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MICRODEBRIS ABUNDANCE, DISTRIBUTION, AND INGESTION BY SARGASSUM—ASSOCIATED JUVENILE FISHES IN THE GULF OF MEXICO

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ABSTRACT: Holopelagic *Sargassum* is a critical nursery habitat for the early life stages of many marine fishes, including several federally managed species in the United States and Caribbean. *Sargassum* is often aggregated along surface convergence features where microdebris (synthetic, semi-synthetic, and naturally-derived particles <5 mm in size) have also been found in relatively high concentrations. In this study, we collected microdebris from *Sargassum* and adjacent open water habitats (in 2018), and juvenile fishes from *Sargassum* (in 2017 and 2018) in the northern Gulf of Mexico to quantify habitat-specific microdebris concentrations and the degree to which *Sargassum*-associated juvenile fishes ingest microdebris. Microdebris concentrations within *Sargassum* habitats were, on average, 180 times greater than those found within adjacent open water habitats. Microdebris concentrations decreased with distance from shore in both *Sargassum* and open water habitats, and generally increased with *Sargassum* biomass. Microdebris ingestion by juvenile (9–320 mm SL) fishes (n = 846) varied by year (all taxa: 24.7% in 2017; 14.7% in 2018) and by taxa, and generally decreased with distance from shore. Small fibers were the dominant type of microdebris observed in stomach contents. The structural complexity of *Sargassum* provides a mechanism for microdebris capture and concentration in surface waters. Since 2011, massive blooms of *Sargassum* have inundated regions in the central Atlantic Ocean and Caribbean Sea. The role of *Sargassum* as a microdebris “sink” has major implications for the transport of microdebris as *Sargassum* drifts within and across basins and eventually strands on beaches and coastal habitats, or subsides to benthic environments.

KEY WORDS: Marine debris, macroalgae, microplastic, essential fish habitat

INTRODUCTION

Sargassum is a holopelagic algal complex (*S. natans* and *S. fluitans*) distributed in the surface waters of the North Atlantic Ocean, including the Gulf of Mexico (GOM; Coston–Clements et al. 1991). *Sargassum* is generally observed in offshore waters where it is often the only structurally complex physical habitat, and the accumulation of *Sargassum* biomass due to small-scale, open ocean convergence processes (e.g., Langmuir circulation; Langmuir 1938) provides refuge and feeding habitat for many marine species, including sea turtles, seabirds, and fishes (Dooley 1972, Rooker et al. 2006, Casazza and Ross 2008, Rothäusler et al. 2012, Van Sebille et al. 2020). Many of the *Sargassum*-associated fishes are larval and juvenile stages, therefore *Sargassum* is widely considered to be a nursery habitat. Over 100 species of fishes are found in association with *Sargassum*, including several federally (U.S. and Caribbean) managed species; as such, *Sargassum* has been designated an Essential Fish Habitat in the U.S. Economic Exclusive Zone (SAFMC 2002).

The first published report of microdebris (synthetic, semi-synthetic, and naturally-derived particles <5 mm in size; Kroon et al. 2018) in the open ocean was from the western Sargasso Sea by researchers studying *Sargassum* and its associated faunal community (Carpenter and Smith 1972); although not chemically analyzed, these particles were described as plastics, mostly consisting of “cylindrical pellets” <5 mm in size. Debris particles that are small in size, have low density, and are positively buoyant can be concentrated and retained within surface waters because of physical oceanographic circulation

and wind patterns (e.g., gyres and eddies; Li et al. 2020). Small marine organisms within these surface waters (e.g., zooplankton; Desforges et al. 2015, Sun et al. 2017, fish larvae; Cole et al. 2013, Steer et al. 2017, juvenile fishes; Hoss and Settle 1990, Ory et al. 2018 and turtles) can ingest microdebris directly through foraging (e.g., confusing microdebris with prey items), or indirectly by consuming prey that have ingested microdebris (Wright et al. 2013).

Most microdebris research has focused specifically on microplastics, although non-synthetic microdebris (particularly microfibers) derived from plant or animal sources make up a significant proportion of the environmental microdebris pool (Suaria et al. 2020). Numerous studies have reported naturally-derived fibers as the primary microdebris in fish guts (e.g., Kroon et al. 2018, Jensen et al. 2019, Muns–Pujadas et al. 2023). Proposed harmful impacts related specifically to the ingestion of microplastics include physical damage, such as internal abrasions and gastrointestinal blockages, as well as physiological impacts related to organic pollutants, toxins, and foreign microbes that adhere to the debris surface (Wright et al. 2013, Mazurais et al. 2015, Koelmans et al. 2016, Vendel et al. 2017, Bucci et al. 2020). Although relatively little research has examined the impacts of naturally-derived microfibers (Athey and Erdle 2022), the same potential impacts related to synthetic microdebris have been proposed (Lusher et al. 2013, Ladewig et al. 2015, Stanton et al. 2019).

Juvenile fishes using *Sargassum* as a nursery habitat may encounter microdebris more frequently than fishes in open water,

because both floating debris and *Sargassum* aggregate in surface convergence features. If *Sargassum* habitats are a microdebris “sink”, then fish exposure to higher concentrations of microdebris may result in higher microdebris ingestion rates (Roch et al. 2020, Santana et al. 2021). Mortality rates are highest during the early life stages of fishes prior to recruitment (Lopez et al. 2014), therefore additional stressors, such as ingestion of non-prey items like microdebris, could negatively impact juvenile fish fitness and condition, and potentially result in low survivorship to recruitment age.

