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Microplastic Abundance, Distribution and Impacts on Sargassum-Associated Juvenile Fishes in the Gulf of Mexico

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MICROPLASTIC ABUNDANCE, DISTRIBUTION AND IMPACTS ON *SARGASSUM*-ASSOCIATED JUVENILE FISHES IN THE GULF OF MEXICO

by

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ABSTRACT

Microplastics are a concern in marine environments because they are highly durable, ubiquitous, and can be mistaken for food and ingested by small organisms. Pelagic *Sargassum*, an important habitat for larval and juvenile stages of many fish species, is found in large surface aggregations, and may provide complex structure in which microplastics become trapped. This could lead to greater risk of microplastic ingestion by fish early life stages associated with *Sargassum* habitats. To better understand the impacts of microplastics within *Sargassum* communities, this study examined 1) microplastic concentrations and ingestion by juvenile fishes associated with *Sargassum; 2)* the microbial communities associated with the *Sargassum* and microplastics; and 3) the influence of microplastic ingestion on the microbiomes of juvenile Gray Triggerfish. Neuston net samples were collected in 2017 and 2018 from open water and *Sargassum* habitats in the Gulf of Mexico to collect microplastics and fishes. Microplastic abundance was significantly higher in *Sargassum* habitats relative to open water habitats. Microplastics were identified in the stomach contents of many species of juvenile fishes with total microplastic frequency of occurrence ranging between 14.7-24.7%. Microplastics had a unique microbiome when compared to the surrounding environment. The microplastic microbiome was found to influence Gray Triggerfish gut microbiomes. The results from this project demonstrate that microplastics are being ingested by juvenile fishes in *Sargassum* and the unique microbiome of microplastics are influencing fish gut microbiomes.

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DEDICATION

I am dedicating my master's thesis to my parents for always encouraging me to go after my dreams, ask questions, explore the world's knowledge, and be creative. Thank you for supporting me throughout my life and through this journey.

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LIST OF ABBREVIATIONS

AFAI	Alternative Floating Algal Index
ANOSIM	Analysis of Similarities
ASV	Amplicon Sequence Variants
FA_Density	Floating Algal Density
FO	Frequency of Occurrence
GoM	Gulf of Mexico
IMR	Integrated Microbiome Resource
KOH	Potassium Hydroxide
MAD	Median Absolute Deviation
MODIS	Moderate Resolution Imaging Spectroradiometer
OTU	Operational Taxonomic Units
PAH	Polycyclic Aromatic Hydrocarbon
PCB	Polychlorinated Biphenyl
SIMPER	Similarity Percentages
QIIME2	Quantitative Insights into Microbial Ecology 2

CHAPTER I - MICROPLASTIC DISTRIBUTION, ABUNDANCE, AND INGESTION BY JUVENILE FISHES ASSOCIATED WITH HOLOPELAGIC *SARGASSUM* HABITATS IN THE NORTHERN GULF OF MEXICO

1.1 Introduction

1.1.1 Microplastics

Marine debris is widely recognized as a major source of pollution in the world's oceans, and understanding the ecosystem impacts of marine debris is an emerging area of research (Coe and Rodgers, 1997; Moore et al., 2001). Marine debris is defined as any solid substance that is manufactured and intentionally or unintentionally disposed into the marine environment (Coe and Rodgers, 1997; Galgani et al., 2010). Plastics are the most dominant form of marine debris, comprising 60-80 % of the total marine debris pool (Gregory and Ryan, 1997; Jambeck et al., 2015). Plastics are highly durable, popularly manufactured, and relatively inexpensive to produce. Some plastic products can be recycled or reused, but a vast majority are deemed ‘end-of-life’ plastics (Barnes et al., 2009). Because plastics are both widely produced and resistant to degradation, their prevalence and persistence in marine environments could lead to continued interactions with organisms and their habitats (Gall and Thompson, 2015; Welden and Cowie, 2017). With respect to organisms, interactions with marine debris can lead to entanglement, ingestion, internal obstruction, and transport of invasive and harmful species (Laist, 1997; Law, 2017). Recent estimates suggest that 4.8 to 12.7 million tons of plastic entered the ocean in 2010, and as of 2014, 5.25 trillion pieces of plastics were estimated to be in the oceans (Eriksen et al., 2014; Jambeck et al., 2015). These estimates are expected to rise with the continued production of plastics (Jambeck et al., 2015).

Plastics in the marine environment undergo many physical and chemical changes. They become brittle and materially degrade from physical actions, solar radiation, and biodegradation (Welden and Cowie, 2017). Degradation of larger plastic pieces results in the formation of microplastics, which are defined as pieces less than 5 mm in size (Arthur et al., 2009). Microplastics are divided into two broad groups. The most common are secondary microplastics, which result from degradation of larger pieces (e.g., soda bottles). Less common are primary microplastics, which are raw plastics originally manufactured at a size less than 5 mm (e.g., microbeads for facial cleaners, nurdles) (Hidalgo-Ruz et al., 2012; Welden and Cowie, 2017). Many microplastics are positively buoyant due to their small size and low density, and are therefore relatively high in abundance in near-surface waters (Lobelle and Cunliffe, 2011).

Microplastic ingestion has been documented in numerous marine organisms (e.g., cetaceans, sea turtles, invertebrates, and fishes) throughout different marine inshore and offshore habitats (Boerger et al., 2010; Lusher et al., 2015; Davidson and Dudas, 2016; Alomar and Deudero, 2017; Courtene-Jones et al., 2017; Vendel et al., 2017; Duncan et al., 2019; Zhu et al., 2019). Because of their small size and surface distribution, microplastics are confused as prey items and ingested, particularly by smaller marine fauna, including zooplankton, ichthyoplankton, and juvenile fishes (Hoss and Settle, 1990; Cole et al., 2013; Desforges et al., 2015; Steer et al., 2017; Sun et al., 2017; Ory et al., 2018), or ingested secondarily through prey (Wright et al., 2013). Microplastics could potentially cause internal physical impacts, such as abrasions and blockages within the gut tract (Wright et al., 2013; Mazurais et al., 2015; Vendel et al., 2017). Because microplastics have a relatively high surface area and an affinity for absorbing

hydrophobic organic chemicals, they become a potential vector for many foreign compounds (Koelmans et al., 2016). Microplastics may therefore transport organic pollutants and toxins from the plastics themselves and present a new pathway for foreign microbial communities to enter organisms; any of these hitchhikers could cause or lead to physiological impacts (Wright et al., 2013; Mazurais et al., 2015). For example, a recent study by Kirstein et al. (2016) reported human pathogenic *Vibrio parahaemolyticus* on microplastics that were similarly seen in the water column, suggesting that microplastics could be another source of pathogenic bacteria. A fish pathogenic bacteria, *V. alginolyticus*, was also observed on microplastics in the study by Kirstein et al. (2016), suggesting that microplastics could be a new source of pathogenic bacteria to fishes (Reed and Francis-Floyd, 1996). These direct and indirect impacts could have negative effects on marine organisms, including fish in early life stages, where lowered health and condition could potentially impact recruitment to adult populations.

1.1.2 *Sargassum*

Two species of brown macroalgae (*Sargassum natans* and *S. fluitans*) combine to form a holopelagic *Sargassum* complex in the surface waters of the Atlantic Ocean, including the Gulf of Mexico (GoM) (Coston-Clements et al., 1991). *Sargassum* distribution at regional scales is highly ephemeral, and often *Sargassum* accumulates in windrows due to convergence processes, such as Langmuir circulation (Langmuir, 1938; Rothäusler et al., 2012). The accumulation of *Sargassum* biomass provides refuge and feeding habitat for many marine species in an otherwise featureless open ocean (Rooker et al., 2006; Dooley, 1972). *Sargassum* has been shown to be a crucial habitat for many fish early life stages, and is designated an Essential Fish Habitat in the U.S. South

Atlantic Economic Exclusive Zone (SAFMC, 2002). Because *Sargassum* habitat provides protection from pelagic predators and an abundant food source, larval and juvenile fish survival is thought to be enhanced by an association with *Sargassum* features (Wells and Rooker, 2004). If so, enhanced survival of early stages should equate to higher recruitment into the adult population (Wells and Rooker, 2004).

There are several reasons to suspect that fishes associated with *Sargassum* may be more susceptible to microplastic ingestion than other open water fishes. First, because *Sargassum* and microplastics are neustonic, they are aggregated in surface features by the same oceanographic processes. Microplastics have been shown to increase in concentrations at convergence features, such as eddies, and more recently in local-scale convergence features that form lines, such as slicks (Brach et al., 2018). A recent study conducted off the coast of Hawaii found that larval fishes within the surface slicks had higher rates of plastic ingestion than fish larvae in adjacent waters (Gove et al., 2019). Second, the complex structure of *Sargassum* may serve to trap other floating debris. Studies have shown the capability of benthic algae and grasses to accumulate and trap microplastics because of epiphyte and biofilm growth (Gutow et al., 2016; Goss et al., 2018). One of the first published descriptions of marine microplastics was based on observations from *Sargassum* suggesting that *Sargassum* may also trap microplastics in similar ways (Carpenter et al., 1972). Lastly, there is also evidence indicating that enclosed and semi-enclosed basins, including the GoM may harbor higher densities of plastics because of greater urbanized coastal inputs (Barnes et al., 2009; Collignon et al., 2012). In the northern GoM, abundances of macroplastics (0.6-2.4 pieces/km^2) and microplastics (5.0-18.4 particles/m^3) in shelf and slope surface waters are comparable to

other semi-enclosed basins, like the Mediterranean Sea (Lecke-Mitchell et al., 1992; Lecke-Mitchell et al., 1997; Di Mauro et al., 2017). These studies suggest that microplastic concentrations throughout the surface waters of the GoM could be found in high concentrations and these concentrations could have variable distributions. Greater densities of microplastics combined with the aggregation and entrapment of microplastics in *Sargassum*, may mean that *Sargassum* could become a sink for microplastics in the GoM. Overall, little is known about the extent and variability of microplastic concentrations in surface waters of the GoM, and how these concentrations vary between *Sargassum* and open water habitats.

The goal of this chapter is to assess the impacts of microplastics on juvenile fishes associated with *Sargassum* habitats in the northern GoM. Specifically, the objectives are to: 1) compare microplastic concentrations in *Sargassum* and adjacent open water habitats; 2) compare the frequency of microplastic ingestion for *Sargassum*-associated juvenile fish species; 3) compare the frequency of microplastic ingestion for *Sargassum*-associated juvenile fish feeding groups; and 4) determine whether the frequency of microplastic ingestion by *Sargassum*-associated juvenile fishes varies spatially (e.g., distance from shore) or with *Sargassum* biomass.

1.2 Materials and Methods

1.2.1 Study Region

Data were collected from floating *Sargassum* and open water neuston habitats in the northern GoM during three cruises aboard R/V *Point Sur* in late spring or early summer (2017-2018) (Table 1.1). *Sargassum* habitats were located using remote sensing products from the University of South Florida's Optical Oceanography Laboratory

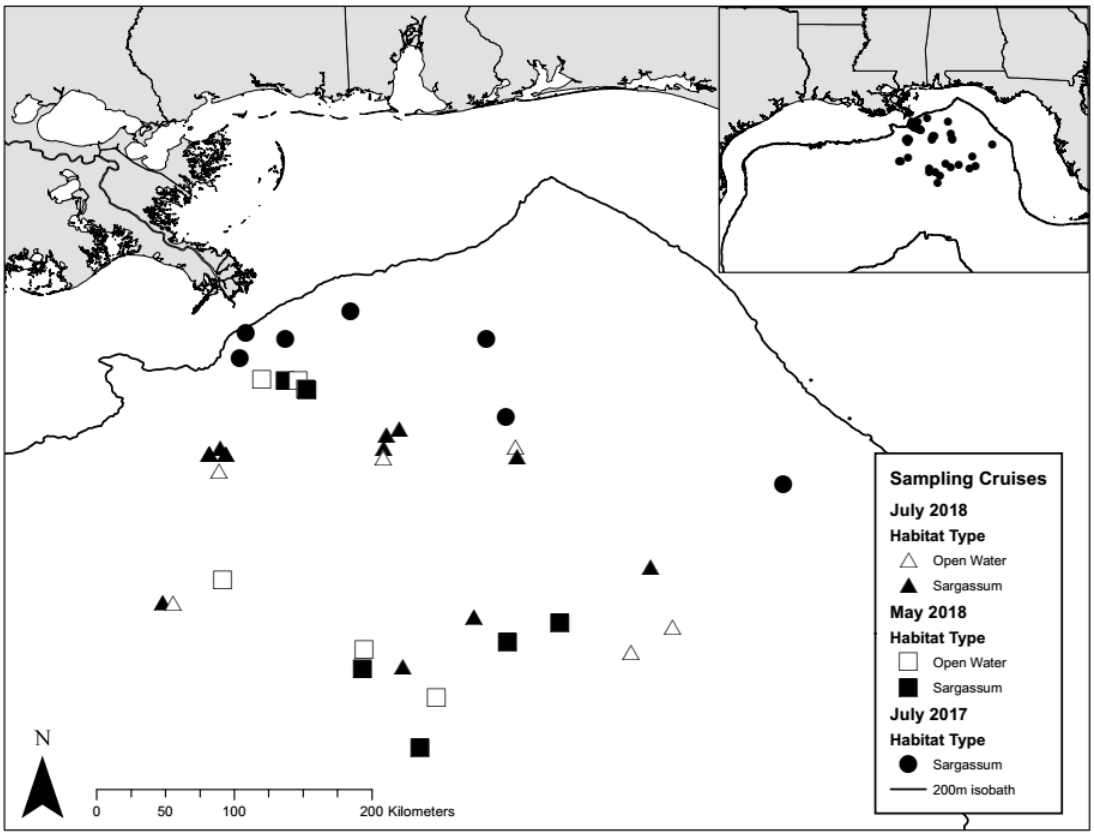

Figure 1.1 Sampling locations for cruises conducted in July 2017, May 2018, and July 2018 in offshore locations of the northern GoM. Symbols (triangle, square, circle) denote cruises. Filled symbols denote *Sargassum* collection stations. Open symbols denote open water collection stations. The solid line indicates the 200 m depth contour.

(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 seven-day 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*

likely occurred. During each cruise, nearly all *Sargassum* sampling stations were located beyond the 200 m isobath (Figure 1.1). For each *Sargassum* station, a paired open water neuston station was sampled by transiting approximately one kilometer from the *Sargassum* station, or until open water with little to no *Sargassum* was present. The paired open water neuston stations from 2018 were used in the following analyses for microplastic concentrations.

1.2.2 Juvenile Fish and Microplastic Collection

A 1x2 m neuston sampler fitted with 505 µm mesh net was towed at each *Sargassum* station to collect *Sargassum* and associated juvenile fish, invertebrates, and microplastics. Each *Sargassum* feature (e.g., mat, weedline) differed in size and morphology, therefore neuston net tow times and the amount of *Sargassum* biomass collected was variable (Table 1.1). 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. The net was retrieved when it appeared to be approximately 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 and more evasive juvenile fishes were collected during a 30-minute hook-and-line fishing set, with four 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.

Table 1.1 Collection data for neuston net samples collected in *Sargassum* (SARG) and open water (OPEN) habitats in the Gulf of Mexico (2017-2018).

Date	Local Time	Sta. No.	Habitat Type	Sample No.	Tow Duration (s)	Distance to shore (km)	Depth (m)	Biomass (kg/m²)
Cruise 1: PS-17-07 (July 20-27, 2017)								
7/20	17:02	02	SARG	02[1]	-	40.0	857	0.20
7/21	11:03	03	SARG	03[1]	-	37.6	405	0.48
7/22	8:47	06	SARG	06[1]	-	20.1	271	0.07
7/23	12:57	09	SARG	09[1]	-	65.4	941	0.69
7/24	10:22	11	SARG	12[1]	-	153.5	1652	0.43
7/26	8:34	14	SARG	16[1]	-	202.5	825	0.43
7/27	13:43	16	SARG	19[1]	-	188.2	2644	0.45
Cruise 2: PS-18-05 (May 30, 2018-June 6, 2018)								
5/30	18:07	20	OPEN	21[2]	512	54.6	837	<0.01
5/31	15:14	21	OPEN	22[2]	633	192.6	2260	<0.01
6/1	10:59	22	SARG	23[2]	21	274.4	2553	3.32
6/1	17:30	23	OPEN	24[2]	300	261.66	2616	0.01
6/2	8:00	24	SARG	25[2]	41	340.52	2971	0.39
6/2	14:50	25	OPEN	27[2]	600	310.70	2809	0.00
6/3	8:04	26	SARG	29[2]	14	62.53	1156	1.27
6/3	14:00	27	OPEN	30[2]	600	66.83	1309	<0.01
6/4	13:41	29	OPEN	33[2]	600	74.44	1403	0.00
6/4	15:54	28	SARG	34[2]	61	75.50	1415	0.18
6/5	13:35	30	SARG	36[2]	137	299.56	2810	0.11
6/6	8:11	31	SARG	38[2]	125	309.72	2939	0.27
Cruise 3: PS-18-07 (July 9-16, 2018)								
7/9	15:09	32	SARG	39[12]	42	137.32	2317	1.89
7/10	8:42	33	SARG	40[12]	57	134.36	2279	1.19
7/10	13:52	33	SARG	41[1]	47	139.61	2279	0.55
7/10	16:02	34	OPEN	42[2]	603	144.76	2423	<0.01
7/11	9:24	36	SARG	43[12]	45	284.45	3124	0.89
7/11	17:07	37	OPEN	44[2]	660	321.21	3156	<0.01
7/11	20:26	38	OPEN	45[2]	605	346.28	3127	0.00
7/12	8:17	39	SARG	46[12]	47	282.02	2762	0.92
7/12	16:14	40	SARG	47[12]	111	272.05	2794	0.57
7/13	10:36	41	OPEN	48[2]	562	204.36	2782	0.00
7/13	13:07	42	SARG	49[12]	54	209.39	2832	1.40
7/14	18:57	43	SARG	50[12]	51	101.34	1288	0.27
7/14	19:52	43	SARG	51[1]	84	102.02	1284	0.22
7/15	9:23	44	SARG	52[12]	135	98.70	1294	0.42
7/15	15:01	44	SARG	53[1]	32	103.16	1290	0.56
7/15	15:21	44	SARG	54[1]	41	103.32	1288	0.73
7/15	16:46	45	OPEN	55[2]	600	114.08	1364	0.00
7/16	9:36	46	SARG	56[12]	56	209.57	2399	0.47
7/16	17:12	47	OPEN	57[2]	660	209.57	1291	0.00

[1]Indicates samples used in analyses of microplastic frequency of occurrence in juvenile fishes.
[2]Indicates samples used in analyses of microplastic concentrations between open water and *Sargassum* habitats.

At each open water station, a 1x2 m neuston net fitted with 505 µm mesh net was towed (10 minute duration) at a speed of approximately 1 kt to collect surface-associated fishes, microplastics, and invertebrates. 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% EtOH for later analyses.

The surface area (m^2) sampled by each neuston net tow during the June 2018 and July 2018 cruise was estimated by using boat speed (m/s), net fishing time (s), and the width of the net (m). Volume was not calculated because flow meters could not be used to measure water flowing through the net when towing through the *Sargassum* habitat because the algae would clog the net. Tow duration was not consistently recorded in July 2017; therefore, no surface area estimates were calculated for that cruise.

1.2.3 Estimates of Microplastic Concentrations

Neuston net samples collected from *Sargassum* and open water stations in June and July 2018 were used to compare microplastic concentrations between the two habitats (Table 1.1). Preserved neuston net samples from *Sargassum* habitats were often large in volume (e.g., multiple 3.8 L jars per sample) because many small fragments of *Sargassum* (e.g., bladders, blades, fronds) remained in the samples after processing at sea. Therefore, *Sargassum* neuston net samples were split using a Motoda plankton splitter, and a one-quarter aliquot of each sample was sorted for microplastics. Open water neuston samples were smaller in overall volume, therefore entire samples were sorted for microplastics. Microplastics 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, avoiding the use of plastic tools where possible, and blank dishes of the same size as the sorting dish were filled with water and placed in the sorting area (Viršek et al., 2016). All microplastics were imaged (Canon, EOS T3i 18MP DSLR) under the microscope in a clean and covered gridded tray, and any questionable pieces and large organic matter were removed. Plastics were 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), then filtered onto Whatman GF/F glass fiber filters using distilled water and allowed to dry completely for 48 h. Once fully dry, an aggregate microplastic weight for each sample was recorded to the nearest 0.1 mg. Blank dishes were processed in the same manner as the plastics and corrected weights were compared to original weights. There was no difference in original and corrected weights, suggesting that contamination of air born plastics was unlikely (Figure A.1). The microplastic weight for each sample was then standardized by the surface area sampled to estimate microplastic concentrations (mg/m^2) at each station. Microplastic 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.

1.2.4 Microplastic Ingestion

Sargassum-associated juvenile fishes collected in July 2017 and July 2018 were examined for evidence of microplastic ingestion (Table 1.2). Due to low abundances, no juvenile fishes from open water neuston samples were examined. All fishes from each *Sargassum* station were used in the gut content analysis; if the total count for a given species exceeded 20, a maximum of 20 individuals was randomly selected from both

Table 1.2 Number (n) of *Sargassum*-associated juvenile fishes dissected for analysis of microplastic ingestion. Size ranges (in mm) are for standard length (SL).

