



Article

Quantifying Spatial and Temporal Trends of Microplastic Pollution in Surface Water and in the Eastern Oyster *Crassostrea virginica* for a Dynamic Florida Estuary

Linda J. Walters ^{1,*}, Casey A. Craig ^{1,2}, Emily Dark ^{3,4}, Jessy Wayles ⁵, Vincent Encomio ^{6,7}, Glenn Coldren ^{7,8}, Tess Sailor-Tynes ⁵, David W. Fox ⁹ and Lei Zhai ⁹

- ¹ Department of Biology, University of Central Florida, 4000 Central Florida Blvd., Orlando, FL 32816, USA
² Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute, 100 Eighth Ave SE, St Peterburg, FL 33701, USA
³ Florida Department of Environmental Protection-Indian River Lagoon Aquatic Preserves, 3300 Lewis St., Fort Pierce, FL 34981, USA
⁴ Martin County Board of County Commissioners, 2401 SE Monterey Rd., Stuart, FL 34996, USA
⁵ Marine Discovery Center, 520 Barracuda Blvd, New Smyrna Beach, FL 32169, USA
⁶ UF IFAS, Florida Sea Grant Martin County Extension, 2014 SE Dixie Highway, Stuart, FL 34996, USA
⁷ Florida Oceanographic Society, 890 NE Ocean Blvd., Stuart, FL 34996, USA
⁸ Department of Biology, Indian River State College, 3209 Virginia Ave., Fort Pierce, FL 34981, USA
⁹ Department of Chemistry and Nanoscience Technology Center, University of Central Florida, 4000 Central Florida Blvd., Orlando, FL 32816, USA
* Correspondence: linda.walters@ucf.edu; Tel.: +1-407-823-2148



Citation: Walters, L.J.; Craig, C.A.; Dark, E.; Wayles, J.; Encomio, V.; Coldren, G.; Sailor-Tynes, T.; Fox, D.W.; Zhai, L. Quantifying Spatial and Temporal Trends of Microplastic Pollution in Surface Water and in the Eastern Oyster *Crassostrea virginica* for a Dynamic Florida Estuary. *Environments* **2022**, *9*, 131. <https://doi.org/10.3390/environments9100131>

Academic Editor: Ioannis K. Kalavrouziotis

Received: 25 August 2022

Accepted: 10 October 2022

Published: 13 October 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: Microplastics (MPs) are a ubiquitous pollutant, emphasizing the need to understand their abundance and the factors that influence these patterns around the globe. In a prior study, high numbers of MPs were found in surface waters and tissues of the oyster *Crassostrea virginica* collected from one location in the Indian River Lagoon (IRL, FL, USA). To better understand spatial and temporal variability of MPs throughout the IRL, for one year, monthly surface water samples were collected from 35 sites, while oysters were collected quarterly from 12 sites. Microscopy and ATR-FTIR were used to quantify MP. In total, 3755 MPs were found in 44% of water samples (mean density \pm CI: 1.47 ± 0.09 MP/L). South IRL water had the most MPs, likely associated with proximity to urbanization, inlets (MP sinks) and tributaries (MP sources). MP ($n = 3181$) were found in 70% of examined *C. virginica* ($n = 1402$). Abundances of MP in oysters were lower in the spring and in north IRL. The overall mean abundance was 2.26 ± 0.16 MP/oyster, and the density was 2.43 ± 0.52 MP/g wet tissue weight. Our results provide a more complete picture of MPs in the IRL, a subtropical, shallow-water estuarine system.

Keywords: citizen-science; Indian River Lagoon; microfiber; Mosquito Lagoon; shellfish restoration

1. Introduction

Plastic is both a common household material and pervasive pollutant despite its relatively short history (e.g., [1–3]). Synthetic plastic was first created by Leo Baekeland in 1907; mass production of plastic, however, did not begin until the 1950s when a new generation of plastics (e.g., PVC, polyvinyl chloride; PS, polystyrene; Nylon; PE, polyethylene; PP, polypropylene; and PET, polyethylene terephthalate) made this feasible [4,5]. Global plastic production continued to increase, with an estimated 8300 million metric tons (Mt) produced up to 2015; 79% is now in landfills or the environment, 9% has been recycled, and 12% was incinerated [6]. In 2015, an estimated 4.8 to 12.7 Mt of plastic debris entered Earth's oceans from a myriad of sources, including ship overspill, container wash-off, coastal development and litter, with a tenfold increase predicted by 2025 [7,8].

Once in the marine environment, plastics are subject to solar, thermal, mechanical and biodegradation, which can weaken or fragment the plastics into smaller dimensions called microplastics (MPs) [9,10]. The National Oceanic and Atmospheric Administration defines an MP as any plastic object less than or equal to 5 mm in size, but can be further categorized into two subclasses, primary and secondary MPs [9]. Primary MPs are those that are manufactured at a small size, whereas secondary MPs form via fragmenting from a larger plastic object [11]. Primary MPs include microbeads in personal care products and ‘nurdles’, raw material formed into small pellets for easy transport that are used to make larger plastic items [12,13]. Secondary MPs include fibers, fragments, foams, and films which vary in shape, depending on how they are formed [11]. Oceanic MPs are predominantly textile fibers (35%), fragments associated with city dust (24%), pieces of tires (28%), or nurdles/beads (3% [7]). Fibers are especially common in estuaries and coastal waters [14]. For example, Simon-Sanchez et al. [15] found fibers were the most abundant type (70%) of MP in the Ebro Delta estuary in Spain. Luo et al. [16] documented similar dominance of microfibers (80%) in coastal waters in the Shanghai area and found that MP abundance increased closer to the city.

Plastic ingestion in marine biota has been documented in hundreds of species at varying trophic levels (e.g., [17,18]). Species of particular interest are filter-feeders such as oysters, clams, and mussels [14,19–21]. The eastern oyster, *Crassostrea virginica*, is an estuarine species along the western Atlantic seaboard known to ingest MPs and negative impacts on its physiology have been reported [22]. To feed, many shellfish species extract particulate matter from the water, encase it in mucous, and then either reject or digest the particles. If rejected, the material is excreted as pseudofeces. If accepted, the material is brought to the mouth opening, passed through the digestive tract, and any remainder is excreted as feces [23]. MPs have been found in both the feces and pseudofeces of *C. virginica* [21]. Additionally, MP ingestion in *Crassostrea* has been documented to reduce reproductive success (e.g., [24]) as well as reduce growth and survival [22].

Microplastics are a ubiquitous pollutant in the marine environment and a potential risk to marine biota, emphasizing the need to understand MP abundance and the factors that influence these patterns in marine systems. Microplastics are transported through coastal systems by a dynamic series of forces such as rain, wind, freshwater discharge, waves, tides, salinity gradients, surface drift, biofouling, and storm events [25,26]. All of these variables can create seasonal trends in MP abundances (e.g., [27]). Identifying what factors influence MP abundances in hydrologically complex coastal landscapes is a defined research gap in the MPs field [28]. Our research goal is to understand spatial and temporal patterns of MPs in surface waters of the Indian River Lagoon system that spans 251 km along the east coast of central Florida, USA. Additionally, we examine spatial and temporal patterns of MPs in tissues of *C. virginica* to better understand how this keystone, filter-feeding species has been impacted by anthropogenic MP pollution. To broaden our impact on the IRL community, we worked with citizen-scientists along the length of the IRL to collect and process samples after extensive training.

2. Methodology

2.1. Study Location

Our focal area is Florida’s largest lagoon, the Indian River Lagoon (IRL), which spans 40% of Florida’s east coast from Ponce de Leon Inlet in the north (29.075898° N, 80.917571° W) to Jupiter Inlet in the south (26.944768° N, 80.073952° W). The IRL is a bar-built lagoon that has limited water exchange through five inlets and is hydrologically complex [29–31]. Four of the five inlets within the lagoon, Ponce de Leon, Sebastian, Ft Pierce, and St Lucie, were included within the defined boundaries of this study. The IRL falls within the boundaries of Volusia, Brevard, Indian River, St. Lucie, Martin, and Palm Beach counties. IRL water (hereafter lagoon water) flows through three interconnected water bodies: Mosquito Lagoon, Indian River, and Banana River, has an average depth of 1.2 m [29]. Saltwater influx comes from the Atlantic Ocean through inlets, while freshwater input is predominantly from rainfall, discharge,

and runoff from nearby land [29]. Currents, tides, and circulation patterns are influenced by factors dependent on location from the nearest inlet; areas closer to inlets have a larger tidal influence while stretches between inlets are primarily driven by wind and freshwater input [29]. This research is focused on IRL oysters and their surrounding waters because *C. virginica* has long been harvested for human consumption and there are long-term efforts to restore these oyster populations (e.g., [32]) for the ecological benefits they provide for fishes [33,34], wading birds [35], and aquatic invertebrates [36,37].

2.2. Sample Collection and Citizen Science

Lagoon water was collected from the IRL over a 12-month sampling period, between March 2019 and February 2020. Water was collected once per month from 35 sites that extended the length of the IRL. All sites were accessible from shore and on public lands (Figure 1, Table 1). Each month, lagoon water samples from all sites were collected within a 4-day time period to limit temporal variation. At each site, five replicate 1 L surface lagoon water samples were collected using a discrete sampling protocol [38,39]. Sample bottles were triple-rinsed in 0.45 μm filtered deionized water in the laboratory, and then again with lagoon water upon site arrival to remove any existing contamination. Bottle rinsing occurred at least 10 m away from the sample collection location. Rinsed bottles were partially submerged to collect the top 5 cm of lagoon water and capped while submerged. At each site, abiotic parameters of air and water temperature were recorded using a thermometer ($^{\circ}\text{C}$), salinity using a refractometer (ppt), and mean wind speed using an anemometer (km/h). Samples were transported back to laboratories and kept at room temperature (20°C) through completion of processing.

