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Low concentrations and low spatial variability of marine microplastics in oysters (*Crassostrea virginica*) in a rural Georgia estuary

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ABSTRACT

Microplastics are an emerging concern for the health of marine ecosystems. In the southeastern US, the filter-feeding Eastern oyster, *Crassostrea virginica*, is susceptible to microplastic ingestion. We quantified the distribution of microplastics within adult oysters (harvestable size > 7.5 cm) from 28 reefs throughout a rural estuary with limited riverine inputs (St. Catherines Sound, Georgia). To determine which variables best predict microplastic concentration in oysters, we also quantified oyster recruitment, distance to ocean, fetch, and water body width. Oysters averaged 0.72 microplastic particles per individual (0.18 particles per gram wet mass); microfragments and microplastics were equally abundant. Although microplastic concentrations were low, multivariate models identified a positive effect of water body width on the site-level concentration of plastic microfibers; average microfragment length was affected by fetch. Our work informs a growing understanding of microplastic distribution in coastal estuaries, providing an important rural contrast to the urbanized estuaries that have been examined.

1. Introduction

Plastic debris has been reported in marine environments since 1975 and has been increasing both in quantity and effects on marine wildlife (Andrady, 2011; Azzarello and Van Vleet, 2007; Eriksson and Burton, 2009; Laist, 2011; Lee et al., 2013). Recent estimates project that between 4.8 and 12.7 million metric tons of plastic enter the ocean from land sources each year (Jambeck et al., 2015), and the breakdown of this waste into microplastics (plastics < 5 mm in length) and potential subsequent ingestion by smaller marine organisms is of rising concern. Microplastics enter ocean systems via two general pathways: (1) in the form of microbeads, capsules, industrial scrubbers, microfibers from textiles, and other particles produced from plastic manufacturing processes, or (2) from the breakdown of larger plastic debris already present in the ocean or beach litter (Lusher et al., 2014).

Although the production, use, and disposal of plastic materials into marine systems continues to increase, the consequences of plastic breakdown and plastic ingestion for marine species is sparsely investigated due to the labor and logistics involved in assessing their distribution and abundance, both within the water column, sediments, and marine organisms themselves (Doyle et al., 2011). Coastal ecosystem hydrodynamics (e.g., wave and tide induced turbulence, freshwater induced stratification, and plume fronts) greatly influence microplastic inputs into the marine environment and determine particle dispersal, suspension, and settling pathways (Krelling et al., 2017; Lima et al., 2015; Sadri and Thompson, 2014; Vermeiren et al., 2016). Specifically, estuaries, which exhibit high suspended inorganic and organic particle content, are subject to these hydrodynamic processes, especially in areas where rivers contribute to microplastic pollution and where additional sediment influx may interact with particle density, size, and charge, leading to increased aggregation and deposition (Besseling et al., 2017). In addition to natural estuarine hydrodynamic processes, microplastic concentration and spatial distribution is influenced by anthropogenic pressures such as high coastal population densities (Frère et al., 2017; Li et al., 2018; Waite et al., 2018).

Other studies suggest there may be little spatial distribution patterns of microplastic concentrations in relation to human population densities. For example, regression analysis conducted by Nel et al. (2017) found no relationship between municipal populations and microplastic density in sediment and water column samples across a gradient of human population densities across 16 sites along the eastern, western, and southern coasts of South Africa. However, water column samples collected from two densely populated estuarine bays showed significantly higher values than sandy beach sites. This weak association between microplastic pollution and population density suggests the importance of long-range distribution via coastal hydrodynamics from point sources, such as harbors, estuarine bays, and metropolitan hubs (Nel et al., 2017; Nel and Froneman, 2015). Similarly, Ling et al. (2017)

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quantified microplastic concentrations in marine sediments across 42 coastal and estuarine sites across south-eastern Australia and found positive correlations between (1) microplastic filaments and wave exposure and (2) microplastic particles with finer sediments, indicating hydrological and sediment-matrix properties are important for particle deposition and retention. However, overall microplastic concentrations were ubiquitous across sampling sites (Ling et al., 2017).