Few microdebris studies have been conducted in the GOM relative to other marine water bodies (see reviews in Shruti et al. 2021 and Grace et al. 2022). Of the studies that examined microdebris ingestion by fishes, most were sampled in coastal or inland waters (e.g., Phillips and Bonner 2015, Peters et al. 2017), and nearly half of the taxa were demersal species; only 5% of fishes were reported to be pelagic (Shruti et al. 2021). To date, there have been no studies examining microdebris distribution and ingestion by fishes in offshore surface waters of the GOM. In this study, we assess the relative exposure to microdebris for juvenile fishes in 2 pelagic habitats in the northern GOM by comparing microdebris concentrations in *Sargassum* and adjacent open water habitats. Further, we quantify the frequency of microdebris ingestion for *Sargassum*-associated juvenile fish species, the spatial patterns associated with microdebris ingestion within *Sargassum*, and the relationships between microdebris ingestion and *Sargassum* biomass.

MATERIALS AND METHODS

Study Region

Data were collected from floating *Sargassum* and open water

neuston habitats in the northern GOM during 3 cruises aboard the R/V *Point Sur* in late spring or early summer (2017–2018; see Supplemental Table S1). *Sargassum* habitats were located using remote sensing products from the University of South Florida’s Optical Oceanography Laboratory (<https://optics.marine.usf.edu/>), specifically the daily Alternative Floating Algal Index (AFAI) and Floating Algal Density (FA_Density) products. The AFAI is an ocean color index which uses data from MODIS (Moderate Resolution Imaging Spectroradiometer) instruments to distinguish floating algae in the open ocean (Hu 2009); the FA_Density is an estimate of the percent *Sargassum* cover (1 km resolution) based on an AFAI 7 d mean (Wang and Hu 2016). When combined with estimated current vectors from HYCOM + NCODA Global 1/12° Analysis (<https://www.hycom.org/>), the resulting remote sensing products identified locations in the northern GOM where *Sargassum* was likely to be found. After reaching a general location with high probability of encountering *Sargassum*, specific *Sargassum* features (mats, weedlines) were identified visually and haphazardly selected for sampling. During each cruise, nearly all *Sargassum* sampling stations were located beyond the 200 m isobath (Figure 1). For each *Sargassum* station, an open water neuston station was sampled by transiting about 1 km from the *Sargassum* station, or until open water with little to no *Sargassum* was present.

Juvenile Fish and Microdebris Collection

A 1 m high by 2 m wide neuston sampler fitted with 505 μm mesh net was towed at each *Sargassum* station to collect *Sargassum* and associated juvenile fishes and debris. Each *Sargassum* feature (e.g., mat, weedline) differed in size and morphology, therefore neuston net tow times and the amount of *Sargassum* biomass collected were variable (Supplemental Table S1). At each *Sargassum* station, the neuston net was lowered into the water as the vessel approached a *Sargassum* weedline or mat such that the upper 0.5 m of the net frame remained above the water surface (Figure 2). *Sargassum* weedlines were sampled along the length of the weedline; *Sargassum* mats were sampled along the widest dimension. The net was retrieved when

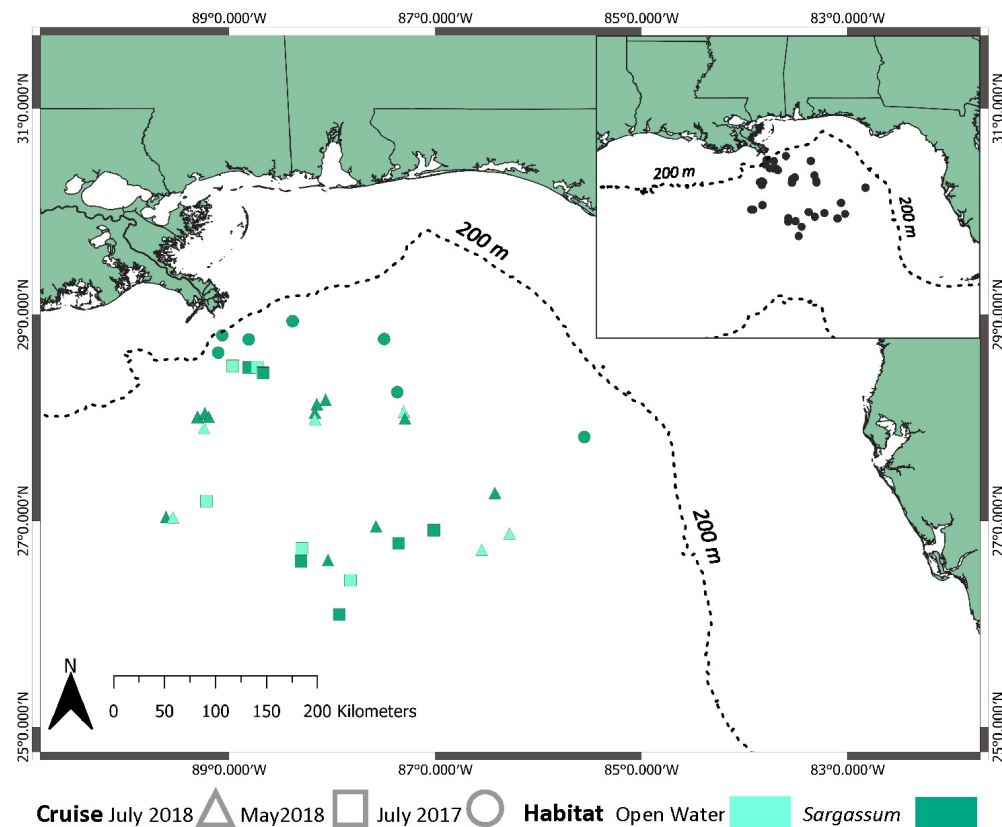


FIGURE 1. Sampling locations for cruises conducted in July 2017, May 2018, and July 2018 in offshore locations of the northern Gulf of Mexico. Symbols denote different cruises; symbol colors denote habitat type. The dashed line indicates the 200 m depth contour. Juvenile fish collected in *Sargassum* during July 2017 and July 2018 were analyzed for microdebris ingestion. *Sargassum* and open water neuston net samples collected in May 2018 and July 2018 were analyzed for microdebris concentrations.