	July 2017		July 2018	
Species	***n***	**SL (mm)**	***n***	**SL (mm)**
Hemiramphidae				
Oxyporhamphus spp.	3	22-34	-	-
Exocoetidae				
Parexocoetus brachypterus	1	26	-	-
Prognichthys occidentalis	1	17	-	-
Syngnathidae				
Syngnathus pelagicus	6	71-153	-	-
Antennariidae				
Histrio histrio	34	9-61	32	10-68
Pomacentridae				
Abudefduf saxatilis	69	9-38	48	12-41
Carangidae				
Caranx bartholomaei	5	20-62	1	27
Caranx crysos	55	11-320	44	12-315
Caranx ruber	6	35-48	5	39-119
Caranx spp.	1	11	-	-
Elagatis bipinnulata	39	16-160	11	20-154
Seriola dumerili	8	148-197	6	152-183
Seriola fasciata	1	115	1	141
Seriola rivoliana	25	16-223	87	16-264
Selar crumenophthalmus	1	98	-	-
Coryphaenidae				
Coryphaena equiselis	3	82-168	-	-
Scombridae				
Euthynnus alletteratus	6	117-157	-	-
Thunnus atlanticus	1	120	-	-
Katsuwonus pelamis	1	148	-	-
Kyphosidae				
Kyphosus spp.	33	10-86	22	16-105
Lobotidae				
Lobotes surinamensis	13	17-174	12	14-76
Nomeidae				
Psenes cyanophrys	-	-	1	63
Balistidae				
Balistes capriscus	112	14-112	49	11-107
Canthidermis maculata	8	56-194	1	24
Canthidermis sufflamen	7	48-192	2	123-161
Diodontidae				
Diodon holocanthus	-	-	1	84
Monacanthidae				
Aluterus monoceros	26	45-167	-	-
Aluterus scriptus	6	66-144	2	27-43
Cantherhines macrocerus	5	48-109	-	-
Cantherhines pullus	16	36-67	5	46-69
Monacanthus spp.	-	-	1	16
Stephanolepis spp.	6	42-75	17	19-78

frozen and ethanol-preserved fishes collected by neuston and hook-and-line sampling. For each cruise, only species with a minimum of three individuals collected were used in diet analyses. Whole guts were dissected from fishes, removed, and weighed (wet) to the nearest 0.0001 g. Entire gut tracts (stomach and intestine) were analyzed under a dissecting microscope using clean techniques for microplastics (Hidalgo-Ruz et al., 2012; Virsek et al., 2016). Microplastics removed from guts were imaged under the microscope, categorized (fiber, fragment, flake, or sphere), and enumerated. Microplastic frequency of occurrence (FO; number of fish with plastic/total number of fish) was calculated for the total of all fish and for each fish species by cruise (July 2017 and July 2018). Differences in FO between species were analyzed using pairwise Fischer's exact tests. To determine if fish size influences the number of microplastics ingested, linear models were used to look at the number of microplastics ingested by fish standard length.

1.2.5 Characterization of Natural Diet Contents

Juvenile fish association with *Sargassum* ranges from obligate species (e.g., *Sargassum*fish) to other species that are presumed to be more transient (e.g., carangids). As such, the relative dependence of a species on *Sargassum* as foraging habitat may be related to its frequency of microplastic ingestion. To examine this association, naturally occurring diet items were also removed from juvenile fishes examined for microplastic ingestion. Prey items were identified to the lowest taxonomic level possible. The proportion of each prey item was calculated for each fish species (sample size >10) based on the number of observations recorded during the gut examination. A hierarchical agglomerative clustering by Ward's Method was then used to group fish species based on the similarity of their prey (Silva et al., 2019). Significant clusters were defined using a

similarity profile analysis (simprof). From these clusters, feeding groups were assigned and then tested using a presence and absence matrix (Jaccard distance) of all individual fishes through analysis of similarities (ANOSIM) to determine if fish feeding groups were significantly different. A similarity percentages (SIMPER) analysis was then run to see the prey driving differences and be able to make inferences about relative dependence of a fish group on *Sargassum* for feeding. Microplastic FO for feeding groups were then tested using Kruskal-Wallis tests.

1.2.6 Spatial and Biomass Comparisons

Variability in gut microplastic FO was examined in relation to distance from shore and *Sargassum* biomass. Microplastic FO was calculated as described above for each 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 were then used to examine microplastic FO relationships between distance from shore and *Sargassum* biomass.

1.3 Results

1.3.1 Microplastic Concentration

A total of 27 neuston net samples was used to examine differences in microplastic concentrations between *Sargassum* and open water neuston habitats (Table 1.1). Microplastic concentrations were significantly lower in open water habitats relative to *Sargassum* habitats for both the May 2018 cruise (Mann Whitney U: W=4, p-value=0.023) and the July 2018 cruise (Mann Whitney U: W=0, p-value=0.0004) (Figure

1.2). Microplastic concentrations were similar across the two cruises (Mann Whitney U: W=70, p-value=0.347) (Figure 1.2). Open water microplastic concentrations ranged from 0.001-0.068 mg/m^2 and *Sargassum* microplastic concentrations ranged from 0.014-22.366 mg/m^2. The mean concentrations of microplastics in open water habitats were the same for each cruise (May 2018: 0.03 mg/m^2; July 2018: 0.03 mg/m^2), and the mean concentration of microplastics from *Sargassum* habitats were similar (May 2018: 5.08 mg/m^2, July 2018: 5.75 mg/m^2).

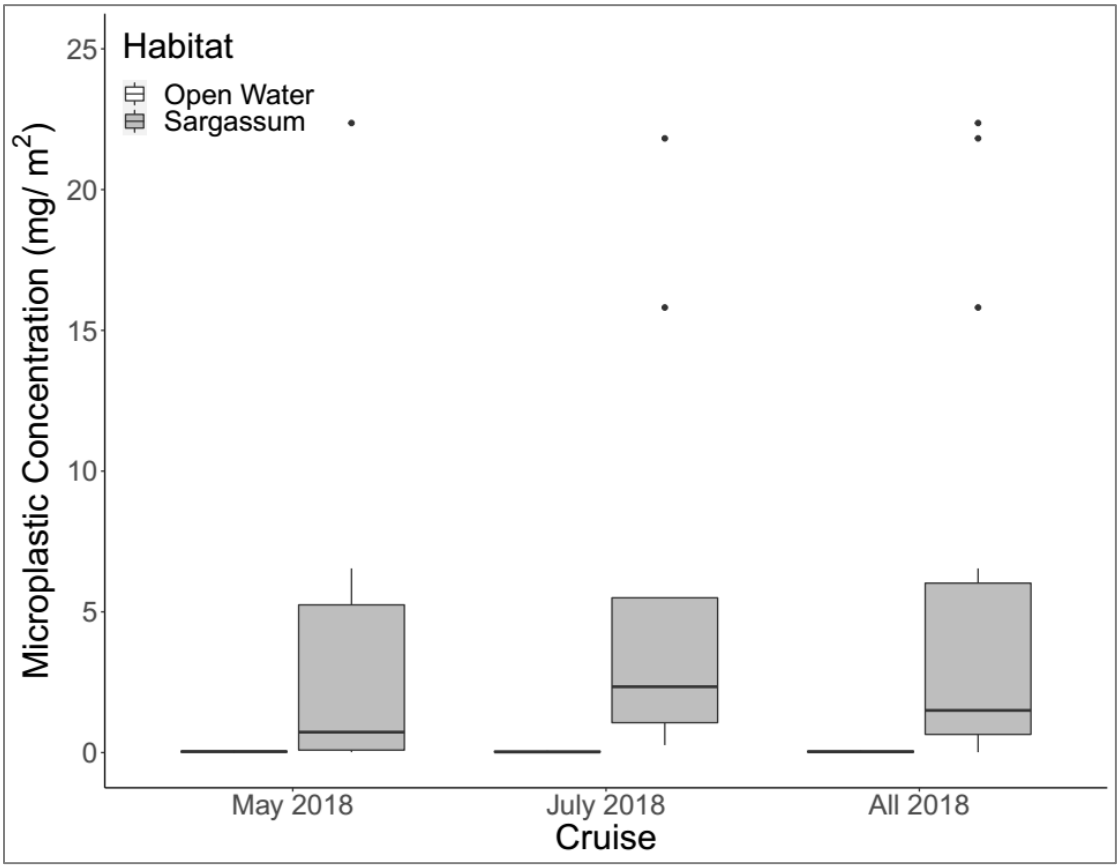

Figure 1.2 Boxplots of microplastic concentrations (mg/m2) for open water and *Sargassum* habitats sampled during research cruises in May 2018 and July 2018, and for both cruises combined. The bold line within each box represents the sample median. The upper and lower portions of each box represent the 25th and 75th percentiles, respectively. Solid vertical lines associated with boxes represent the highest and lowest values with 1.5 times the interquartile range. Points above or below a box represent outliers.

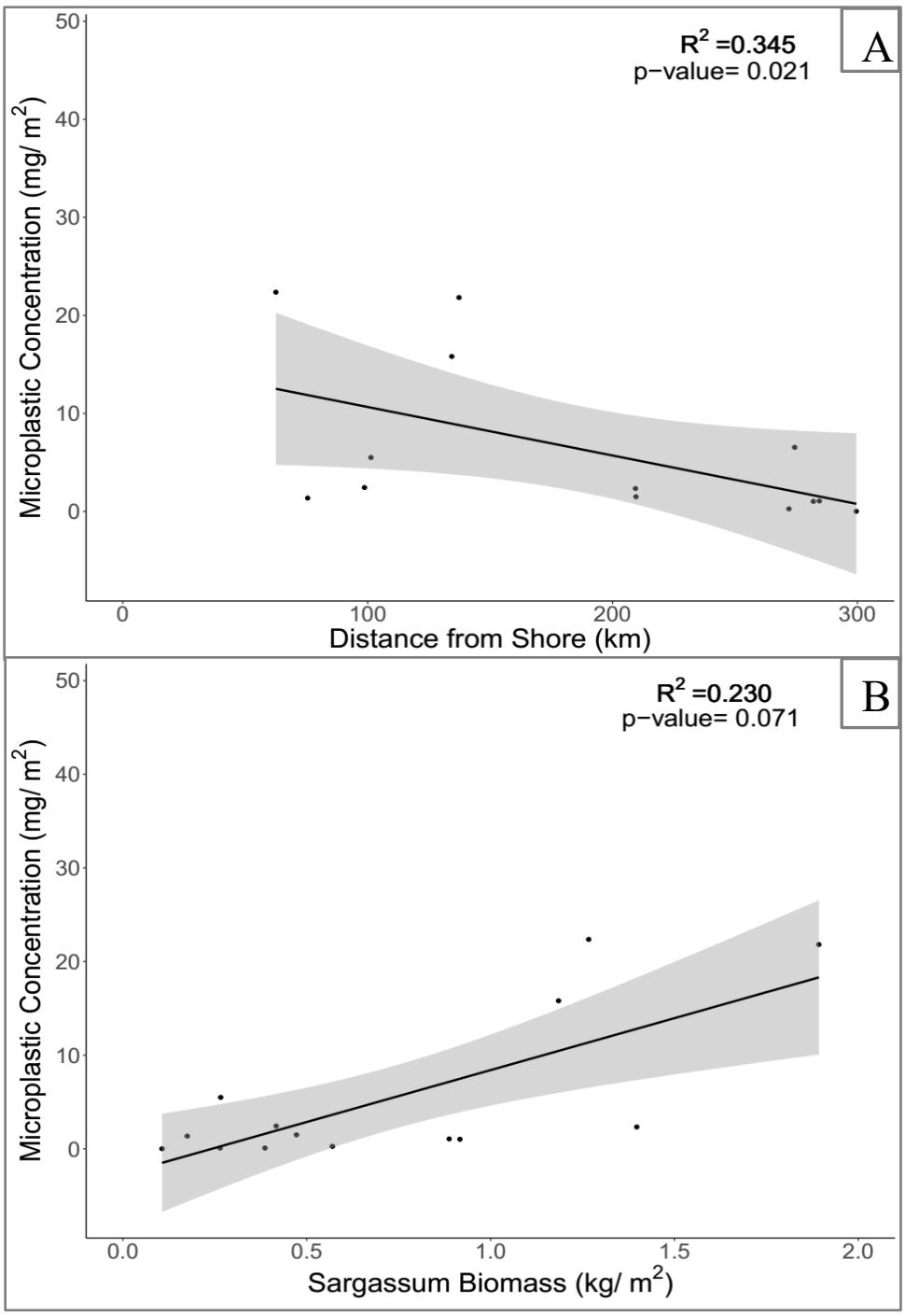

Figure 1.3 Linear regressions of microplastic concentrations in *Sargassum* habitats with A) distance from shore and B) *Sargassum* sample biomass for collections made in May 2018 and July 2018. Shaded regions denote 95% confidence.

Microplastic concentrations from *Sargassum* habitats were calculated from sampling stations that ranged from approximately 99-340 km from shore (Figure 1.1; Table 1.1). Microplastic concentrations in *Sargassum* habitats decreased with distance from shore ($F=6.854$, $R^2=0.345$, p-value=0.021) (Figure 1.3a). The biomass of *Sargassum* collected in these samples ranged from 0.11-3.32 kg/m^2. Although not significant ($F=3.878$, $R^2=0.230$, p-value=0.071), microplastic concentrations generally increased with *Sargassum* biomass (Figure 1.3b). Microplastic concentrations from open water habitats were calculated from sampling stations that ranged from approximately 55-346 km from shore (Figure 1.1; Table 1.1). Microplastic concentrations in open water habitats decreased with distance from shore ($F=5.217$, $R^2=0.343$, p-value=0.045) (Figure1.4).

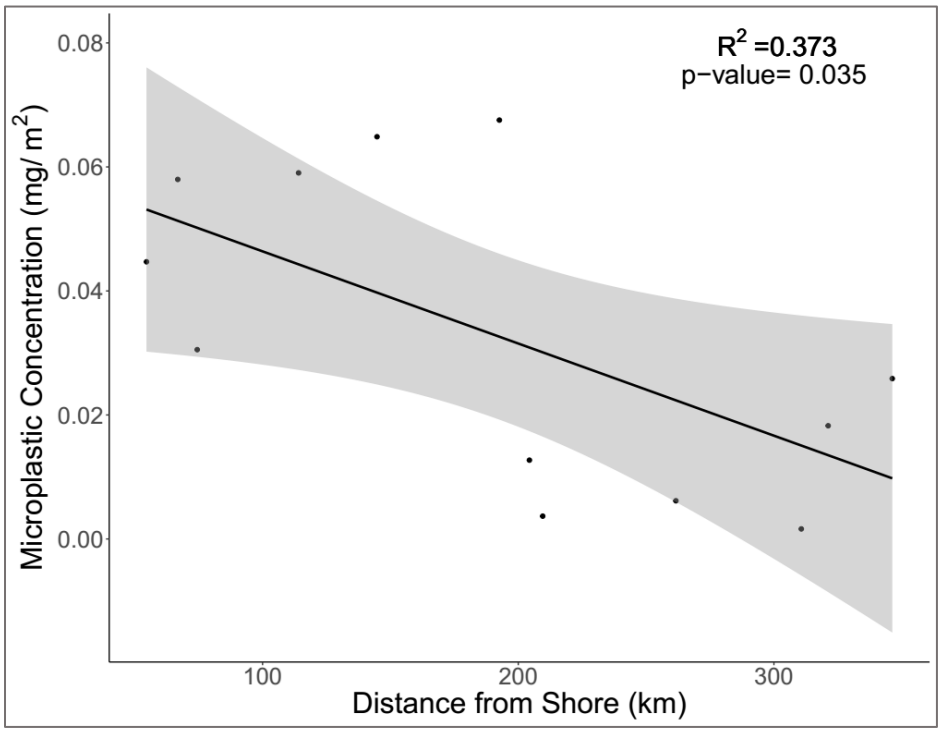

Figure 1.4 Linear regression of microplastic concentrations in open water habitats with distance from shore for collections made in May 2018 and July 2018. Shaded regions denote 95% confidence.

1.3.2 Microplastic Ingestion

Juveniles of 29 species of *Sargassum*-associated fishes were collected during July 2017 (n=502 individuals); of these, 22 species met the criteria for microplastic ingestion analyses (i.e., minimum of three individuals) (Table 1.2). Microplastic FO varied by taxa, ranging from 0% (5 taxa) to 50% (*Aluterus scriptus*) (Figure 1.5a). Approximately half of the species examined had a microplastic FO of 20% or higher. For all taxa combined, the overall microplastic FO was 24.7% (Figure 1.5a).

Juveniles of 20 species of *Sargassum*-associated fishes were collected during July 2018 (n=348 individuals); of these, 12 species met the criteria for microplastic ingestion analyses (Table1.2). The overall microplastic FO in 2018 was 14.7%, which was lower than 2017 (Figure 1.5b). Microplastic FO varied by taxa, ranging from 0% (*Caranx ruber*) to 33% (2 species) (Figure 1.5b). Nearly half of the species examined had a microplastic FO of 20% or higher.

Results of a Fischer's exact test for all species examined from July 2017 suggested some taxa differed in microplastic FO (p-value=0.01). Posthoc pairwise Fischer's exact tests identified differences in microplastic FO among several species (Table 1.3). *S. rivoliana* (FO= 40%), *B. capriscus* (FO= 39.3%), and *Kyphosus* spp. (FO= 36.4%) all had significantly higher microplastic FO than *C. pullus* (FO= 0%), *H. histrio* (8.8%), *A. saxatilis* (FO= 14.5%), and *C. crysos* (FO= 16.4%). *B. capriscus* also had a significantly higher FO of microplastic than *A. monoceros* (FO= 15.4%). Within the fish with lower FO of microplastic, *A. saxatilis* had a significantly lower FO than *C. crysos*. *C. pullus* also had a significantly lower FO of microplastic from *A. scriptus* (FO= 50%), *C. macrocerus* (FO= 40%), and *E. bipinnulata* (FO= 25.6%).

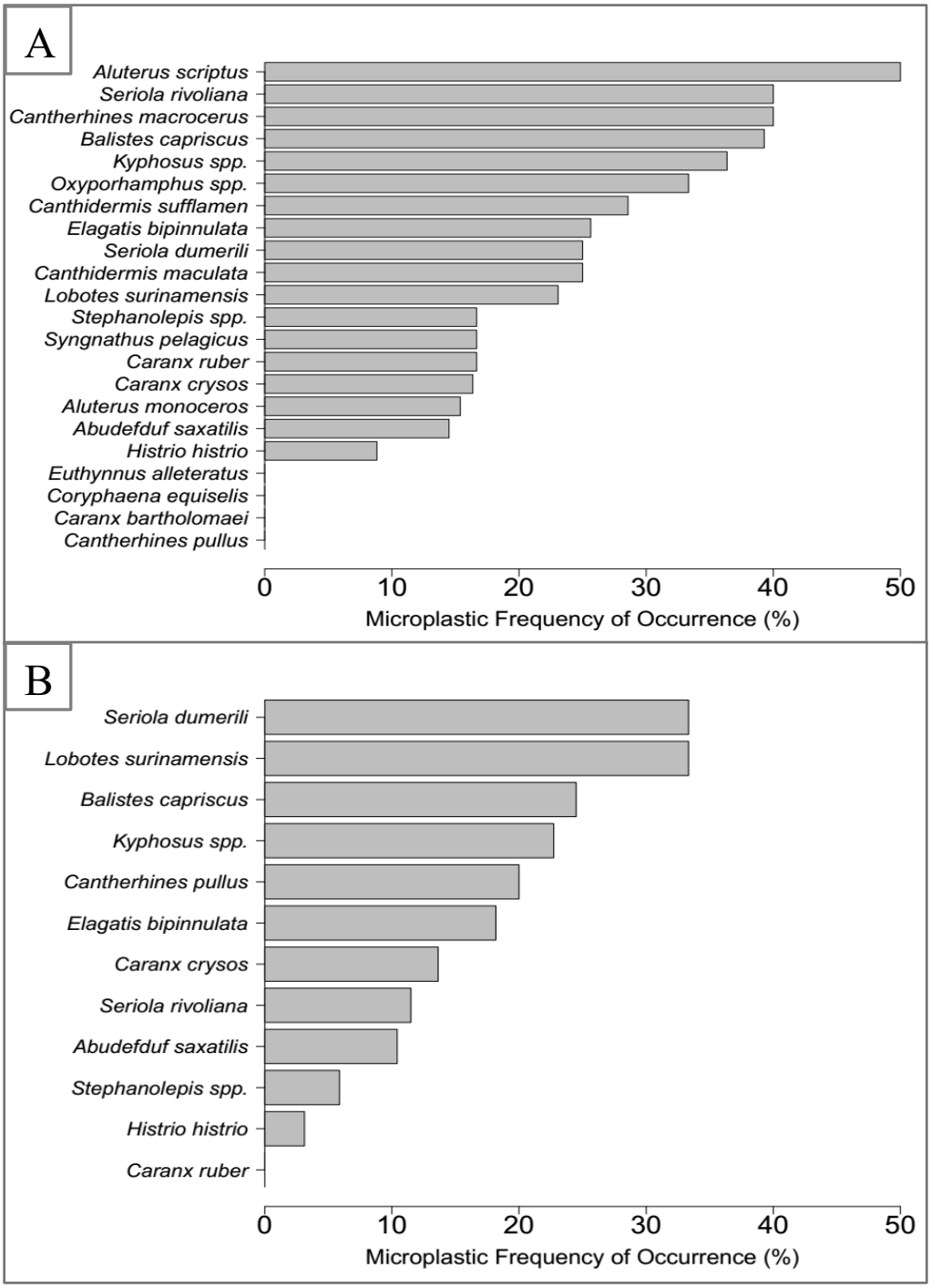

Figure 1.5 Microplastic frequency of occurrence plotted for *Sargassum*-associated fish species collected in A) July 2017 and B) July 2018. Only fish species with a minimum sample size of three individuals were used in analyses.