Due to their small sizes and various densities, microplastics have the potential to enter biological systems at various trophic levels, disrupting physiological processes and affecting organismal health (Wright et al., 2013). Because microplastics have a potential to infiltrate plankton and sediments, both suspension and deposit feeders face the risk of ingesting microplastic particles, either directly from the water column or indirectly through the consumption of microalgae containing microplastics (Avio et al., 2015; Browne et al., 2008). In marine species including phytoplankton (*Scenedesmus spp.*), polychaete worms (*Arenicola marina*), blue mussels (*Mytilus edulis*), and a variety of fish and fish larvae, ingested microplastic particles have been shown to compromise feeding and digestion by directly blocking digestive routes and reducing uptake of viable food sources, or indirectly via adverse effects on other biological processes such as reproduction, metabolism, and liver filtration, which can shift energetic allocation from feeding to immune maintenance (Bhattacharya et al., 2010; Köhler, 2010; Lee et al., 2013; Mazurais et al., 2015; Oliveira et al., 2013; Rochman et al., 2013; Sussarellu et al., 2016; Wright et al., 2013). Despite concerns regarding ingestion, and a limited but growing number of studies that have examined the microplastic ingestion of filter-feeding bivalves, including oysters (e.g., Cole and Galloway, 2015; Sussarellu et al., 2016), we still know little about the factors contributing to accumulated microplastic concentrations in organisms.

Oyster reefs in estuarine systems have important ecological and economic value. They provide structured habitat for other species, protect shorelines from erosion by stabilizing sediments and providing wave attenuation, and are an important commercial shellfish for seafood industries (Coen et al., 2007; Grabowski et al., 2012). However, oysters have been negatively affected by numerous anthropogenic factors over the last few decades including overharvesting, habitat loss, ocean acidification and toxin exposure (MacInnes and Calabrese, 1979; Kurochkin et al., 2009). As the ubiquity of microplastics and their effects on oysters are increasingly understood, understanding the factors driving microplastic ingestion will be critical for maintaining healthy ecosystems. Certain types of microplastics have been shown to affect oysters' energy uptake and allocation, reproduction, and offspring performance (Sussarellu et al., 2016). However, in other cases, oyster growth, respiration, and filtration rates have been robust to effects from microplastics, especially compared to other benthic fauna that have been shown to decrease in abundance with exposure to microplastics (Green, 2016). Maintaining the health of this important ecosystem engineer is critical for estuarine ecosystem functioning (Byers et al., 2006; Gutiérrez et al., 2003).

Oyster populations (*Crassostrea virginica*) in Georgia are buoyed by high density and recruitment, which exhibit the highest values in the Atlantic southeastern US (Byers et al., 2015). Oysters in Georgia may benefit from inhabiting a largely undeveloped coastline that is sparsely populated relative to other coastlines of the southeastern US. A sparser human population along the coastline may also contribute lower ambient microplastic concentrations to the water system since rural coastlines have been shown to have lower concentrations of primary and secondary microplastics (Li et al., 2018).

