	5 (27)	0
Chlorurus microrhinos	Parrotfish	39.7	1	0 (0)	0 (0)	0
Chlorurus sordidus	Parrotfish	33.7 ± 10.4 (22.0–60.2)	17	16 (1123)	12 (22)	0
Epinephelus polyphekadion	Carnivore	33.5 ± 5.1 (26.2–43)	18	12 (178)	6 (21)	0
Lutjanus bohar	Carnivore	66.7	1	1 (16)	1 (7)	0
Lutjanus gibbus	Benthic Invertivore	28.5 ± 5.0 (19.3–36.4)	23	18 (490)	11 (21)	0
Naso lituratus	Browser	34.0 ± 5.2 (22.2–41.5)	21	15 (167)	10 (17)	0
Plectropomus laevis	Carnivore	69.8	1	1 (1)	0 (0)	0
Variola louti	Carnivore	45.0 ± 2.0 (43.0–47.0)	3	2 (2)	0 (0)	0

Table 3
Literature review of ingestion rates of microplastics by coral reef fish in the Pacific Ocean. %FO = % frequency of occurrence. N/A = data not available.

	Location	*Species*	Common Name	n	% FO	#plastic particles ingested/fish (mean, mean ± SD)	Average or range of plastic size ingested or analyzed for	Reference
Scraper-parrotfish	Eastern French Polynesia	*Chlorurus sordidus*	Daisy/Bullethead parrotfish	4	25	N/A	> 1 mm	Forrest and Hindell, 2018
	South China Sea	*Scarus tricolore*	Tricolour parrotfish	12	58	1.42	< 1 mm	Huang et al., 2023
	South China Sea	*Scarus schlegeli*	Yellowband parrotfish	3	100	9.33	< 1 mm	Huang et al., 2023
	South China Sea	*Scarus sordidus*	Darktail parrotfish	4	100	3.5	< 1 mm	Huang et al., 2023
	Samoa	*Scarus niger*	Dusky parrotfish	30	23	0.27	63 μm - 5 mm	Markic et al., 2018
	Samoa	*Scarus oviceps*	Dark capped parrotfish	45	11	0.31	63 μm - 5 mm	Markic et al., 2018
	Tahiti	*Scarus psittacus*	Common parrotfish	30	17	0.17	63 μm - 5 mm	Markic et al., 2018
	Marshall Islands	*Chlorurus sordidus*	Daisy/Bullethead parrotfish	17	0	0	> 1 mm	This Study
	Marshall Islands	*Chlorurus microrhinos*	Steephead parrotfish	1	0	0	> 1 mm	This Study
Grazer	Rapa Nui	*Kyphosus sandwicensis*	Pacific chub	39	51	2.1	63 μm - 5 mm	Markic et al., 2018
	Tahiti	*Ellochelon vaigiensis*	Squaretail mullet	33	48	2.06	63 μm - 5 mm	Markic et al., 2018
	Samoa	*Acanthurus lineatus*	Lined surgeonfish	24	17	0.25	63 μm - 5 mm	Markic et al., 2018
	Samoa, Tahiti	*Ctenochaetus striatus*	Striated surgeonfish	56	23	0.3	63 μm - 5 mm	Markic et al., 2018
	South China Sea	*Siganus puellus*	Masked rabbitfish	8	25	0.38	< 1 mm	Huang et al., 2023
	South China Sea	*Siganus argenteus*	Silver rabbitfish	10	30	1.3	< 1 mm	Huang et al., 2023
	South China Sea	*Siganus punctatissimus*	Fine-spotted rabbitfish	10	10	0.1	< 1 mm	Huang et al., 2023
	South China Sea	*Acanthurus pyroferus*	Chocolate surgeonfish	N/A	N/A	0–4	0.45 μm −5 mm	Nie et al., 2019
	South China Sea	*Acanthurus japonicus*	Japan surgeonfish	N/A	100	1–6	0.45 μm −5 mm	Nie et al., 2019
	French Polynesia	*Siganus spp*	N/A	33	15	0.15 ± 0.10	31 μm −2.4 mm	Garnier et al., 2019
	Marshall Islands	*Acanthurus triostegus*	Convict Tang	12	0	0	> 1 mm	This Study
Browser	South China Sea	*Naso lituratus*	Orange-spined unicornfish	N/A	0	0	0.45 μm −5 mm	Nie et al., 2019
	South China Sea	*Naso brevirostris*	Spotted unicornfish	1	100	6	0.45 μm −5 mm	Nie et al., 2019
	Samoa	*Naso lituratus*	Orangespine unicornfish	28	14	0.25	63 μm - 5 mm	Markic et al., 2018
	Samoa	*Naso unicornis*	Bluespine unicornfish	30	17	0.23	63 μm - 5 mm	Markic et al., 2018
	French Polynesia, Lord Howe Island, Henderson Island	*Naso brachycentron*	Humpback unicornfish	1	0	0	> 1 mm	Forrest and Hindell, 2018
	Marshall Islands	*Naso lituratus*	Orange-spined unicornfish	21	0	0	> 1 mm	This Study
Benthic Invertivore	Auckland	*Pagrus auratus*	Silver seabream	22	5	0.05	63 μm - 5 mm	Markic et al., 2018
	Samoa	*Lethrinus amboinensis*	Ambon emperor	26	23	0.38	63 μm - 5 mm	Markic et al., 2018
	Tahiti	*Gnathodentex aureolineatus*	Striped large-eye bream	29	7	0.07	63 μm - 5 mm	Markic et al., 2018
	Samoa	*Lethrinus obsoletus*	Orange-striped emperor	30	13	0.17	63 μm - 5 mm	Markic et al., 2018
	Samoa	*Lutjanus gibbus*	Humpback red snapper	29	21	0.41	63 μm - 5 mm	Markic et al., 2018
	Rapa Nui	*Heteropriacanthus cruentatus*	Glasseye	10	30	0.3	63 μm - 5 mm	Markic et al., 2018
	South China Sea	*Lethrinus rubrioperculatus*	Spotcheek Emperor	10	0	0	< 1 mm	Huang et al., 2023
	South China Sea	*Plectorhynchus orientalis*	Oriental Sweetlips	6	0	0	< 1 mm	Huang et al., 2023
	Henderson Island	*Thalassoma purpureum*	Surge wrasse	2	50	N/A	> 1 mm	Forrest and Hindell, 2018