Table 1.3 Results (p-values) of multiple pairwise Fisher's Exact tests comparing microplastic frequency of occurrence (FO) in the guts of *Sargassum*-associated juvenile fishes collected in July 2017. Species listed under the species column are abbreviated across the top row in the same order. Bold values indicate significant differences between species pairs at α=0.05.

Species*	Hihi	Oxsp	Exsp	Sype	Cacr	Caru	Caba	Elbi	Seri	Sedu	Cosp	Losu	Kysp	Absa	Eual	Baca	Cdma	Cdsu	Almo	Alsc	Chma	Chpu	Stsp
Hihi	-																						
Oxsp	0.29	-																					
Exsp	1.00	1.00	-																				
Sype	0.49	1.00	1.00	-																			
Cacr	0.35	0.44	1.00	1.00	-																		
Caru	0.49	1.00	1.00	1.00	1.00	-																	
Caba	1.00	0.37	1.00	1.00	1.00	1.000	-																
Elbi	0.07	1.00	1.00	1.00	0.30	1.000	0.573	-															
Seri	**0.00**	1.00	0.51	0.38	**0.04**	0.383	0.140	0.27	-														
Sedu	0.23	1.00	1.00	1.00	0.62	1.000	0.487	1.00	0.67	-													
Cosp	1.00	1.00	1.00	1.00	1.00	1.000	1.000	1.00	0.53	1.000	-												
Losu	0.32	1.00	1.00	1.00	0.68	1.000	0.522	1.00	0.47	1.000	1.00	-											
Kysp	**0.00**	1.00	0.53	0.64	**0.04**	0.643	0.158	0.44	0.79	0.693	0.53	0.49	-										
Absa	0.53	0.39	1.00	1.00	0.80	1.000	1.000	0.19	**0.01**	0.603	1.00	0.42	**0.01**	-									
Eual	1.00	0.33	1.00	1.00	0.57	1.000	1.000	0.31	0.14	0.473	1.00	0.51	0.15	1.000	-								
Baca	**0.00**	1.00	0.52	0.40	**0.00**	0.405	0.155	0.17	1.00	0.709	0.28	0.36	0.84	**0.000**	0.08	-							
Cdma	0.23	1.00	1.00	1.00	0.62	1.000	0.487	1.00	0.67	1.000	1.00	1.00	0.69	0.603	0.47	0.709	-						
Cdsu	0.19	1.00	1.00	1.00	0.59	1.000	0.470	1.00	0.68	1.000	1.00	1.00	1.00	0.304	0.46	0.705	1.000	-					
Almo	0.43	0.47	1.00	1.00	1.00	1.000	1.000	0.53	0.11	0.625	1.00	0.67	0.13	0.751	0.55	**0.037**	0.625	0.59	-				
Alsc	**0.03**	1.00	0.46	0.54	0.08	0.546	0.182	0.33	0.67	0.580	0.46	0.32	0.65	0.061	0.18	0.681	0.580	0.59	0.120	-			
Chma	0.11	1.00	1.00	0.54	0.23	0.546	0.444	0.60	1.00	1.000	0.46	0.58	1.00	0.183	0.18	1.000	1.000	1.00	0.269	1.00	-		
Chpu	0.54	0.15	1.00	0.27	0.10	0.273	1.000	**0.02**	**0.00**	0.101	1.00	0.07	**0.00**	0.197	1.00	**0.001**	0.101	0.08	0.136	**0.01**	**0.048**	-	
Stsp	0.49	1.00	1.00	1.00	1.00	1.000	1.000	1.00	0.38	1.000	1.00	1.00	0.64	1.000	1.00	0.405	1.000	1.00	1.000	0.54	0.546	0.273	-

* Abbreviations: Hihi=*Histrio histrio,* Oxsp=*Oxyporhamphus* spp., Exsp=Exocoetidae spp., Sype=*Syngnathus pelagicus,* Cacr=*Caranx crysos,* Caru=*Caranx ruber,* Caba=*Caranx bartholomaei,* Elbi=*Elagatis bipinnulata,* Seri=*Seriola rivoliana,* Sedu=*Seriola dumerili,* Cosp=*Coryphaena* spp., Losu=*Lobotes surinamensis,* Kysp=*Kyphosus spp.,* Absa=*Abudefduf saxatilis,* Eual=*Euthynnus alleteratus,* Baca=*Balistes capriscus,* Cdma=*Canthidermis maculata,* Cdsu=*Canthidermis sufflamen,* Almo=*Aluterus Monoceros,* Alsc=*Aluterus scriptus,* Chma=*Cantherhines macrocerus,* Chpu=*Cantherhines pullus,* Stsp=*Stephanolepis* spp.

Results of a Fischer's exact test for all species examined from July 2018 suggested no significant differences in microplastic FO between species (p-value=1).

All four microplastic types 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 1.6a). 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 two or fewer (Figure 1.6b). The maximum numbers of fibers observed in a single individual were 9 (*B. capriscus* individual in July 2017) and 7 (*B. capriscus* individual and *E. bipinnulata* individual July 2018). Results from linear models examining the number of microplastics ingested by fish standard length (mm) were not significant for fishes collected in July 2017 (R^2=<0.001, p-value=0.996) and July 2018 (R^2=0.005, p-value=0.621), suggesting that fish size does not influence the number of microplastics ingested.

Hierarchal agglomerative clustering and simprof analyses grouped fish species into five distinct feeding groups based on observed prey in July 2017 (Figure 1.7a). ANOSIM results for July 2017 (R=0.282, p-value=0.001) suggest that the fish feeding groups were different. SIMPER results (Table 1.4) for July 2017 suggest that there was overlap of influential prey items in most fish groups, however some prey items were influential in predator group assignments, including shrimp and fish (Group B17), planktivorous prey (e.g., fish eggs, copepods, salp, and chaetognath; Group E17 and Group D17), and epiphytes (e.g., bryozoans, and hydroids) and *Sargassum* (Groups A17 and C17). Diets for Groups D17 and E17 differed in relative contributions of gelatinous organisms and amphipods (Group D17) and copepods and fish eggs (Group E17). Diets

for Groups A17 and C17 differed in the relative contributions of shrimp (Group A17) and gelatinous organisms, amphipods, and gastropods (Group C17). Kruskal-Wallis test results indicate that there are no differences in microplastic FO by feeding groups for July 2017 (Chi-squared=4, p-value=0.406).

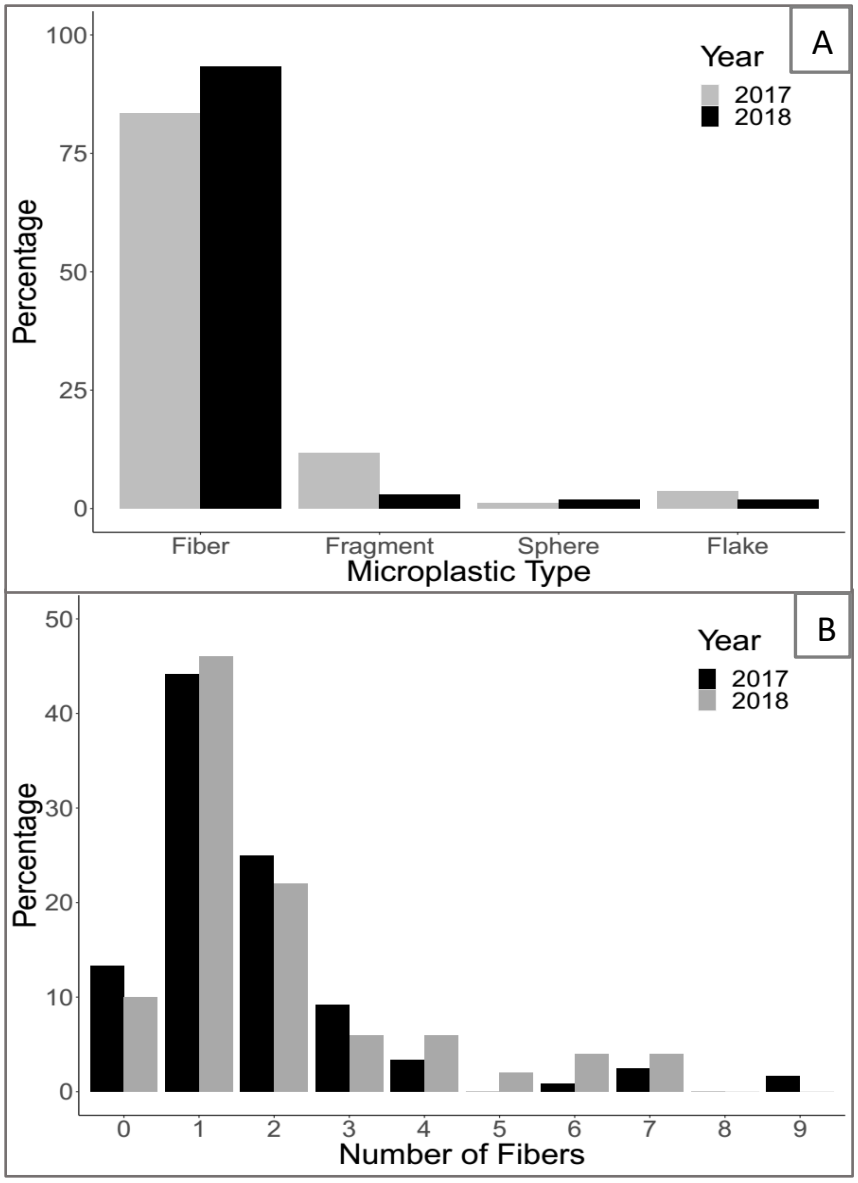

Figure 1.6 *A)* Percentage of microplastic types found in the guts of *Sargassum*-associated fishes collected in July 2017 and July 29=018. B) Percentage of fiber pieces ingested per individual fish collected in July 2017 and July 2018.

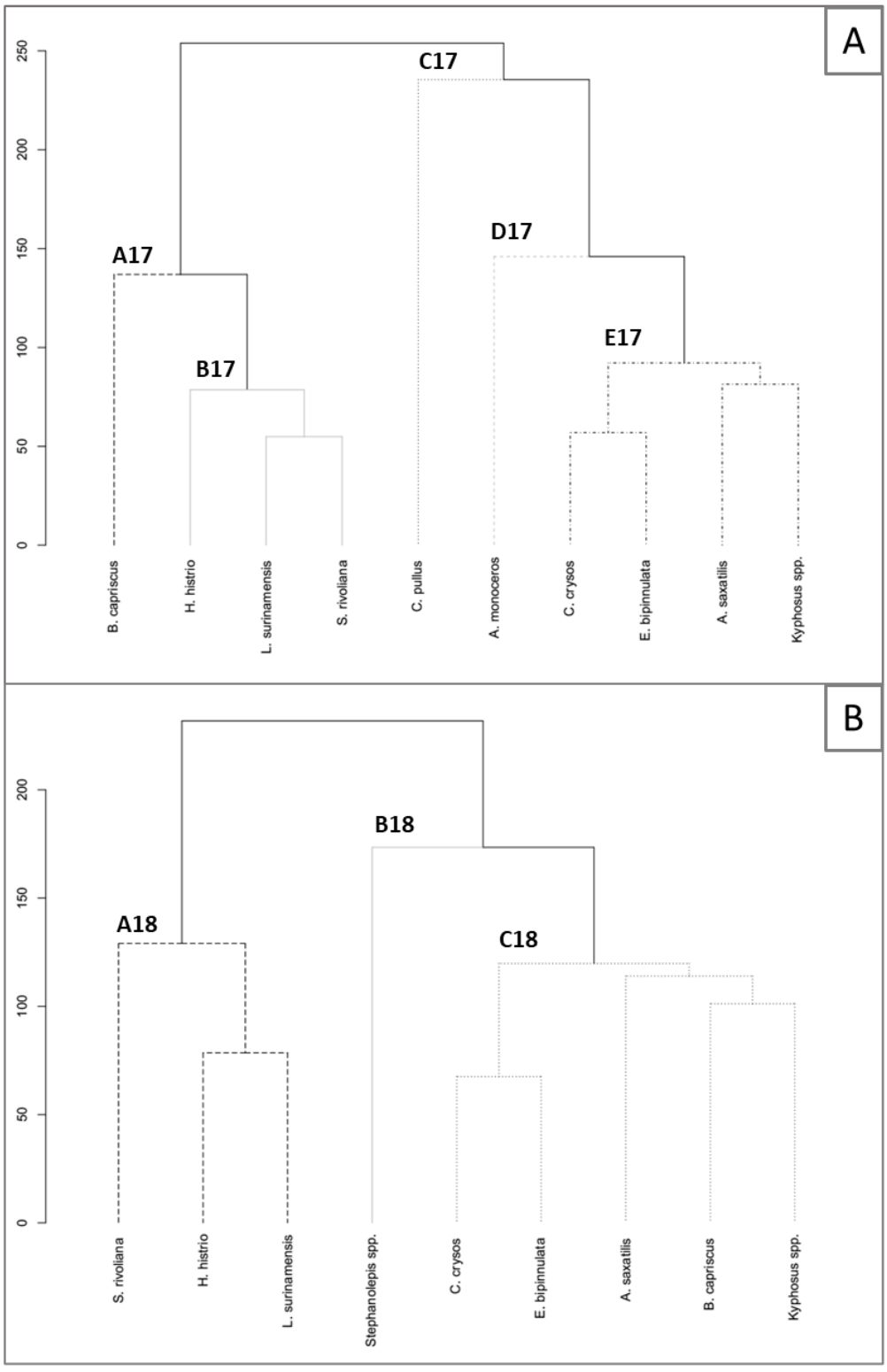

Figure 1.7 Cluster analysis using the combined prey FO for each species for juvenile fishes collected in July 2017 (A) and July 2018 (B). Clusters were based on Euclidean distance and Ward's method. Significant clusters were defined using simprof analyses.

Hierarchal agglomerative clustering and simprof analyses for July 2018 grouped fish into three distinct groups based on observed diet (Figure 1.7b). ANOSIM results for July 2018 (R=0.328, p-value=0.001) suggesting that the fish feeding groups were different. SIMPER results (Table 1.5) for 2018 suggests that there were differences between groups based on the influential prey items. The influential prey items that defined the different predator groups were shrimp and fish (Group A18), epiphytes (e.g., hydroids and *Spirorbis* spp.) and *Sargassum* (Group B18), and planktivorous prey (e.g., copepods and chaetognath; Group C18). Kruskal-Wallis test results also indicate that there were no differences in microplastic FO by feeding groups for July 2018 (Chi-squared=2, p-value=0.368).

Sargassum-associated fishes examined for microplastic FO were collected from *Sargassum* habitats that ranged from approximately 20-284 km from shore (Figure 1.1). Although not statistically significant (F=3.452, p-value=0.076), microplastic FO in juvenile fishes generally decreased with distance from shore (Figure 1.8a). The biomass of *Sargassum* collected in these samples ranged from 0.07-1.7 kg/m^2. No relationship was found between microplastic FO in juvenile fishes and *Sargassum* biomass (F=0.148, p-value=0.705) (Figure 1.8b).

Table 1.4 Results from SIMPER analyses of fish feeding groups for July 2017.
Top ten contributing prey taxa are listed in order of most contributing to least for each pairwise feeding group comparison.

Groups A17 & B17
Overall dissimilarity= 0.80805

Prey Taxa	Group A17 avg. abund.	Group B17 avg. abund.	Contrib%	Cum.%
Shrimp	0.5769	0.7463	6.04	7.47
Shrimp_*L_fucorum*	0.2788	0.4776	5.79	14.64
Bryozoan	0.5192	0.0448	5.69	21.68
Sargassum	0.4423	0.1642	5.13	28.03
Copepod_Calanoid	0.4327	0.1642	4.98	34.20
Copepod	0.4231	0.0299	4.38	39.62
Fish_eggs	0.3365	0.1194	4.26	44.89
Copepod_Poecilostomatoid	0.4038	0.0746	4.19	50.07
Copepod_Harpacticoid	0.3462	0.0299	3.79	54.76
Polychaeta	0.3462	0.0299	3.73	59.38

Groups A17 & C17
Overall dissimilarity= 0.7485

Prey Taxa	Group A17 avg. abund.	Group C17 avg. abund.	Contrib%	Cum.%
Amphipod	0.2500	0.8125	5.18	6.92
Gastropod_UnID	0.3173	0.8125	4.35	12.73
Gelatinous_organism	0.0192	0.5625	4.20	18.34
Shrimp	0.5769	0.2500	3.93	23.59
Copepod	0.4231	0.6250	3.87	28.77
Sargassum	0.4423	0.7500	3.87	33.93
Bryozoan	0.5192	0.4375	3.60	38.74
Hydroid	0.2019	0.5625	3.59	43.54
Copepod_Calanoid	0.4327	0.2500	3.29	47.93
Gastropod_Pteropoda	0.1923	0.4375	3.21	52.22

Groups C17 & B17
Overall dissimilarity= 0.9174

Prey Taxa	Group C17 avg. abund.	Group B17 avg. abund.	Contrib%	Cum.%
Amphipod	0.8125	0.0000	8.66	9.44
Gastropod_UnID	0.8125	0.0299	6.81	16.86
Shrimp	0.2500	0.7463	6.56	24.01
Copepod	0.6250	0.0299	6.03	30.58
Sargassum	0.7500	0.1642	5.98	37.11
Gelatinous_organism	0.5625	0.0000	5.87	43.50
Hydroid	0.5625	0.0149	4.61	48.53
Shrimp_*L_fucorum*	0.0625	0.4776	4.50	53.43
Gastropod_Pteropoda	0.4375	0.0000	3.96	57.74
UnID_content	0.4375	0.0746	3.90	61.99

Groups D17 & A17
Overall dissimilarity= 0.8983

Prey Taxa	Group D17 avg. abund.	Group A17 avg. abund.	Contrib%	Cum.%
Gelatinous_organism	0.7273	0.0192	7.67	8.54
Amphipod	0.6364	0.2500	6.07	15.30
Shrimp	0.1818	0.5769	6.05	22.04
Bryozoan	0.0000	0.5192	5.16	27.78
Sargassum	0.1364	0.4423	4.63	32.93
Copepod	0.2727	0.4231	4.62	38.08
UnID_content	0.3182	0.2115	4.47	43.06
Copepod_Calanoid	0.0000	0.4327	4.12	47.64
Isopod	0.3636	0.0673	3.64	51.69
Copepod_Poecilostomatoid	0.0000	0.4038	3.57	55.67

Table 1.4 (Continued.)

Groups D17 & B17
Overall dissimilarity= 0.9480

Prey Taxa	Group D17 avg. abund.	Group B17 avg. abund.	Contrib%	Cum.%
Shrimp	0.1818	0.7463	12.63	13.32
Gelatinous_organism	0.7273	0.0000	12.16	26.14
Amphipod	0.6364	0.0000	9.90	36.58
Shrimp_*L_fucorum*	0.0000	0.4776	7.61	44.61
UnID_content	0.3182	0.0746	6.72	51.69
Isopod	0.3636	0.0448	5.24	57.22
Fish	0.0909	0.1642	4.08	61.52
Copepod	0.2727	0.0299	3.92	65.66
Sargassum	0.1364	0.1642	3.54	69.40
Salp	0.2727	0.0000	3.39	72.98

Groups D17 & C17
Overall dissimilarity= 0.7289

Prey Taxa	Group D17 avg. abund.	Group C17 avg. abund.	Contrib%	Cum.%
Gastropod_UnID	0.0909	0.8125	6.12	8.39
Sargassum	0.1364	0.7500	5.63	16.12
Copepod	0.2727	0.6250	5.12	23.14
Gelatinous_organism	0.7273	0.5625	4.39	29.17
Hydroid	0.0455	0.5625	4.29	35.06
UnID_content	0.3182	0.4375	4.29	40.95
Amphipod	0.6364	0.8125	4.15	46.64
Gastropod_Pteropoda	0.1364	0.4375	3.91	52.01
Isopod	0.3636	0.1250	3.29	56.52
Bryozoan	0.0000	0.4375	3.23	60.96

Group D17 & E17
Overall dissimilarity= 0.9274

Prey Taxa	Group D17 avg. abund.	Group E17 avg. abund.	Contrib%	Cum.%
Gelatinous_organism	0.7273	0.0000	12.49	13.47
Amphipod	0.6364	0.0324	10.09	24.36
Copepod	0.2727	0.4973	8.95	34.00
Fish_eggs	0.0000	0.4270	8.32	42.97
UnID_content	0.3182	0.1676	7.56	51.12
Isopod	0.3636	0.0162	5.25	56.78
Decapod	0.0455	0.1946	3.49	60.54
Salp	0.2727	0.0000	3.47	64.27
Chaetognath	0.0000	0.2378	3.40	67.94
Shrimp	0.1818	0.0703	2.88	71.05

Groups E17 & A17
Overall dissimilarity= 0.8682

Prey Taxa	Group E17 avg. abund.	Group A17 avg. abund.	Contrib%	Cum.%
Shrimp	0.0703	0.5769	6.99	8.05
Copepod	0.4973	0.4231	6.25	15.26
Fish_eggs	0.4270	0.3365	6.10	22.28
Bryozoan	0.0054	0.5192	5.79	28.96
Sargassum	0.0162	0.4423	4.93	34.63
Copepod_Calanoid	0.1243	0.4330	4.88	40.26
Copepod_Harpacticoid	0.1135	0.3462	4.24	45.15
Copepod_Poecilostomatoid	0.0757	0.4039	4.19	49.97
Polychaeta	0.0541	0.3462	3.94	54.52
UnID_content	0.1676	0.2115	3.51	58.55

Table 1.4 (Continued.)