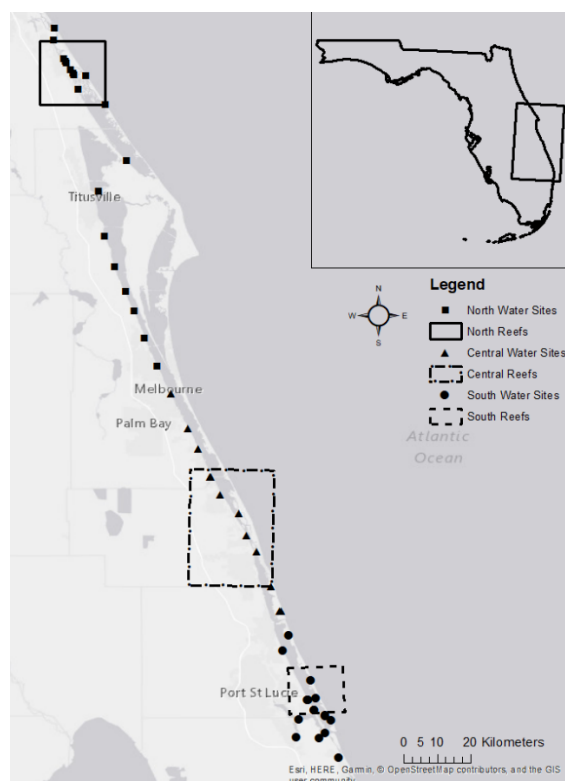


Figure 1. Indian River Lagoon microplastic water sampling sites (points) and oyster reef areas (boxes).

Table 1. Indian River Lagoon water sampling sites from north to south.

Site No.	Site Name	Abbreviation	Region	Latitude	Longitude
1	Smyrna Dunes Park	SDP	N	29.063822	−80.915744
2	Marine Discovery Center	MDC	N	29.030158	−80.917641
3	River Breeze Park	RBP	N	28.898601	−80.85174
4	CANA Boat Ramp	CANAB	N	28.934251	−80.829475
5	CANA Parking Lot #5	CANA5	N	28.857672	−80.777248
6	Haulover Canal	HOC	N	28.706285	−80.720657
7	Parrish Park	PPK	N	28.623625	−80.794767
8	Campground	CAMP	N	28.504	−80.7801
9	Briarwood	BW	N	28.42123	−80.75245
10	Lee Wenner Boat Ramp	LWBR	N	28.355086	−80.722994
11	Rockledge	ROCK	N	28.3014	−80.7005
12	Rotary Park	RPK	N	28.2295	−80.6714
13	Pineapple	PINE	C	28.154	−80.6382
14	Front Street	FS	C	28.079558	−80.599847
15	Malabar	MAL	C	27.9862	−80.5532
16	Christensen	CHR	C	27.93112	−80.526022
17	Outriggers	OUT	C	27.855367	−80.492992
18	Sebastian	SEB	C	27.80892	−80.466215
19	Environmental Learning Center	ELC	C	27.758069	−80.415706
20	Vero	VERO	C	27.654303	−80.368983
21	Round Island	RI	C	27.561131	−80.328635
22	Wildcat	WC	C	27.495292	−80.303114
23	Bear Point	BP	S	27.429391	−80.281382
24	Midway	MID	S	27.38723	−80.297868
25	Jensen Beach	JEN	S	27.308302	−80.22226
26	Palm City Bridge	PCB	S	27.155333	−80.261
27	Riverwalk	RW	S	27.20225	−80.253883
28	Fish House	FH	S	27.151083	−80.199867
29	Twin Rivers	TR	S	27.164933	−80.18215
30	Driftwood	DW	S	27.255533	−80.23085
31	Jensen Beach Impound	JB	S	27.260117	−80.209233
32	River Cove	RC	S	27.21435	−80.183983
33	House of Refuge	HOR	S	27.199617	−80.166283
34	Indian Riverside Park	IRP	S	27.228535	−80.212716
35	Jimmy Graham Boat Ramp	JGBR	S	27.09958	−80.145616

Citizen scientists associated with the University of Central Florida (UCF; current students and alumni) and partnering non-profit conservation agencies situated along the IRL assisted with water sample collection and microscopic identifications. Citizens who ranged in age from 16 to 80 were recruited through existing agencies' volunteer networks, social media postings, and by word of mouth. To be included as a citizen scientist, individuals had to be available for the 12 months of the project and have their own transportation. Citizen recruits underwent extensive MP training where they were educated about MP generation and pollution, scientific procedures used for field sampling of MP, and sample inspection protocols in the laboratory. Once successfully trained, citizen scientists were independently deployed to collect water samples each month at pre-selected sites that were near their homes. Lagoon water samples were processed and inspected for MPs in laboratories at UCF or the associated conservation agencies under direct supervision of university/agency investigators. A minimum of 10% of all samples were then re-checked by university personnel as required by our EPA QAPP (Quality Assurance Project Plan). On all sample collection and processing dates, all participants were requested to wear only natural fiber clothing.

To compare to the collected water samples, the oyster *C. virginica* was collected quarterly for one year from 12 intertidal reefs in the IRL. This included 6 reefs from the north, 3 from the central, and 3 from the south IRL (Figure 1). Sampling reefs in the north region were randomly selected using a random number generator (www.random.org) (accessed on 1 December 2018), while central and southern reefs were the only sustainable, intertidal reefs accessible in each respective region (E. Dark, pers. comm.; [40]). Additionally, the 3 southern reefs were part of oyster restoration efforts. Sampling distribution was skewed to the north to be representative of *C. virginica* abundance in the IRL, as there is

a historical downward trend in oyster abundance as latitude decreases [40,41]). At each reef, 30 individual *C. virginica* were collected. Fifteen large (shell length ≥ 36 mm) and fifteen small (shell length < 36 mm) *C. virginica* were haphazardly collected from each reef, wrapped in aluminum foil, bagged, and placed on ice. Oysters from all 12 reefs were collected within a 7-day window to limit temporal variation. Samples were brought to the University of Central Florida Department of Biology laboratory for storage in a -20 °C freezer until processing.

2.3. Sample Processing

IRL water samples were vacuum-filtered at room temperature using Whatman nitrocellulose membrane filter paper (47 mm, 0.45 μm pore size) to extract MP, and placed in triple-rinsed, sealed, 60×15 mm Petri dishes. Filters were inspected once dry using a dissecting microscope (Leica EZ4, Morrisville, NC, USA) at $20\times$ – $40\times$ magnification. MP type, color, and size (mm) were recorded following protocol established by the Shaw Institute [42]. To distinguish between natural and synthetic items, potential MPs were prodded using forceps to test breakage, and examined for discrete variation in color, shape, and margins (smooth, jagged, frayed) along their lengths.

Individual *C. virginica* were thawed and shell heights (mm) were recorded using calipers. After each oyster was shucked, the blotted-dry, wet-weight of soft tissue weighed (g) was determined using a top-loading balance (Ohaus Scout Pro, Parsippany, NJ, USA), and then placed in individual glass Erlenmeyer flasks (125 mL for small, 250 mL for large oysters). Digestion protocol followed procedures established by [43] for the optimal extraction of MPs from bivalve tissues. A 10% potassium hydroxide (KOH) solution was added to each flask at a ratio of 3:1 volume (mL) to wet tissue weight. Flasks were covered and placed in a shaking incubator at 40 °C at 60 rpm for 24 h, and then removed and left at room temperature for an additional 24 h where tissue digestion was completed. A 1.0 M citric acid solution was added to the digested tissue solution until a neutral pH (7.0) was reached to prevent an interaction with filters [20]. The neutralized solution was vacuum-filtered under a fume hood using Whatman glass microfiber filters (90 mm, 1.2 μm pore size) and placed in triple-rinsed Petri dishes for later quantification.

2.4. Limiting Polymer Contamination and Degradation

Procedural MP contamination was controlled for by triple-rinsing all equipment used during digestion and filtration with 0.45 μm filtered deionized (DI) water prior to each use (M.M. Patterson, pers. comm.). Solutions used during digestions were also made with 0.45 μm filtered DI water. Chemical digestion of oysters was conducted in a fume hood to prevent polymer contamination during the filtration process [44]. KOH was preferable to digest bivalve soft tissue and extract MP particles as small as 1 μm in size because it preserves major polymers, including rayon [43].

Aerial contamination was quantified during microscopy by using five filter-control blanks (filters dampened with 0.45 μm filtered, deionized water placed in triple-rinsed Petri dishes) [44,45]. Blanks (exposed filters) were haphazardly placed on the table immediately around the microscopy station at all times during inspection to quantify potential air contamination while samples were exposed [45]. Blanks were inspected for MP, then normalized (Abundance_N) to a mean contamination rate per minute.

2.5. Fourier-Transform Infrared Spectroscopy

To supplement MP identification, polymer composition was determined using attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR) at the University of Central Florida Nanoscience Technology Center using a Shimadzu IRSpirit-T instrument. A subset of samples containing MPs (10% of water and 10% of *C. virginica* samples with MPs) were randomly selected (www.random.org) (accessed on 1 March 2020). And all potential MPs larger than 0.5 mm in size were scanned [46]. MPs were scanned in the 600 to 4000 cm^{-1} range and spectra were matched to the reference library from Shimadzu

using differential derivative point matching (ATR-FTIR Polymer and Polymer Additives Database #220-93143-07, 2020). A score, also known as hit quality index (HQI), for each spectrum was calculated to measure percent match using the equation:

$$\text{HQI} = \frac{\left(\left(1 - \frac{D}{S} \right)^{\frac{1}{3}} + 1 \right)}{2} \quad (1)$$

where D is the summation of the primary and reference spectra by calculation of a fitting method, and S is the area of the primary derivative curve of the sample spectrum. Spectra were automatically included as a polymer if score match was 700 or higher, ambiguous scores of 600–700 were manually sorted for inclusion in analysis, and scores of 600 or below were excluded [2,47]. Ambiguously scored spectra were inspected and differentiated by visual peak matching. Polyester is predominantly PET and could not be elucidated as a distinctly different polymer, so polyester signals were classified as PET [6]. A subset of signals (10%) on MP identified in control blanks were scanned using ATR-FTIR to determine any overlap between polymers found in the IRL and aerial contamination.

