

Biomonitoring of microplastics in the Caribbean Sea with invasive lionfish

Sahara M. Ríos-Bonilla^{a,b}, Jaaziel E. García-Hernandez^{b,c}, Daniel A. Toledo-Rodríguez^{b,c},
Nikolaos V. Schizas^c, Lisa J. Rodrigues^{a*}

^aDepartment of Geography and the Environment, Villanova University, 800 Lancaster Avenue,
Villanova, PA, 19085, USA

^bEcoAzul, HC01 Box 4729, Lajas, PR 00667, USA

^cDepartment of Marine Sciences, Marine Genomic Biodiversity Laboratory, University of Puerto
Rico Carr. 108 Mayagüez, PR, 00681, USA

*Corresponding author:

E-mail: lisa.rodrigues@villanova.edu (L. J. Rodrigues)

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Abstract

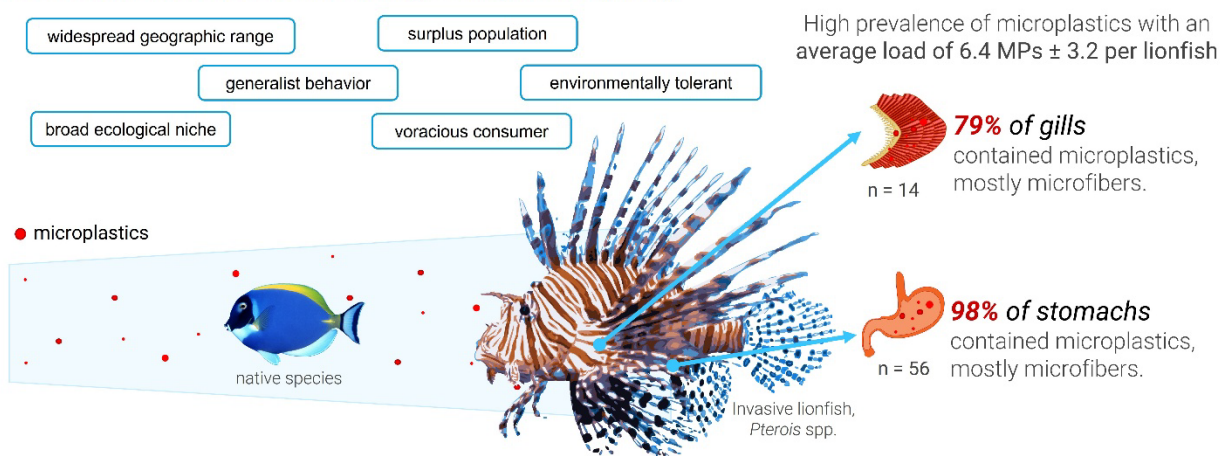
Biomonitoring or using organisms to assess the distribution and bioavailability of microplastics (MPs) is one approach for improving the management of MPs that are abundant and ubiquitous in marine ecosystems. The Caribbean region lacks a target species for biomonitoring MPs. Here, we propose using the introduced lionfish (*Pterois* spp.) that have successfully invaded the Caribbean Sea and Western Atlantic Ocean. With their widespread population and broad ecological niche, the life history characteristics of invasive lionfish are well suited for biomonitoring. To evaluate whether invasive lionfish uptake MPs, we collected 56 lionfish from different depths and reefs in the same region and examined their stomach and gills for MPs. Using both dissection and acid digestion techniques to evaluate the lionfish tissues, we found a high abundance of MPs with an average load of $6.4 \text{ MPs} \pm 3.2$ per lionfish with no pattern associated with depth, reef, or size of lionfish. Most MPs were microfibers and were blue, black, or clear in appearance. There was also a high prevalence of MPs, 98% of lionfish had MPs in their stomach and 79% had MPs in their gills. Our data verify the suitability of the invasive lionfish as a biomonitoring tool for MPs given their generalist behavior, wide habitat, and surplus population, with the added benefits of culling invasive species without disturbing native fish communities.

Keywords

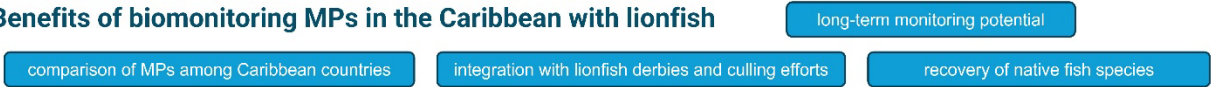
Lionfish, Microplastic, Biomonitoring, Caribbean Sea, Invasive species, Microfibers

Graphical Abstract

Lionfish are ideal for biomonitoring microplastics (MPs)