We quantified estuarine-scale spatial variation in the concentration of microplastic particles in oysters within a relatively isolated estuary with minor riverine input compared to other Georgia estuaries, and determined environmental variables associated with microplastic variation. We measured five environmental variables at each reef where we collected oysters that we thought would influence, or covary with, microplastic concentrations in oysters, including width of the water body, fetch, intertidal elevation, distance from the ocean, and oyster recruitment. First, we predicted that oysters on reefs located within wider, more exposed parts of the estuary would contain a greater quantity of microplastics than reefs located in smaller constricted areas. Water body width may affect microplastics directly by controlling the volume or velocity of water flow, which could affect the concentration of particulates such as microplastics and also influence oyster filtration (Lenihan et al., 1996). Water body width could also indirectly affect microplastic concentrations by affecting the size and shape of the reef since larger water bodies generally have larger reefs with more complex shapes that could trap more settling particles. Second, fetch, or the distance traveled by wind across open water, influences how much wave energy reaches a reef, which determines the volume of water and seston delivered, as well as the flushing potential (Nordstrom and Jackson, 2012). Third, intertidal elevation could affect microplastic concentrations in oysters because it controls the flow speed of water to which oysters are subjected, how long oysters are submerged and can feed, and the settlement propensities of microplastics of various densities. Fourth, we predicted that distance to the ocean would reflect the degree of oceanic influence, such as energy, water density and microplastic concentrations themselves. Reefs that are farther from the open ocean may have greater exposure to microplastics that often wash into estuaries from riverine sources. Fifth, oyster recruitment might mirror the deposition of other waterborne particulates like microplastics if hydrodynamics accumulate larvae and microplastics in the same places. Alternatively, microplastics might be negatively correlated with recruitment if the densities of microplastics are sufficiently different from larvae and get flushed out of high flow areas that oyster larvae prefer. All of the above hypothesized associations of microplastic concentration with environmental variables, however, may be muted if the overall abundance of microplastics is low due to the estuary's rural setting.

2. Methods and materials

Site Selection and Field Data Collection — To quantify microplastic content in oyster tissue and its association with biological and physical habitat characteristics, in summer 2017 we collected adult oysters of harvestable size (shell length $>$ 7.5 cm, which are usually at least two years old) from 30 intertidal reefs within 90 km^2 of estuary between St. Catherines and Sapelo Sounds (31.664845° N, 81.219921° W) (Harding et al., 2008; Southworth et al., 2010). This estuary (hereafter referred to as St. Catherines estuary) is situated within a non-urbanized area of the Georgia coast and has no major riverine inputs. Oysters are strictly intertidal in Georgia, and they range from approximately 0.155 m to 1.20 m above MLLW. For each reef, we took the elevational band and roughly divided it into thirds (high, medium, and low tidal elevation). To ensure we had enough oysters for processing 14 g of tissue, at each reef we collected 6 to 10 oysters from each of the three categorical intertidal elevations on the reef (high, medium, and low). We wrapped the oysters in industrial strength tinfoil and placed them in a cooler to transport to the lab for further processing. The collections came from reefs across the full range of water body widths, from creeks to sounds, that had been sampled the previous year for oyster larvae recruitment (see below). At each site, we took GPS coordinates using the Avenza Maps application for smartphones (Avenza Systems Inc., 2017) and imported them in ArcGIS 10.5 software to enable spatial analysis and visualization.

From July to October 2016 we measured monthly oyster recruitment over its predominant recruitment period throughout the study area using larval collection devices called "spat sticks" that were mounted vertically approximately 0.2 m above each reef. Spat sticks were corrugated PVC pipes infused with calcium carbonate that through their rugose surface and composition mimic adult oyster shells, attracting oyster larvae to settle (Johnson and Smee, 2014, 2012; Byers et al., 2015). They were 15 cm long and 2 cm in diameter (surface

area = 0.0094 m^2). The spat sticks were deployed for one month, removed for counting recruits, and replaced with clean spat sticks for the next month. At the end of the 4-month measurement period, the average monthly oyster recruitment was calculated for each reef.

Predictor Variables obtained through GIS Analysis — In addition to oyster recruitment, which was measured in the field, we also quantified three other predictor variables at each collection site through GIS analyses, creating data layers (rasters) in ArcGIS 10.5. First, we calculated water body width by (1) creating an overwater-distance-to-land layer, (2) using arcHydro (Leonardo, 2017) to create a water body centerline, (3) creating an overwater-distance-to-centerline layer, and (4) multiplying every layer pixel by two. Second, we calculated fetch using the Waves ArcGIS toolbox (Rohweder et al., 2012). Wind speed and direction model input was calculated using wind data (2006–2015, Weather Underground.com) from St. Simons Island, GA for 16 compass arcs (22.5° each). Third, we quantified distance to the ocean by classifying water east of the barrier islands in our study area as ocean, and then creating the shortest overwater-distance-to-ocean layer. Oyster reef polygons were converted to points and all layer data (water body width, fetch and distance to ocean) were extracted to reef points.