2.6. Statistical Methods

Abundance data for MP in lagoon water and *C. virginica* MPs were broadly dispersed and had a high presence of zeros, so each were analyzed using negative binomial generalized linear modelling (GLM) for zero-inflated data (R package “pscl”). To quantify spatial and temporal variations in MP abundance in lagoon water, predictor variables tested included region, site, and season. Seasons were defined by standard meteorological seasons in Florida (Spring = March–May, Summer = June–August, Fall = September–November, Winter = December–February). To determine what factors may influence MP abundance in water, distance to the nearest tributary (km) and distance to the nearest inlet (km) were tested as predictor variables in models. Distinct regional differences in MP abundance were apparent, so both whole-IRL and independent regional model analyses were incorporated to distinguish trends more precisely. The IRL is very expansive; therefore, models with predictors of distance to an inlet and tributary were only included within the individual regional analyses.

Control blanks were normalized to a contamination rate per minute (C_M) using the formula:

$$C_M = \frac{MP_B}{T_B} \quad (2)$$

where MP_B is the mean number of MP per blank and T_B is the time that blanks were exposed in minutes [21]. Contamination per minute values then were used to calculate the contamination per sample (C_S) using the formula:

$$C_S = C_M \times T_E \quad (3)$$

where T_E is the length of time each filter was exposed during inspection [21]. Contamination per sample values were incorporated as a covariate in water models but only included if significant in the model.

To quantify MP abundance and fluctuations in IRL oysters, predictor variables tested in models included region, site, season, and shell height. To determine what factors may influence MP abundance in oysters, distance to the nearest tributary, and distance to the nearest inlet were also tested as predictor variables. Contamination per sample and tissue weight were incorporated in all oyster models but only included in analyses if significant.

Post hoc model selection using all possible predictor variable combination for Akaike information criterion (AIC) was used to determine which variables best predicted MP abundance in both lagoon water and oyster samples from the IRL. Regional differences in MP abundance were apparent so both whole-IRL and independent regional model analyses

of *C. virginica* were incorporated. Regional models used the same predictor variables as the whole-IRL models to distinguish trends.

Linear regressions were used to determine differences in air and water temperature between IRL regions, and seasons. Regressions were also used to determine wind speed and salinity differences between IRL regions, sites, and months. All statistical analyses were performed using R, version 4.0.3 [48].

3. Results

3.1. Microplastics in Lagoon Water

Overall, a total of 3755 MPs were observed in 44% of all lagoon water samples. When separated by regions, 70.7% of north IRL samples contained MP, while 29.6% and 66.0% of samples contained MP for the central and south regions, respectively. These data were collected by 84 citizen-scientists who collectively donated 1600 h to this MP water effort. Lagoon water MP density ranged from 0 to 25.0 MP/L and had a mean (\pm CI) of 1.47 ± 0.09 MP/L (Figure 2). Mean normalized abundance reduced the range slightly to 0–24.6 MP and the mean to 1.42 ± 0.10 . MP abundance differed between the IRL regions. Central sites had the lowest MP abundance, followed by the north, then south regions ($p < 0.001$ for all, Figure 2). Fibers, fragments, films, and foams were found in water, with fibers comprising 95.6% MP. Fragments, foams, and films comprised the remaining 3.9%, 0.3%, and 0.2% MPs, respectively. Plastics ranged in size from 0.1 mm to 30.0 mm and had a mean longest dimension (\pm CI) of 1.9 ± 0.1 mm.

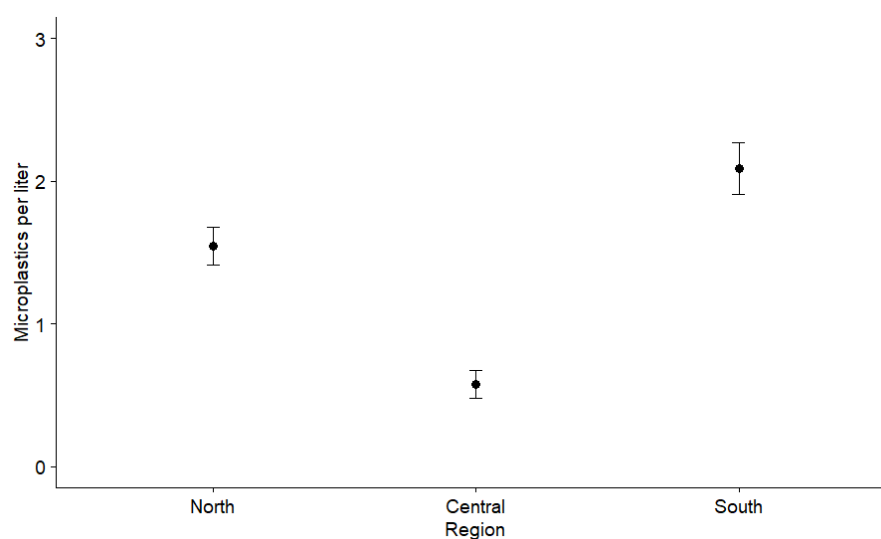


Figure 2. Microplastic abundance per liter of water from the north, central, and south Indian River Lagoon. Values are mean abundance (point) and 95% confidence interval of the mean. (GLM, $p < 0.05$, North = 716, Central = 598, South = 776).

The most plausible model to predict lagoon-wide MP abundance in IRL water included site and season as predictor variables (AIC = 6283.6; McFadden pseudoR² = 0.12, Table 2). For the entire IRL and the north IRL, MP abundance was higher in lagoon water in fall and winter ($p \leq 0.02$). In central and south regions, the most plausible predictors of MP abundance were site and season (AIC = 1131.2, McFadden pseudoR² = 0.10, Table 2) and (AIC = 2710.5; McFadden pseudoR² = 0.12), respectively (Table 2). Abundance was also higher in fall and winter ($p < 0.001$) for both regions. When the presence/absence of MPs was considered for IRL water overall, winter was a significant predictor of MP presence ($p = 0.005$). Despite this lagoon-wide pattern, MP presence was not predicted by any season when regions were considered separately.

Table 2. Zero-inflated negative binomial GLM models of MP abundance in lagoon water overall and by region (north, central, south). Values reported are AIC, delta AIC, degrees of freedom, and AIC weight.

Indian River Lagoon Water	AIC	ΔAIC	df	AIC Weight
Site + season	6283.6	0	77	1
Site	6306.6	23	71	<0.001
Tributary + region + season	6569.9	286.3	15	<0.001
Tributary + region	6584.1	300.5	9	<0.001
Region + season	6644.6	361	13	<0.001
Region	6660.2	376.6	7	<0.001
Season	6938.3	654.8	9	<0.001
Inlet	6946.8	663.2	7	<0.001
Tributary	6951.4	667.8	5	<0.001
North Lagoon	AIC	ΔAIC	df	AIC Weight
Season	2422.9	0	9	0.8221
Site + season	2426.0	3.1	31	0.1748
Site	2434.1	11.2	25	0.0031
Central Lagoon	AIC	ΔAIC	df	AIC Weight
Site + season	1131.2	0	27	0.7207
Site	1133.1	1.9	21	0.2771
Inlet + season	1143.6	12.4	11	0.0015
Inlet	1145.0	13.8	5	<0.001
Tributary	1179.1	47.9	5	<0.001
Season	1209.9	78.7	9	<0.001
South Lagoon	AIC	ΔAIC	df	AIC Weight
Site + season	2710.5	0	33	1
Site	2742.7	32.2	27	<0.001
Tributary + season	2912.9	202.5	11	<0.001
Tributary	2920.5	210	5	<0.001
Season	3003.5	293	9	<0.001

MP abundances differed between IRL regions with regard to oceanic and freshwater influences. In the north IRL, MP abundance did not vary with distance to a tributary or inlet. There are no tributaries in the north and one oceanic inlet (Ponce de Leon Inlet). A different trend was apparent in the central IRL, where MP abundance decreased with increasing distance from a tributary ($p < 0.001$) and increased with increasing distance from an inlet ($p = 0.001$). Abundance decreased by 0.99 MP/L for every 1 km increase in distance from a tributary and increased by 0.82 MP/L for every 1 km increase in distance from an inlet in the central IRL. In the southern region, no oceanic influence was apparent; however, there was tributary influence and MP abundance decreased by 0.88 MP/L for every 1 km increase in distance from a tributary ($p < 0.001$).

3.2. Microplastics in Oysters

Crassostrea virginica ($n = 1402$) from the IRL contained a total of 3181 MPs. The composition of MP was dominantly fibers (95.0%), while fragments comprised 4.4%, and films and foams comprising less than 1% MP combined. Seventy percent ($n = 981$) of *C. virginica* contained MP in their tissues. When considered by region, 65.5% of north samples, 70.4% of central samples, and 76.7% of south samples contained MP. The dominant color of MPs lagoon-wide and for each region was black. Plastics ranged in size from 0.1 to 35 mm, with a mean size (\pm CI) of 2.79 ± 0.10 mm. Oysters had a mean MP abundance (\pm CI) of 2.26 ± 0.16 MP/individual and density of 2.43 ± 0.52 MP/g wet tissue weight. MP abundance differed between *C. virginica* from IRL regions; northern oysters contained less MP than central and south oysters ($p < 0.001$, Figure 3), but abundance did not differ between the central and south oysters.