Groups E17 $ B17
Overall dissimilarity= 0.9359

Prey Taxa	Group E17 avg. abund.	Group B17 avg. abund.	Contrib%	Cum.%
Shrimp	0.0703	0.7463	15.90	16.99
Fish_eggs	0.4270	0.1194	10.19	27.88
Copepod	0.4973	0.0299	10.17	38.75
Shrimp_*L_fucorum*	0.0054	0.4776	9.06	48.43
Copepod_Calanoid	0.1243	0.1642	4.29	53.02
UnID_content	0.1676	0.0746	4.25	57.56
Chaetognath	0.2378	0.0149	3.99	61.82
Decapod	0.1946	0.0597	3.92	66.01
Shrimp_*L_tenuicornis*	0.0000	0.2239	3.89	70.16
Fish	0.0378	0.1642	3.42	73.82

Groups E17 & C17
Overall dissimilarity= 0.8742

Prey Taxa	Group E17 avg. abund.	Group C17 avg. abund.	Contrib%	Cum.%
Amphipod	0.0324	0.8125	8.63	9.87
Gastropod_UnID	0.0595	0.8125	6.81	17.66
Sargassum	0.0162	0.7500	6.46	25.06
Gelatinous_organism	0.0000	0.5625	5.98	31.90
Copepod	0.4973	0.6250	5.22	37.87
Hydroid	0.0811	0.5625	4.74	43.30
Fish_eggs	0.4270	0.1875	4.68	48.65
UnID_content	0.1676	0.4375	4.25	53.51
Gastropod_Pteropoda	0.0162	0.4375	4.04	58.14
Bryozoan	0.0054	0.4375	3.51	62.16

Table 1.5 Results from SIMPER analyses of fish feeding groups for July 2018.
Top ten contributing prey taxa are listed in order of most contributing to least for each pairwise feeding group comparison.

Groups A18 & B18
Overall dissimilarity= 0.8830

Prey Taxa	Group A18 avg. abund.	Group B18 avg. abund.	Contrib%	Cum.%
Shrimp_*L_fucorum*	0.570	0.000	7.60	8.60
Shrimp	0.648	0.294	7.35	16.93
Sargassum	0.375	0.706	6.93	24.77
Polychaeta	0.016	0.588	6.53	32.17
UnID_content	0.047	0.353	5.16	38.01
Hydroid	0.000	0.471	4.95	43.61
Polychaeta_*Spirorbis* spp.	0.016	0.412	4.51	48.72
Gastropod_UnID	0.047	0.353	4.04	53.30
Fish	0.383	0.000	3.96	57.79
Decapod	0.211	0.235	3.54	61.80

Groups C18 & A18
Overall dissimilarity= 0.9039

Prey Taxa	Group A18 avg. abund.	Group B18 avg. abund.	Contrib%	Cum.%
Shrimp	0.136	0.648	8.20	9.07
Shrimp_*L_fucorum*	0.019	0.570	7.99	17.91
Copepod_Calanoid	0.395	0.211	5.40	23.88
Copepod_Poecilostomatoid	0.346	0.242	5.33	29.78
UnID_content	0.333	0.047	4.67	34.95
Fish	0.086	0.383	4.55	39.99
Sargassum	0.080	0.375	4.19	44.63
Copepod	0.247	0.078	3.70	48.72
Copepod_Cyclopoid	0.284	0.000	3.68	52.79
Chaetognath	0.204	0.211	3.48	56.64

Groups C18 & B18
Overall dissimilarity= 0.8966

Prey Taxa	Group A18 avg. abund.	Group B18 avg. abund.	Contrib%	Cum.%
Sargassum	0.080	0.706	8.14	9.07
Polychaeta	0.105	0.588	6.89	16.76
UnID_content	0.333	0.353	6.46	23.96
Hydroid	0.056	0.471	5.36	29.94
Gastropod_UnID	0.216	0.353	4.97	35.48
Shrimp	0.136	0.294	4.78	40.81
Polychaeta_*Spirorbis* spp.	0.006	0.412	4.75	46.11
Copepod_Calanoid	0.395	0.000	4.72	51.37
Copepod_Poecilostomatoid	0.346	0.000	4.42	56.29
Copepod_Cyclopoid	0.284	0.059	4.14	60.91

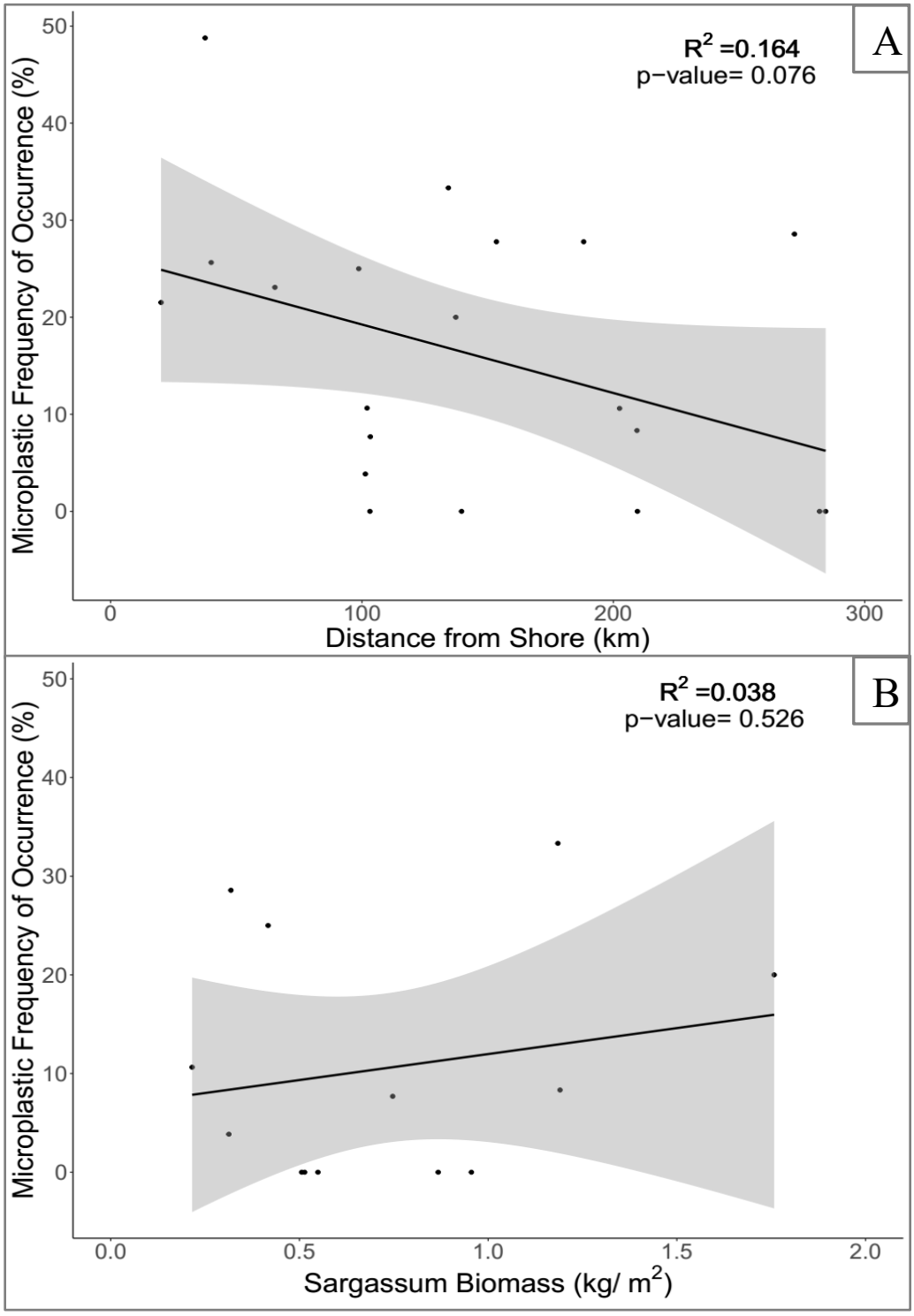

Figure 1.8 Linear regressions of microplastic FO in juvenile fish guts collected by neuston net tows per station with A) distance from shore for collections made in July 2017 and July 2018 and B) *Sargassum* sample biomass for collections made in July 2018. Shaded regions denote 95% confidence.

1.4 Discussion

The results from this study provide the first quantitative estimates of microplastic concentrations within *Sargassum* and adjacent open water habitats of the GoM, and demonstrate that juvenile fishes associated with *Sargassum* encounter higher microplastic concentrations than fishes inhabiting open water habitats surrounding it. In addition, this study presents some of the first insights on marine pelagic juvenile fish microplastic FO. Microplastic ingestion by *Sargassum*-associated juvenile fishes was observed across nine families and 19 species of fishes and covered a range of feeding types specific to the *Sargassum* community including shrimp eaters, epiphyte generalists, and planktivorous feeders. These combined results allow for some of the first observations of microplastic impacts on juvenile fishes using *Sargassum* habitats within the GoM and aid in understanding microplastic aggregation and concentrations in offshore locations.

1.4.1 Microplastic Concentration

Microplastic concentrations within individual *Sargassum* features were highly variable, but on average were 180 times greater than those found in open water. A recent study conducted off the coast of Hawaii Island also found that microplastic concentrations were about 130 times greater within slicks or areas of ocean convergence than outside of them (Gove et al., 2019). While previous studies have shown that microplastics aggregate in large scale ocean gyres, these results and other recent studies have shown that smaller scale oceanographic surface features of convergence also serve to concentrate microplastics at the surface (Brach et al., 2018; Gove et al., 2019). In addition, the complexity of the *Sargassum* habitat itself provides a mechanism for trapping microplastics. *Sargassum* morphology and density are known to be highly

variable in nature from small floating clumps (scales of cm) to large mats (scales of m to km), as was evident in this study (Table 1.1). My results suggest that as *Sargassum* biomass increases, so does microplastic concentration (Fig 1.3). This could be attributed to the complex structure the algae provide for microplastics to adhere and become trapped. Previous studies have found microplastics (0.7-4.4 pieces/ per blade) trapped within the epibiont communities on seagrasses and macroalgae including a benthic *Sargassum* spp. (Goss et al., 2018; Seng et al., 2020). These results suggest that the ability of *Sargassum* to physically collect microplastics and its inherent aggregation with microplastics, could mean that fish using this habitat could be more impacted than those living outside of it.

While microplastic concentrations within *Sargassum* were significantly higher than those found in adjacent open water habitats, both habitats followed a similar spatial trend where microplastic concentrations decreased with distance from shore. This suggests that there could be a cross-shelf gradient of microplastics in the GoM, with greater concentrations of microplastics inshore and lower concentrations offshore. 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 microplastics entering the basin. For example, 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, like the Mediterranean Sea. Mediterranean Sea studies have seen higher concentrations of microplastics closer to drainage systems and near highly populated coastal cities (Schmidt et al., 2018; Vianello et al., 2018). The open water

microplastic concentrations observed in this study (0.03 mg/m^2) fell in the lower range of concentrations found in the Mediterranean Sea (0-9.298 mg/m^2) (Collignon et al., 2012; Ruiz-Crejón et al., 2016; Schmidt et al., 2018). This result is not unexpected, because most of the samples taken in this study were in offshore waters of the GoM, and relatively far from coastal sources of microplastics. The results presented here provide some of the first offshore estimates of microplastic concentrations in the northern GoM, and suggest that organisms inhabiting nearshore surface waters are more likely to encounter microplastics than offshore species.

1.4.2 Microplastic Ingestion

The overall microplastic FO in juvenile fishes associated with *Sargassum* (14.7-24.7%) were much lower than those reported for other juvenile fishes (52-59%) from other habitats (Kazour et al., 2018; Collicutt et al., 2019; Naidoo et al., 2020). Many of the previous studies sampled juvenile fishes in nearshore nursery habitats (e.g., mangroves, estuaries) and include many benthic and benthopelagic species (e.g., Salmonidae, Pleuronectidae, Cichlidae, Terapontidae, Mugilidae, and Ambassidae) (Kazour et al., 2018; Collicutt et al., 2019; Naidoo et al., 2020). In contrast, the fishes collected in *Sargassum* were pelagic species, and were collected at least 20 km offshore. As noted above, microplastic plastic concentrations were found to decrease with distance from shore. Microplastic FO in fish was also found to decrease with distance from shore, which likely explains the lower microplastic FO observed in the guts of juvenile *Sargassum*-associated fishes in offshore waters. Recent studies conducted on pelagic fishes in the Pacific Ocean found overall microplastic FO that were more similar to the microplastic FO found in the GoM (8.6-24.3%) (Goven et al., 2019; Markic et al., 2019).

These studies included fish of similar sizes (5-1,386 mm TL) and families (Balistidae, Carangidae, Pomacentridae, Kyphosidae, and Monacanthidae) of those found associated with *Sargassum* (Goven et al., 2019; Markic et al., 2019). This suggests that pelagic fish potentially have lower microplastic FO because they are farther away from coastal sources of microplastics. The lower microplastic FO in pelagic fish could also be attributed to the absence of seafloor sediment microplastics. Benthic and benthopelagic fish are also subject to potentially high concentrations of microplastics found in the seafloor sediments which could explain their higher microplastic FO (Ling et al., 2017). Even though overall microplastic FO was lower for pelagic fish, micro-fibers (83.5-93.3%) were found to be the dominant microplastic type ingested by both benthopelagic and pelagic juvenile fishes (micro-fibers=68-90%) (Kazour et al., 2018; Collicutt et al., 2019; Naidoo et al., 2020). Similarly, individual benthopelagic juvenile fishes were found to have ingested between 1-2 microplastics on average and the pelagic juvenile fishes were found to have ingested about 2 microplastics on average (Kazour et al., 2018; Collicutt et al., 2019). I saw that the number of microplastic observed in the gut were not related to the size of the fish, and I observed microplastics in both the stomach and intestine. This and the results above would suggest that microplastics are not accumulating in the juvenile fishes, but are moving through the fish gastrointestinal tract.

Microplastics aggregating within the *Sargassum* habitat are being ingested by several juvenile fish species and at varying frequencies. It was hypothesized that obligate *Sargassum* residents (e.g., *Histrio histrio, Syngnathus pelagicus*) would have higher microplastic FO than more transient species, such as *Seriola* spp. and *B. capriscus*. However, *H. histrio* had one of the lowest microplastic FO (3-9%) overall, in contrast to

Seriola spp. (33-40% FO), *B. capriscus* (25-39% FO), and *Kyphosus* spp. (23-36% FO), which had some of the highest observed microplastic FO. Therefore, being obligate to the *Sargassum* habitat is not a driving factor for higher microplastic FO, and in contrast, transient juvenile fishes using this habitat as a nursery may be at higher risk from microplastic impacts.

Feeding behavior could also influence the ingestion of microplastics. It was hypothesized that fish foraging in close association with *Sargassum* would have higher microplastic FO. More specifically, this would affect fish eating epiphytes (e.g., bryozoans, hydroids, *Spirorbis* spp.) found directly on *Sargassum* or eating invertebrates living within *Sargassum* (e.g., *Latreutes fucorum*, *Leander tenuicornis*, *Portunis sayi*). Although distinct feeding groups were observed in 2017 and 2018 (including shrimp eaters, epiphyte generalists, and planktivorous fishes), there were no significant differences among feeding groups and their microplastic FO. However, there was a trend in July 2017 of higher microplastic FO for fish feeding more directly on *Sargassum* (34% FO), followed by fish feeding in close association with *Sargassum* (e.g., *L. fucorum, L. tenuicornis*) (22% FO), and planktivorous fishes feeding further afield (e.g., copepods, chaetognaths) (20% FO). A different pattern was observed in July 2018, in part because the feeding group membership varied; for example, *B. capriscus* was grouped with epiphyte feeders in 2017 and with planktivorous fishes in 2018. Variations in feeding group assignment between years may reflect size-related or ontogenetic differences in foraging; *B. capriscus* collected in 2017 were larger on average (and fed primarily on epiphytes), whereas those sampled in 2018 were generally smaller (and fed on zooplankton). These results suggest that microplastic FO may change through ontogeny

as foraging modes change. Another possible cause for differences in microplastic FO seen among different feeding groups in *Sargassum* could be the foraging preference. Recent studies have found that fish eating a more generalist diet have had higher microplastic FO than those fish that are more specialized (Mizraji et al., 2017; Peters et al., 2017). Both generalist (*B. capriscus*) and specialist (*Seriola* spp.) feeders within *Sargassum* were found to have higher microplastic FO. Also, the microplastic FO for generalist (*B. capriscus*, *C. pullus*, and *Stephanolepis* spp.; FO 0-39%) and specialist (*Seriola* spp., *L. surinamensis*, *H. histrio*; FO 3-40%) feeders ranged from low to high across both sampling years. There are likely other factors (e.g. feeding strategy, such as ambush predator vs. grazer, and secondary ingestion of microplastics via prey) not examined in my study that may drive differences in microplastic FO for juvenile fishes associated with *Sargassum,* which leaves room for further investigation.

1.4.3 Conclusions and Future Directions

This study provides the first quantitative descriptions of microplastic concentrations, distribution, and impacts to offshore *Sargassum* habitats within the northern GoM. Although microplastic concentrations and microplastic FO in fish guts decreased with distance from shore, *Sargassum* appears to be a sink for surface microplastics in offshore locations because concentrations are greater within *Sargassum* than the adjacent open water habitats. Future work should examine the gut contents of juvenile fishes collected in near-surface, open water habitats for comparison. Attempts were made in this study to collect open water juveniles with a Methot frame trawl (Methot 1986), but with limited success. Additional methods of collecting small open water fishes, such as mid-water trawls (e.g., Tanabe and Niu 1998) and microtrolling

(Duguid and Juanes 2017), may be more successful. Future research should also focus on the potential for secondary microplastic ingestion through prey (Romeo et al., 2015). Several of the juvenile *Sargassum*-associated fishes fed on invertebrates, such as shrimp and polychaetes, which could be potential vectors for microplastics. This would also lend itself to comparing open water and *Sargassum* habitats in order to better understand the differences in microplastic ingestion by juvenile fish predators between habitats.

Finally, *Sargassum* acts as a nursery habitat for many juvenile fishes and has shown the potential to accumulate larger concentrations of microplastics because of its complex structure and biomass. A diverse range of juvenile fish species associated with *Sargassum* are consuming microplastics, but further investigations into the implications of this need to be studied. Recent 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). Future studies would benefit from looking at how microplastics could impact the overall condition of juvenile fishes associated with *Sargassum*. This would allow for a better understanding of how microplastics impact juvenile fishes developing within the *Sargassum* habitat and potentially identify a source for decreased recruitment to the adult population.

CHAPTER II - TAXONOMIC RELATIONSHIPS AMONG THE MICROBIAL COMMUNITIES OF *SARGASSUM*, MICROPLASTICS, AND THE GASTROINTESTINAL TRACTS OF ASSOCIATED GRAY TRIGGERFISH

2.1 Introduction

Microplastic ingestion has been documented for a range of marine organisms, including juvenile fishes (Hoss and Settle, 1990; Boerger et al., 2010; Cole et al., 2013; Lusher et al., 2015; Davidson and Dudas, 2016; Alomar and Deudero, 2017; Courtene-Jones et al., 2017; Steer et al., 2017; Collicutt et al., 2018; Ory et al., 2018; Duncan et al., 2019). Laboratory experiments with captive fishes have documented negative physical impacts of microplastics, including abrasions, intestinal lesions, and alterations to intestinal structure because of ingestion (Pedà et al., 2016; Jovanović, 2017; Jabeen et al., 2018; Ahrendt et al., 2020). Similar studies have also investigated microplastic toxicity on fish physiological activities, and have reported changes in behavior, reduced condition, and mortality among various life stages (Oliveira et al., 2013; Rochman et al., 2013; Karami et al., 2016; Lu et al., 2016; Rainieri et al., 2018). Specifically, the additives associated with microplastics, such as polycyclic aromatic hydrocarbon (PAH) and polychlorinated biphenyl (PCB), interact with and alter fish internal physiology (Oliveira et al., 2013; Rochman et al., 2013). Although lab experiments provide insight into the physical and physiological impacts of microplastics, quantifying harmful impacts that affect the fitness or survival of fishes and other marine animals *in situ* has been challenging. The difficulties lie in attributing cause directly to microplastics outside of a controlled environment.

The surface area of microplastics allow for the colonization of microorganisms, some of which may be harmful to fishes if ingested (Carpenter et al., 1972; Moore, 2008; Webb et al., 2009; Lobelle and Cunliffe, 2011; Kirstein et al., 2016). Microbes are highly abundant and ubiquitous in the marine environment (Landry and Kirchman, 2002; Walsh et al., 2016; Easson and Lopez, 2019), and microbial assemblages can be highly variable among habitat types (Sullam et al., 2012). Microplastics can have microbial communities distinct from surrounding marine and fresh waters, and distinct communities across marine water basins (Zettler et al., 2013; McCormick et al., 2014; Amaral-Zettler et al., 2015). Putative human pathogenic bacteria (*Vibrio parahaemolyticus*) and a fish pathogenic bacteria (*V. alginolyticus*) have been observed on microplastics suggesting that microplastics could also be a new vector for pathogenic bacteria to enter fishes (Kirstein et al., 2016). Recent mesocosm studies on larval and adult zebrafish have shown that exposures of pristine microplastics can cause microbial dysbiosis, oxidative stress, and changes in relative metabolite abundances in the gut (Jin et al., 2018; Wan et al., 2018; Qiao et al., 2019). These results demonstrate the potential of microplastics to affect the microbiomes of fishes, but questions remain as to how microplastic microbial biofilms could impact fish gut microbiomes after ingestion.