(*continued on next page*)

Table 3 (*continued*)

	Location	*Species*	Common Name	n	% FO	#plastic particles ingested/fish (mean, mean ± SD)	Average or range of plastic size ingested or analyzed for	Reference
	French Polynesia, Lord Howe Island, Henderson Island	*Thalassoma trilobatum*	Christmas wrasse	4	25	N/A	> 1 mm	Forrest and Hindell, 2018
	French Polynesia, Lord Howe Island, Henderson Island	*Oxycheilinus unifasciatus*	Ringtail wrasse	1	0	0	> 1 mm	Forrest and Hindell, 2018
	French Polynesia, Lord Howe Island, Henderson Island	*Lutjanus gibbus*	Paddletail snapper	1	0	0	> 1 mm	Forrest and Hindell, 2018
	Marshall Islands	*Lutjanus gibbus*	Humpback Red snapper	23	0	0	> 1 mm	This Study
Carnivore	Henderson Island	*Variola louti*	Coronation grouper	15	20	N/A	> 1 mm	Forrest and Hindell, 2018
	French Polynesia, Lord Howe Island, Henderson Island	*Epinephelus fasciatus*	Blacktip grouper	17	0	0	> 1 mm	Forrest and Hindell, 2018
	French Polynesia	*Epinephelus merra*	Honeycomb grouper	33	30	0.39 ± 0.14	31 μm −2.4 mm	Garnier et al., 2019
	South China Sea	*Cephalopholis argus*	Peacock grouper	10	0	0	< 1 mm	Huang et al., 2023
	South China Sea	*Epinephelus fasciatus*	Blacktip grouper	12	33	0.08	< 1 mm	Huang et al., 2023
	South China Sea	*Variola louti*	Red louti grouper	10	20	0.7	< 1 mm	Huang et al., 2023
	South China Sea	*Epinephelus merra*	Mottled grouper	13	23	0.15	< 1 mm	Huang et al., 2023
	Marshall Islands	*Epinephelus polyphekadion*	Camoflauge grouper	18	0	0	> 1 mm	This Study
	Marshall Islands	*Lutjanus bohar*	Two-spot snapper	1	0	0	> 1 mm	This Study
	Marshall Islands	*Plectropomus laevis*	Saddleback grouper	1	0	0	> 1 mm	This Study
	Marshall Islands	*Variola louti*	Yellow-edged lyretail	3	0	0	> 1 mm	This Study