Laboratory Analysis — After collection, we shucked the oysters using prewashed oyster knives inside a ventilation chamber to prevent aerial microplastic contamination. The chamber was constructed to cover the work bench using PVC pipes, plastic sheets, and duct tape (Fig. S1). Glass panels were inserted to provide a clear view inside the benchtop chamber. A fan was placed above a central opening at the top of the chamber to continuously ventilate the chamber and move air upwards, minimizing contamination during the shucking process. To standardize the biomass of tissue analyzed and to account for variability in the size of individual oysters, for each of the three tidal elevations on each reef, we haphazardly selected and shucked a subsample of 3 to 5 adult oysters from the total collected in the field to yield a single sample of ~14 g of oyster wet mass. One site was an extreme case where 7 adult oysters were used to reach the ~14 g wet mass. The entirety of oyster tissue from each oyster shucked was used. The tissue was placed in a pre-washed 200-mL glass jar and stored in a freezer for further processing.

To separate the microplastics from the oyster tissue, we followed a protocol to dissolve tissue (Karami et al., 2017). After thawing each pooled oyster tissue sample, we added 10% KOH solution (Oakwood Chemical) in a 1:10 ratio (oyster mass/volume of solution). Thus, a 15.0 g tissue sample received 150 mL of 10% KOH solution. The samples were incubated at 40 °C for 72 h in an incubator. Each sample was stirred with a metal knife once a day to ensure complete mixing and efficient digestion. The knife was rinsed with DI water in between each sample and dried with a kimwipe. After incubation, each sample was poured over a 35-μm sieve and rinsed thoroughly with deionized water. We included an additional density separation step to further isolate microplastic pieces, because enough digestion resistant materials (e.g., shell fragments) remained in the samples after the KOH digestion and sieving process. We therefore transferred contents collected on the sieve to a 15 mL falcon tube, added 1.5 g/ml of 4.4 M NaI solution (Alfa Aesar), and centrifuged it at 1610 G for 5 min (Karami et al., 2017). Afterwards, we poured the supernatant over a vacuum filtration apparatus with a Whatman No. 540 filter paper. Filter papers were removed and placed into a labeled tinfoil packet and briefly placed in a drying oven (70 °C, 5–10 min) to prevent wet particles from sticking to the foil packets. The tin foil packets were then sealed and stored at room temperature for further examination. Given the available space in the incubator, the samples were processed in three batches. For each batch, two procedural control blanks were processed starting with a clean 200-mL glass jar at the KOH digestion stage to account for contamination in each batch.

Next, we did a visual inspection of each filter paper to count and categorize microplastics. To minimize airborne contamination, we constructed a wooden viewing station using standard plywood (Fig. S2). We prepared the viewing station by positioning a dissecting microscope (3.5X-90X magnification), a light source, a candle, and a watch glass. The entire viewing station was thoroughly cleaned before visual inspection using a compressed air cleaner to remove any loose particles from the area. Additionally, forceps and a sewing needle were cleaned with deionized water and dried before placing into the viewing station.

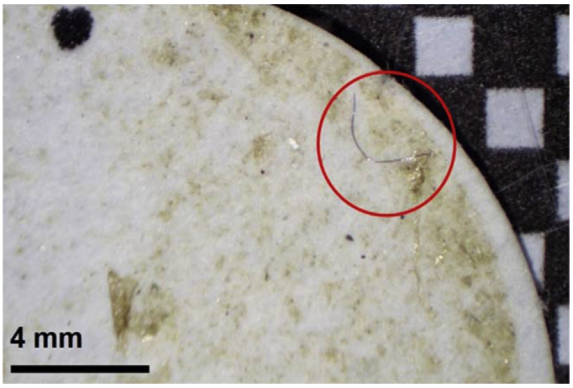

Fig. 1. Filter paper section containing a microfiber particle, circled in red. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