Bacteria are known to have specific symbiotic associations and can be beneficial and sometimes critical to functions within their host (Sullam et al., 2012). Specifically, it has been shown that bacteria have a role in digestive processes, such as enzymatic activities, within several species of aquatic organisms (Stickney and Shumway, 1974; Harris, 1993). The bacterial microbiome within invertebrates and mammals and their symbiotic role has been well-studied, but aquatic vertebrates, particularly fish

microbiomes and their symbiotic roles, are underrepresented in the literature (Harris, 1993; Ray et al., 2012; Sullam et al., 2012). From these studies, there has been evidence of the importance of these symbiotic relationships within fish guts, and that gut microbiota in fish contribute to immune response and possibly nutritional uptake (Lauzon et al., 2010; Ray et al., 2012).

Previous studies have examined microbial communities in fishes with respect to building gut microbiomes and the influx of diseases through ingesting sea water (Hansen and Olafsen, 1999; Vadstein et al., 2013; Llewellyn et al., 2014). The development of a microbiome during the early life stages is thought to be established at random from the surrounding environment, and then becomes more structured and stable as the fish develop and reach adulthood (Vadstein et al., 2013; Llewellyn et al., 2014). Because the microbiome can be influenced by the surrounding environment and is important to fish digestion and immune functions, fishes could be impacted by harmful microbes and changes in gut microbiome structure. These changes in microbiome structure could lead to changes in individual fishes physiology, growth, and nutrition (Lauzon et al., 2010). Describing relationships between the community of microorganisms in the external environment (e.g., surrounding water, habitats) and within fishes is critical to understanding how microbes are introduced to fish guts and their potential impacts.

As documented in Chapter 1, microplastic concentrations are significantly higher in *Sargassum* than in adjacent open water habitats (Figure 1.2), and there is evidence that *Sargassum*-associated fishes are ingesting microplastics (Figure 1.5). The microbiome associated with *Sargassum natans*, *S. fluitans*, and other brown macroalgae in general has not been well studied (Susilowati et al., 2015; Torralba et al., 2016; Serebryakova et al.,

2018). Therefore, the pelagic *Sargassum* community presents an opportunity to examine how *Sargassum*, microplastics, and their associated microbiomes potentially impact juvenile fishes foraging in this habitat.

The goal of this chapter is to assess the impacts of microplastic ingestion on the microbiome of juvenile Gray Triggerfish (*Balistes capriscus*), a common *Sargassum*-associated fish species in the northern Gulf of Mexico (GoM). Specific objectives are to: 1) describe the microbial community of marine microplastics associated with *Sargassum* and ambient water; 2) describe the gut microbial community of juvenile Gray Triggerfish collected in *Sargassum* habitats; and 3) compare the gut microbial communities of juvenile Gray Triggerfish observed with and without evidence of microplastic ingestion.

2.2 Materials and Methods

2.2.1 Study Region

Samples were collected from *Sargassum* habitats in the northern GoM during two cruises aboard *R/V Point Sur* (July 9-16, 2018; May 28- June 4, 2019) (Figure 2.1). The locations of *Sargassum* features were determined using daily Alternative Floating Algal Index (AFAI) and Floating Algal Density (FA_Density) products from the University of South Florida's Optical Oceanography Laboratory (https://optics.marine.usf.edu/). 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 seven-day mean (Wang and Hu 2016). Likely distributions of *Sargassum* were gathered from the combined vectors of HYCOM + NCODA Global

1/12° Analysis (https://www.hycom.org/). During each cruise, all *Sargassum* sampling stations were located beyond the 200 m isobath (Figure 2.1).

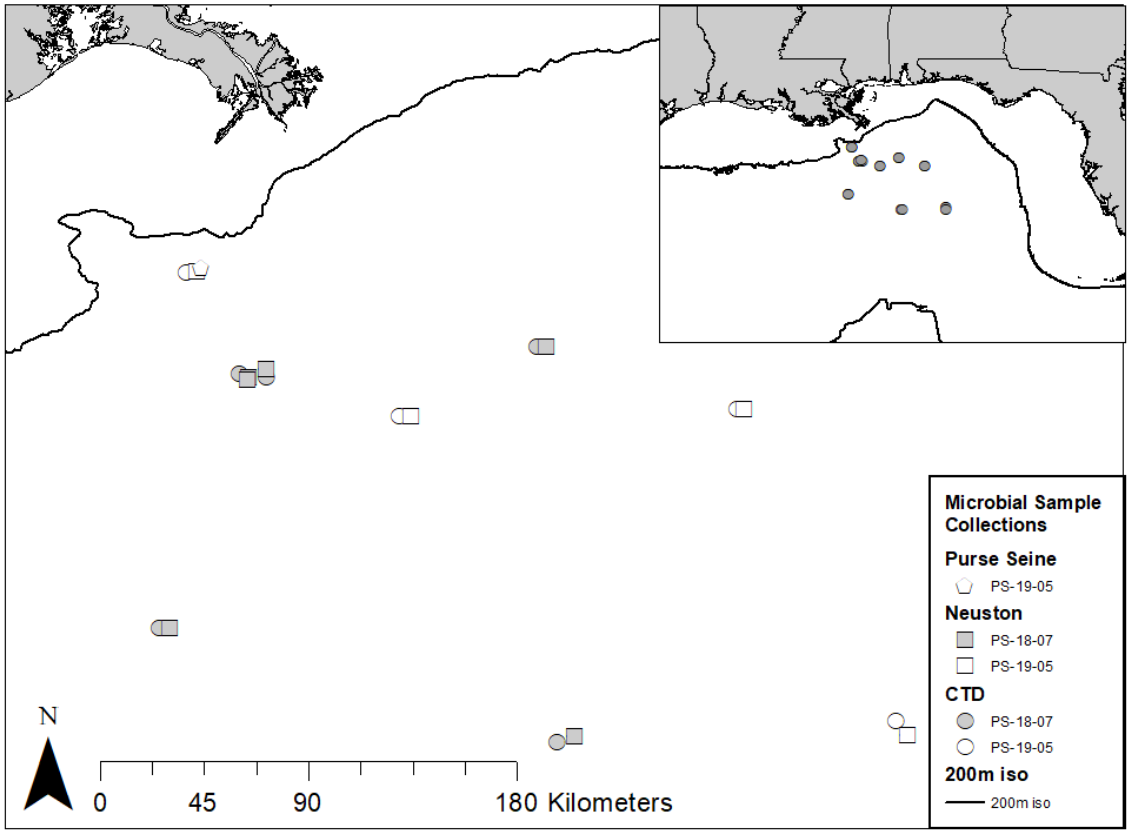

Figure 2.1 Sampling locations for cruises conducted in July 2018 and May 2019 in offshore locations of the northern GoM. Colors (grey and white) denote cruises. Symbols (pentagon, square, and circle) denote microbial sample collection methods. Water samples were collected from CTD casts, fish were collected from neuston and purse seine nets, and microplastics and *Sargassum* were collected from neuston nets. The solid line indicates the 200 m depth contour.

2.2.2 Microbial Sample Collection

A 1x2 m neuston sampler fitted with a 505 µm mesh net was towed at each *Sargassum* station to collect *Sargassum*, microplastics, and *Sargassum*-associated juvenile fishes, including Gray Triggerfish. The neuston net was lowered into the water as the vessel approached a *Sargassum* feature and fished with the upper 0.5 m of the net above the water surface. The net was retrieved when *Sargassum* filled one-quarter to one-

third of the net. Once on board, *Sargassum* was removed from the net and placed in holding bins until they could be rinsed for organisms and debris. Samples of each *Sargassum* species (*S. fluitans* and *S. natans*) were collected using sterile forceps, rinsed, and then stored in cryogenic vials at -20 °C. Microplastics were collected using sterile forceps from contents rinsed into a 333 µm sieve with sea water out of the *Sargassum* and placed in cryogenic vials on ice until the net tow sample processing was completed, at which point the vials were stored at -20 °C. Gray Triggerfish was identified as the target species for this study because they consume prey that are closely associated with the *Sargassum* community (e.g., *Sargassum* epiphytes), and have a relatively high frequency of occurrence of microplastics in their guts (Table 1.4, Figure 1.5). Juvenile Gray Triggerfish were collected in the *Sargassum* neuston net tows, as well as from hook-and-line sampling and opportunistic dip netting; all specimens used in this analysis were frozen whole at -20 °C. Ambient water samples were collected from the sea surface near *Sargassum* or just below the *Sargassum* canopy using Niskin bottles. Sea water samples (2.5 L) were filtered using a peristaltic pump and cells were concentrated on Sterivex-GP filters (0.22 µm) (Hamdan et al., 2013). Filters were then stored at -20° C.

Ambient water, *Sargassum*, and microplastic samples were stored in -80° C after returning to shore. Gray Triggerfish specimens were stored at -20° C until hind guts could be extracted. For gut extraction, fish were removed from the freezer and hind guts were removed using sterile equipment and stored in cryogenic vials at -80° C. The hind gut was chosen for microbial characterization, so that the foregut could be examined for evidence of microplastic ingestion.

2.2.3 DNA Extraction

DNA was extracted from *Sargassum*, water, microplastic, and fish hindgut samples using the FastDNA^{TM} SPIN Kit for Soil (MP Biomedicals). All samples were extracted following the modified protocol of Hamdan et al. (2013). The biofilm of microplastics was extracted from samples ranging in weight from 8-87 mg and were comprised of microplastic fragments, flakes, and fibers (Mugge et al., 2019; Salerno et al., 2018). To account for both epiphytic and endophytic bacteria, sprigs of *Sargassum natans* and *S. fluitans* (including stem, blades, and bladders) were crushed and homogenized while frozen using a sterile mortar and pestle, and a subsample between 100-200 mg was used for extraction (Serebryakova et al., 2018). To account for all bacteria associated with the hindgut wall and ingested gut contents, Gray Triggerfish hindguts were similarly crushed while frozen and homogenized using a sterile mortar and pestle before extraction. Because most fish were small, entire crushed guts could be run in one tube. Large hindgut samples were processed in multiple tubes (maximum 300 mg of tissue per tube), then recombined at the filtering step. All extracted DNA samples were quantified through a Qubit 2.0 Fluorometric Quantitation system following manufacturer's protocol (Invitrogen).

2.2.4 16S rRNA Amplification and Sequencing

Samples were sequenced at the Integrated Microbiome Resource (IMR) facility using Illumina MiSeq amplicon sequencing of the 16S rRNA genes (Comeau et al., 2011). V6-V8 variable regions of the 16s rRNA gene were amplified using the B969F (ACGCGHNRAACCTTACC) and BA1406R (ACGGGCRGTGWGTRCAA) primer set for bacteria. Because fish hindgut samples returned low sequence counts, samples were

concentrated and re-sequenced targeting the V4-V5 variable regions of the 16s rRNA gene with the universal primer set of 515FB (GTGYCAGCMGCCGCGGTAA) and 926R (CCGYCAATTYMTTTRAGTTT). Sequences were run through the Quantitative Insights into Microbial Ecology 2 (QIIME2) pipeline following the protocols outlined by Bolyen et al. (2019). Demultiplexed sequences were merged, denoised, and dereplicated into amplicon sequence variants (ASVs). Operational taxonomic units (OTUs) were de novo clustered at >97% similarity using VSEARCH. Taxonomy was assigned to all sequence samples for the combined V4-V8 variable regions using VSEARCH and the SILVA 132 reference database. Outliers were determined using median absolute deviation (MAD) analyses on the quality-controlled sequence count by sample type (water, *Sargassum*, microplastic, and Gray Triggerfish hindguts) (Leys et al., 2013). Based on these analyses, two samples of microplastics, two samples of *Sargassum*, and three samples of V6-V8 fish gut sequences were dropped from analyses. Although only three fish gut samples were considered outliers for V4-V5 fish gut sequences, all V4-V5 fish gut sequences were dropped from analyses because of overall lower sequence counts after quality control.

2.2.5 Statistical Analyses

A rarefied Shannon diversity index, Chao1 richness estimator, and Good's coverage index were calculated at the species level within QIIME2. Good's coverage index compares the number of singleton OTUs to the sum of abundances for all OTUs in order to determine how well sequencing covered the observable OTUs from the environment (Good, 1953). Chao1 estimates richness based on OTU abundances, which allows for sampling effort to be taken into account (Chao, 1984). Non-metric

multidimensional scaling (NMDS) analyses using Bray Curtis dissimilarity matrices and weighted-UniFrac matrices were run using the R statistical packages vegan and phyloseq to examine community differences among sample types. Differences in alpha diversity were examined using Kruskal-Wallis Rank sum tests followed by Dunn's test of multiple comparisons. Analysis of similarities (ANOSIM) were calculated on beta diversity metrics followed by similarity percentages (SIMPER) in order to determine significance and the taxa driving those differences. SourceTracker2 was used to calculate the proportions of different environmental microbial source samples (*Sargassum*, microplastics, and water) for each Gray Triggerfish gut sample (Knights et al., 2011).

2.3 Results

2.3.1 Microbiome Composition in Microplastics, *Sargassum*, Water, and Fish Guts

A total of 788,022 sequences (average length 394 bp) for microplastic, *Sargassum*, water, and Gray Triggerfish gut samples remained after quality filtering (Table 2.1). Bacterial sequences were higher in microplastic, *Sargassum*, and water samples than in Gray Triggerfish hindgut samples. Gray Triggerfish gut sequences comprised 1.8% (13,839) of the total sequence count for all sample types (Table 2.1). Overall, microplastic, *Sargassum*, and water samples had higher diversity than Gray Triggerfish guts (Chi-squared=21.1, p-value=0.0001) (Figure 2.2 a; Table 2.1). Species richness was also higher (Chi-squared=34.8, p-value=1.332e-07) for microplastic, *Sargassum*, and water samples; however, Chao1 richness for *Sargassum* and microplastic samples was higher than in the water samples (Figure 2.2 b). Based on a 0.99 average of Good's coverage, water, *Sargassum*, microplastic, and Gray Triggerfish gut microbial communities were sufficiently sampled from the environment.

Table 2.1 Descriptive statistics for water (WATER), *Sargassum* (SARG), microplastic (MICRO), and Gray Triggerfish gut (GUT) samples collected in *Sargassum* habitats in the Gulf of Mexico (2018-2019).

Station	Sample Date	Sample Type	Gear	Sample ID	Raw Sequences	Quality* Controlled Sequences	Bacterial OTUs	Shannon Diversity (rarefied	Goods Coverage Index	Chao1
					Cruise 3 (July 9-16, 2018)					
33	7/10	WATER	CTD	A14	30639	16428	39	4.74	1	209
	7/10	WATER	CTD	PA14	43161	18488	30	4.23	1	169
	7/10	SARG	NEU	40S	29397	14876	58	5.62	1	390
	7/10	MICRO	NEU	40MP	43813	24870	46	5.02	1	378
	7/10	GUT	NEU	40-01	259	85	15	3.86	1	15
39	7/12	WATER	CTD	A16	42962	21070	28	3.90	1	156
	7/12	WATER	CTD	PA16	53581	24058	29	4.01	1	149.07
	7/12	SARG	NEU	46S	27650	17964	37	4.05	1	321
43	7/14	WATER	CTD	A19	26928	15120	33	4.29	1	153
	7/14	WATER	CTD	PA19	41294	17109	26	3.71	1	117
	7/14	SARG	NEU	50S	24405	14720	41	4.55	1	271
	7/14	MICRO	NEU	50MP	24660	16025	54	5.46	1	376
	7/14	GUT	NEU	50-03	552	292	25	4.20	1	32
	7/14	GUT	NEU	50-13	521	269	14	2.79	1	16
	7/14	GUT	NEU	51-04	330	185	10	2.70	1	10
	7/14	GUT	NEU	51-05	320	154	12	2.07	1	13
	7/14	GUT	NEU	51-06	1086	359	11	2.56	1	14
	7/14	GUT	NEU	51-07	7519	5969	8	0.93	1	25
	7/14	GUT	NEU	51-08	682	186	12	2.76	1	12
44	7/15	WATER	CTD	A21	28063	17635	33	4.42	1	168
	7/15	WATER	CTD	PA21	66540	31550	32	4.14	1	173
	7/15	MICRO	NEU	52MP	16515	11085	47	5.19	1	272
	7/15	GUT	NEU	52-03	514	166	14	3.33	1	15
	7/15	GUT	NEU	52-04	705	179	12	2.61	1	13
	7/15	GUT	NEU	52-05	2229	1129	21	3.83	1	43
	7/15	GUT	NEU	52-07	2136	748	17	2.54	1	33
46	7/16	WATER	CTD	A22	49265	23241	28	3.96	1	175
	7/16	WATER	CTD	PA22	82013	34831	27	3.85	1	174.43
	7/16	SARG	NEU	56S	46181	24166	57	5.59	1	560.05
	7/16	MICRO	NEU	56MP	35362	21356	58	5.66	1	452
	7/16	GUT	RS	RS17	1043	393	14	3.11	1	22

Table 2.1 Continued.

Station	Sample Date	Sample Type	Gear	Sample ID	Raw Sequences	Quality* Controlled Sequences	Bacterial OTUs	Shannon Diversity (rarefied	Goods Coverage Index	Chao1
					Cruise 4 (May 28- June 4, 2019)					
54	5/31	WATER	CTD	A26	61171	29473	30	4.11	1	173
	5/31	WATER	CTD	PA26	59170	26986	22	3.10	1	134
	5/31	SARG	NEU	65S	38196	29201	21	2.65	1	201
	5/31	MICRO	NEU	65MP	24122	16719	25	3.94	1	186
	5/31	GUT	NEU	65-02	2818	1710	19	3.26	1	38
56	6/1	WATER	CTD	A27	51674	24183	40	4.63	1	217
	6/1	WATER	CTD	PA27	48066	25152	26	3.97	1	176
	6/1	SARG	NEU	67S	23408	16788	22	2.90	1	226
	6/1	MICRO	NEU	67MP	12359	7699	30	3.48	1	202.33
	6/1	GUT	NEU	67-16	1969	910	26	3.93	1	45
60	6/3	WATER	CTD	A29	92133	42447	29	3.97	1	191
	6/3	WATER	CTD	PA29	60894	29918	36	4.25	1	143
	6/3	SARG	NEU	72S	30665	17624	52	5.40	1	398
	6/3	MICRO	NEU	72MP	69718	47250	47	5.21	1	396.05
	6/3	GUT	NEU	72-01	529	216	6	2.24	1	6
62	6/4	WATER	CTD	A30	66715	33078	32	4.23	1	238.03
	6/4	WATER	CTD	PA30	57936	27574	28	3.71	1	149
	6/4	SARG	NEU	75S	29668	17792	52	5.15	1	382
	6/4	MICRO	NEU	75MP	28793	17707	54	5.42	1	373.13
	6/4	GUT	PS	02-01	1268	538	21	3.62	1	31
	6/4	GUT	RS	RS63	1370	351	29	4.56	1	34

*Sequences filtered, denoised, and chimeric sequences removed.

Proteobacteria were dominant across all microplastic biofilm samples, with Alpha-, Gamma-, and Delta- making up approximately 77% of the total observed abundances. Alphaproteobacteria were the most abundant (71.2%), followed by Gammaproteobacteria (5.3%), and Deltaproteobacteria (0.8%) (Figure 2.3). The second most abundant bacteria found on microplastics were Bacteroidetes (16.8%), which was largely comprised of class Bacteroidia (16.6%). Finally, Cyanobacteria of the class Oxyphotobacteria (3.8%) comprised a relatively small portion of the observed microplastic biofilms.