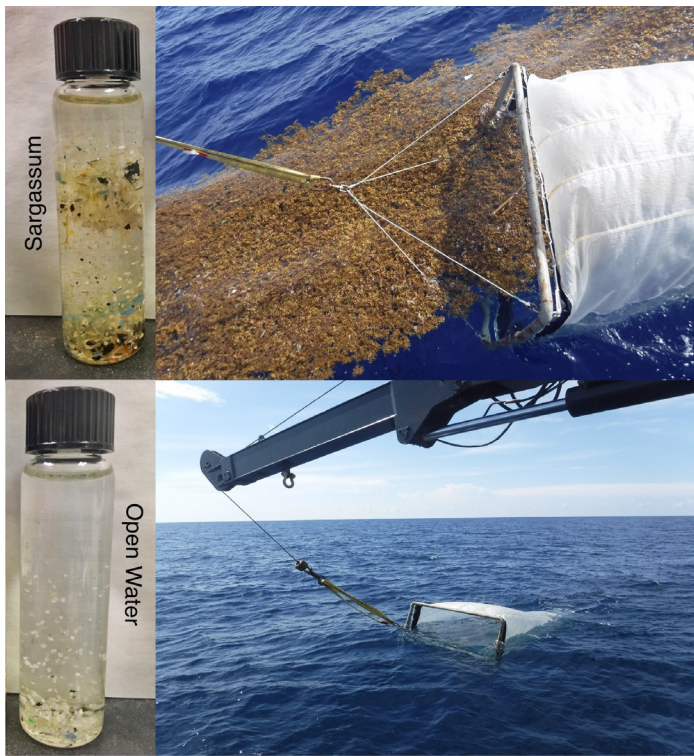


FIGURE 2. Images of microdebris collections in various northern Gulf of Mexico habitats via surface neuston net tows. Top: *Sargassum* collections. Bottom: Open water.

it appeared to be about one quarter to one third full. Once recovered, *Sargassum* was removed from the net, rinsed of organisms and debris, weighed to the nearest 0.1 kg, and returned to sea. Fishes, invertebrates, and debris rinsed from *Sargassum* were collected in a 333 μm sieve and preserved in 95% ethanol or frozen for later sorting and analyses. In addition, larger (7–32 cm), more evasive juvenile fishes were collected during a 30 min hook-and-line fishing set, with 4 anglers fishing along the edge of the *Sargassum* habitat using small hook (sizes 4, 8) Sabiki rigs. Fishes collected via hook-and-line sampling, along with those collected opportunistically with a long-handle dipnet, were preserved in 95% ethanol or frozen for later analyses.

At each open water station, a 1 m high by 2 m wide neuston net fitted with 505 μm mesh net was towed for 10 min at a speed of about 2 kt to collect surface-associated fishes, debris, and invertebrates (Lyczkowski–Shultz et al. 2013). As before, the net was towed such that the upper 0.5 m of the frame remained above the water surface. Once on board, net contents were rinsed and collected in a 333 μm sieve. All contents were preserved in 95% ethanol for later analyses.

The surface area (m^2) sampled by each neuston net tow was estimated by using ship speed (m/s), net fishing time (s), and the width of the net (m). Tow duration was not consistently recorded in July 2017, so surface areas were not calculated for this year. The volume of water filtered by neuston nets was not calculated; standard plankton net flowmeters are ineffective when towed through *Sargassum* (due to entanglement), and although we attempted to fish the net frame half in the water, variable sea states impacted the depth of fishing during each tow.

Estimates of Microdebris Concentrations

Neuston net samples collected from *Sargassum* and open water stations in May and July 2018 were used to compare microdebris concentrations between the 2 habitats (Supplemental Table S1). Preserved neuston net samples from *Sargassum* habitats were often large in volume (e.g., multiple 3.8 L jars per sample) because many fragments of *Sargassum* (e.g., bladders, blades, fronds) remained in the samples after processing at sea. Therefore, all jars of a single *Sargassum* neuston net sample were combined and then split using a Motoda plankton splitter, and a one-quarter aliquot of each sample was sorted for microdebris. Open water neuston samples were smaller in overall volume (<1.0 L per sample), therefore entire samples were sorted for microdebris. Microdebris were sorted from samples under a dissecting microscope using clean techniques, which included wearing 100% cotton lab coats, maintaining a clean work surface, using covered dishes, and avoiding the use of plastic tools where possible. To estimate possible contamination from the lab environment during sample sorting, blank dishes of the same size as the sorting dish were filled with water and placed in the sorting area (Viršek et al. 2016).

Microdebris were identified by visual inspection (Hidalgo–Ruz et al. 2012, Gove et al. 2019) and determined to be likely synthetic in origin using a combination of the following visual references: shape, color, lack of internal organic structures (e.g., cellular structure), no external organic structures (e.g., spines or hairs), and malleability (i.e., effort to break). Even though we followed established protocols for the visual determination of microplastics collected in plankton samples, we acknowledge that without polymer analyses (e.g., FT–IR or Raman characterization), which were unavailable to us, there may be some error associated with our classification, particularly with respect to microfibers (Suaria et al. 2020). Therefore, we conservatively refer to the putative microplastics identified in the results of our study as “microdebris” (Duarte et al. 2020, Rapp et al. 2021), a more encompassing terminology that includes microplastics and other potentially harmful non-prey items. Microdebris types were classified as microfibers, spheres, flakes or fragments, following the descriptions of Li et al. (2016). Any organic particles or particles of unknown origin were removed and not included in further analyses. All microdebris particles were imaged (Canon, EOS T3i 18MP DSLR) under the microscope in a clean and covered sorting tray (36 square grids). In order to estimate a microdebris concentration size range, 4 random grids from the 36-grid sorting tray for each neuston sample were selected and microdebris pieces within the grid square were measured using iSolution Lite software. Each microdebris sample and control blank was then treated with a 1 M potassium hydroxide (KOH) solution for 24 h in order to remove any remaining organic material (Kühn et al. 2017).