Benefits of biomonitoring MPs in the Caribbean with lionfish



Microplastics (MPs) or plastic pieces that are <5 to 0.001 mm are known to be a widespread and persistent marine pollutant (Li et al., 2025). Both primary MPs that are manufactured to be a small size (Osman et al., 2023) and secondary MPs derived from fragmentation of larger plastics (Duan et al., 2021) are prevalent and pose risks to aquatic organisms that encounter or ingest them (Li et al., 2025). Biomonitoring MPs in marine trophic webs by using organisms to assess the abundance and bioavailability of MPs, is an important step in mitigating and managing the pollution crisis (Savoca et al., 2024). Organisms that are ideal for biomonitoring have a widespread occurrence, ease of availability, tolerance to environmental fluctuations and pollutants, a broad niche, a stable population, and the ability to retain MPs (Vaid et al., 2024). In the Caribbean Sea, MPs have been a problem for decades (Law et al., 2010), but monitoring of MPs in Caribbean species has been limited compared to other global marine regions (Orona-Návar et al., 2022). Studies of MPs in the region have focused on benthic invertebrates (Aranda et al., 2022; Coc et al., 2021) or native fish (Calderon et al., 2019;

Garcés-Ordóñez et al., 2020, 2022; Jimenez-Cárdenas et al., 2022; Lozada-Gómez & Pérez-Reyes, 2023) species. The biomonitoring potential of these species is limited by ecosystem they inhabit, population size, and geographic range, with most fish species from the Caribbean coastline of Colombia. In the only broad analysis to date, fecal samples from the queen conch (*Aliger gigas*) from eleven sites throughout the Caribbean Sea found 100% incidence of MPs (n=175), ranging from 42-270 MPs per queen conch (Aranda et al., 2022). As the first Caribbean-wide survey of MPs, Aranda et al., (2022) provided baseline values for benthic MPs with non-destructive sampling of a CITES-listed species. However, MPs found in *A. gigas* may not represent MPs in the water column and biomonitoring of MPs should incorporate a widely available nekton species.

Native to the Indo-Pacific, lionfish (*Pterois volitans* and *Pterois miles*) are invasive in the Caribbean Sea and western Atlantic Ocean. They exhibit all the characteristics of an ideal biomonitoring species (Vaid et al., 2024), while overcoming the limitations of previously suggested biomonitoring species from the Caribbean. They have a wide geographic range encompassing the entire Caribbean Sea and Gulf of Mexico, extending north to Bermuda and North Carolina (Betancur-R et al., 2011; Johnston & Purkis, 2011), and recently, extending south along the coastline of Brazil (Soares et al., 2022). Similarly, lionfish exhibit a wide depth range that extends from the surface to mesophotic (30-150 m) depths (Andradi-Brown et al., 2017) with wide home ranges of >100,000 m² on average (Green et al., 2021). Lionfish are environmentally tolerant species that have successfully colonized the western Atlantic Ocean with rapid growth rates, resistance to parasites, lack of natural predators, and an ability to evade anti-predator defenses of their prey (Côté & Smith, 2018; del Río et al., 2023). Often described as voracious consumers, lionfish have a wide-ranging diet as opportunistic generalist carnivores,

including both vertebrate and invertebrate prey (Harms-Tuohy et al., 2016). Thus, they occupy a broad ecological niche in the Caribbean Sea. Using an invasive species for biomonitoring MPs has the added benefit of managing a detrimental species in the region, while avoiding some of the challenges associated with harming native species. While invasive lionfish from the Mediterranean Sea have been reported with MPs in their gastrointestinal tracts (Kılıç et al., 2022), it is not yet known whether lionfish in the Caribbean readily uptake MPs.

To assess the biomonitoring potential of lionfish, we collected lionfish from within La Parguera Marine Natural Reserve, a 3,500-acre protected area in southwestern Puerto Rico. The reserve supports critical marine habitats, serves as a food source, and attracts tourism. However, La Parguera and the southwest region of Puerto Rico are subject to high concentrations of pollution from macroplastic (>5 mm) and MPs deposited in coastal ecosystems (Bruffey & Rodrigues, 2025; Ríos-Bonilla, 2024). A total of 56 lionfish (≥ 13 cm in length) were collected between June and August 2023 by spearfishing at 2–44 m depth on SCUBA (Fig. 1; Table 1). The majority were collected from deep offshore reefs ($n=51$; >18 m), with a few from shallow reefs ($n=5$; <15 m). The collection date, site, depth (m), wet body weight (g) and total length (cm) of each lionfish were recorded in the field. Stomachs ($n=42$) were removed and labeled, frozen at -20°C , and shipped to Villanova University on dry ice, where they were stored at -80°C prior to analysis. We collected additional lionfish to assess the potential for uptake of MPs by different physiological pathways and removed both their stomachs and gills ($n=14$ for each); these were similarly frozen and shipped to Villanova University.