We divided each filter paper into six sections to allow for optimal image capturing and examination. We counted microplastics and categorized them in three categories: fibers, beads, and fragments of irregular shape. If it was unclear whether the particle was made of plastic (e.g., if it had a clear or non-descript color), we performed the "hot needle test", whereby plastic particles will move or melt in reaction to the heat of the needle, and natural fibers will not react to the heat (De Witte et al., 2014; Hidalgo-Ruz et al., 2012; Karlsson et al., 2017). We then took pictures of the six sections of each filter paper using a Nikon digital camera to measure lengths of the identified microfibers. From the images, we measured microfiber and fragment lengths (mm) using the "freehand line" tool in ImageJ (Abramoff et al., 2004). A standard scale of 4 mm was included in every picture (Fig. 1).

For statistical analyses, we used as response variables the number of fibers and the number of fragments, as well as the total number of microplastics. Because micro-bead abundance was negligible (n = 1), we did not use microbeads as a separate response variable. The average microfiber length and average microfragment length were each calculated by averaging the lengths of all fibers and fragments, respectively, in a sample.

Quality Control — To prevent contamination throughout our procedure, all glassware was washed with a commercial dishwashing liquid, rinsed with deionized water, and dried in a drying oven (70 °C, 24 h). Cotton lab coats, nitrile gloves, and 100% cotton clothing were worn during the entire experiment. The lab procedures were carried out in a fume-hood or ventilation chamber to prevent potential contamination with airborne microplastics. As aforementioned, two procedural blanks (controls) were performed without oyster tissue in parallel with each of the three batches of oyster samples processed to evaluate background contamination.

For the procedural controls (n = 6) we found an average (± SD) of 1.33 (± 1.51) microplastic particles per filter paper, composed of 1.17 (± 1.47) microfibers and 0.167 (± 0.41) microfragments. Most of this contamination was observed in the first batch of samples, representing eight reefs. Specifically, in the two blanks for batch one, we found 5 fibers and 1 fragment, out of the total 7 fibers and 1 fragment across all six blanks. Although the number of particles found across all the blanks was low and similar to other studies (e.g., Su et al., 2018), batch one was the only batch that contained any appreciable signs of contamination. Thus, for each oyster filter paper sample in batch one, we

Table 1
Summary of microplastic distribution. A total of 1192.28 g of oyster tissue and 308 oysters were collected from 28 reefs in St. Catherines estuary, Georgia, USA. The average mass of oyster tissue per site (± SD) was 42.58 g (± 1.796) and the average number of oysters per site was 11 (± 2.13). The mean wet tissue mass per oyster (calculated by dividing collective mass at the site level by the number of oysters sampled and averaging over the 28 sample sites) was 3.86 g (± 0.947). One micro-bead was found throughout the entire study. Total microplastic and microfiber numbers reported here reflect the correction for the low microfiber contamination in the first processed batch of oysters.

	Total particle #	Total abundance per site (42.58 g oyster tissue) (± SD)	Site-level average abundance per g oyster tissue (± SD)	Site-level average abundance per oyster (± SD)
Total Microplastics	213	7.61 (± 3.26)	0.178 (± 0.074)	0.724 (± 0.339)
Microfibers	105	3.75 (± 2.41)	0.088 (± 0.055)	0.361 (± 0.246)
Microfragments	107	3.82 (± 2.07)	0.089 (± 0.048)	0.359 (± 0.202)

subtracted out the average number of microfibers found on the two blanks and rounded down to the nearest whole integer (i.e., 2). For a third of the samples in batch one, values were lower than the average blank value, so the final value was bounded at zero. Average length of microfibers and microfragments was not altered since an average of the individual pieces should be robust to a small level of extraneous entries.