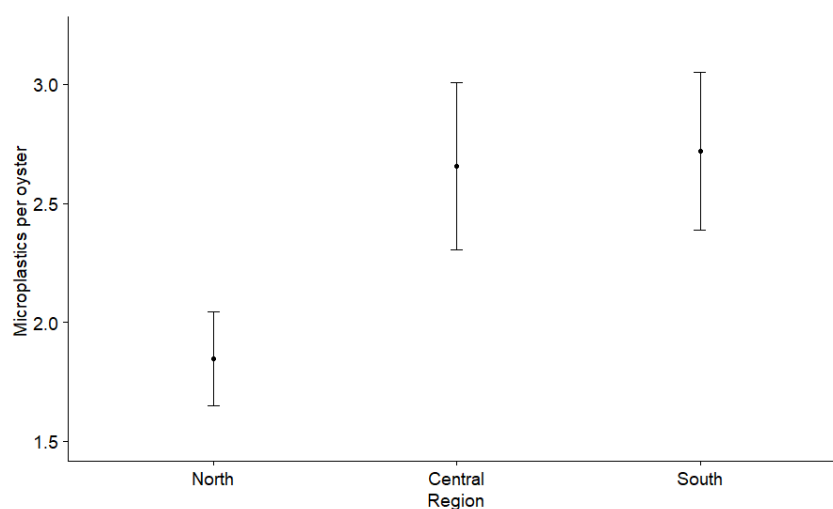


Figure 3. Microplastic abundance by region in Indian River Lagoon oysters. Values reported are the mean (point) and the 95% confidence interval of the mean (error bar). (GLM, $p = 0.01$, North = 710, Central = 345, South = 347).

The most plausible model to predict MP abundance throughout the IRL for *C. virginica* included season, site, and shell height as predictor variables (Table 3). MP abundance was higher in oysters sampled in summer, fall, and winter ($p < 0.03$ for all) than in spring. Within each region, there was variability in MP abundances ($p < 0.05$). In north IRL, the most plausible model included site + season (AIC = 2376.0; McFadden pseudo $R^2 = 0.11$, Table 3), while in the central and south IRL, season + shell height produced the most plausible models (Table 3). Abundance was higher in central *C. virginica* in summer and fall ($p < 0.001$). The model indicated MP abundance increased by 1.4 MP/individual for every 1 mm increase in shell height ($p < 0.001$) in the north. The same model indicated that MP abundance increased by 1.51 MP/individual for every 1 mm increase in shell height in *C. virginica* from the central IRL, and abundance increased by 1.45 MP for every 1 mm increase in shell height ($p \leq 0.002$) in the south. When the presence/absence of MPs was considered by region, summer, fall, and winter were significant predictors of MP presence in oysters from the northern region ($p \leq 0.04$). Similarly, in central IRL, summer, fall, and winter were all predictors of MP presence ($p \leq 0.04$) for *C. virginica*. No variable was a significant predictor of MP presence for south oysters.

Microplastic abundance trends in *C. virginica* differed between IRL regions with regard to oceanic and freshwater influence. In the northern and central IRL, MP abundance in oysters decreased with every 1 km increase in distance from a freshwater tributary, by 0.77 MP/individual and 0.52 MP/individual, respectively ($p \leq 0.01$ for both). However, no oceanic influence on MP abundance was detected in any region, and in the southern IRL, there was also no tributary influence.

3.3. Abiotic Parameters

Air and water temperature differed significantly between IRL regions and seasons ($p < 0.005$ for all), but did not differ between sampling sites for water and oyster collections. As expected, temperatures were higher in summer and spring, and lower in winter. The water temperature was also higher in the southern region. Salinity did not vary between regions or seasons but did vary between sites ($p < 0.05$ for all). Wind speed varied between regions and seasons, but not months. Wind speed was slower in the central IRL region ($p = 0.004$), and faster at northern water sampling sites that were more exposed ($p < 0.01$).

Table 3. Zero-inflated negative binomial GLM models of MP abundance in Indian River Lagoon oysters, overall and by region. Values reported are AIC, delta AIC, degrees of freedom, and AIC weight.

Indian River Lagoon Oysters	AIC	ΔAIC	df	AIC Weight
Site + season + shell height	4984.0	0.0	35	1
Season + shell height	5020.6	32.6	13	<0.001
Site + shell height	5053.1	69.1	29	<0.001
Shell height	5088.2	104.2	7	<0.001
Site + season	5158.6	174.6	35	<0.001
Season	5208.4	224.4	13	<0.001
Site	5248.5	264.5	29	<0.001
Tributary	5277.0	293.0	9	<0.001
Region	5289.1	305.1	11	<0.001
North Lagoon	AIC	ΔAIC	df	AIC Weight
Site + season	2376.0	0.0	23	0.63
Site + season + shell height	2377.1	1.0	23	0.37
Season + shell height	2404.2	28.2	13	<0.001
Season	2407.2	31.2	13	<0.001
Site	2411.0	34.9	17	<0.001
Site + shell height	2413.5	36.5	17	<0.001
Tributary	2435.6	59.6	9	<0.001
Inlet	2435.6	59.6	9	<0.001
Shell height	2436.5	60.5	7	<0.001
Central Lagoon	AIC	ΔAIC	df	AIC Weight
Season + shell height	1203.5	0.0	13	1
Shell height	1263.2	59.7	7	<0.001
Site + season	1289.7	86.2	15	<0.001
Season	1310.2	106.7	13	<0.001
Site	1364.5	161.0	9	<0.001
Tributary	1371.7	168.2	9	<0.001
South Lagoon	AIC	ΔAIC	df	AIC Weight
Season + shell height	1335.8	0.0	13	1
Shell height	1372.0	36.2	7	<0.001
Season	1408.7	72.9	13	<0.001

3.4. Polymer Composition and Contamination

In total, 122 signals of suspected MPs were obtained using ATR-FTIR spectroscopy, and 78 (64%) were confirmed synthetic polymers. Fibers, fragments, foams, and films were found in both lagoon water and *C. virginica*. Fibers dominated type composition, comprising 95.6% and 95.0% MPs in water and *C. virginica*, respectively. Colors varied across the spectrum, but black MPs were the most common. It is possible that the citizen scientists who collected field samples may have contaminated their samples if their clothing was not created from natural materials [49,50]. We acknowledge this potential source of error. Project scientists ensured that all individuals involved in laboratory MP microscopy wore only natural fiber garments.

Polyethylene terephthalate (PET) was the most abundant polymer in lagoon water and *C. virginica* in the IRL, and comprised 50%, and 56% MPs, respectively. Polypropylene (PP), polyethylene (PE), polystyrene (PS), and polyamide (PA) were also found in lagoon water in differing proportions (Figure 4). All scanned MPs were fibers, except for two clear fragments. One fragment was PE and the other was a synthetic wax. There was one rayon fiber found and it was in an oyster. Miscellaneous (“other”) polymers found in both water and oyster samples were polymer blends, with the exception of one acrylic adhesive confirmed in lagoon water, and one polyacrylate fiber confirmed in *C. virginica*.

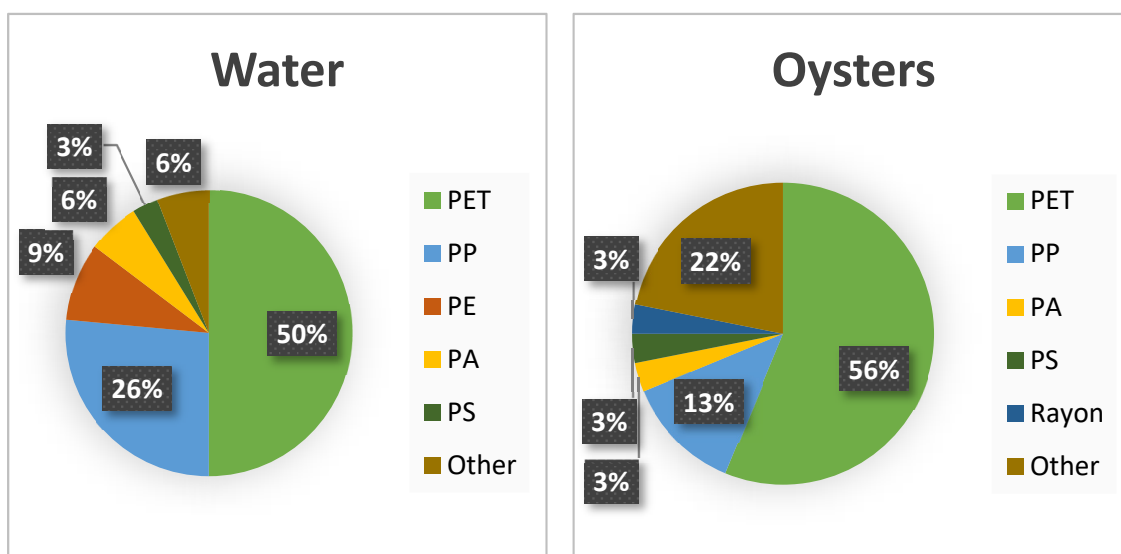


Figure 4. Synthetic polymers in microplastics from water and oysters from the Indian River Lagoon. Polymers are polyethylene terephthalate (PET), polypropylene (PP), polyethylene (PE), polyamide (PA), polystyrene (PS), rayon, and miscellaneous polymers (Other).

Aerial control blanks associated with lagoon water had a mean contamination rate of 0.016 MP/min. The mean time of exposure associated with microscopy for water samples was 8 min. Mean aerial contamination rate for *C. virginica* samples was 0.020 MP/min and samples were exposed for a mean time of 5 min. There was a 15% overlap (three PET particles) between polymers found in samples and polymers from aerial control blanks.

4. Discussion

A growing body of research, including our results, suggest oceans function as sinks for MPs, while coastal surface waters entering estuaries are sources [51–53]. The current study quantified MP abundance in surface water and *C. virginica* from the IRL to determine if spatial and temporal factors influence MP abundances within this system. Overall, *C. virginica* had an average of 2.26 MP/individual, or 2.43 MP/g tissue weight, and IRL water had 1.47 MP/L. Significant variations and trends in MP abundance were detected across seasons, and within spatial extents less than 5 km, indicating both site and season should be incorporated into MP research designs. For example, our research determined that the south IRL was a hotspot for MP pollution.