Gills and stomachs were analyzed separately with different methodologies to ensure accurate identification of MPs in different tissues (Fig. 2). Thawed samples were first inspected under a dissecting microscope (10x–40x magnification) to identify any fragile MPs that may be

destroyed by acid digestion and to quantify the recovery of MPs following acid digestion. Dissection also allowed us to assess the potential for biomagnification of MPs or MPs within prey items consumed whole by lionfish (Harms-Tuohy et al., 2016). We used an ultraviolet (UV, 315–400 nm) flashlight to enhance the visibility of clear MPs that may fluoresce (Ehlers et al., 2020; Fig. 3). Probable MPs (>0.25 mm) were recorded based on their appearance, flexibility, brittleness, texture, color, and thickness.

Stomach samples were acid digested to isolate MPs from organic content. Each stomach was placed in a 230-ml glass jar and digested in 15 ml of 70% nitric acid (HNO_3) per gram of tissue (Naidoo et al., 2017). Samples were heated to 40°C for 2–2.5 hours on a hotplate, dissolving organic matter while preserving synthetic polymers. This shorter timeframe (compared to 24 hours) was selected to minimize the known destructive impact of HNO_3 on nylon (Naidoo et al., 2017). MPs identified during the dissection were left in situ and all were recovered following the acid digestion, indicating minimal to no loss of MPs. Following digestion, liquified samples were diluted with deionized water (1:10 v/v) and filtered through 1.6- μm glass fiber filters using a vacuum filtration system in a disinfected hood. Filters were dried at 60°C for 48 hours and subsequently analyzed under a microscope with both white and UV light to identify and characterize MPs (Fig. 3). We used SAS 9.4 for Windows statistical software to conduct an analysis of variance (ANOVA) test to compare total MPs by reef site and a multiple regression analysis to determine any relationship among lionfish depth, length and/or weight with total MPs.

A subset of visually identified MPs >2.5 mm² ($\sim 4\%$, $n=15$) isolated from either stomach or gills was analyzed using Attenuated Total Reflectance (ATR) Fourier Transform Infrared Spectroscopy (FTIR; Agilent Cary 630) to determine polymer types. The instrument was fitted

with a diamond-ATR detector (1.8 mm diameter), with a spectral range of 4000-650 cm^{-1} and a spectral resolution of 4 cm^{-1} . A total of 8 background and 8 samples scans were taken. We conducted a similarity search and compared samples to spectra in PODEAM518–1, POTRAM514–2, and our in-house laboratory libraries. The size restriction ensured coverage of the FTIR crystal but limited the samples we could analyze. The top sample match and its quality was recorded and we compared spectra to published FTIR spectra to account for variations due to degradation (Brandon et al., 2016; Syakti, 2017). Positive matches had quality scores ≥ 0.82 (Bruffey & Rodrigues, 2025). Non-MPs were mostly natural molecules, including resins, lipid or protein globules (Table 2), were removed from the dataset and not included in further analyses.

Quality assurance and quality control (QA/QC) guidelines were followed as reviewed in Grillo et al., (2022). Metal tools and glassware were exclusively used during all laboratory procedures to avoid plastic contamination. Laboratory countertops and all surfaces in the fume hood were cleaned and inspected with UV light before use and 100% cotton lab coats were worn during analyses. Blanks were processed following the same procedure as described with every batch of 10 samples during acid digestions, for a total of $n=6$ filter blanks. Each was inspected under a dissecting microscope with a white light and a UV light following acid digestion and filtration. MPs were not detected on any blanks.

A total of 55 out of 56 lionfish had MPs in their stomach and 11 out of 14 lionfish had MPs in their gills (Fig. 4A; Table 1). Altogether, 98.2% had MPs in their stomachs and 78.6% had MPs in their gills, emphasizing the ubiquity of MPs in the trophic web of southwest Puerto Rico. Lionfish had an average load of 6.4 ± 3.2 MPs, ranging from 0 to 17 MPs per lionfish (Fig. 4; Table 1). When both stomachs and gills were analyzed from a subset of lionfish ($n=14$), two to five times more MPs per lionfish were found in the stomach compared to the gills (Table 1).

Lionfish from southwest Puerto Rico had higher concentrations of MPs than lionfish from the Mediterranean; the only other study to assess MPs in lionfish found that 71% had MPs in their gastrointestinal tract with an average load of 1.5 ± 1.8 MPs per lionfish (Kılıç et al., 2022). These data indicate that lionfish can bioaccumulate MPs by both ingestion and water filtration and/or respiration, with regional differences in load of MPs, suggesting their effectiveness as a biomonitoring species. Interestingly, the freshwater fish, *Pterygoplichthys multiradiatus*, that is invasive in northern Puerto Rican rivers had average loads ranging from 2.8-4.9 MPs per fish and prevalence ranging from 83-87% (Lozada-Gómez & Pérez-Reyes, 2023). The relatively higher abundance and prevalence of MPs we report for lionfish near Puerto Rico suggest that lionfish are exposed to more MPs or preferentially ingest more MPs than invasive inland fish.