Statistical Analysis — All of our sampled reefs had data on water body width, fetch, and distance to ocean. Out of the 30 reefs sampled, 28 had available oyster recruitment data. To include this additional independent variable in complete-case model analyses, we excluded the two reefs missing recruitment data. Because intertidal elevation was a categorical variable, we conducted an initial, separate analysis for the effect of tidal elevation (high, medium, or low) on each of the five response variables: 1) the total number of microplastic particles, 2) the number of microfiber particles, 3) the number of microfragment particles, 4) the average length of microfibers, and 5) the average length of microfragments per 14 g oyster sample from each tidal elevation on each reef. We ran generalized linear regression models (GLMs) on the effect of intertidal height, including site as a random variable, on total microplastics, microfibers, and microfragments using a Poisson distribution and a log link function, and on ln (average microfiber length + 1) and ln (average microfragment length + 1) with a Gaussian distribution and an identity link function. These analyses indicated that intertidal elevation was not a significant predictor of total microplastics ($\chi^2_{2,\ 81} = 2.73$; $p = 0.255$), microfibers ($\chi^2_{2,\ 81} = 2.92$; $p = 0.232$), microfragments ($\chi^2_{2,81} = 0.499$; $p = 0.778$), ln average microfiber length per site ($\chi^2_{2,\ 81} = 0.966$; $p = 0.617$), or ln average microfragment length per site ($\chi^2_{2,\ 81} = 0.207$; $p = 0.902$). Thus, for the remaining analyses we dropped tidal elevation as a variable and pooled the number of microplastic particles for each tidal elevation (high, medium, low) to get overall counts per site (~42 g oyster tissue) for each response variable.

Next, we checked the normality of the distribution for each predictor variable (water body width, fetch, distance to ocean, and oyster recruitment) using the Shapiro–Wilk test in RStudio, Version 1.1.419 (R Studio Team, 2016). We determined that the $\log_{10}$ transformation resulted in more normally distributed data for most of the predictor variables. We also tested for collinearity among our predictor variables and found $\log_{10}$(fetch) and $\log_{10}$(water body width) to be correlated with $R > 0.7$; however, we decided to keep both variables in the model because of their possible complementarity in predicting microplastic concentration. The final predictor variables included in our models were distance to ocean, $\log_{10}$(fetch), $\log_{10}$(water body width), $\log_{10}$(average oyster recruitment). We also found that ln (average microfiber length +1) and ln (average microfragment length +1) were more normally distributed and we used these transformed response variables for analyses.

To determine the most influential physical and biological variables associated with microplastic distribution, we used each of the four independent predictor variables in GLMs with stepwise AIC model competition to select the most parsimonious models as informed by model weights. We ran a GLM on each of the five response variables—total microplastic particles, microfragment particles, microfiber particles, ln (average microfiber length + 1), ln (average microfragment length +1)—using a Poisson distribution and a log link function for the first three, and a Gaussian distribution for the fourth and fifth. We used Kolmogorov-Smirnov tests to verify that the count data were best described with Poisson distributions and the ln $(x+1)$-transformed length data were best described with Gaussian distributions. To help visualize patterns, for each top fitting model we explored the univariate effects of the identified predictor variables on each response variable using log-linear regression models.

3. Results

Microplastic Quantification — Across the 28 sites, in each 42.58 g (± 1.79, SD) oyster tissue sample we found an average (± SD) of 7.61 (± 3.26) microplastic particles, comprised of 3.75 (± 2.41) microfibers, 3.82 (± 2.11) microfragments, and 0.0357 (± 0.189) microbeads. The average length of microfibers was 1.64 mm (± 1.28) and the average length of microfragments was 0.405 (± 0.521) (Table 1). Table 1 also presents these abundances standardized by g of oyster tissue and by the number of oysters included in the collective 42 g sample. Of the corrected total microplastic particles observed (n = 213), microfragments (n = 107) were only slightly more abundant (50.2%) than microfibers (49.3%). Means and ranges for predictor