Microdebris weight was chosen over microdebris counts for habitat comparisons because many debris pieces were fragile and broke into several smaller pieces during sorting and handling. Therefore, count data would have artificially and inconsistently overestimated microdebris abundance. After treatment in 1 M KOH, microdebris samples and control blanks were fil-

tered onto pre-weighed Whatman GF/F glass fiber filters using distilled water and allowed to dry completely for 48 h. Once fully dry, an aggregate microdebris weight for each sample and blank was recorded to the nearest 0.1 mg. To assess airborne microdebris contamination, a corrected weight was derived by subtracting the weight recorded for each blank from the weight of its microdebris sample complement. There was no statistical difference between the uncorrected and corrected microdebris sample weights (Supplemental Figure S1), which suggested no contamination due to airborne microdebris. The microdebris weight for each sample was then standardized by the surface area sampled to estimate microdebris concentrations (mg/m^2) at each station. Microdebris concentrations between *Sargassum* and open water neuston habitats were then compared (within cruise and both cruises combined) using independent 2-group Mann Whitney U tests (R Core Team 2022).

Microdebris Ingestion

Sargassum-associated juvenile fishes collected in July 2017 and July 2018 were examined for evidence of microdebris ingestion (Supplemental Table S2). *Sargassum*-associated fish assemblages are generally distinct from open water habitats (Casazza and Ross 2008), and our attempts to collect juvenile fishes of similar taxonomic composition and abundance from open water habitats were unsuccessful. Therefore, we were unable to examine microdebris ingestion by open water juveniles in our analysis. All fishes from each *Sargassum* station were used in the gut content analysis; if the total count for a given species exceeded 20 at a single station, a maximum of 20 individuals were randomly selected from both frozen and ethanol-preserved fishes collected by neuston and hook-and-line sampling. For each cruise, only species with a minimum of 3 individuals collected were used in diet analyses. Whole guts were dissected from fishes, removed, and weighed (wet) to the nearest 0.1 mg. Entire gut tracts (stomach and intestine) were analyzed under a dissecting microscope using clean techniques for microdebris as stated above (Hidalgo-Ruz et al. 2012, Viršek et al. 2016). Microdebris removed from guts were imaged under the microscope, categorized (fiber, fragment, flake, or sphere) following Li et al. (2016), enumerated, and measured (iSolution Lite). The weights of individual microdebris pieces were not considered in this analysis, because the very small mass could not be accurately weighed with the available equipment. Microdebris frequency of occurrence (FO; number of fish with microdebris/total number of fish) was calculated for total fish and for each fish species by cruise (July 2017 and July 2018). Differences in FO between species were analyzed using pairwise Fisher's exact tests (R Core Team 2022), which is more appropriate for small sample sizes than Chi-Square tests. For fishes that ingested microdebris, linear models (R Core Team 2022) were used to examine the relationship between fish size (standard length) and the number of microdebris particles ingested for dominant taxa (*Seriola rivoliana*, *Balistes capriscus*, *Abudefduf saxatilis*) and for all species combined.

Spatial and Biomass Comparisons

Microdebris FO was calculated as described above for all fish species collected in a neuston net by station. Distance

from shore was calculated using the proximity tool in ArcMap through ArcGIS. The closest distance in any direction was calculated from a station point to the continental shore line. *Sargassum* biomass (kg) from each neuston net tow was standardized to the surface area (m^2) sampled (kg/m^2). Linear regression models (R Core Team 2022) were used to examine the relationship between microdebris concentrations in *Sargassum* and open water habitats relative to distance from shore (using samples from May 2018 and July 2018; microdebris concentration data were not available for July 2017). Linear regression models (R Core Team 2022) were also used to examine the relationships between microdebris FO relative to distance from shore (using samples from July 2017 and July 2018; diet analyses were not available for May 2018), and *Sargassum* biomass (using samples from July 2018; *Sargassum* biomass data was not available for July 2017).

RESULTS

Microdebris Concentration

Twenty-seven neuston net samples from 2 research cruises (May 2018, July 2018) in the northern GOM were used to compare microdebris concentrations between *Sargassum* and open water neuston habitats (Supplemental Table S1). Microdebris concentrations were higher in *Sargassum* habitats relative to adjacent open water habitats for both the May 2018 cruise (Mann Whitney U, $W = 4$, $p = 0.023$, 95% CI $[-22.298, -0.013]$, $n=12$) and the July 2018 cruise (Mann Whitney U, $W = 0$, $p = 0.0004$, 95% CI $[-15.753, -0.992]$, $n=15$) (Figure 3A). The range of microdebris concentrations from both habitats was similar across the 2 cruises (Mann Whitney U, $W = 70$, $p = 0.347$, 95% CI $[-0.045, 2.249]$, $n=27$). Open water microdebris concentrations ranged from 0.001 – 0.068 mg/m^2 and *Sargassum* microdebris concentrations ranged from 0.014 – 22.366 mg/m^2 . The mean concentration of microdebris in open water habitats was the same for each cruise (May 2018: 0.03 mg/m^2 ; July 2018: 0.03 mg/m^2), and the mean concentration of microdebris from *Sargassum* habitats was similar (May 2018: 5.08 mg/m^2 , July 2018: 5.75 mg/m^2). Microdebris had estimated sizes ranging from 0.1 – 25.36 mm in length for *Sargassum* and 0.06 – 28.7 mm in length for open water (Supplemental Figure S2A). The majority of measured pieces were <3 mm in length (Supplemental Figure S2A).