Studies of estuarine and coastal bivalves have reported variable MP abundances (e.g., [27,54,55]). Comparisons of MP abundance in Florida's IRL to abundances of other estuaries in the United States are summarized in Table 4. Compared to a previous study on MPs in the north IRL by [56], our abundance values for both lagoon water and adult oysters were lower even though the oyster reefs were in close proximity (separated by < 1 km). This difference can likely be attributed to the different MP collection and extraction procedures between studies, as FTIR was not included in [56], and we now know that large numbers of natural fibers are present in the IRL system (C.A.C., pers. obs.). Additionally, aerial contamination was not reported in the Waite et al. [56] publication. MP abundance in IRL water was comparable to mean abundances in a surface water study from Tampa Bay (FL), published in 2019, but not to earlier studies from highly urbanized Charleston Harbor, SC or less urbanized Winyah Bay, SC [55,57]. It is important to note that the south IRL, where mean MP abundance exceeded 2 MP/L, is the most highly urbanized area within the IRL system and some of the sampling locations were along human-built canals. When oysters were compared, MP abundance in *C. virginica* from the IRL was less than MP abundance in *C. gigas* from the Oregon coast, but was similar to *C. gigas* in the Salish Sea, WA [58,59]. This may be related to oyster filtration rates, month or time of sampling, MP protocols,

or localized urbanization. Filtration rates for *C. gigas* from the Pacific Ocean have been measured in the field at 0.35–0.73 L g⁻¹ h⁻¹ and 2.5–12 L g⁻¹ h⁻¹ in laboratory trials (dry tissue weight; [60–64]). There are reports that this species can filter in “high gear” vs. “low gear”, with a 3-fold difference in filtration [65]. Galimany et al. [66] reported that adult IRL *C. virginica* filter 18 L day⁻¹ (0.75 L h⁻¹) in flow-through chambers using lagoon water, while Grizzle et al. [67] reported 1.2 L h⁻¹ filtration rates in shallow IRL field conditions. The MP abundances reported in this study were also comparable to those from *C. gigas* collected along the French Atlantic coast (1.7 MP/individual; [68]).

Table 4. Comparison of microplastic abundance in water and oysters from the Indian River Lagoon and other estuaries in the United States. Values reported are mean abundance per liter of water, microplastics per individual oyster, and standard error of the mean.

Water	Location	Abundance ± S.E.	Reference	
	Indian River Lagoon, FL	1.46 ± 0.05	Present study	
	Mosquito Lagoon, FL	23.1	[56]	
	Tampa Bay Estuary, FL	0.94 ± 0.52	[55]	
	Charleston Harbor, SC	6.6 ± 1.3	[57]	
	Winyah Bay, SC	30.8 ± 12.1	[57]	
Oysters	Location	Abundance ± S.E.	Reference	Species
	Indian River Lagoon, FL	2.26 ± 0.08	Present study	<i>Crassostrea virginica</i>
	Mosquito Lagoon, FL	16.5	[56]	<i>Crassostrea virginica</i>
	Salish Sea, WA	1.75	[59]	<i>Crassostrea gigas</i>
	Oregon Coast	10.95 ± 0.77	[58]	<i>Crassostrea gigas</i>

4.1. Spatial Microplastic Fluctuations and Influences

MP abundance in both lagoon water and *C. virginica* were influenced differently by hydrological factors of distance to a tributary or inlet. Freshwater contribution to the IRL comes from land runoff and a dynamic matrix of rivers, drainage canals, creeks, and ditches, which are unevenly distributed throughout the lagoon [29]. In the northern IRL, there was no tributary or inlet influence detected on MP abundance in water or oysters. There are no tributaries and the north IRL is considered microtidal with water residence times (50% renewal time) within the region varying greatly with distance from inlet; for example, residence time for the northernmost and southernmost portions of Mosquito Lagoon (north IRL) are 15 days and 172 days, respectively [30]. Within the northern IRL, MP abundance in water did not differ between sites; however, abundance was different in oysters from different reefs. All northern sampling reefs were located within 20 km of Ponce de Leon Inlet and within 5 km of each other so spatial influences on MP abundances in *C. virginica* were hard to distinguish. All reefs were located in the central Mosquito Lagoon region, where water residence time is low (~15 days; [30]). Likewise, abiotic conditions between sampled reefs in the north IRL did not differ. It is likely that the variation in MP abundance in the north IRL is not explained by variables captured in this study, and additional research is needed. One possibility is that the location of stormwater outfalls, not incorporated in this study, are important sources of MP pollution in this region of the IRL (L.J.W., pers. comm.; [3,69]).

In the central IRL, a positive trend of freshwater influence on MP abundance was found in both water and *C. virginica*, as abundance decreased with increasing distance from a tributary. Tributaries within the boundaries of the central IRL are the Sebastian and Eau Gallie Rivers, Turkey and Crane Creeks, and numerous manmade canals (e.g., Vero Main, Vero North, Vero South, Taylor; [29]). The Sebastian River is the second largest tributary into the IRL [70]. Despite the greater tributary presence, water from the central IRL contained the lowest MP abundances, which may be attributed to the inlets flushing MPs out of the region. There are two inlets within the boundaries of the central IRL, Sebastian and Fort Pierce, which contribute to lower water residence times and increased tidal flushing in the

region [71]. In oysters from the central IRL, however, there was no inlet influence while MP abundance increased with closer proximity to freshwater tributaries. This may be attributed to the proximity of two of the three reefs to tributaries; both were adjacent to tributaries that empty into the lagoon, while the third reef was 3 km away from Fort Pierce Inlet. Additionally, this third reef was located on the western side of the central IRL, where there is a lessened tidal influence compared to the eastern shore [72]. Another important factor for MP abundance in water and oysters in the central IRL was discharge from the Sebastian River, which had a mean annual discharge rate of $\sim 100 \text{ m}^3 \text{ s}^{-1}$ throughout this study [72]. Law et al. [73] found that MPs accumulate in areas with water velocities slower than 2 cm s^{-1} , suggesting MPs in this area are flushed away by the water velocity of the Sebastian River.

Southern IRL MP abundance was high for both *C. virginica* and lagoon water. MP abundance in southern lagoon water decreased with increasing distance from a tributary; however, MP abundance in *C. virginica* did not. There is one primary tributary in the south IRL, the St. Lucie Estuary (SLE), which is also the largest tributary to the IRL and connects the lagoon to Lake Okeechobee through the C44 canal [29]. MP abundance was not influenced by distance to an inlet, in either lagoon water or *C. virginica*. There is one inlet within the southern IRL boundary defined in this study, the St. Lucie Inlet, which has constricted water flow into the area [74]. As a result, there is less tidal influence in this area and in the SLE [74]. This suggests the St. Lucie Inlet is not flushing MP out of the southern IRL at rates fast enough to accommodate deposition from the tributary. Abundance in *C. virginica* in the southern IRL did not differ between sampling reefs. Similar abundances in southern reefs may be attributed to their location, as they were all north of the SLE and St. Lucie Inlet (>5 km) where water circulation patterns are less impacted by either the tributary or inlet [49,74].

4.2. Temporal Microplastic Fluctuations and Influences

Our sampling design included one collection per month (IRL water) or per season (oysters), so it is essential to consider this limitation to the study when examining temporal trends in MPs in the IRL, especially with oysters. With IRL water overall and for all three regions, MP abundance was higher in the fall and winter during our study. Temporal variation in MP abundance was likely impacted by extreme events that happened during the study period. Hurricane Dorian, which paralleled Florida's east coast between 1 and 3 September 2019, may have impacted MP abundance in the lagoon. Hitchcock [25] found MP abundance levels in the Cooks River Estuary (Australia) were 40-fold higher during a storm event. Around the time of Hurricane Dorian, discharge out of the Sebastian River and SLE increased to $\sim 305 \text{ m}^3 \text{ s}^{-1}$ [73]. There were also prolonged high-water levels associated with a new lunar cycle and slowing of the Atlantic current in November 2019, which may also have influenced water discharges, and thus, MP abundance [73].

With IRL oysters, the overall pattern suggests that spring had lower MP abundances than other seasons. This pattern may be related to seasonal oyster eco-physiology and seasonal differences in their pumping rates. Pumping rates increase with temperature [75]. Likewise, oyster reproduction increases with temperature and Florida oysters begin to spawn each year when temperatures reach or exceed $25 \text{ }^\circ\text{C}$ [40]. In the north IRL, *C. virginica* recruited to reefs in all months of the year except January–March throughout a 6-year study; ≥ 5 spat were recorded per 0.25 m^2 as long as the water temperature from nearby continuous monitoring was $\geq 19.3 \text{ }^\circ\text{C}$ [76]. Spring oysters were collected in March 2019 when water temperatures were below this temperature threshold. Combined, this suggests that lowered pumping and reproductive activity by oysters may result in lower MP abundance. This could have implications for harvesting and human consumption, but more detailed studies on this topic are required. Craig et al. [21] found that *C. virginica*, regardless of size, were able to egest MPs at a mean rate of 1 MP per 1 h through feces, and 1 MP per 2 h through pseudofeces, and that egestion efficiency decreased by 0.8% per 1 g increase in tissue weight.

This corresponds to the current finding that size is important as MP abundance increased by 1.4–1.5 MP/individual for every 1 mm increase in shell height.