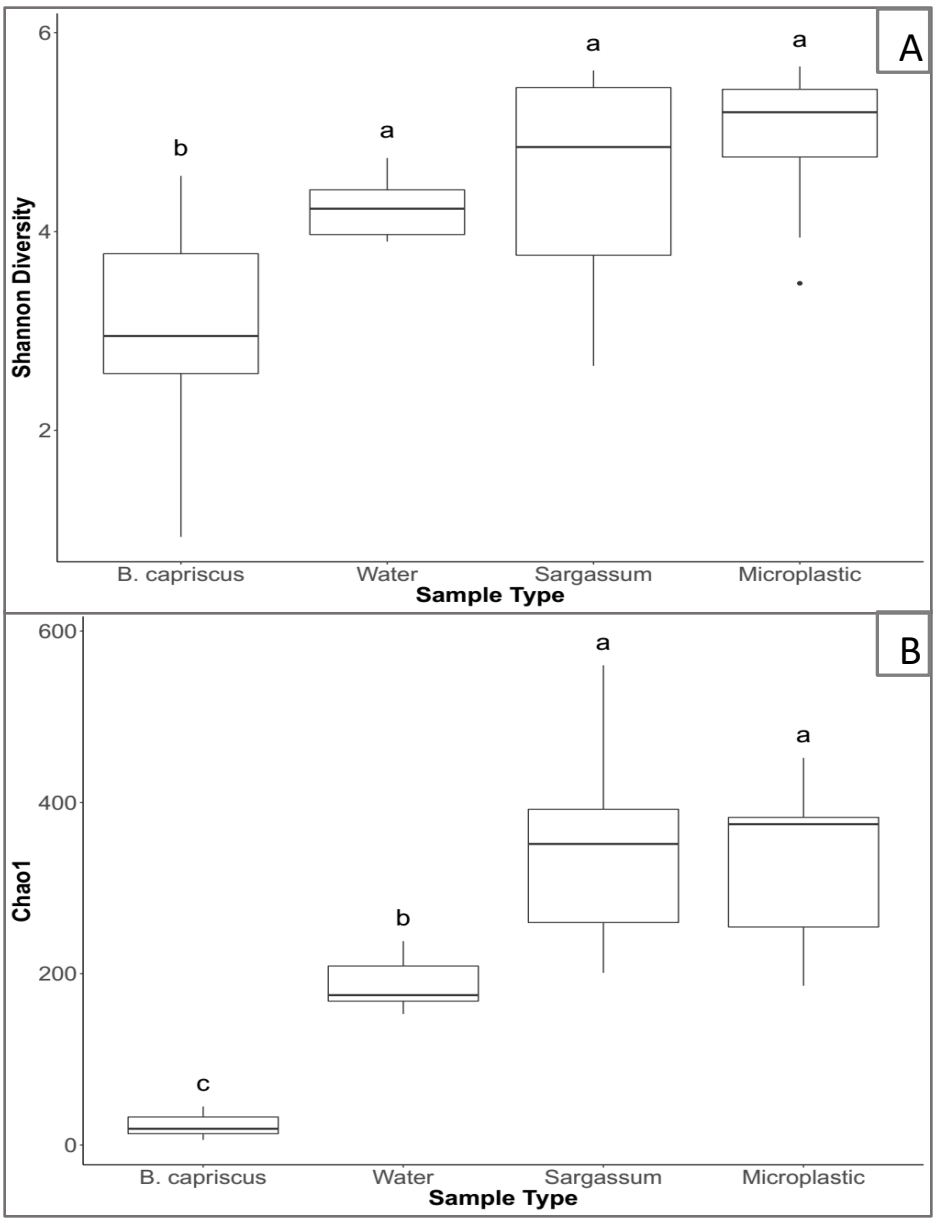

Figure 2.2 Boxplots of a) Shannon diversity and b) Chao1 for microbiomes of Gray Triggerfish guts, water, *Sargassum*, and microplastic samples collected in July 2018 and May 2019. The bold line within each box represents the sample median. The upper and lower portions represent the 25th and 75th percentiles. Solid vertical lines represent the highest and lowest values with 1.5 times the interquartile range. Points outside of these lines represent outliers. Letters above the boxes represent significance based on Dunn's test of multiple comparisons.

The observed microplastic biofilm community was similar to the associated ambient water and *Sargassum* biofilms at the class level. Proteobacteria were dominant in both water (74.8%) and *Sargassum* (52.8%) samples, with the class Alphaproteobacteria comprising 63.3% and 49.7% of the community, respectively (Figure 2.3). Gammaproteobacteria were the second most abundant group, comprising up 10.7% for water and 2.2% for *Sargassum*. Deltaproteobacteria was equally abundance in water, *Sargassum* and microplastic samples. *Sargassum* samples had lower abundances of Proteobacteria than microplastic biofilms and water samples and had higher abundances of Cyanobacteria of the class Oxyphotobacteria (31.8%) relative to microplastic biofilm (3.8%) and water (12.3%). Microplastic biofilms had higher abundances of Bacteroidetes of the class Bacteroidia than *Sargassum* biofilms (9.9%) and water (9.6%).

The Gray Triggerfish gut community was also dominated by Proteobacteria (50.9%); however, the class Gammaproteobacteria was most abundant (31.6%), followed by Alphaproteobacteria (17.4%) and Deltaproteobacteria (1.9%) (Figure 2.3). Cyanobacteria of the class Oxyphotobacteria (19.6%) were the second most abundant taxa, similar to *Sargassum* and water samples. Gray Triggerfish hindgut microbial communities were unique in that they included Tenericutes of the class Mollicutes (4.5%) and Actinobacteria of the class Actinobacteria (4.3%). Finally, 14.3% of the overall abundance for hindgut samples were classified to Kingdom Bacteria, but could not be further identified.

Figure 2.3 Microbiome community plots for each individual sample of water, microplastic, *Sargassum*, and Gray Triggerfish guts at class level. Proportions represent relative abundance for each sample.

Results from Bray Curtis dissimilarity (R=0.87, p-value=0.001) and weighted UniFrac (R=0.20, p-value=0.004) analyses showed that communities differed phylogenetically and in abundance among microplastic, *Sargassum*, water, and Gray Triggerfish hindgut samples (Figure 2.4). Even though microplastics were closely grouped with *Sargassum* for beta diversity, each group was defined by specific OTUs and were phylogenetically distinct. SIMPER analyses (Table 2.2) indicated water samples differed from all other samples because of the presence of OTUs related to Alphaproteobacteria of the order SAR11 and Oxyphotobacteria of the order Synechococcales. *Sargassum* samples differed from all others by having OTUs related to Oxyphotobacteria chloroplast and Alphaproteobacteria of the order Rickettsiales. Microplastic samples differed from all others with OTUs related to Alphaproteobacteria of Caulobacterales (specifically Hyphomonadaceae) and Rhodobacterales. While Gray Triggerfish gut samples had much lower abundances than the other samples, they had abundances of the order Synechococcales which were similarly seen in water samples and made them differ from *Sargassum* samples. *Sargassum* samples were different from Gray Triggerfish guts and microplastic samples because of OTUs related to the orders Phormidesmiales and Nostocales (Gray Triggerfish guts only). Microplastic samples differed from *Sargassum* and Gray Triggerfish gut samples because of OTUs related to the orders Sphingomonadales and Rhizobiales (Gray Triggerfish guts only).

2.3.2 Microplastic Impacts on Gray Triggerfish Gut Communities

Of the twelve Gray Triggerfish analyzed, seven fish were observed with microplastics in the foregut. Gut communities did not differ in Shannon diversity (Chi-squared=0.086, p-value=0.770) or Chao1 richness (Chi-squared=0.910, p-value=0.34)

between fishes with and without microplastics in the foregut (Figure 2.5). On average, Shannon diversity was 2.9 for all Gray Triggerfish microbiomes. Although not significant, fish with microplastics had higher Chao1 richness (22) on average than fish without microplastics (17). Bray Curtis dissimilarity (R=0.116, p-value=0.188) and weighted UniFrac (R=-0.130, p-value=0.852) results also suggested no differences in the

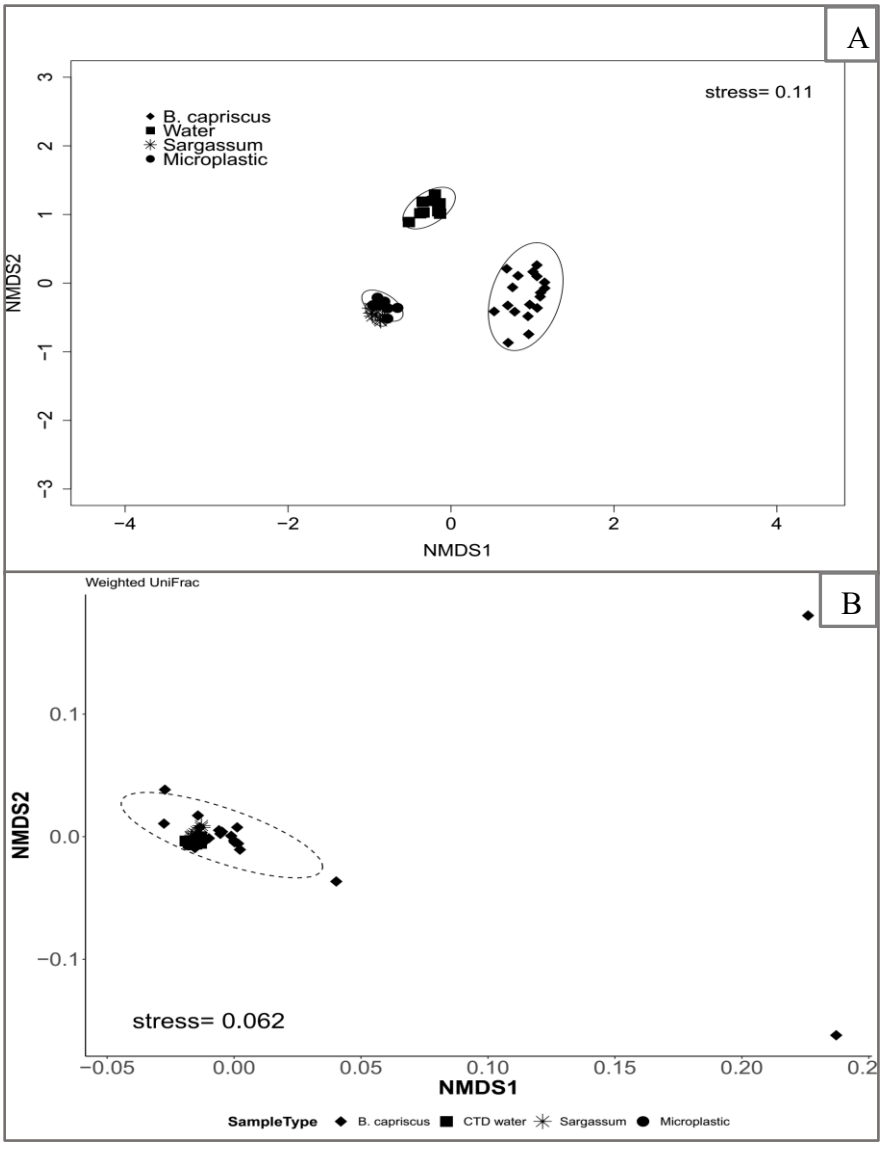

Figure 2.4 NMDS plots for a) Bray Curtis dissimilarity and b) weighted UniFrac for microbiomes of Gray Triggerfish guts, water, *Sargassum*, and microplastic samples from July 2018 and May 2019. Ellipses represent 95% confidence.

Table 2.2 Results from SIMPER analyses of Gray Triggerfish guts, water, *Sargassum*, and microplastic microbial community samples. Top ten contributing OTU taxa are listed in order of most contributing to least for each pairwise sample type comparison.

Water and Gray Triggerfish Gut
Overall dissimilarity= 0.9873

Taxanomy	WATER avg. abund.	GUT avg. abund.	Contrib%	Cum. %
Proteobacteria;Alphaproteobacteria;SAR11 clade	4544.33	7.33	16.80%	17.01
Proteobacteria;Alphaproteobacteria;SAR11 clade;Clade I	2768.44	1.50	9.80%	26.94
Cyanobacteria;Oxyphotobacteria;Synechococcales;Cyanobiaceae	1330.22	318.11	6.74%	33.77
Cyanobacteria;Oxyphotobacteria;Synechococcales;Cyanobiaceae;Prochlorococcus MIT9313	2016.78	1.67	6.73%	40.59
Proteobacteria;Alphaproteobacteria;SAR11 clade	1327.44	0.00	5.43%	46.09
Proteobacteria;Alphaproteobacteria;Rhodospirillales;AEGEAN-169 marine group	906.44	0.56	3.66%	49.80
Bacteroidetes;Bacteroidia;Flavobacteriales;Flavobacteriaceae;NS5 marine group	565.56	0.22	2.85%	52.69
Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae	591.44	0.00	2.70%	55.42
Proteobacteria;Gammaproteobacteria;SAR86 clade;uncultured;uncultured;uncultured	610.89	0.00	2.42%	57.87
Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;uncultured;uncultured	647.11	0.00	2.36%	60.27

Water and *Sargassum*
Overall dissimilarity= 0.9812

Taxanomy	WATER avg. abund.	SARG avg. abund.	Contrib%	Cum. %
Proteobacteria;Alphaproteobacteria;SAR11 clade	4544.33	4.75	9.88%	10.07
Cyanobacteria;Oxyphotobacteria;Chloroplast	0.00	4319.75	9.54%	19.79
Proteobacteria;Alphaproteobacteria;SAR11 clade;Clade I	2768.44	0.00	5.89%	25.79
Proteobacteria;Alphaproteobacteria;Rickettsiales;Mitochondria	0.00	2579.38	5.84%	31.75
Cyanobacteria;Oxyphotobacteria;Synechococcales;Cyanobiaceae;Prochlorococcus MIT9313	2016.78	0.00	4.15%	35.97
Cyanobacteria;Oxyphotobacteria;Synechococcales;Cyanobiaceae	1330.22	1.13	3.39%	39.43
Proteobacteria;Alphaproteobacteria;SAR11 clade	1327.44	0.00	3.07%	42.56
Proteobacteria;Alphaproteobacteria;Rhodospirillales;AEGEAN-169 marine group	906.44	0.00	2.06%	44.66
Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae	1.11	658.63	1.59%	46.28
Bacteroidetes;Bacteroidia;Flavobacteriales;Flavobacteriaceae;NS5 marine group	565.56	0.00	1.47%	47.78

Table (2.2 Continued.)

Water and Microplastic
Overall dissimilarity= 0.9820

Taxanomy	WATER avg. abund.	MICRO avg. abund.	Contrib%	Cum. %
Bacteria;Proteobacteria;Alphaproteobacteria;SAR11 clade	4544.33	2.63	10.01%	10.20
Bacteria;Proteobacteria;Alphaproteobacteria;SAR11 clade;Clade I	2768.44	0.00	5.96%	16.27
Bacteria;Cyanobacteria;Oxyphotobacteria;Synechococcales;Cyanobiaceae;Prochlorococcus	2016.78	0.00	4.19%	20.53
Bacteria;Cyanobacteria;Oxyphotobacteria;Synechococcales;Cyanobiaceae	1330.22	0.63	3.47%	24.06
Bacteria;Proteobacteria;Alphaproteobacteria;SAR11 clade	1327.44	0.00	3.12%	27.24
Bacteria;Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;uncultured	2.67	1116.00	2.10%	29.38
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodospirillales;AEGEAN-169 marine group	906.44	0.00	2.09%	31.51
Bacteria;Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;uncultured	12.33	899.88	2.00%	33.55
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae	64.89	790.25	1.54%	35.11
Bacteria;Bacteroidetes;Bacteroidia;Flavobacteriales;Flavobacteriaceae;NS5 marine group	565.56	0.00	1.51%	36.65

Gray Triggerfish Gut and *Sargassum*
Overall dissimilarity= 0.9919

Taxanomy	GUT avg. abund.	SARG avg. abund.	Contrib%	Cum. %
Cyanobacteria;Oxyphotobacteria;Chloroplast	16.83	4319.75	19.71%	19.87
Proteobacteria;Alphaproteobacteria;Rickettsiales;Mitochondria	18.61	2579.38	12.29%	32.26
Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae	1.39	658.63	3.59%	35.87
Cyanobacteria;Oxyphotobacteria;Phormidesmiales;Phormidesmiaceae	1.00	590.13	3.26%	39.17
Cyanobacteria;Oxyphotobacteria;Nostocales;Nostocaceae	0.22	345.00	1.87%	41.05
Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae	0.00	289.63	1.58%	42.64
Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae	0.00	291.38	1.51%	44.17
Cyanobacteria;Oxyphotobacteria;Synechococcales;Cyanobiaceae	318.11	1.13	1.35%	45.53
Cyanobacteria;Oxyphotobacteria;Phormidesmiales;Phormidesmiaceae	0.00	229.00	1.25%	46.79
Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Tateyamaria	0.56	233.75	1.23%	48.03

Table (2.2 Continued.)

Gray Triggerfish Gut and Microplastic
Overall dissimilarity= 0.9929

Taxanomy	GUT avg. abund.	MICRO avg. abund.	Contrib%	Cum. %
Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;uncultured	0.00	899.88	5.38%	5.42%
Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;uncultured	0.00	1116.00	3.88%	9.33%
Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae	1.44	790.25	3.73%	13.08
Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Ruegeria	14.28	299.00	2.86%	15.97
Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae	0.56	610.38	2.86%	18.84
Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae	0.00	538.00	2.71%	21.57
Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae	0.00	471.38	2.63%	24.22
Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae	0.00	530.88	2.23%	26.47
Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae	0.44	353.25	2.16%	28.65
Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae	0.89	525.25	2.12%	30.78

***Sargassum* and Microplastic**
Overall dissimilarity= 0.7914

Taxanomy	SARG avg. abund.	MICRO avg. abund.	Contrib%	Cum. %
Cyanobacteria;Oxyphotobacteria;Chloroplast	4319.75	4.13	10.86%	13.73
Proteobacteria;Alphaproteobacteria;Rickettsiales;Mitochondria	2579.38	5.00	6.67%	22.16
Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;uncultured	0.00	1116.00	2.32%	25.09
Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;uncultured	149.50	899.88	1.93%	27.53
Cyanobacteria;Oxyphotobacteria;Phormidesmiales;Phormidesmiaceae	590.13	22.50	1.61%	29.57
Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae	164.13	790.25	1.48%	31.44
Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae	658.63	193.13	1.36%	33.17
Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae	11.88	471.38	1.31%	34.82
Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae	224.88	353.25	1.17%	36.30
Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae	109.75	610.38	1.16%	37.76

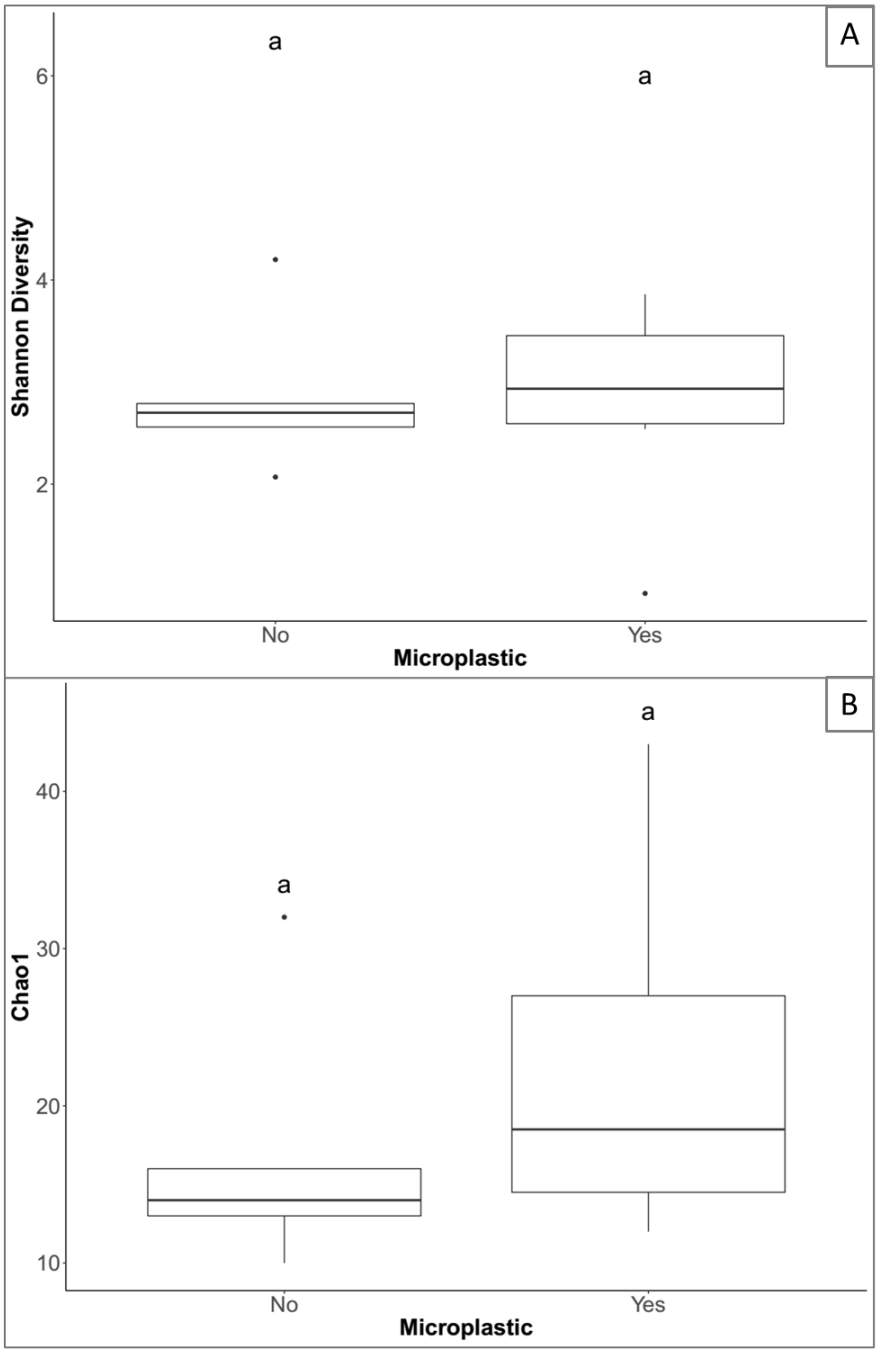

Figure 2.5 Boxplots of a) Shannon diversity and b) Chao1 for microbiomes of Gray Triggerfish guts with (Yes) and without (No) microplastic observations from July 2018.
The bold line within each box represents the sample median. The upper and lower portions represent the 25th and 75th percentiles. Solid vertical lines represent the highest and lowest values with 1.5 times the interquartile range. Points outside of these lines represent outliers. Letters above the boxes represent significance based on Dunn's test of multiple comparisons.

abundances or taxonomic composition of Gray Triggerfish microbiomes for individuals with and without microplastics (Figure 2.6). There were differences seen in the relative abundances of specific taxa between fish with and without microplastics observed. Overall, fish with observed microplastics in their guts had greater abundances of Ralstonia (15.3 %), Burkholderia-Calleronia-Paraburkholderia (13.6 %), and Rhodobacteraceae (7.2 %) compared to fish without microplastics observed (6 %, 2.9 %, and 0.97 %). Fish without microplastics observed in their guts had greater abundances of Cyanobiaceae (25.3 %), Cutibacterium (6 %), Enterobacteriaceae (4 %), and Vibrio spp. (1.68 %) compared to fish with microplastics observed (12.8 %, 1.1 %, 2.3 %, and 0 %).