Microdebris concentrations from open water habitats were calculated from sampling stations that ranged from 55–346 km from shore (Figure 1; Supplemental Table S1). Microdebris concentrations in open water habitats decreased with distance from shore (Linear regression, $r^2 = 0.343$, $F_{1,10} = 5.217$, $p = 0.045$, CI $[-0.0003, -1.292]$; Figure 3B). Microdebris concentrations from *Sargassum* habitats were calculated from sampling stations that ranged from 99–340 km from shore (Figure 1; Supplemental Table S1). Microdebris concentrations in *Sargassum* habitats decreased with distance from shore (Linear regression, $r^2 = 0.345$, $F_{1,13} = 6.854$, $p = 0.021$, CI $[-0.088, -0.009]$; Figure 3C). The biomass of *Sargassum* collected in these samples ranged from 0.11 – 3.32 kg/m^2 . Microdebris concentrations generally increased with *Sargassum* biomass (Linear regression, $r^2 =$

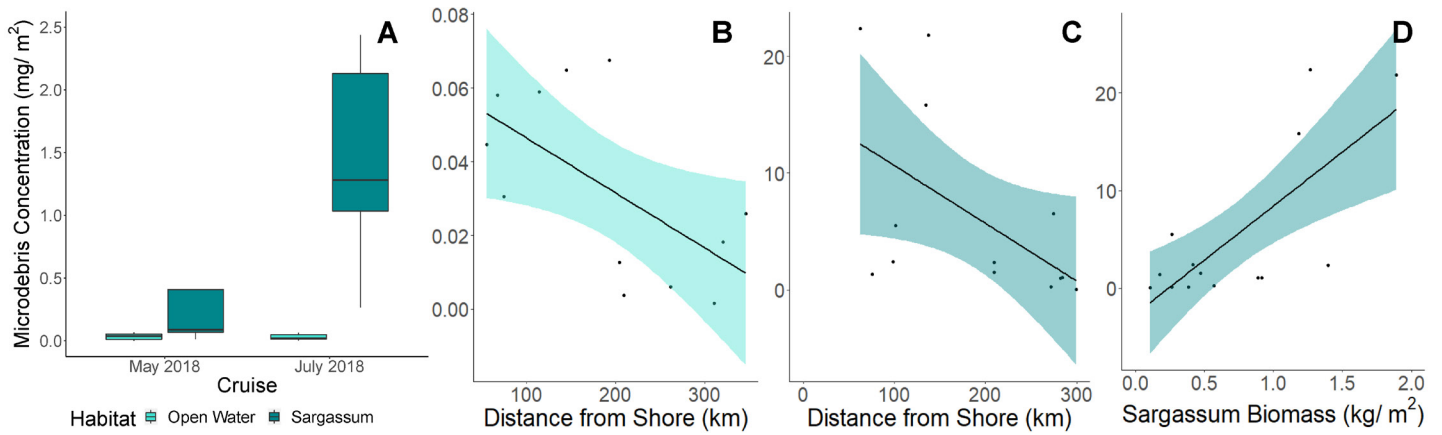


FIGURE 3. Microdebris concentrations for Sargassum and open water habitats sampled during May 2018 and July 2018 cruises in the northern Gulf of Mexico. A. Microdebris concentrations (mg/m²) for open water and Sargassum. The bold line within each box represents the sample median. The upper and lower portions of each box represent the 25th and 75th percentiles, respectively. Solid vertical lines associated with boxes represent the highest and lowest values with 1.5 times the interquartile range. B. Linear regression of microdebris concentrations in open water habitats with distance from shore (km). C. Linear regression of microdebris concentrations in Sargassum habitats with distance from shore (km). D. Linear regression of microdebris concentrations related to Sargassum biomass (kg/m²). Shaded regions denote 95% confidence (B, C, D).

0.230, $F_{1,13} = 3.878$, $p = 0.071$, CI [-0.442, 9.280]; Figure 3D).

Microdebris Ingestion

Sargassum-associated juvenile fishes were collected for gut content analysis during research cruises in July 2017 and July 2018. In total, 29 species (n = 502 individuals) and 20 species (n = 348 individuals) were collected during the July 2017 and July 2018 cruises, respectively. Of these, 22 taxa from July 2017 and 12 taxa from July 2018 met the criteria (minimum of 3 individuals) for microdebris ingestion analyses (Supplemental Table S2).

Microdebris FO in the guts of Sargassum-associated fishes varied by taxa, ranging from 0% (5 taxa) to 50% (*Aluterus scriptus*) in July 2017 and 0% (*Caranx ruber*) to 33% (*Seriola dumerili*, *Lobotes surinamensis*) in July 2018 (Figures 4A, B). About half of the species examined had a microdebris FO

of 20% or higher for both years. For all taxa combined, the overall microdebris FO was 24.7% (July 2017) and 14.7% (July 2018) (Figures 4A, B).

Results of a Fisher’s exact test for all species examined from July 2017 suggested some taxa differed in microdebris FO ($p = 0.01$). Posthoc pairwise Fisher’s exact tests identified differences in microdebris FO among several species (Supplemental Table S3). *Seriola rivoliana* (FO = 40%), *Balistes capricus* (FO =