4.3. Polymers and Coastal Restoration

Development and field testing of non-plastic materials have become important topics in the field of coastal restoration to reduce any unintended consequences of plastic restoration materials on the environment [77]. Negative assumptions continue to be made about plastic-based restoration materials contributing to overall MP abundances without supporting data. Materials traditionally used for restoration borrowed heavily from the aquaculture industry and many areas around the globe use black, Naltex (PE) mesh bags for oyster reef restoration and living shoreline stabilization (e.g., [78,79]). The mesh bags are filled with shells, often from shell recycling programs, and deployed as the bases for reefs or as wave breaks along shorelines. This made sense as this bagging mesh was non-toxic, volunteer-friendly, and low cost. However, while aquaculturists may leave materials in water bodies for finite time frames, this was not the plan for many restoration projects. Hence, there is a community concern that these PE bags deployed for restoration are now adding to the MP problem in the IRL and elsewhere. We were able to directly test if *C. virginica* collected from the south IRL had a large number of black PE fibers or fragments as all three study reefs were previously restored using Naltex bags (V.E., pers. Comm.). Although black was the dominant color of fibers that dominated both water and oyster MP in south IRL, it is unlikely that the source was the Naltex mesh bags as PE signatures were limited (9% in water, < 2% in oysters). However, the minimum sample size for FTIR was 0.5 mm; thus, the number of Naltex mesh bag MP of smaller dimensions is not known. Craig et al. [21] likewise found very few (<5%) PE MPs in oyster biodeposits (feces, pseudofeces) and 8% in the soft tissues of 280 individuals of *C. virginica* collected throughout the IRL in a separate study.

There are many alternative sources of MPs in the IRL and around the globe. Polyethylene terephthalate (PET) was the dominant polymer found in both *C. virginica* and water in our study and the IRL oyster biodeposits study by Craig et al. [20]. PET was the most abundant polymer in feces, pseudofeces, and oyster tissue, and comprised 80%, 50%, and 58% confirmed MP, respectively [21]. PET is prominent in the single-use plastic industry, particularly plastic drink bottles [80–82]. Polyester, also known as PET, is the most produced synthetic textile material in the world and is common in clothing [83]. Since 95% of MPs in the IRL were fibers, it is possible that some MPs originated from wastewater treatment plants or septic systems. Another source is stormwater outfalls, and IRL outfall locations have higher MP abundances than other regions (Walters et al., unpublished data). Globally, Boucher and Friot [7] estimate 35% of plastics in oceans are from synthetic textiles associated with laundry.

Of the 44 misidentified non-polymers, 39% were natural textile fibers, including wool, cotton, and silk fibroin [84]. An additional 30% were cellulose derivatives (e.g., microcrystalline, microfibrillated cellulose), and 10% were ramie fiber. These are fibers engineered to be resistant to breakage, suggesting that there may be a weakness in identification procedures where resistance to breakage may be too heavily relied upon as a characteristic to classify a particle as an MP.

5. Conclusions

Over the period of this study, 84 trained citizens participated in water sampling, processing, and inspecting, and contributed 1600 h of their time to Indian River Lagoon MP research. In the IRL, MP abundance was variable, both spatially and temporally, which can be attributed to the unique hydrology of this 251 km long estuary along the east coast of central Florida. In total, 6936 MPs were found in the IRL (water + oysters) collections, 95% of which were fibers, and the majority was PET. The southern IRL was a hotspot for microplastic pollution; this region is highly urbanized and also includes the largest tributary to the IRL. Overall, freshwater tributaries in the central and south IRL were the suggested

sources of MP pollution, while the Sebastian and Ft. Pierce inlets flushed MPs out of this system. Using a mean abundance of 1.5 MP/L and lagoon volume of 953,000,000 cm³ [85], we estimate there are ~1.4 trillion MPs in the Indian River Lagoon. This research builds on previous IRL MP research as well as the rapidly expanding global body of research of spatial and temporal variations in MP abundance in dynamic estuarine systems that include both highly urbanized and undeveloped areas.

Author Contributions: Conceptualization, L.J.W., E.D., J.W. and V.E.; methodology, L.J.W., C.A.C., E.D. and J.W.; validation, L.J.W. and C.A.C.; formal analysis, C.A.C.; investigation, L.J.W., C.A.C., E.D., T.S.-T. and G.C.; resources, L.J.W., E.D., J.W., V.E. and L.Z.; data curation, C.A.C.; writing—original draft preparation, C.A.C.; writing—review and editing, L.J.W., E.D., J.W., V.E., T.S.-T., G.C., D.W.F. and L.Z.; visualization, C.A.C.; supervision, L.J.W., and L.Z.; project administration, L.J.W.; funding acquisition, L.J.W., E.D., J.W. and V.E. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Indian River Lagoon National Estuary Program and National Science Foundation award #1617374.

Data Availability Statement: Data are available on UCF STARS digital repository at <https://stars.library.ucf.edu> (accessed on 24 August 2022).

Acknowledgments: We thank all the citizen-scientists, staff and research assistants from University of Central Florida, Florida Oceanographic Society, Marine Discovery Center, Indian River Lagoon Aquatic Preserve, Oxbow Eco-Center, and Environmental Learning Center who dedicated their time, space and equipment to complete this project. In particular, we thank K. Fusco, P. Sacks, A. Wright, W. Giles, S. Busch, M. Keplinger, S. Krulis and J. Copertino for field and laboratory assistance. Finally, we thank the National Park Service (Canaveral National Seashore) for providing access to north IRL sites.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Sekudewicz, I.; Dabrowska, A.M.; Syczewski, M.D. Microplastic Pollution in Surface Water and Sediments in the Urban Section of the Vistula River (Poland). *Sci. Total Environ.* **2021**, *762*, 143111. [[CrossRef](#)] [[PubMed](#)]
2. Zhao, J.; Ran, W.; Teng, J.; Liu, Y.; Liu, H.; Yin, X.; Cao, R.; Wang, Q. Microplastic Pollution in Sediments from the Bohai Sea and the Yellow Sea, China. *Sci. Total Environ.* **2018**, *640*, 637–645. [[CrossRef](#)] [[PubMed](#)]
3. Kalavrouziotis, I.; Karapanagioti, H. *Microplastics in Water and Wastewater*, 2nd ed.; IWA Publishing: London, UK, 2020.
4. Baekeland, L.H. The Synthesis, Constitution, and Uses of Bakelite. *Ind. Eng. Chem.* **1909**, *1*, 149–161. [[CrossRef](#)]
5. Crespy, D.; Bozonnet, M.; Meier, M. 100 years of Bakelite, the Material of 1000 uses. *ChemInform* **2008**, *39*, 3322–3328. [[CrossRef](#)]
6. Geyer, R.; Jambeck, J.R.; Law, K.L. Production, Use, and Fate of All Plastics Ever Made. *Sci. Adv.* **2017**, *3*, e1700782. [[CrossRef](#)]
7. Boucher, J.; Friot, D. *Primary Microplastics in the Oceans: A Global Evaluation of Sources*; IUCN: Gland, Switzerland, 2017; pp. 227–229.
8. Jambeck, J.R.; Geyer, R.; Wilcox, C.; Siegler, T.R.; Andrady, A.; Narayan, R.; Law, K.L. Plastic Waste Inputs from Land into the Ocean. *Science* **2015**, *347*, 768–771. [[CrossRef](#)]
9. Arthur, C.; Baker, J.; Bamford, H. (Eds.) Proceedings of the International Research Workshop on the Occurrence, Effects and Fate of Microplastic Marine Debris. In *NOAA Technical Memorandum NOS-OR&R-30*; University of Washington Tacoma: Tacoma, Washington, DC, USA, 2009.
10. Guo, X.; Wang, J. The Chemical Behaviors of Microplastics in Marine Environment: A Review. *Mar. Pollut. Bull.* **2019**, *142*, 1–14. [[CrossRef](#)]
11. Barnes, D.K.; Galgani, F.; Thompson, R.C.; Barlaz, M. Accumulation and Fragmentation of Plastic Debris in Global Environments. *Phil. Trans. R. Soc. B Biol. Sci.* **2009**, *364*, 1985–1998. [[CrossRef](#)]
12. Auta, H.S.; Emenike, C.U.; Fauziah, S.H. Distribution and Importance of Microplastics in the Marine Environment: A Review of the Sources, Fate, Effects, and Potential Solutions. *Environ. Int.* **2017**, *102*, 165–176. [[CrossRef](#)]
13. Ellison, K. The Trouble with Nurdles. *Front. Ecol. Environ.* **2007**, *5*, 396. [[CrossRef](#)]
14. Li, H.X.; Ma, L.S.; Lin, L.; Ni, Z.X.; Xu, X.R.; Shi, H.H.; Yan, Y.; Zheng, G.; Rittschof, D. Microplastics in Oyster *Saccostrea cucullata* Along the Pearl River Estuary, China. *Environ. Pollut.* **2018**, *236*, 619–625. [[CrossRef](#)]
15. Simon-Sanchez, L.; Grelaud, M.; Garcia-Orellana, J.; Ziveri, P. River Deltas as Hotspots of Microplastic Accumulation: The Case Study of the Ebro River (NW Mediterranean). *Sci. Total Environ.* **2019**, *687*, 1186–1196. [[CrossRef](#)]
16. Luo, W.; Su, L.; Craig, N.J.; Du, F.; Wu, C.; Shi, H. Comparison of Microplastic Pollution in Different Water Bodies from Urban Creeks to Coastal Waters. *Environ. Pollut.* **2019**, *246*, 174–182. [[CrossRef](#)]