Studies of other fish species from the Caribbean report a wide range of prevalence of MPs from 3% to 100% with an average prevalence of 55% (Supplemental Table 1). In Caribbean fish species, MPs have been found in diverse tissues, including stomachs and gastrointestinal tracts (Calderon et al., 2019; Garcés-Ordóñez et al., 2020, 2022; Jimenez-Cárdenas et al., 2022; Lozada-Gómez & Pérez-Reyes, 2023; McGirl et al., 2024; Sánchez-Hernández et al., 2021), livers (McGill et al., 2024), and gills (Alfred et al., 2022). There was no identifiable factor that correlated with number of MPs in lionfish we studied, with no pattern associated with reef site ($df=5$, $F=0.74$, $p=0.5981$), depth ($F=0.23$, $p=0.6339$), lionfish length ($F=0.13$, $p=0.7225$), total wet weight ($F=0.05$, $p=0.8315$), or dry stomach weight ($F=1.55$, $p=0.2186$), indicating that they are exposed to MPs in most environments they inhabit and across size classes. Similarly, Avio et al., (2020) found no relationship between length or weight and ingested MPs for thirteen fish species from the Adriatic Sea, suggesting that fish size is not an important factor in accumulation of MPs. No correlation in MPs by depth in Puerto Rico may be related to overlapping diets

across habitats (Harms-Tuohy et al., 2016), an important characteristic for selecting a biomonitoring species (Vaid et al., 2024).

Of the 359 MPs collected from 56 individuals, 78.5% were microfibers (Fig. 4B) with an average length of $4.1 \text{ mm} \pm 4.7$. Most were colored blue (28.2%), black (24.1%), or clear (23.7%); the remaining microfibers were white (7.3%), red (6.1%), gray (4.6%), green (3.1%), yellow (2.7%) or orange (0.4%). After microfibers, nurdles and small fragments of hard plastic made up the remaining 21.4% of total MPs recovered, along with 1 film. Most MPs (94%) found in lionfish from the Mediterranean were also microfibers and were mostly black (55%) or blue (32%) (Kılıç et al., 2022). Microfibers make up the majority of MPs in marine ecosystems (Mishra et al., 2019) and were the majority of MPs in the gastrointestinal tracts of fish from the Caribbean (Supplemental Table 1), Adriatic (>80%) (Avio et al., 2020), English Channel (68.3%) (Lusher et al., 2013), and coastal Brazil (Garcia et al., 2020). Microfibers in the ocean likely derive from textile waste in wastewater discharge and the in situ degradation of fishing nets, lines, and ropes (Mishra et al., 2019).

Of the 15 potential MPs analyzed by FTIR, 53.3% were identified as synthetic polymers, including polyethylene (2 hard fragments, 1 pellet), polypropylene (1 hard fragment), polyamide nylon (3 fibers) and cellulose acetate (1 film) (Table 2; Supplemental Figure 1). While we only analyzed samples that were large enough for FTIR and our quality matches were good (>0.8200), if available, Raman Spectroscopy would allow us to improve detection and provide more definitive confirmation of polymer types (Silva et al., 2024). Polyethylene and polyamide nylon are typically found as common polymer types in other fish from the Caribbean Sea (Sánchez-Hernández et al., 2021). Six potential MPs (40%) were identified as organic components, including tannic acid and proteins, and were removed from subsequent analyses. A plastic

additive (6%) was identified as EFKA® FA 4644, a polyamide liquid additive used for pigment dispersion

(<https://chemicalsafety.com/sds1/sdsviewer.php?id=30185698&name=Efka%20FA%204644>).

While the additive is for commercial use, it is a known eye and skin irritant and considered toxic to aquatic organisms.

Dissections recovered 32.5% MPs from stomachs (n=338 total) (Fig. 4). While the method was time-consuming, two prey fish consumed whole by lionfish were also dissected. Both contained MPs, providing evidence of biomagnification through the trophic web. Acid digestion recovered more MPs (67.5%) than dissection alone. The UV light identified MPs at all steps, especially clear microfibers in the gills (Fig. 3), and is a low-cost, accessible tool (Ehlers et al., 2020). Numerous authors have called for a standardized approach for MPs analyses (e.g., Savoca et al., 2024). While most studies of MPs in fish from the Caribbean use either dissection or digestion (Supplemental Table 1), our results suggest that combining both methods can improve the accuracy and efficiency of estimating MP loads in fish, especially for predators that consume prey whole.