Results from the SourceTracker2 analysis identified microplastic, *Sargassum*, and ambient water as potential sources for Gray Triggerfish microbiomes, with the relative proportions of each source varying among individual fish hindguts (Figure 2.7). Overall, fish with microplastics observed in their guts had on average 10.7% of their gut community sourced to the microplastic microbial community. Fish without microplastics observed in their guts had on average 5.3% of their gut community sourced to the microplastic microbial community. Three fish had between 62-87 % of their gut community sourced to the ambient water microbial community. The *Sargassum* community was less sourced, with only two fish sourcing above 10% of their gut community from *Sargassum*.

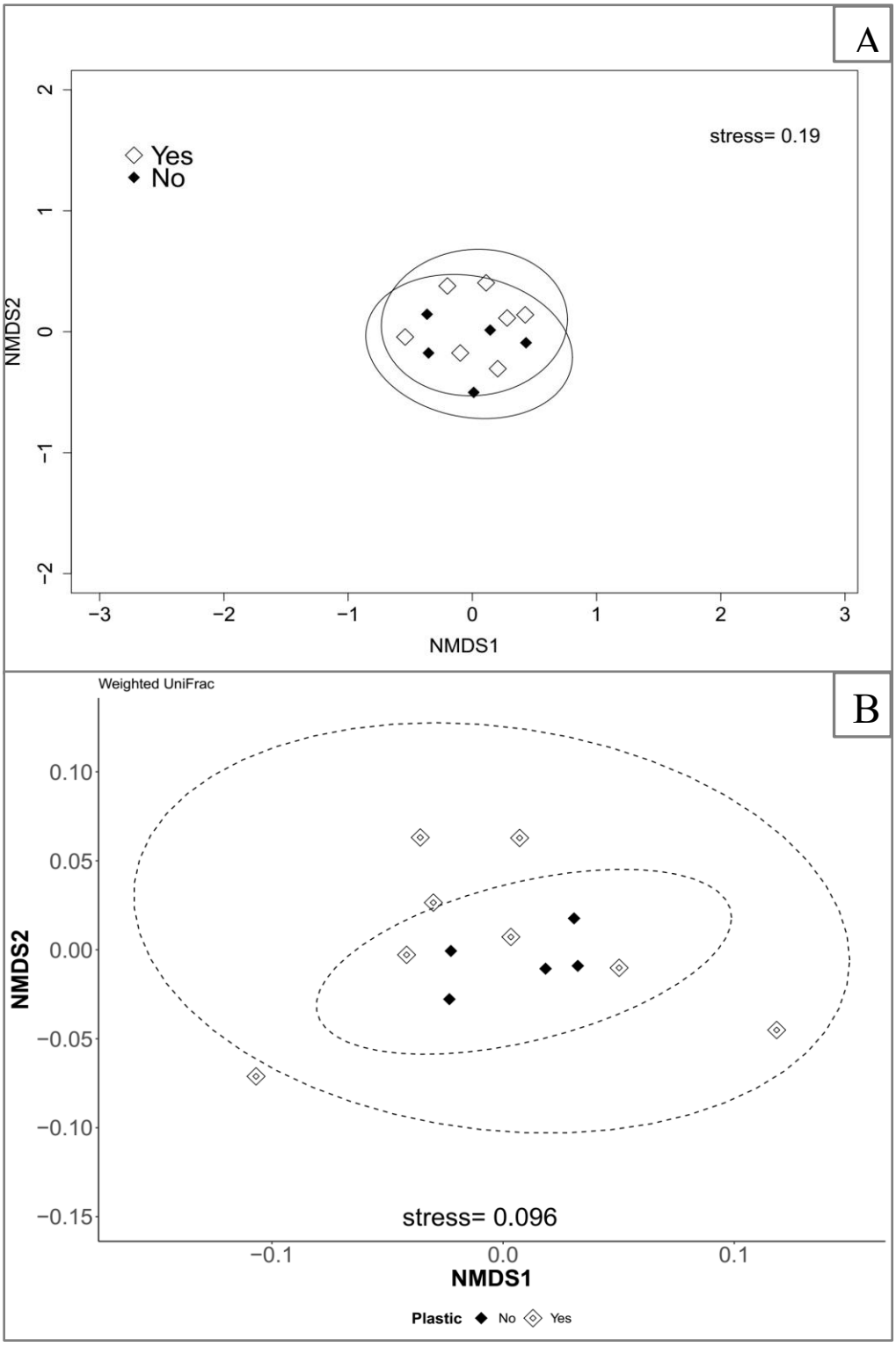

Figure 2.6 NMDS plots for a) Bray Curtis dissimilarity and b) weighted UniFrac for microbiomes of Gray Triggerfish guts with (Yes) and without (No) microplastics observed from July 2018. Ellipses represent 95% confidence.

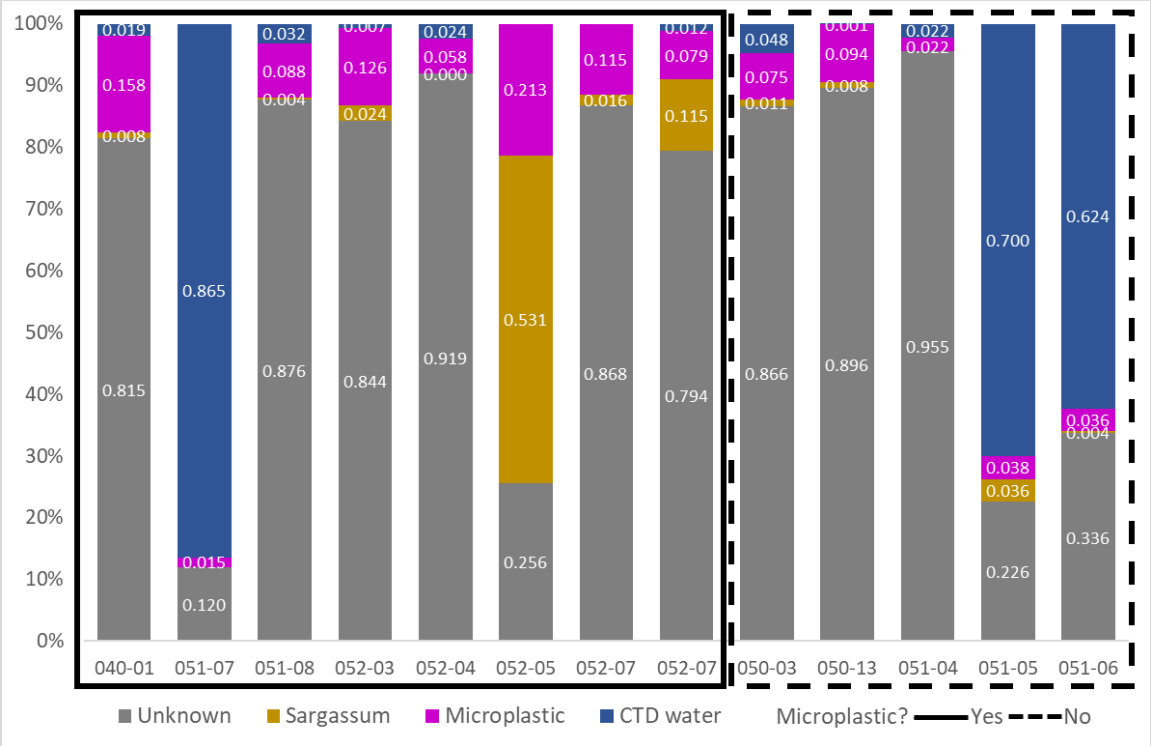

Figure 2.7 Microbiome mixing proportions for Gray Triggerfish gut samples (sink) compared to water, microplastic, and *Sargassum* samples (sources) from July 2018 and May 2019. Undetermined sources represented by the unknown column.

2.4 Discussion

This study provides the first quantitative estimates of microplastic biofilm and Gray Triggerfish gut microbial communities associated with pelagic *Sargassum* in the GoM. The microplastic biofilm community was distinct from both the ambient water and Gray Triggerfish hindguts; however, microplastics shared similar bacteria and were closely clustered to *Sargassum*. This study also provides the first quantitative estimates of microplastic biofilm impacts on juvenile fishes associated with *Sargassum*, and demonstrated that microplastics can influence fish gut microbial communities in early life fishes.

2.4.1 Microplastic Microbial Community

The microbial community of marine microplastics associated with *Sargassum* are dominated by Proteobacteria (Alpha-) and Bacteroidetes (Bacteroidia) similar to other studies on the plastisphere (Jiang et al., 2018; Xu et al., 2019). Both families of bacteria are key biofilm formers (Lee et al., 2008). Proteobacteria and Bacteroidetes are often in succession of each other when it comes to biofilm formations and relative abundances of these have been used to discriminate biofilm stages (De Tender et al., 2015). Although Proteobacteria and Bacteroidetes were both abundant on *Sargassum* and microplastics, both taxa were 1.5 times more abundant on microplastics. Alphaproteobacteria were the most abundant class of bacteria associated with microplastics and included primary surface colonizers like Rhodobacterales and Caulobacterales (Jiang et al., 2018). Specifically, the families Rhodobacteraceae and Hyphomonadaceae were found in high abundances on microplastics in this study. Although both *Sargassum* and microplastics showed similar abundances of Rhodobacteraceae, Hyphomonadaceae was seen in abundances almost seven times greater on microplastics than on *Sargassum*. This family has been previously seen as a distinct microbial group associated with microplastics and because of holdfast-like appendages that allow them to anchor to surfaces, they may have an affinity for microplastics because they are able to attach more securely to the smoother plastic surface (Oberbeckmann et al., 2018). Microplastics provide complex structure and surface area for biofilm creating bacteria, but they also provide a source of energy for bacteria that can break down hydrocarbon compounds (Kertesz et al., 2018; Li et al., 2020). Sphingomonadales (3.14 %) and Hyphomonadaceae (21.46%) were both observed on microplastics associated with *Sargassum* and are known to break down hydrocarbons

(Kertesz et al., 2018; Oberbeckmann et al., 2018). Both of these bacteria groups have been recorded on microplastics by previous studies (Kertesz et al., 2018; Oberbeckmann et al., 2018). This suggests that the microplastics within *Sargassum* are being utilized as an energy source and not just a habitat.

While microplastics are being colonized by key biofilm forming taxa, they have also been shown to be vectors for potentially harmful pathogens that could affect organisms ingesting them (Zettler et al., 2013; Kirstein et al., 2016). Pathogenic and toxic taxa like *Vibrio* spp. have been seen in high abundances up to 24% by Zettler et al. (2013) and low abundances less than 0.01% by Bryant et al. (2016). *Vibrio* spp. on microplastics from this study were observed in low abundances on average 0.67%. While *Vibrio* spp. were seen in less than 1% abundance across all *Sargassum* habitat types, water and *Sargassum* samples had on average 0.1% abundance compared to microplastic samples. Taxa capable of causing diseases in fish such as Flavobacterium (0.007 %) and Tenacibaculum (0.32 %) were also observed on microplastics. Abundances of Tenacibaculum on microplastics (0.32%) were in similar abundance on *Sargassum* (0.37%) and both taxa were seen in low proportions (Loch and Faisal, 2015; Smage et al., 2016). This suggests that microplastics are supporting abundances of potentially pathogenic bacteria in similar proportions to the ambient environment around them and provide a new vector for potentially pathogenic bacteria to be transferred into marine organisms, specifically fish.

2.4.2 Gray Triggerfish Gut Microbial Community

This study includes the first descriptions of Gray Triggerfish microbiomes, and adds to a growing literature on the microbial communities associated with fishes (Egerton et al., 2018; Gao et al., 2020; Le and Wang, 2020). As identified in other species, Proteobacteria were the dominant taxa in Gray Triggerfish hindguts, comprising approximately 50% of the gut microbial community, with the class Gammaproteobacteria being dominant (Ingersleve et al., 2014; Givens et al., 2015; Parris et al., 2016). The Gammaproteobacteria in highest abundances were the families Burkholderiaceae (19.5 %), Vibrionaceae (4 %), and Enterobacteriaceae (3.5 %) which have all been documented as members of the fish gut community (Carda-Dieguez et al., 2014; Egerton et al., 2018; Soriano et al., 2018). Members of these families can be important in digestion (Enterobacteriaceae, Vibrionaceae) and contain potential fish pathogens (Burkholderiaceae, Vibrionaceae) (Soriano et al., 2018; Egerton et al., 2018).

The second most abundant taxa represented in Gray Triggerfish guts were from the class Oxyphotobacteria (Cyanobacteria), more specifically chloroplasts and the order Synechococcales. Cyanobacteria have been observed on the surface of *Sargassum* and were the second most abundant class seen on *Sargassum* (32 %) from this study (Philips et al., 1986; Jean Lopez et al., 2020). Furthermore, Synechococcales are among one of the most abundant picoplankton in the marine environment and comprised 13.7% of the bacteria observed in ambient water samples from the current study (Scanlan and West, 2002). Therefore, it is not surprising to see abundances of chloroplasts and cyanobacteria within the Gray Triggerfish gut microbial community because Gray Triggerfish eat directly on the *Sargassum* algae often ingesting it because their epiphytic prey

(Bryozoans and hydroids) are attached to *Sargassum*. Cyanobacteria are considered an important food source for fishes and chloroplasts can be a sign of an herbivorous diet (Currin et al., 2011; Li et al., 2018).

2.4.3 Microplastic Microbiome Impacts on Gray Triggerfish Gut Microbiome

The ambient environment and fish diet can influence the structure of the fish gut microbiol community (Michl et al., 2017; Perez-Pascual et al., 2020). Also, fish microbiomes change through ontogeny, which suggests the gut microbial community structure is susceptible to the introduction of foreign microbes throughout the early life of a fish (Parris et al., 2016; Yan et al., 2016; Le and Wang, 2020). We also know that pristine microplastics have the ability to disrupt homeostasis of the fish gut microbial community by altering the proportions of specific taxa present (Jin et al., 2018). In my study, the diversity and richness of hindgut microbiomes did not differ among Gray Triggerfish with microplastics observed in their foreguts relative to those with no microplastics in their foreguts. Ideally, an entire fish gut should be analyzed for both the presence of microplastics and the microbiome community composition; however, this is not possible, as dissecting the gut for microplastics would risk contamination by ambient microbes. Therefore, as a compromise, the gut was partitioned as described in the methods, which leads to a few uncertainties in our interpretation of results. First, it is not possible to know if there were microplastics in the hindguts, which would have influenced the microbial community analyses. Also, it is not possible to know if microplastics were previously in an "empty" gut and passed to the hindgut or excreted prior to capture.

Even with these caveats, I observed that the relative abundances of bacteria differed between Gray Triggerfish with microplastics observed in their foreguts relative to those with no microplastics in their foreguts. One family, Rhodobacteraceae, stood out because this was the only bacterial group in high abundances on microplastics that had differing abundances between fish with and without microplastic observations. Fish with microplastics observed had seven times more Rhodobacteraceae than fish without. There was also evidence that fish hindgut microbiome sourced from the microplastic biofilm community twice as much in fishes with microplastics observed compared to fish without microplastics observed. Finally, even though we saw close clustering of the *Sargassum* and microplastic communities for beta diversity, each group was dominated by unique OTUs that made them phylogenetically distinct. Therefore, the results from the source tracker analysis would suggest that unique microplastic biofilms can influence the structure of juvenile fish gut communities.

2.4.4 Conclusions and Future Directions

The results from this study indicate that the microbiomes of *Sargassum* and associated microplastics are similar; however, the biofilm-forming Hyphomonadaceae show a preference for microplastics. The Gray triggerfish gut microbial community was distinct from the ambient environment around it. However, environmental habitats (water, *Sargassum*, and microplastics) that are being ingested by Gray triggerfish are also being sourced for proportions of the fish gut microbial community. Microplastics have a consistent influence on the juvenile Gray Triggerfish gut microbial community across all samples; however, fish with microplastic observed in their guts had twice as much microplastic microbial community sourced than those without microplastics observed.

This could be related to the high abundances of Rhodobacteraceae seen in fish with microplastics. While differences in communities were seen, future studies would benefit from larger samples sizes and replicates of the various communities associated with *Sargassum* in order to better understand variability in community structure between sample sites. While this study gives new information related to the *Sargassum* community microbiota and first looks into Gray triggerfish gut microbiome, further investigations should be made.

APPENDIX A

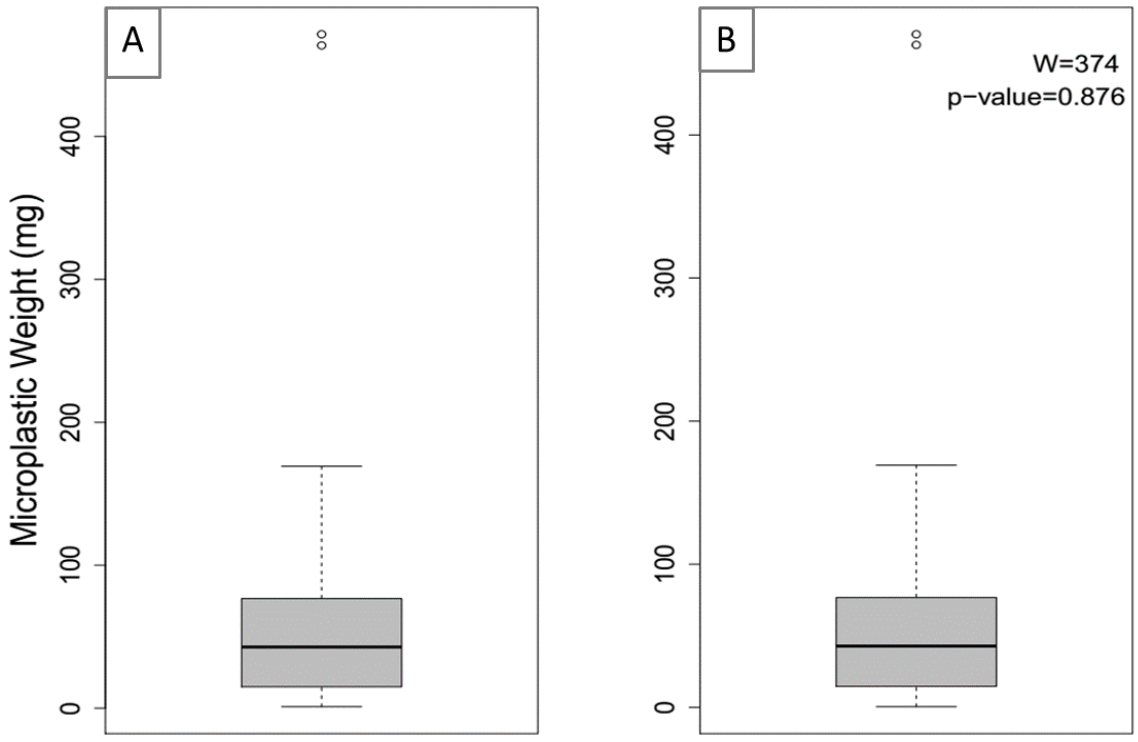

Figure A.1 A) Original microplastic concentration weights and B) blank-corrected microplastic concentrations weights. Significance tested with Wilcoxon Rank Sum test.

REFERENCES

Ahrendt, C., Perez-Venegas, D. J., Urbina, M., Gonzalez, C., Echeveste, P., Aldana, M., ... & Galbán-Malagón, C. (2020). Microplastic ingestion cause intestinal lesions in the intertidal fish Girella laevifrons. Marine Pollution Bulletin, 151, 110795.

Alomar, C., & Deudero, S. (2017). Evidence of microplastic ingestion in the shark Galeus melastomus Rafinesque, 1810 in the continental shelf off the western Mediterranean Sea. Environmental pollution, 223, 223-229.

Amaral-Zettler, L. A., Zettler, E. R., Slikas, B., Boyd, G. D., Melvin, D. W., Morrall, C. E., ... & Mincer, T. J. (2015). The biogeography of the Plastisphere: implications for policy. Frontiers in Ecology and the Environment, 13(10), 541-546.

Arthur, C., Baker, J. E., & Bamford, H. A. (2009). Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris, September 9-11, 2008, University of Washington Tacoma, Tacoma, WA, USA.

Barnes, D. K., Galgani, F., Thompson, R. C., & Barlaz, M. (2009). Accumulation and fragmentation of plastic debris in global environments. Philosophical Transactions of the Royal Society B: Biological Sciences, 364(1526), 1985-1998.

Boerger, C. M., Lattin, G. L., Moore, S. L., & Moore, C. J. (2010). Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. Marine pollution bulletin, 60(12), 2275-2278.

Brach, L., Deixonne, P., Bernard, M. F., Durand, E., Desjean, M. C., Perez, E., ... & ter Halle, A. (2018). Anticyclonic eddies increase accumulation of microplastic in the North Atlantic subtropical gyre. Marine pollution bulletin, 126, 191-196.

Carpenter, E. J., & Smith, K. L. (1972). Plastics on the Sargasso Sea surface. Science, 175(4027), 1240-1241.