2023). Therefore, as a follow-up confirmatory analysis, all particles identified as potentially synthetic plastic polymers by reflectance on the μFTIR were analyzed again individually using ATR mode. The final ATR-FTIR screening is more time intensive, so the μFTIR stage was helpful at reducing the 2100+ suspected particles to a more manageable number of 115. By analyzing these 115 particles using the more accurate ATR-FTIR method, we are confident that the Marshall Island reef fish analyzed here had no ingested microplastics (Table S2). Had only visual inspection been performed, the plastic ingestion incidence and quantities would have been greatly overestimated. The following two stages in the process eliminated false positive identifications of microplastics.

No plastics >1 mm were found in the guts of the fish examined in this study, which makes for interesting comparisons to the few previous studies on microplastic ingestion by reef fish elsewhere in the Pacific Ocean (Table 3). Fish should be from the same trophic level when making comparisons, as foraging patterns have been shown to influence species differences in microplastic ingestion (Peters et al., 2017). Among parrotfish outside of RMI, plastic ingestion incidence ranged from 11 % to 100 % (Forrest and Hindell, 2018; Huang et al., 2023; Markic et al., 2018). For best comparisons, all studies would use the same methods (particle size class, polymer identification methods), but this is not possible at this early stage in the microplastic research field because standardized methods have not been suggested or adopted yet. As for targeted particle sizes, two parrotfish studies assessed >1 mm (this study; Forrest and Hindell, 2018). One out of four parrotfish sampled from Eastern Polynesia, much closer to the South Pacific Garbage Patch, was found to have ingested plastic (Forrest and Hindell, 2018). Conversely, the other two studies included particles <1 mm (Huang et al., 2023; Markic et al., 2018). Assessing different size classes of particles will lead to drastic differences. For example, studies assessing particles <0.2 mm often observe high or 100 % frequency of occurrence (Savoca et al., 2021), because microfibers are ubiquitous and small particles are more abundant by count than larger particles (Shim et al., 2022). Parrotfish from the South China Sea had 100 % FO of only microfibers (Huang et al., 2023). The variability in plastic ingestion rates within a fish guild across the Pacific Ocean could be caused by many reasons, including but not limited to different sources and quantities of plastic in the respective environments, different foraging habits of the species within a guild, different methods of isolating and identifying plastic particles. The latter is likely to be a large source of the variability. Studies that did not do chemical polymer identification could have numerous false positives. In addition, this study only included up to three fish of each species per sampling site. We acknowledge that this small sample size could cause bias in the study. However, if the fish from the Marshall Islands are considered together, five of the nine species have >12 individuals. These sample sizes are comparable to other studies in the Pacific Ocean (Table 3). The species with only 1 to 3 individuals total from the Marshall Islands Region cannot be considered representative of their species as a whole. An increased number of individuals is needed to draw conclusions about these species.