Invasive lionfish have been proposed for their biomedical potential (Uyan, 2022) and as a biomonitor for organic pollutants (Horricks et al., 2019) but not for biomonitoring MPs. Based on the results of our study, we recommend using lionfish for biomonitoring MPs throughout the Caribbean region. Beyond simply detecting MPs, a focus on lionfish as a biomonitoring species will allow for comparisons among countries in the Caribbean and between the Caribbean and the Mediterranean regions where lionfish are also invasive (Savoca et al., 2024). Many countries have existing culling programs and/or lionfish derbies, providing access to large quantities of lionfish from a wide spatial range (Andradi-Brown et al., 2017) that could be used to integrate

and coordinate biomonitoring efforts. As successful invasives, lionfish have the potential to provide long-term monitoring of MPs wherever lionfish spread. Combining these strategies with existing waste management programs can reduce some barriers associated with assessing and mitigating MPs in the Caribbean region (Orona-Návar et al., 2022).

CRedit Authorship Contribution Statement

Sahara M. Ríos-Bonilla: Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jaaziel E. García-Hernandez:** Writing – review & editing, Investigation. **Daniel A. Toledo-Rodríguez:** Investigation. **Nikolaos V. Schizas:** Writing – review & editing, Resources. **Lisa J. Rodrigues:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Funding acquisition, Formal analysis, Conceptualization.

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Declaration of Competing Interest

The authors declare that there are no competing financial interests or personal relationships that could have influenced the research, analysis, or conclusions presented in this paper.

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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.2025.118947>.

Data Availability

Data are available on Mendeley Data: <https://data.mendeley.com/datasets/hy827zcvzx/1>

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399 **Table 1.** Sites and average depth (m) where lionfish were collected, and the sample size, average length (cm), wet body weight (g),
400 dry stomach weight (g) and abundance of MPs per lionfish and the prevalence of MPs in lionfish stomachs and gills for each site. Site
401 numbers correspond to labels on the map in Fig. 1. Averages are shown ± 1 standard deviation.

Site Information			Lionfish Biometric Data			MPs in Stomachs			MPs in Gills	
Number	Name	Depth (m)	Lionfish (n)	Length (cm)	Wet Body Weight (g)	Dry Stomach Weight (g)	Abundance (MPs per lionfish)	Prevalence (%)	Abundance (MPs per lionfish)	Prevalence (%)
1	Isla Magueyes	1.83 ± 0.0	3	19.0 ± 4.8	103.3 ± 61.7	0.2 ± 0.2	5.0 ± 1.7	100	N/A	N/A
2	Cayo Mario	10.1	1	24.0	175.0	0.2	6	100	N/A	N/A
3	Cayo Turromote	14.3	1	20.0	95.0	0.2	10	100	N/A	N/A
4	El Hoyo	23.8 ± 0.6	6 ^a	24.7 ± 2.6	286.3 ± 117.5	0.3 ± 0.1	6.8 ± 1.4	100	1.2 ± 0.4	66.6
5	Weinberg (Veril)	24.8 ± 3.0	37	27.1 ± 3.9	270.6 ± 110.0	0.3 ± 0.2	6.1 ± 3.3	97.3	N/A	N/A
6	Hole in the Wall	41.7 ± 2.9	8 ^a	26.8 ± 3.7	254.4 ± 116.8	0.2 ± 0.1	5.0 ± 0.7	100	1.8 ± 0.5	87.5

402 ^a Both stomachs and gills were analyzed from lionfish collected from El Hoyo and Hole in the Wall only.

403 Table 2. Microplastics (MPs) analyzed by Attenuated Total Reflectance (ATR) Fourier Transform
 404 Infrared Spectroscopy (FTIR) to determine their polymer type. For each, their visual description
 405 prior to analysis, best match and quality match value (unitless), library, resulting polymer type
 406 and length (mm) are included. CAS = Chemical Abstracts Service number; PA = polyamide; PE
 407 = polyethylene; PP = polypropylene

Description	Match Name	Quality	Library	Polymer Type	Length (mm) ^a
		Match (unitless)			
Film	EKFA FA 4644	0.8308	PODEAM514-2	Chemical Additive	>1.8
Film	Synthetic leather lot#6 polyamide based	0.85534	PODEAM518-1	PA	>1.8
Film	Synthetic leather lot#6 polyamide based	0.83484	PODEAM518-1	PA	>1.8
Film	Synthetic leather lot#6 polyamide based	0.86397	PODEAM518-1	PA	>1.8
Film	Poly(oxyethylene)sorbitan monolaurate, (CAS#: 9005-64-5)	0.85485	PODEAM514-2	Surfactant	>1/8
Film	Myrrh (Brown part)	0.9375	PODEAM514-2	Natural Resin	>1/8
Film	Myrrh (Brown part)	0.94199	PODEAM514-2	Natural Resin	>1/8
Film	Olibanum	0.92154	PODEAM518-1	Natural Resin	>1/8
Hard fragment ^b	Plastic cup	0.94028	Internal	PP	2.0
Hard fragment ^b	Food container	0.9818	Internal	PE	3.0

Hard fragment ^b	PF-51, black garbage bag (exterior)	0.93896	Internal	PE	2.0
Pellet ^b	Milk jug cap	0.93503	Internal	PE	2.2
Pellet	Tannalbin, (CAS #: 9006- 52-4)	0.91345	PODEAM518-1	Protein globule	>1/8
Pellet	Tannalbin, (CAS #: 9006- 52-4)	0.92433	PODEAM514-2	Protein globule	>1/8
Pellet	Coleseed oil	0.87322	PODEAM518-1	Lipid globule	>1/8

408 ^a Length >1.8 mm were films or pellets that were too fragile to expand to their full length. At a
409 minimum, these were large enough to cover the ATR-FTIR detector (1.8 mm in diameter or 2.5
410 mm²).