Coe, J. M., & Rogers, D. (Eds.). (1997). Marine debris: sources, impacts, and solutions. Springer-Verlag

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., & Galloway, T. S. (2013). Microplastic ingestion by zooplankton. Environmental science & technology, 47(12), 6646-6655.

Collicutt, B., Juanes, F., & Dudas, S. E. (2019). Microplastics in juvenile Chinook salmon and their nearshore environments on the east coast of Vancouver Island. Environmental Pollution, 244, 135-142.

Collignon, A., Hecq, J. H., Glagani, F., Voisin, P., Collard, F., & Goffart, A. (2012). Neustonic microplastic and zooplankton in the North Western Mediterranean Sea. Marine pollution bulletin, 64(4), 861-864.

Coston-Clements, L., Settle, L. R., Hoss, D. E., & Cross, F. A. (1991). Utilization of the *Sargassum* habitat by marine invertebrates and vertebrates, a review (Vol. 296). US Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southeast Fisheries Science Center, Beaufort Laboratory.

Courtene-Jones, W., Quinn, B., Gary, S. F., Mogg, A. O., & Narayanaswamy, B. E. (2017). Microplastic pollution identified in deep-sea water and ingested by benthic invertebrates in the Rockall Trough, North Atlantic Ocean. Environmental Pollution, 231, 271-280.

Da Silva, V. E. L., Firmiano, L. P. S. D. S., Teresa, F. B., BATISTA, V. D. S., Ladle, R. J., & Fabré, N. N. (2019). Functional traits of fish species: Adjusting resolution to accurately express resource partitioning. Frontiers in Marine Science, 6, 303.

Davidson, K., & Dudas, S. E. (2016). Microplastic ingestion by wild and cultured Manila clams (Venerupis philippinarum) from Baynes Sound, British Columbia. Archives of Environmental Contamination and Toxicology, 71(2), 147-156.

Desforges, J. P. W., Galbraith, M., & Ross, P. S. (2015). Ingestion of microplastics by zooplankton in the Northeast Pacific Ocean. Archives of environmental contamination and toxicology, 69(3), 320-330.

Di Mauro, R., Kupchik, M. J., & Benfield, M. C. (2017). Abundant plankton-sized microplastic particles in shelf waters of the northern Gulf of Mexico. Environmental Pollution, 230, 798-809.

Dooley, J. K. (1972). Fishes associated with the pelagic *Sargassum* complex, with a discussion of the *Sargassum* community. Contrib. Mar. Sci., 16, 1-32.

Duguid, W. D., & Juanes, F. (2017). Microtrolling: an Economical Method to Nonlethally Sample and Tag Juvenile Pacific Salmon at Sea. Transactions of the American Fisheries Society, 146(2), 359-369.

Duncan, E. M., Broderick, A. C., Fuller, W. J., Galloway, T. S., Godfrey, M. H., Hamann, M., ... & Santillo, D. (2019). Microplastic ingestion ubiquitous in marine turtles. Global change biology, 25(2), 744-752.

Easson, C. G., & Lopez, J. V. (2019). Depth-Dependent environmental drivers of microbial plankton community structure in the Northern Gulf of Mexico. Frontiers in microbiology, 9, 3175.

Eriksen, M., Lebreton, L. C., Carson, H. S., Thiel, M., Moore, C. J., Borerro, J. C., ... & Reisser, J. (2014). Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PloS one, 9(12), e111913.

Galgani, F., Fleet, D., Van Franeker, J. A., Katsanevakis, S., Maes, T., Mouat, J., ... & Amato, E. (2010). Marine Strategy Framework directive-Task Group 10 Report marine litter do not cause harm to the coastal and marine environment. Report on the identification of descriptors for the Good Environmental Status of European Seas regarding marine litter under the Marine Strategy Framework Directive. Office for Official Publications of the European Communities.

Gall, S. C., & Thompson, R. C. (2015). The impact of debris on marine life. Marine pollution bulletin, 92(1-2), 170-179.

Ghanbari, M., Kneifel, W., & Domig, K. J. (2015). A new view of the fish gut microbiome: advances from next-generation sequencing. Aquaculture, 448, 464-475.

Goss, H., Jaskiel, J., & Rotjan, R. (2018). Thalassia testudinum as a potential vector for incorporating microplastics into benthic marine food webs. Marine pollution bulletin, 135, 1085-1089.

Gove, J. M., Whitney, J. L., McManus, M. A., Lecky, J., Carvalho, F. C., Lynch, J. M., ... & Kobayashi, D. R. (2019). Prey-size plastics are invading larval fish nurseries. Proceedings of the National Academy of Sciences, 116(48), 24143-24149.

Gregory, M. R., & Ryan, P. G. (1997). Pelagic plastics and other seaborne persistent synthetic debris: a review of Southern Hemisphere perspectives. In Marine debris (pp. 49-66). Springer, New York, NY.

Gutow, L., Eckerlebe, A., Giménez, L., & Saborowski, R. (2016). Experimental evaluation of seaweeds as a vector for microplastics into marine food webs. Environmental science & technology, 50(2), 915-923.

Hamdan, L. J., Coffin, R. B., Sikaroodi, M., Greinert, J., Treude, T., & Gillevet, P. M. (2013). Ocean currents shape the microbiome of Arctic marine sediments. The ISME journal, 7(4), 685-696.

Hansen, G. H., & Olafsen, J. A. (1999). Bacterial interactions in early life stages of marine cold water fish. Microbial ecology, 38(1), 1-26.

Harris, J. M. (1993). The presence, nature, and role of gut microflora in aquatic invertebrates: a synthesis. Microbial ecology, 25(3), 195-231.

Hidalgo-Ruz, V., Gutow, L., Thompson, R. C., & Thiel, M. (2012). Microplastics in the marine environment: a review of the methods used for identification and quantification. Environmental science & technology, 46(6), 3060-3075.

Hoss, D. E., & Settle, L. R. (1990). Ingestion of plastics by teleost fishes. In Proceedings of the Second International Conference on Marine Debris. NOAA Technical Memorandum. NOAA-TM-NMFS-SWFSC-154. Miami, FL (pp. 693-709).

Hu, C. (2009). A novel ocean color index to detect floating algae in the global oceans. Remote Sensing of Environment, 113(10), 2118-2129.

Jabeen, K., Li, B., Chen, Q., Su, L., Wu, C., Hollert, H., & Shi, H. (2018). Effects of virgin microplastics on goldfish (Carassius auratus). Chemosphere, 213, 323-332.

Jambeck, J. R., Geyer, R., Wilcox, C., Siegler, T. R., Perryman, M., Andrady, A., ... & Law, K. L. (2015). Plastic waste inputs from land into the ocean. Science, 347(6223), 768-771.

Jin, Y., Xia, J., Pan, Z., Yang, J., Wang, W., & Fu, Z. (2018). Polystyrene microplastics induce microbiota dysbiosis and inflammation in the gut of adult zebrafish. Environmental Pollution, 235, 322-329.

Jovanović, B. (2017). Ingestion of microplastics by fish and its potential consequences from a physical perspective. Integrated Environmental Assessment and Management, 13(3), 510-515.

Karami, A., Romano, N., Galloway, T., & Hamzah, H. (2016). Virgin microplastics cause toxicity and modulate the impacts of phenanthrene on biomarker responses in African catfish (Clarias gariepinus). Environmental research, 151, 58-70.

Kazour, M., Jemaa, S., El Rakwe, M., Duflos, G., Hermabassiere, L., Dehaut, A., ... & Khalaf, G. (2020). Juvenile fish caging as a tool for assessing microplastics contamination in estuarine fish nursery grounds. Environmental Science and Pollution Research, 27(4), 3548-3559.

Kirstein, I. V., Kirmizi, S., Wichels, A., Garin-Fernandez, A., Erler, R., Löder, M., & Gerdts, G. (2016). Dangerous hitchhikers? Evidence for potentially pathogenic Vibrio spp. on microplastic particles. Marine environmental research, 120, 1-8.

Knights, D., Kuczynski, J., Charlson, E. S., Zaneveld, J., Mozer, M. C., Collman, R. G., ... & Kelley, S. T. (2011). Bayesian community-wide culture-independent microbial source tracking. Nature methods, 8(9), 761-763.

Koelmans, A. A., Bakir, A., Burton, G. A., & Janssen, C. R. (2016). Microplastic as a vector for chemicals in the aquatic environment: critical review and model-supported reinterpretation of empirical studies. Environmental science & technology, 50(7), 3315-3326.

Kühn, S., Van Werven, B., Van Oyen, A., Meijboom, A., Rebolledo, E. L. B., & Van Franeker, J. A. (2017). The use of potassium hydroxide (KOH) solution as a suitable approach to isolate plastics ingested by marine organisms. Marine Pollution Bulletin, 115(1-2), 86-90.

Laist, D. W. (1997). Impacts of marine debris: entanglement of marine life in marine debris including a comprehensive list of species with entanglement and ingestion records. In Marine debris (pp. 99-139). Springer, New York, NY.

Landry, M. R., & Kirchman, D. L. (2002). Microbial community structure and variability in the tropical Pacific. Deep Sea Research Part II: Topical Studies in Oceanography, 49(13-14), 2669-2693.

Langmuir, I. (1938). Surface motion of water induced by wind. Science, 87(2250), 119-123.

Lauzon, H. L., Gudmundsdottir, S., Steinarsson, A., Oddgeirsson, M., Martinsdottir, E., & Gudmundsdottir, B. K. (2010). Impact of probiotic intervention on microbial load and performance of Atlantic cod (Gadus morhua L.) juveniles. Aquaculture, 310(1-2), 139-144.

Law, K. L. (2017). Plastics in the marine environment. Annual review of marine science, 9, 205-229.

Lecke-Mitchell, K. M., & Mullin, K. (1992). Distribution and abundance of large floating plastic in the north-central Gulf of Mexico. Marine Pollution Bulletin, 24(12), 598-601.

Lecke-Mitchell, K. M., & Mullin, K. (1997). Floating marine debris in the US Gulf of Mexico. Marine Pollution Bulletin, 34(9), 702-705.

Ling, S. D., Sinclair, M., Levi, C. J., Reeves, S. E., & Edgar, G. J. (2017). Ubiquity of microplastics in coastal seafloor sediments. Marine Pollution Bulletin, 121(1-2), 104-110.

Llewellyn, M. S., Boutin, S., Hoseinifar, S. H., & Derome, N. (2014). Teleost microbiomes: the state of the art in their characterization, manipulation and importance in aquaculture and fisheries. Frontiers in microbiology, 5, 207.

Lobelle, D., & Cunliffe, M. (2011). Early microbial biofilm formation on marine plastic debris. Marine pollution bulletin, 62(1), 197-200.

Lu, Y., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., ... & Ren, H. (2016). Uptake and accumulation of polystyrene microplastics in zebrafish (Danio rerio) and toxic effects in liver. Environmental science & technology, 50(7), 4054-4060.

Lusher, A. L., Hernandez-Milian, G., O'Brien, J., Berrow, S., O'Connor, I., & Officer, R. (2015). Microplastic and macroplastic ingestion by a deep diving, oceanic cetacean: the True's beaked whale Mesoplodon mirus. Environmental Pollution, 199, 185-191.

Markic, A., Niemand, C., Bridson, J. H., Mazouni-Gaertner, N., Gaertner, J. C., Eriksen, M., & Bowen, M. (2018). Double trouble in the South Pacific subtropical gyre:

Increased plastic ingestion by fish in the oceanic accumulation zone. Marine pollution bulletin, 136, 547-564.

Mazurais, D., Ernande, B., Quazuguel, P., Severe, A., Huelvan, C., Madec, L., ... & Zambonino-Infante, J. (2015). Evaluation of the impact of polyethylene microbeads ingestion in European sea bass (Dicentrarchus labrax) larvae. Marine environmental research, 112, 78-85.

McCormick, A., Hoellein, T. J., Mason, S. A., Schluep, J., & Kelly, J. J. (2014). Microplastic is an abundant and distinct microbial habitat in an urban river. Environmental science & technology, 48(20), 11863-11871.

Methot, R. D. (1986). Frame trawl for sampling pelagic juvenile fish. CalCOFI Rep, 27, 267-278.

Mizraji, R., Ahrendt, C., Perez-Venegas, D., Vargas, J., Pulgar, J., Aldana, M., ... & Galbán-Malagón, C. (2017). Is the feeding type related with the content of microplastics in intertidal fish gut?. Marine pollution bulletin, 116(1-2), 498-500.

Moore, C. J. (2008). Synthetic polymers in the marine environment: a rapidly increasing, long-term threat. Environmental research, 108(2), 131-139.

Moore, C. J., Moore, S. L., Leecaster, M. K., & Weisberg, S. B. (2001). A comparison of plastic and plankton in the North Pacific central gyre. Marine pollution bulletin, 42(12), 1297-1300.

Mugge, R. L., Brock, M. L., Salerno, J. L., Damour, M., Church, R. A., Lee, J. S., & Hamdan, L. J. (2019). Deep-Sea Biofilms, Historic Shipwreck Preservation and the Deepwater Horizon Spill. Frontiers in Marine Science, 6, 48.

Naidoo, T., Thompson, R. C., & Rajkaran, A. (2020). Quantification and characterisation of microplastics ingested by selected juvenile fish species associated with mangroves in KwaZulu-Natal, South Africa. Environmental Pollution, 257, 113635.

Niu, K. (1998). Sampling juvenile skipjack tuna, Katsuwonus pelamis, and other tunas. Fishery Bulletin, 96, 641-646.

Oliveira, M., Ribeiro, A., Hylland, K., & Guilhermino, L. (2013). Single and combined effects of microplastics and pyrene on juveniles (0+ group) of the common goby Pomatoschistus microps (Teleostei, Gobiidae). Ecological Indicators, 34, 641-647.

Ory, N. C., Gallardo, C., Lenz, M., & Thiel, M. (2018). Capture, swallowing, and egestion of microplastics by a planktivorous juvenile fish. Environmental Pollution, 240, 566-573.

Peda, C., Caccamo, L., Fossi, M. C., Gai, F., Andaloro, F., Genovese, L., ... & Maricchiolo, G. (2016). Intestinal alterations in European sea bass Dicentrarchus labrax (Linnaeus, 1758) exposed to microplastics: preliminary results. Environmental pollution, 212, 251-256.

Peters, C. A., Thomas, P. A., Rieper, K. B., & Bratton, S. P. (2017). Foraging preferences influence microplastic ingestion by six marine fish species from the Texas Gulf Coast. Marine pollution bulletin, 124(1), 82-88.

Qiang, L., & Cheng, J. (2019). Exposure to microplastics decreases swimming competence in larval zebrafish (Danio rerio). Ecotoxicology and environmental safety, 176, 226-233.

Qiao, R., Sheng, C., Lu, Y., Zhang, Y., Ren, H., & Lemos, B. (2019). Microplastics induce intestinal inflammation, oxidative stress, and disorders of metabolome and microbiome in zebrafish. Science of the Total Environment, 662, 246-253.

Rainieri, S., Conlledo, N., Larsen, B. K., Granby, K., & Barranco, A. (2018). Combined effects of microplastics and chemical contaminants on the organ toxicity of zebrafish (Danio rerio). Environmental research, 162, 135-143.

Ray, A. K., Ghosh, K., & Ringø, E. (2012). Enzyme-producing bacteria isolated from fish gut: a review. Aquaculture Nutrition, 18(5), 465-492.

Reed, P. A., & Francis-Floyd, R. (1996). Vibrio infections of fish. University of Florida Cooperative Extension Service, Institute of Food and Agriculture Sciences, EDIS.

Rochman, C. M., Hoh, E., Kurobe, T., & Teh, S. J. (2013). Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Scientific reports, 3, 3263.

Romeo, T., Pietro, B., Pedà, C., Consoli, P., Andaloro, F., & Fossi, M. C. (2015). First evidence of presence of plastic debris in stomach of large pelagic fish in the Mediterranean Sea. Marine pollution bulletin, 95(1), 358-361.

Rooker, J. R., Turner, J. P., & Holt, S. A. (2006). Trophic ecology of *Sargassum*-associated fishes in the Gulf of Mexico determined from stable isotopes and fatty acids. Marine Ecology Progress Series, 313, 249-259.

Rothäusler, E., Gutow, L., & Thiel, M. (2012). Floating seaweeds and their communities. In Seaweed biology (pp. 359-380). Springer, Berlin, Heidelberg.

Ruiz-Orejón, L. F., Sardá, R., & Ramis-Pujol, J. (2016). Floating plastic debris in the Central and Western Mediterranean Sea. Marine Environmental Research, 120, 136-144.

Salerno, J. L., Little, B., Lee, J., & Hamdan, L. J. (2018). Exposure to crude oil and chemical dispersant may impact marine microbial biofilm composition and steel corrosion. Frontiers in Marine Science, 5, 196.

Schmidt, N., Thibault, D., Galgani, F., Paluselli, A., & Sempéré, R. (2018). Occurrence of microplastics in surface waters of the Gulf of Lion (NW Mediterranean Sea). Progress in Oceanography, 163, 214-220.

Seng, N., Lai, S., Fong, J., Saleh, M. F., Cheng, C., Cheok, Z. Y., & Todd, P. A. (2020). Early evidence of microplastics on seagrass and macroalgae. Marine and Freshwater Research.

Serebryakova, A., Aires, T., Viard, F., Serrão, E. A., & Engelen, A. H. (2018). Summer shifts of bacterial communities associated with the invasive brown seaweed *Sargassum* muticum are location and tissue dependent. PLoS One, 13(12), e0206734.

Steer, M., Cole, M., Thompson, R. C., & Lindeque, P. K. (2017). Microplastic ingestion in fish larvae in the western English Channel. Environmental Pollution, 226, 250-259.

Stickney, R. R., & Shumway, S. E. (1974). Occurrence of cellulase activity in the stomachs of fishes. Journal of Fish Biology, 6(6), 779-790.

Sullam, K. E., Essinger, S. D., Lozupone, C. A., O'CONNOR, M. P., Rosen, G. L., Knight, R. O. B., ... & Russell, J. A. (2012). Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis. Molecular ecology, 21(13), 3363-3378.

Sun, X., Li, Q., Zhu, M., Liang, J., Zheng, S., & Zhao, Y. (2017). Ingestion of microplastics by natural zooplankton groups in the northern South China Sea. Marine pollution bulletin, 115(1-2), 217-224.

Susilowati, R., Sabdono, A., & Widowati, I. (2015). Isolation and characterization of bacteria associated with brown algae *Sargassum* spp. from Panjang Island and their antibacterial activities. Procedia Environ. Sci, 23, 240-246.

Torralba, M. G., Franks, J. S., Gomez, A., Yooseph, S., Nelson, K. E., & Grimes, D. J. (2017). Effect of Macondo Prospect 252 oil on microbiota associated with pelagic *Sargassum* in the northern Gulf of Mexico. Microbial ecology, 73(1), 91-100.

Vadstein, O., Bergh, Ø., Gatesoupe, F. J., Galindo-Villegas, J., Mulero, V., Picchietti, S., ... & Defoirdt, T. (2013). Microbiology and immunology of fish larvae. Reviews in Aquaculture, 5, S1-S25.

Vendel, A. L., Bessa, F., Alves, V. E. N., Amorim, A. L. A., Patrício, J., & Palma, A. R. T. (2017). Widespread microplastic ingestion by fish assemblages in tropical estuaries subjected to anthropogenic pressures. Marine Pollution Bulletin, 117(1-2), 448-455.

Vianello, A., Da Ros, L., Boldrin, A., Marceta, T., & Moschino, V. (2018). First evaluation of floating microplastics in the Northwestern Adriatic Sea. Environmental Science and Pollution Research, 25(28), 28546-28561.

Viršek, M. K., Palatinus, A., Koren, Š., Peterlin, M., Horvat, P., & Kržan, A. (2016). Protocol for microplastics sampling on the sea surface and sample analysis. JoVE (Journal of Visualized Experiments), (118), e55161.

Walsh, E. A., Kirkpatrick, J. B., Rutherford, S. D., Smith, D. C., Sogin, M., & D'Hondt, S. (2016). Bacterial diversity and community composition from seasurface to subseafloor. The ISME journal, 10(4), 979-989.

Wan, Z., Wang, C., Zhou, J., Shen, M., Wang, X., Fu, Z., & Jin, Y. (2019). Effects of polystyrene microplastics on the composition of the microbiome and metabolism in larval zebrafish. Chemosphere, 217, 646-658.

Wang, M., & Hu, C. (2016). Mapping and quantifying *Sargassum* distribution and coverage in the Central West Atlantic using MODIS observations. Remote sensing of environment, 183, 350-367.

Welden, N. A., & Cowie, P. R. (2017). Degradation of common polymer ropes in a sublittoral marine environment. Marine pollution bulletin, 118(1-2), 248-253.

Wells, R. J., & Rooker, J. R. (2004). Spatial and temporal patterns of habitat use by fishes associated with *Sargassum* mats in the northwestern Gulf of Mexico. Bulletin of Marine Science, 74(1), 81-99.

Wright, S. L., Thompson, R. C., & Galloway, T. S. (2013). The physical impacts of microplastics on marine organisms: a review. Environmental pollution, 178, 483-492.

Zettler, E. R., Mincer, T. J., & Amaral-Zettler, L. A. (2013). Life in the “plastisphere”: microbial communities on plastic marine debris. Environmental science & technology, 47(13), 7137-7146.

Zhu, L., Wang, H., Chen, B., Sun, X., Qu, K., & Xia, B. (2019). Microplastic ingestion in deep-sea fish from the South China Sea. Science of The Total Environment, 677, 493-501.