17. Wang, F.J.; Tan, Z.; Peng, J.; Qiu, Q.; Li, M. The Behaviors of Microplastics in the Marine Environment. *Mar. Environ. Res.* **2016**, *113*, 7–17. [[CrossRef](#)]
18. Zhang, F.; Man, Y.B.; Mo, W.Y.; Man, K.Y.; Wong, M.H. Direct and Indirect Effects of Microplastics on Bivalves, with a Focus on Edible Species: A Mini-Review. *Crit. Rev. Environ. Sci. Technol.* **2020**, *50*, 2109–2143. [[CrossRef](#)]
19. Cho, Y.; Shim, W.J.; Jang, M.; Han, G.M.; Hong, S.H. Nationwide Monitoring of Microplastics in Bivalves from the Coastal Environment of Korea. *Environ. Pollut.* **2021**, *270*, 116175. [[CrossRef](#)]
20. Ward, J.E.; Zhao, S.; Holohan, B.A.; Mladinich, K.M.; Griffin, T.W.; Wozniak, J.; Shumway, S.E. Selective Ingestion and Egestion of Plastic Particles by the Blue Mussel (*Mytilus edulis*) and Eastern Oyster (*Crassostrea virginica*): Implications for Using Bivalves as Bioindicators of Microplastic Pollution. *Environ. Sci. Technol.* **2019**, *53*, 8776–8784. [[CrossRef](#)]
21. Craig, C.A.; Fox, D.W.; Zhai, L.; Walters, L.J. In-Situ Microplastic Egestion Efficiency of the Eastern Oyster *Crassostrea virginica*. *Mar. Pollut. Bull.* **2022**, *178*, 113653. [[CrossRef](#)]
22. Eierman, L. Multiple Negative Impacts of Polyethylene Terephthalate Plastic (PET) Exposure on Juvenile Eastern Oysters (*Crassostrea virginica*). In Proceedings of the Coastal & Estuarine Research Federation 25th Biennial Conference, Mobile, AL, USA, 3–7 November 2019.
23. Newell, R.I.; Jordan, S.J. Preferential Ingestion of Organic Material by the American Oyster *Crassostrea virginica*. *Mar. Ecol. Prog. Ser.* **1983**, *13*, 47–53. [[CrossRef](#)]
24. Sussarellu, R.; Suquet, M.; Thomas, Y.; Lambert, C.; Fabioux, C.; Pernet, M.; Le Goic, N.; Quillien, V.; Mignant, C.; Epelboin, Y.; et al. Oyster Reproduction is Affected by Exposure to Polystyrene Microplastics. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 2430–2435. [[CrossRef](#)]
25. Hitchcock, J.N. Storm Events as Key Moments of Microplastic Contamination in Aquatic Ecosystems. *Sci. Total Environ.* **2020**, *734*, 139436. [[CrossRef](#)]
26. Xia, W.; Rao, Q.; Deng, X.; Chen, J.; Xie, P. Rainfall is a Significant Environmental Factor of Microplastic Pollution in Inland Waters. *Sci. Total Environ.* **2020**, *732*, 139065. [[CrossRef](#)]
27. Lima, A.R.A.; Barletta, M.; Costa, M.F. Seasonal Distribution and Interactions between Plankton and Microplastics in a Tropical Estuary. *Estuar. Coast. Shelf Sci.* **2015**, *165*, 213–225. [[CrossRef](#)]
28. Zhang, Y.L.; Wang, F.X.; Shock, C.C.; Yang, K.J.; Kang, S.Z.; Qin, J.T.; Li, S.E. Effects of Plastic Mulch on the Radiative and Thermal Conditions and Potato Growth under Drip Irrigation in Arid Northwest China. *Soil Tillage Res.* **2017**, *172*, 1–11. [[CrossRef](#)]
29. Indian River Lagoon National Estuary Program. 2030 Comprehensive Conservation and Management Plan. 2019. Available online: <https://onelagoon.org/management-plan/> (accessed on 19 August 2022).
30. Rosario-Llantín, J.A.; Zarillo, G.A. Flushing Rates and Hydrodynamical Characteristics of Mosquito Lagoon (Florida, USA). *Environ. Sci. Pollut. Res.* **2021**, *28*, 30019–30034. [[CrossRef](#)]
31. Smith, N.P. Tidal and Nontidal Flushing of Florida’s Indian River Lagoon. *Estuaries* **1993**, *16*, 739–746. [[CrossRef](#)]
32. Walters, L.; Sacks, P.; Campbell, D. Boating Impacts and Boat-Wake Resilient Restoration of the Eastern Oyster *Crassostrea virginica* in Mosquito Lagoon, Florida, USA. *Fla. Sci.* **2021**, *84*, 173–199.
33. Lewis, D.; Durham, K.; Walters, L.; Cook, G. Resident Fishes as Higher Trophic Level Indicators of Oyster Reef Restoration Success. *Sustainability* **2021**, *13*, 13004. [[CrossRef](#)]
34. Loch, J.M.H.; Walters, L.J.; Donnelly, M.L.; Cook, G.S. Restored Coastal Habitat Can “Reel In” Juvenile Sportfish: Population and Community Responses in the Indian River Lagoon, Florida, USA. *Sustainability* **2021**, *13*, 12832. [[CrossRef](#)]
35. Copertino, J.; Harris, K.; Chute, L.; Walters, L. Impact of Oyster (*Crassostrea virginica*) Reef Restoration on Benthic Invertebrates and Coastal Birds in a Subtropical Estuary. *Sustainability* **2022**, *14*, 2371. [[CrossRef](#)]
36. Barber, A.; Walters, L.; Birch, A. Potential for Restoring Biodiversity of Macroflora and Macrofauna on Oyster Reefs in Mosquito Lagoon, Florida. *Fla. Sci.* **2010**, *73*, 47–62.
37. Searles, A.; Gipson, E.; Walters, L.; Cook, G. Oyster Reef Restoration Facilitates the Recovery of Marine Invertebrate Abundance, Diversity, and Composition in Estuarine Communities. *Nat. Sci. Rep.* **2022**, *12*, 8163. [[CrossRef](#)] [[PubMed](#)]
38. Cutroneo, L.; Reboa, A.; Besio, G.; Borgogno, F.; Canesi, L.; Canuto, S.; Dara, M.; Enrile, F.; Forioso, I.; Greco, G.; et al. Microplastics in Seawater: Sampling Strategies, Laboratory Methodologies, and Identification Techniques Applied to Port Environment. *Environ. Sci. Pollut. Res.* **2020**, *27*, 8938–8952. [[CrossRef](#)] [[PubMed](#)]
39. Zhu, J.; Zhang, Q.; Li, Y.; Tan, S.; Kang, Z.; Yu, X.; Lan, W.; Cai, L.; Wang, J.; Shi, H. Microplastic Pollution in the Maowei Sea, a Typical Mariculture Bay of China. *Sci. Total Environ.* **2019**, *658*, 62–68. [[CrossRef](#)]
40. Radabaugh, K.R.; Geiger, S.P.; Moyer, R.P. (Eds.) *Oyster Integrated Mapping and Monitoring Program Report for the State of Florida*; Technical Report 22; Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute: St. Petersburg, FL, USA, 2019.
41. Garvis, S.K.; Sacks, P.E.; Walters, L.J. Formation, Movement, and Restoration of Dead Intertidal Oyster Reefs in Canaveral National Seashore and Mosquito Lagoon, Florida. *J. Shellfish Res.* **2015**, *34*, 251–258. [[CrossRef](#)]
42. Shaw Institute. Guide to Microplastics Identification, A Comprehensive Methods Guide for Microplastics Identification and Quantification in the Laboratory. Shaw Institute: Blue Hill, ME, USA, 2019. Available online: <https://www.shawinstitute.org/> (accessed on 1 September 2019).
43. Thiele, C.J.; Hudson, M.D.; Russell, A.E. Evaluation of Existing Methods to Extract Microplastics from Bivalve Tissue: Adapted KOH Digestion Protocol Improves Filtration at Single-Digit Pore Size. *Mar. Pollut. Bull.* **2019**, *142*, 384–393. [[CrossRef](#)]