411 ^b FTIR spectra for sample compared to library reference spectra are shown in Supplemental Fig.
412 1.

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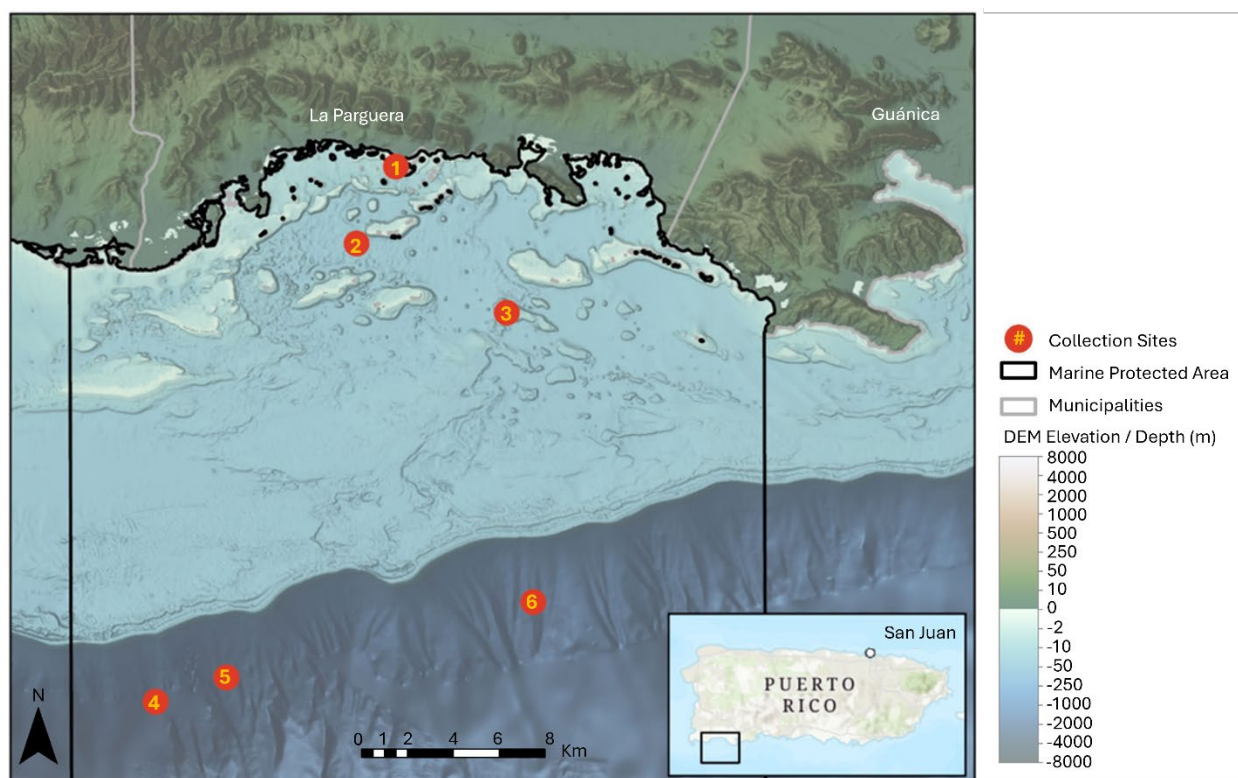
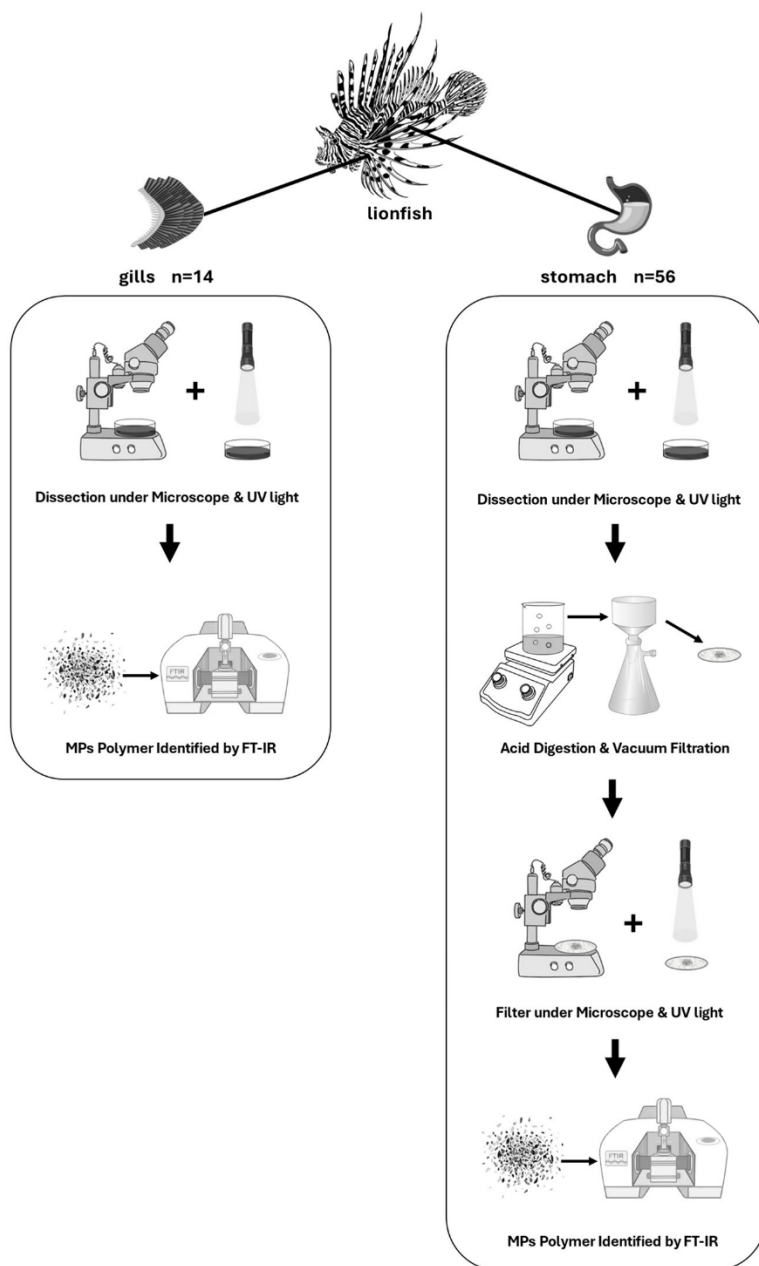