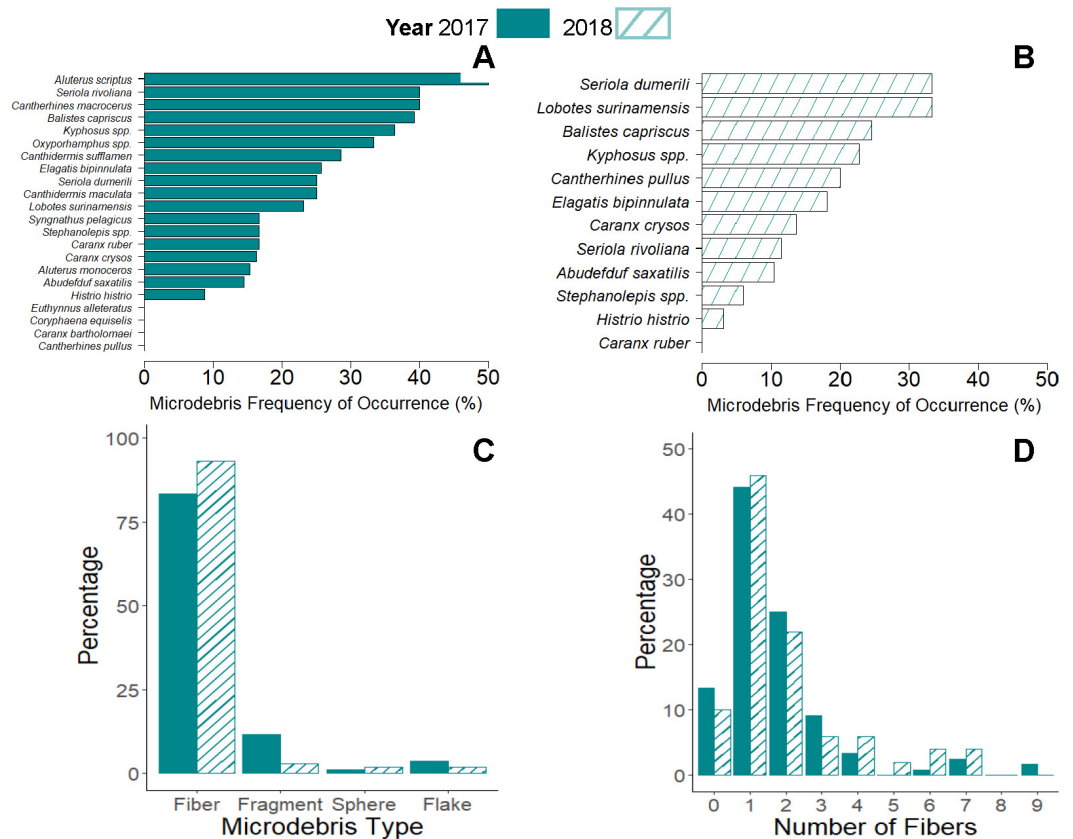


FIGURE 4. Quantifications of microdebris occurrence in juvenile Sargassum-associated fish species in the northern Gulf of Mexico. A. Microdebris frequency of occurrence in fish species in July 2017. B. Microdebris frequency of occurrence in fish species in July 2018. C. Microdebris types found in the guts of Sargassum-associated fishes in 2017 and 2018. D. Percent frequency distribution of microfibers (number ingested per individual) for 2017 and 2018.

39.3%), and *Kyphosus* spp. (FO = 36.4%) all had higher microdebris FO than *Cantherhines pullus* (FO = 0%), *Histrio* (FO = 8.8%), *Abudefduf saxatilis* (FO = 14.5%), and *Caranx crysos* (FO = 16.4%). *Balistes capriscus* also had a higher FO of microdebris than *Aluterus monoceros* (FO = 15.4%). Within the fish with lower FO of microdebris, *A. saxatilis* had a lower FO than *C. crysos*. *Cantherhines pullus* also had a lower FO of microdebris from *Aluterus scriptus* (FO = 50%), *Cantherhines macrocerus* (FO = 40%), and *Elagatis bipinnulata* (FO = 25.6%). Results of a Fisher's exact test for all species examined from July 2018 suggested no differences in microdebris FO between species ($p = 1$).

Four microdebris types (fiber, fragment, flake, or sphere) were observed in fish guts from both years. However fibers were the dominant form, comprising 83.5% and 93.3% in July 2017 and July 2018, respectively (Figure 4C). Microdebris ingested by *Sargassum*-associated juvenile fishes ranged from 0.14–46.28 mm in length for July 2017 and 0.16–6.15 mm in length for July 2018 (Supplemental Figure S2B). Over half of all ingested microdebris pieces were <3 mm in length (Supplemental Figure S2B). Of the fishes with fibers in their guts, most had a single fiber (44.2% and 46% in July 2017 and July 2018, respectively), and nearly all had 2 or fewer (Figure 4D). The maximum numbers of fibers observed in a single fish specimen were 9 (*B. capriscus* in July 2017) and 7 (*B. capriscus* and *E. bipinnulata* in July 2018). Results from linear models examining the number of microdebris particles ingested in relation to fish size (all species combined) were not different for fishes collected in July 2017 (Linear regression, $r^2 = <0.001$, $F_{1,118} = 0.001$, $p = 0.996$, CI [−0.006, 0.006]) or July 2018 (Linear regression, $r^2 = 0.005$, $F_{1,48} = 0.248$, $p = 0.621$, CI [−0.006, 0.010]). When examined by taxon (across both years), results from linear models examining the number of microdebris particles ingested in relation to fish size varied. Relatively weak, positive relationships were observed for *Balistes capriscus* (Linear regression, $r^2 = 0.080$, $F_{1,54} = 4.697$, $p = 0.035$, CI [0.002, 0.048]) and *A. saxatilis* (Linear regression, $r^2 = 0.215$, $F_{1,13} = 3.564$, $p = 0.082$, CI [−0.014, 0.202]),

whereas a weakly negative relationship was observed for *S. rivoliana* (Linear regression, $r^2 = 0.250$, $F_{1,17} = 5.673$, $p = 0.029$, CI [−0.033, −0.002]).

Sargassum-associated fishes examined for microdebris FO were collected from *Sargassum* habitats that ranged from 20–284 km from shore (Supplemental Table S1). Microdebris FO in juvenile fishes generally decreased with distance from shore (Linear regression, $r^2 = 0.164$, $F_{1,18} = 3.452$, $p = 0.076$, CI [−0.149, 0.008]; Figure 5A). The biomass of *Sargassum* collected in these samples ranged from 0.22–1.76 kg/m² (Supplemental Table S1). No relationship was found between microdebris FO in juvenile fishes and *Sargassum* biomass (Linear regression, $r^2 = 0.038$, $F_{1,11} = 0.429$, $p = 0.526$, CI [−12.419, 22.946]; Figure 5B).