44. Foekema, E.M.; de Gruijter, C.; Mergia, M.T.; van Franeker, J.A.; Murk, A.J.; Koelmans, A.A. Plastic in North Sea Fish. *Environ. Sci. Technol.* **2013**, *47*, 8818–8824. [[CrossRef](#)]
45. Granek, E.F.; Brander, S.; Holland, E.B. Microplastics in Aquatic Organisms: Improving Understanding and Identifying Directions for the Next Decade. *Limnol. Oceanogr. Lett.* **2020**, *5*, 1–4. [[CrossRef](#)]
46. Gago, J.; Galgani, F.; Maes, T.; Thompson, R.C. Microplastics in Seawater: Recommendations from the Marine Strategy Framework Directive Implementation Process. *Front. Mar. Sci.* **2016**, *3*, 219. [[CrossRef](#)]
47. Frias, J.P.G.L.; Gago, J.; Otero, V.; Sobral, P. Microplastics in Coastal Sediments from Southern Portuguese Shelf Waters. *Mar. Environ. Res.* **2016**, *114*, 24–30. [[CrossRef](#)]
48. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2019; Available online: <https://www.R-project.org/> (accessed on 1 December 2018).
49. Gwinnett, C.; Miller, R.Z. Are We Contaminating Our Samples? A Preliminary Study to Investigate Procedural Contamination During Field Sampling and Processing for Microplastic and Anthropogenic Microparticles. *Mar. Pollut. Bull.* **2021**, *173*, 113095. [[CrossRef](#)]
50. Rota, E.; Bergami, E.; Corsi, I.; Bargagli, R. Macro- and Microplastics in the Antarctic Environment: Ongoing Assessment and Perspectives. *Environments* **2022**, *9*, 93. [[CrossRef](#)]
51. Siegfried, M.; Koelmans, A.A.; Besseling, E.; Kroeze, C. Export of Microplastics from Land to Sea. A Modelling Approach. *Water Res.* **2017**, *127*, 249–257. [[CrossRef](#)]
52. van Franeker, J.A.; Law, K.L. Seabirds, Gyres and Global Trends in Plastic Pollution. *Environ. Pollut.* **2015**, *203*, 89–96. [[CrossRef](#)]
53. Wooddall, L.C.; Sanchez-Vidal, A.; Canals, M.; Paterson, G.L.; Coppock, R.; Sleight, V.; Calafat, A.; Rogers, A.D.; Narayanaswamy, B.E.; Thompson, R.C. The Deep Sea is a Major Sink for Microplastic Debris. *R. Soc. Open Sci.* **2014**, *1*, 140317. [[CrossRef](#)]
54. Han, M.; Niu, X.; Tang, M.; Zhang, B.T.; Wang, G.; Yue, W.; Kong, X.; Zhu, J. Distribution of Microplastics in Surface Water of the Lower Yellow River Near Estuary. *Sci. Total Environ.* **2020**, *707*, 135601. [[CrossRef](#)]
55. McEachern, K.; Alegria, H.; Kalagher, A.L.; Hanse, C.; Morrison, S.; Hastings, D. Microplastics in Tampa Bay, Florida: Abundance and Variability in Estuarine Waters and Sediments. *Mar. Pollut. Bull.* **2019**, *148*, 97–106. [[CrossRef](#)]
56. Waite, H.; Donnelly, M.; Walters, L. Quantity and Types of Microplastics in the Organic Tissues of the Eastern Oyster *Crassostrea virginica* and Atlantic Mud Crab *Panopeus herbstii* from a Florida Estuary. *Mar. Pollut. Bull.* **2018**, *129*, 179–185. [[CrossRef](#)]
57. Gray, A.D.; Wertz, H.; Leads, R.R.; Weinstein, J.E. Microplastics in Two South Carolina Estuaries: Occurrence, Distribution, and Composition. *Mar. Pollut. Bull.* **2018**, *128*, 223–233. [[CrossRef](#)]
58. Baechler, B.R.; Stienbarger, C.D.; Horn, D.A.; Joseph, J.; Taylor, A.R.; Granek, E.F.; Brandre, S.M. Microplastic Occurrence and Effects in Commercially Harvested Finfish and Shellfish: Current Knowledge and Future Directions. *Limnol. Oceanogr. Lett.* **2020**, *5*, 113–136. [[CrossRef](#)]
59. Martinelli, J.C.; Phan, S.; Luscombe, C.K.; Padilla-Gamino, J.L. Low Incidence of Microplastic Contaminants in Pacific Oysters (*Crassostrea gigas* Thunberg) from the Salish Sea, USA. *Sci. Total Environ.* **2020**, *715*, 136826. [[CrossRef](#)]
60. Wheat, E.; Ruesink, J.L. Commercially-Cultured Oysters (*Crassostrea gigas*) Exert Top-Down Control on Intertidal Pelagic Resources in Willapa Bay, Washington, USA. *J. Sea Res.* **2013**, *81*, 33–39. [[CrossRef](#)]
61. Bougrier, S.; Geairon, P.; Deslous-Paoli, J.M.; Bacher, C.; Jonquieres, G. Allometric Relationships and Effects of Temperature on Clearance and Oxygen-Consumption Rates of *Crassostrea gigas* (Thunberg). *Aquaculture* **1995**, *134*, 143–154. [[CrossRef](#)]
62. Coughlan, J. The Estimation of Filtering Rate from the Clearance of Suspensions. *Mar. Biol.* **1969**, *2*, 356–358. [[CrossRef](#)]
63. Dupuy, C.; Vaquer, A.; Lam-Hoai, T.; Rougier, C.; Mazouni, N.; Lautier, J.; Collos, Y.; Le Gall, S. Feeding Rate of the Oyster *Crassostrea gigas* in a Natural Planktonic Community of the Mediterranean Thau Lagoon. *Mar. Ecol. Prog. Ser.* **2000**, *205*, 171–184. [[CrossRef](#)]
64. Kobayashi, M.; Hofmann, E.; Powell, E.N.; Klinck, J.; Kusaka, K. A Population Dynamics Model for the Japanese Oyster, *Crassostrea gigas*. *Aquaculture* **1997**, *149*, 285–321. [[CrossRef](#)]
65. Powell, E.; Hofmann, E.; Klinck, J.; Ray, S. Modeling Oyster Populations: I. A Commentary on Filtration Rate: Is Faster Always Better? *J. Shellfish Res.* **1992**, *11*, 387–398.
66. Galimany, E.; Freeman, C.J.; Lunt, J.; Domingos, A.; Sacks, P.; Walters, L. Feeding Competition between the Native Oyster *Crassostrea virginica* and the Invasive Mussel *Mytella charruana*. *Mar. Ecol. Prog. Ser.* **2017**, *564*, 57–66. [[CrossRef](#)]
67. Grizzle, R.E.; Greene, J.K.; Coen, L.D. Seston Removal by Natural and Constructed Intertidal Eastern Oyster (*Crassostrea virginica*) Reefs: A Comparison with Previous Laboratory Studies, and the Value of In Situ Methods. *Estuar. Coasts* **2008**, *31*, 1208–1220. [[CrossRef](#)]
68. Phuong, N.N.; Poirier, L.; Pham, Q.T.; Lagarde, F.; Zalouk-Vergnoux, A. Factors Influencing the Microplastic Contamination of Bivalves from the French Atlantic Coast: Location, Season and/or Mode of Life? *Mar. Pollut. Bull.* **2018**, *129*, 664–674. [[CrossRef](#)]
69. Mak, C.W.; Tsang, Y.Y.; Leung, M.M.L.; Fang, J.K.H.; Chan, K.M. Microplastics from Effluents of Sewage Treatment Works and Stormwater Discharging into the Victoria Harbor, Hong Kong. *Mar. Pollut. Bull.* **2020**, *157*, 111181. [[CrossRef](#)] [[PubMed](#)]
70. Bergman, M.J.; Donnangelo, L.J. Simulation of Freshwater Discharges from Ungauged Areas to the Sebastian River, Florida. *J. Amer. Water Res. Assoc.* **2000**, *36*, 1121–1132. [[CrossRef](#)]
71. Kim, Y.T. Water Balance and Flushing Time in the Restricted Indian River Lagoon (IRL), Florida USA. *Ocean Polar Res.* **2003**, *25*, 75–87. [[CrossRef](#)]

72. USGS Surface Water Data for the Nation. Available online: <https://waterdata.usgs.gov/nwis/sw> (accessed on 21 August 2022).
73. Law, K.L.; Morét-Ferguson, S.; Maximenko, N.A.; Proskurowski, G.; Peacock, E.E.; Hafner, J.; Reddy, C.M. Plastic Accumulation in the North Atlantic Subtropical Gyre. *Science* **2010**, *329*, 1185–1188. [[CrossRef](#)]
74. Ji, Z.G.; Hu, G.; Shen, J.; Wan, Y. Three-Dimensional Modeling of Hydrodynamic Processes in the St. Lucie Estuary. *Estuar. Coast Shelf Sci.* **2007**, *73*, 188200. [[CrossRef](#)]
75. Loosanoff, V.L. Some Aspects of Behavior of Oysters at Different Temperatures. *Biol. Bull.* **1958**, *114*, 57–70. [[CrossRef](#)]
76. Walters, L.; Phlips, E.; Badylak, S.; McClenachan, G.; Sacks, P.; Donnelly, M. Field Observations of the Negative Association between Recruitment of the Eastern Oyster *Crassostrea virginica* and the Brown Tide *Aureoumbra lagunensis* in Mosquito Lagoon, FL. *Fla. Sci.* **2021**, *84*, 81–91.
77. Walters, L.; Roddenberry, A.; Crandall, C.; Wayles, J.; Donnelly, M.; Barry, S.; Clark, M.; Escandell, O.; Hansen, J.C.; Laakkonen, K.; et al. The Use of Non-Plastic Materials for Oyster Reef and Shoreline Restoration: Understanding What is Needed and Where the Field is Headed. *Sustainability* **2022**, *14*, 8055. [[CrossRef](#)]
78. Morris, R.; Bilkovic, D.; Boswell, M.; Bushek, D.; Cebrian, J.; Goff, J.; Kibler, K.; La Peyre, M.; McClenachan, G.; Moody, J.; et al. The Application of Oyster Reefs in Shoreline Protection: Are We Over-Engineering for an Ecosystem Engineer? *J. Appl. Ecol.* **2019**, *56*, 1703–1711. [[CrossRef](#)]
79. Morris, R.; Bilkovic, D.; Boswell, M.; Bushek, D.; Cebrian, J.; Goff, J.; Kibler, K.; Le Peyre, M.; McClenachan, G.; Moody, J.; et al. Large-Scale Variation in Wave Attenuation of Oyster Reef Living Shorelines and the Influence of Inundation Duration. *Ecol. Appl.* **2021**, *31*, e02382. [[CrossRef](#)]
80. Beck, R.W. *National Post-Consumer Plastics Recycling Report*; American Plastics Council, Division of American Chemistry Council: Arlington, VA, USA, 2004.
81. Delle Chiaie, K.R.; McMahon, F.R.; Williams, E.J.; Price, M.J.; Dove, A.P. Dual-Catalytic Depolymerization of Polyethylene Terephthalate. *Polym. Chem.* **2020**, *11*, 1450–1453. [[CrossRef](#)]
82. Moore, C.J. Synthetic Polymers in the Marine Environment: A Rapidly Increasing, Long-Term Threat. *Environ. Res.* **2008**, *108*, 131–139. [[CrossRef](#)]
83. Jaffe, M.; Easts, A.J.; Feng, X. Polyester Fibers. In *Thermal Analysis of Textiles and Fibers. The Textile Institute Book Series*; Woodhead Publishing: Sawston, UK, 2020; pp. 133–149.
84. Koh, L.D.; Cheng, Y.; Teng, C.P.; Khin, Y.W.; Loh, X.J.; Tee, S.Y.; Low, M.; Ye, E.; Yu, H.D.; Zhang, Y.W.; et al. Structures, Mechanical Properties and Applications of Silk Fibroin Materials. *Prog. Polym. Sci.* **2015**, *46*, 86–110. [[CrossRef](#)]
85. Smith, N. The Intertidal Volume of Florida’s Indian River Lagoon. *Fla. Sci.* **1992**, *55*, 209–218.