Fig. 1. Map of the study area in southwest Puerto Rico, showing the collection sites against a coastal digital elevation model (DEM) of elevation and bathymetry (m) from National Centers for Environmental Information, National Oceanic and Atmospheric Administration (NCEI-NOAA). Details for each site are included in Table 1.



420

421 **Fig. 2.** Flowchart of the protocols used for isolating microplastics (MPs) in the gills (left) and
 422 stomachs (right) of lionfish. In brief, both gills and stomachs were dissected under a microscope
 423 with white and ultraviolet (UV) light; stomachs were then acid digestion and resulting filtrate
 424 was inspected for MPs; and isolated MPs were identified by Fourier Transform Infrared (FTIR)
 425 spectroscopy.

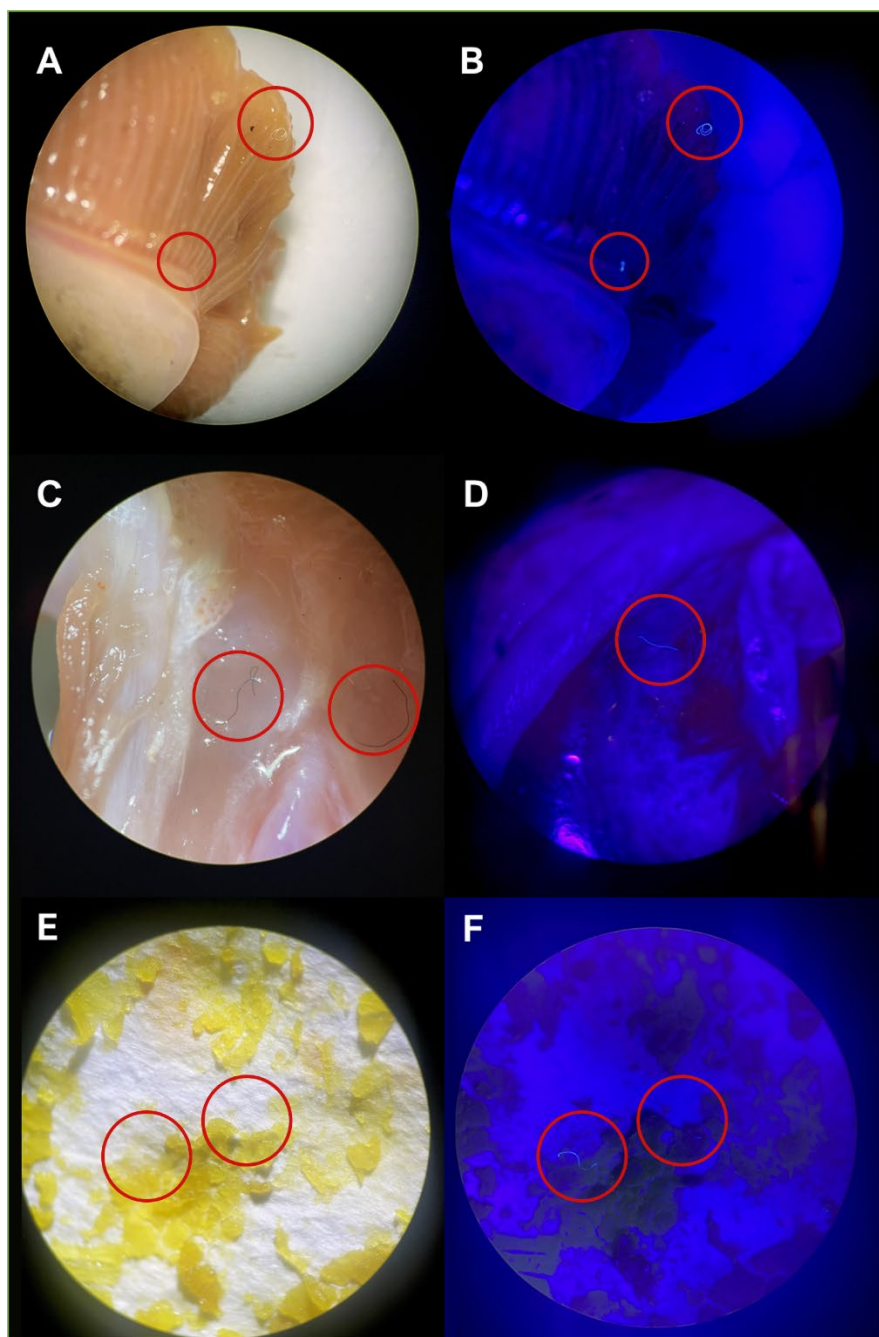


Fig. 3. Detection of microplastics (MPs) was enhanced using ultraviolet (UV) light under a dissection microscope. **(A, B)** Two clear microfibers were identified on gills with UV light in panel B. Different microfibers detected in stomach samples with **(C)** white light and **(D)** UV light. **(E, F)** Two clear microfibers on a filter after acid digestion were identified with UV light in panel F.

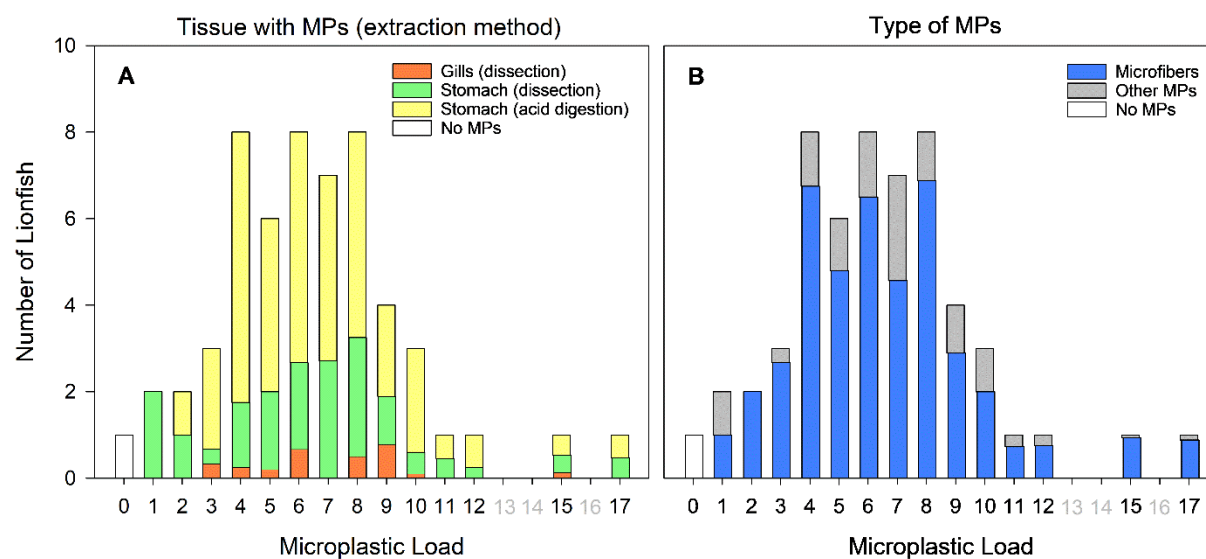


Fig. 4. Histograms of total load of microplastics (MPs) per lionfish by **(A)** tissue where the MPs were found and the extraction method used for isolation; and **(B)** type of MPs. Grey numbers on the x-axis indicate total load of MPs not found in any lionfish.