DISCUSSION

Microdebris Concentration

Microdebris concentrations within *Sargassum* habitats were highly variable, but on average were 180 times greater than those found in adjacent open water habitats, which suggest that *Sargassum* habitats are microdebris sinks in offshore surface waters. Our results are similar to those from a study conducted off the coast of Hawaii that reported microplastic concentrations to be 130 times greater within surface slicks relative to areas without ocean convergence features (Gove et al. 2019). Gallardo et al. (2021) also reported greater microplastic densities inside surface slicks relative to outside surface slicks in the coastal waters of Rapa Nui, however, the difference in magnitude in their study was much lower (about 3–fold). One possible explanation for this disparity in magnitude is the exclusion of microfibers from their analysis, as fibers are often the largest component of microdebris surveys (Gago et al. 2018, Suaria et al. 2020). Although previous studies have shown that microdebris is aggregated in large scale ocean gyres, our results support the hypothesis that smaller scale oceanographic surface features of convergence, like those that form *Sargassum* aggregations, also serve to concentrate microdebris at the surface (Brach et

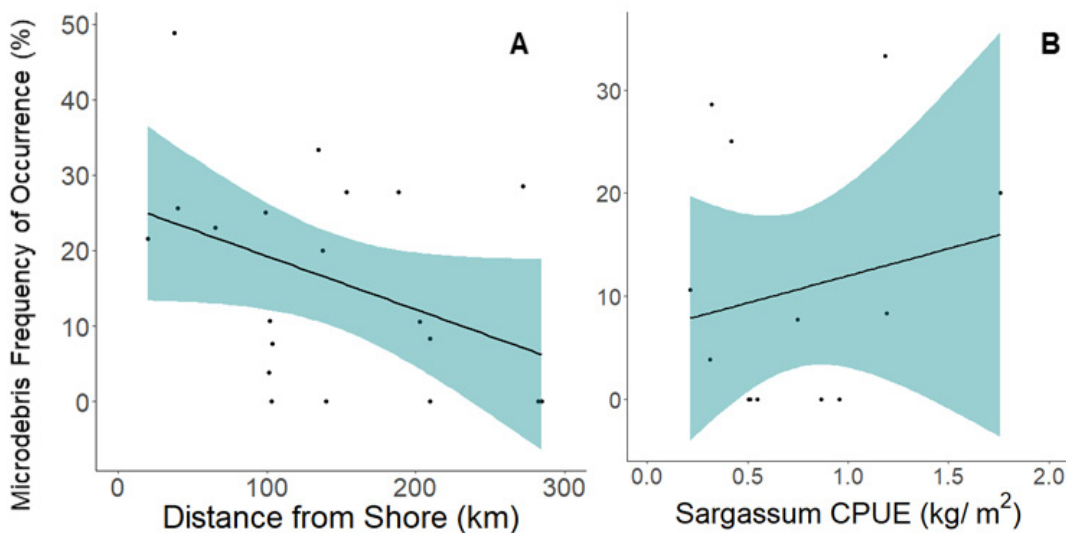


FIGURE 5. Linear regressions of microdebris frequency of occurrence in juvenile fish guts collected in the northern Gulf of Mexico by neuston net tows. A. Microdebris frequency relative to distance from shore for July 2017 and July 2018. B. Microdebris frequency relative to *Sargassum* biomass for July 2018. Shaded regions denote 95% confidence. Individual points are the frequency of occurrence for all fish species collected in a single neuston net tow.

al. 2018, Gove et al. 2019, Van Sebille et al. 2020, Cózar et al. 2021).

Sargassum morphology and density are known to be highly variable in nature, ranging from small floating clumps (scales of cm) to large mats and weedlines (scales of m to km), as was evident in this study. Our results suggest that as *Sargassum* biomass increases, so does microdebris concentration. This could be attributed to the complex structure the algae provide for microdebris to adhere and become trapped. Previous studies have found microplastics trapped within the epibiont communities on seagrasses and benthic macroalgae, including a benthic *Sargassum* species (Gutow et al. 2016, Goss et al. 2018, Seng et al. 2020). Combined, the aggregation of *Sargassum* and microdebris along frontal features, and the tendency for *Sargassum* to physically retain microdebris, increases the likelihood that *Sargassum*-associated juvenile fishes and other organisms will encounter microdebris. Previous laboratory studies have demonstrated that fish ingestion of microdebris increases with the concentration of microdebris in the ambient water (e.g., Roch et al. 2020). Although we were not able to test it here, the higher microdebris encounter rate in *Sargassum* also likely increases the probability of microdebris ingestion for some species relative to open water habitats.

Spatial variability in microdebris concentration was observed for both *Sargassum* and open water habitats, with microdebris concentrations generally decreasing with distance from shore. This relationship could be attributed to the semi-enclosed nature of the GOM, where large populations of urbanized coastal communities and large freshwater tributaries (e.g., Mississippi River, Mobile Bay) influence the amount of microdebris entering the basin. For example, Di Mauro et al. (2017) reported high concentrations of microplastics in the nearshore slope waters west of the Mississippi River mouth similar to those reported in other semi-enclosed basins. In the Mediterranean Sea, higher concentrations of microplastics have been reported closer to drainage systems and near highly populated coastal cities (Schmidt et al. 2018, Vianello et al. 2018). The open water microdebris concentrations observed in our study (0.03 mg/m²) fall within the lower reported range of concentrations found in the Mediterranean Sea (0–9.298 mg/m²; Collignon et al. 2012, Ruiz-Oregon et al. 2016, Schmidt et al. 2018). This result is not unexpected, because most of the samples taken in our study were in offshore waters (beyond the 200 m isobath) of the GOM, and relatively far from coastal sources of microdebris. The results presented here provide some of the first offshore estimates of microdebris concentrations in the northern GOM, demonstrating that *Sargassum* is a sink for microdebris in surface water habitats, and suggests that marine organisms encounter spatially variable surface concentrations of microdebris that decrease with distance from terrestrial inputs.