Other marine animals have also had no evidence of microplastic ingestion including Antarctic fur seals, penguin chicks, and many species of fish (Garcia-Garin et al., 2020; Savoca et al., 2021; Leistenschneider et al., 2022). Plastic ingestion, or lack thereof, can be impacted by the amount of marine plastic debris in the location of the species studied or the feeding style of the animal (Leistenschneider et al., 2022). The occurrence of plastic ingestion in marine fish has increased by 2 % per year since 2010 and the average frequency of occurrence is modeled to increase to >50 % by 2030 (Savoca et al., 2021). While the fish included in this study were not observed to ingest plastic, they may in the future as plastic pollution is likely to increase.

The absence of microplastics in the GI tract of nearly 100 fish samples from the RMI is reassuring for human consumers in this country and elsewhere. Fish are an important source of protein and micronutrients (O'Meara et al., 2023) and are consumed in large quantities in the RMI

(Pinca et al., 2009; Echigo, 2010; Gillet, 2016). Furthermore, reef fishes are commonly exported to friends and family throughout the Pacific, so the impact on human health is broader than just within RMI (Chen, 2018; Dacks et al., 2020). While the scientific literature on the human health risks associated with microplastic ingestion is inconclusive (Carbery et al., 2018), based on the precautionary principle, zero exposure is preferable. However, micro- and nano-plastics <1 μm can potentially leave the GI tract and be translocated into fish gills, liver, and muscle tissue, potentially harming the human consumers of these fish (Lu et al., 2016; Collard et al., 2017; Karami et al., 2018; Abbasi et al., 2018; McIlwraith et al., 2021). In addition, this project did not examine microplastics in marine invertebrates like mussels, clams, or giant clams, as this was beyond the scope of the study. However, studies suggest that bivalve mollusks, such as mussels and clams, are one of the greatest dietary exposure routes to microplastics, especially microfibers, for humans (Van Cauwenberghe and Janssen, 2014; Li et al., 2015; Wright and Kelly, 2017). Future research on microplastics in the RMI should sample commonly consumed invertebrates, particularly filter-feeding bivalves (e.g., clams) as well as analyze the fish gut content liquids for microfibers and particles <1 mm. This study may have underestimated plastic ingestion by only examining particles >1 mm. Additionally, we recommend assessing microplastics in frequently consumed and exported pelagic species that were not examined in this study. For example, mahi-mahi (*Coryphaena* spp.) are of specific interest as they feed at the surface of the water column and may ingest floating plastic debris, as well as other smaller pelagic species like anchovies (*Engraulidae*). Existing public health guidelines advise consuming smaller foraging pelagic species instead of larger species like swordfish (*Xiphias gladius*) and marlin (*Istiophoridae*) to reduce the risk of exposure to mercury and persistent organic pollutants from fish, especially during pregnancy, since these species tend to bioaccumulate and biomagnify these toxicants (DeWailly and Rouja, 2009; McLean Pirkle et al., 2015). However, assessing the safety in consuming these smaller fish should be better evaluated; if they are found to have elevated concentrations of microplastics, public health recommendations, especially those targeting pregnant women, may need to be revised.

5. Conclusion

The reef fish sampled within the Marshall Islands as part of this study did not contain microplastics >1 mm. The lack of ingested microplastics by the same or similar fish species has also been observed globally. Microplastic ingestion may be heavily dependent on the trophic level of the fish and the feeding style, and/or the plastic contamination of the region in which they are foraging. The results of this study suggest plastics >1 mm in size do not appear to pose a significant ecotoxicological risk to these reef species nor to the health of people in the RMI who may be consuming them. Plastic ingestion by other species not studied here but regularly consumed by people in the RMI should be examined, along with microplastics <1 mm. Future research should especially include pelagic fish species and invertebrates, particularly filter-feeding bivalves, to better understand the ecotoxicological risks of plastic ingestion to human health in these island communities.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Megan Donahue reports financial support was provided by World Bank Group. If there are other authors, they 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

Data is available in supplementary information.

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NIST Disclaimer: Certain commercial equipment, instruments, or materials are identified in the present study to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

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