Microdebris Ingestion

The overall microdebris FO in juvenile fishes associated with *Sargassum* (14.7–24.7%) was lower than reported FO for juvenile fishes from other nursery habitats (52–59%); these previous studies sampled juvenile fishes in nearshore nursery

habitats (e.g., mangroves, estuaries) and included benthic and benthopelagic species (e.g., Salmonidae, Pleuronectidae, Cichlidae, Terapontidae, Mugilidae, and Ambassidae; Collicutt et al. 2019, Kazour et al. 2020, Naidoo et al. 2020, Alfred et al. 2022). In contrast, the fishes collected in *Sargassum* were pelagic species, and were collected at least 20 km offshore. The spatial relationship we observed was variable, although there was a general trend of lower microdebris FO in the guts of juvenile *Sargassum*-associated fishes with distance from shore. Our results are more similar to those from studies conducted on pelagic fishes in the Pacific Ocean, which reported overall microdebris FO ranging from 8.6–24.3% (Markic et al. 2018, Gove et al. 2019). The Pacific Ocean studies included fishes of similar sizes (5–1,386 mm TL) and families (Balistidae, Carangidae, Pomacentridae, Kyphosidae, and Monacanthidae) to those found associated with *Sargassum* (Markic et al. 2018, Gove et al. 2019). Overall, offshore pelagic fishes, even those associated with *Sargassum* (a microdebris sink), generally have lower microdebris FO because they are farther away from coastal sources of microdebris.

The lower microdebris FO in pelagic fish could also be attributed to the absence of seafloor sediment microdebris. Benthic and benthopelagic fish are subject to potentially higher concentrations of microdebris found in seafloor sediments which could explain their higher microdebris FO (Ling et al. 2017). Even though overall microdebris FO was lower for pelagic fish, micro-fibers (83.5–93.3%) were found to be the dominant microdebris type ingested by both benthopelagic and pelagic juvenile fishes (micro-fibers = 68–90%; Collicutt et al. 2019, Kazour et al. 2020, Naidoo et al. 2020). Similarly, individual benthopelagic juvenile fishes were found to have ingested between 1–2 microdebris pieces on average and the pelagic juvenile fishes were found to have ingested about 2 microdebris pieces on average (Collicutt et al. 2019, Kazour et al. 2020).

Similar to our findings, results from previous studies reporting relationships between juvenile fish size and the number of microdebris particles ingested have been equivocal. In general, when taxa are aggregated for analyses, no relationships between size and microdebris ingestion have been reported (Kazour et al. 2020, Naidoo et al. 2020, Nanninga et al. 2021). However, similar to our study, taxon-specific variation exists. For example, Hajovsky (2019) reported negative (e.g., *Mugil* spp.), positive (e.g., *Sciaenops ocellatus*), and neutral (e.g., *Leiostomus xanthurus*) relationships between the number of microdebris particles ingested and the size of juvenile fishes collected in the northern GOM. These observations suggest that the number of microdebris particles being ingested by juvenile fishes is not driven by size alone, and that other considerations (e.g., feeding guild, foraging strategies) should be considered for future studies.

Microdebris aggregating within the *Sargassum* habitat are being ingested by a wide range of species and at varying frequencies. It was hypothesized that obligate (e.g., *Histrio histrio*, *Syngnathus pelagicus*) and closely associated (e.g., presettlement *Abudefduf saxatilis*) *Sargassum* fishes would be predicted to have

higher microdebris FO than more transient species, such as *Seriola* spp. and *Balistes capricus*. However, *H. histrio* (FO = 8.8%) and *A. saxatilis* (FO=14.5%) were among the species in our study with the lowest observed microdebris FO overall, in contrast to *Seriola* spp. (FO = 35.3%), *B. capricus* (FO = 39.3%), and *Kyphosus* spp. (FO = 36.4%), which had some of the highest observed microdebris FO. Therefore, the degree of association with *Sargassum* (obligate vs. transient) is not a predictor of microdebris FO. One hypothesis may be that taxon-specific feeding ecology may explain variability in microdebris FO. For example, *H. histrio* is a cryptic ambush predator that swallows prey (e.g., shrimp, crabs, fish) whole, whereas *B. capricus* is a grazer that selectively bites and crushes encrusting organisms and crustaceans that live directly on and within the *Sargassum* fronds (Brooks et al. 2007, Ballard and Rakocinski 2012). Further investigations into the differences in feeding styles and diet of *Sargassum*-associated juvenile fishes may explain why some species have higher observed microdebris FO than others.

CONCLUSIONS

Our study provides the first quantitative estimates of microdebris concentrations within *Sargassum* and adjacent open water habitats of the northern GOM, and provides evidence that juvenile fishes associated with *Sargassum* encounter higher microdebris concentrations than fishes inhabiting open water habitats surrounding it. There was a general trend of lower ambient concentrations of microdebris, and lower microdebris FO, in the guts of juvenile *Sargassum*-associated fishes with distance from shore. A diverse range of juvenile fish species associ-

ated with *Sargassum* are consuming microdebris. Mesocosm experiments have shown the potential for microplastics to cause physical, physiological, and behavioral impacts to fishes once ingested (Qiang and Cheng 2019, Qiao et al. 2019, Ahrendt et al. 2020). While we were unable to confirm through polymer analysis that the microdebris ingested by juvenile fishes associated with *Sargassum* were in fact plastic, these pieces of microdebris (and in most cases microfibers) are not a part of the natural diet, and like confirmed microplastics could have the potential to cause harm to the fishes ingesting them. Indeed, many of the same detrimental impacts associated with microplastics (e.g., pseudo-satiation, physical blockage or damage of alimentary canals, etc.) apply to other non-food particles. With regard to toxicity, many natural fibers (e.g., wool, cotton) are treated with flame retardants, synthetic dyes and other chemical processes that could be harmful (Suaria et al. 2020). Further investigations into impacts of microplastic and microdebris ingestion on *Sargassum*-associated fish growth, condition and survival are needed to assess implications for recruitment. Since 2011, *Sargassum* "blooms" have been increasingly common along the equatorial Atlantic and Caribbean Sea (Wang et al. 2019). With increasing biomass, *Sargassum* will be a major source of microplastic and microdebris collection and transport as it moves within and across basins and eventually strands on beaches and coastal habitats, or subsides to benthic environments (Wang et al. 2019). This has major implications for not only the juvenile fishes living in and around the *Sargassum* habitat, but also for the juvenile fishes living in the areas to which *Sargassum* will be transported.

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