Table 2
Multivariate GLM Results. Model response variables are total (corrected) microplastic (MP) particles, microfiber particles, microfragment particles, ln (average microfiber length+1), and ln (average microfragment length +1). Model predictor variables are $1og_{10}$water body width (wbw); distance to ocean (distance); $1og_{10}$fetch (fetch); and $1og_{10}$average oyster recruitment (recruit). Pseudo-R^2 values are both the Cragg-Uhler and McFadden pseudo R^2's. logLik is the Log Likelihood. Values displayed in bold indicate a significant result. Table S1 reports the relative rankings of the top three models for each response variable.

Response Variable	Predictor Variables	χ^2	Model Significance	Pseudo-R^2	logLik	Variable Estimate (SE)	Variable Significance
Total MP Particles	Wbw	2.36	p = 0.12	0.08; 0.02	−71.96	0.187 (0.122)	p = 0.126
Microfiber Particles	Wbw	4.04	**p = 0.04**	0.14; 0.03	−62.22	0.352 (0.176)	**p = 0.0461**
Microfragment Particles[a]	Distance	0.70	p = 0.40	0.03; 0.01	−59.29	2.32e-05 (2.77e-05)	p = 0.402
Ln Average Microfiber Length	Wbw	0.49	p = 0.17	0.21; 0.15	−10.36	0.290 (0.159)	p = 0.0792
	Recruit					−0.360 (0.239)	p = 0.145
Ln Average Microfragment Length	Fetch	0.15	**p = 0.04**	−0.26; −0.35	7.78	0.109 (0.054)	p = 0.0551

[a] For the microfragment particles model the intercept-only model was the best, so the second-best model is displayed.

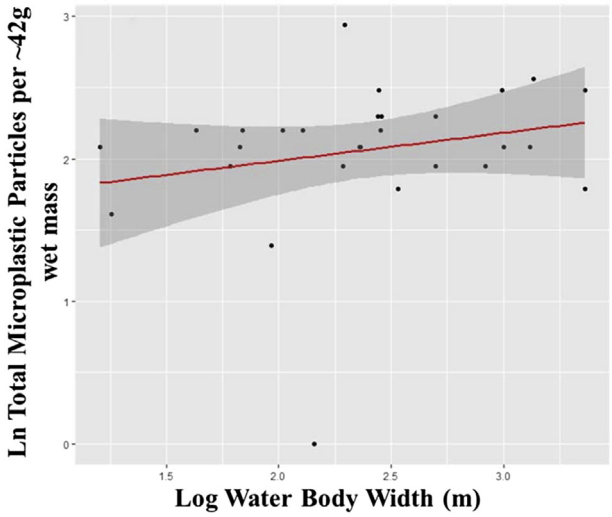

Fig. 2. Total corrected microplastic particles per ~42.5 g of oyster tissue [ln(x +1) transformed] as a function of log water body width (R^2 = 0.0499; p = 0.253). The 95% confidence interval is shown in gray.

variables are reported in Table S2.

The most parsimonious models for total microplastic particles and microfibers contained a single variable—a positive effect of $\log_{10}$(water body width) (Table 2, Figs. 2–3). The model for ln (average microfiber length +1) contained two variables—a positive effect of $\log_{10}$(water body width) and a negative effect of $\log_{10}$(oyster recruitment) (Table 2, Fig. 4a,b). The model for ln (average microfragment length +1) contained a single variable—a positive effect of $\log_{10}$(fetch) (Table 2, Fig. 5). For each of these four response variables, the top model was heavily weighted above other candidate models (Table S1). For total microfragment particles the intercept-only model fit more than twice as well as the next best model, indicating that no variables explained this response well (Table S1). The second-best model had a weak, non-significant effect of distance from the ocean (Table 2).

Although the models identified significant predictor variables, the amount of variability they explained in microplastic abundances was low (Table 2, Figs. 2–5). The range of microplastic particles for sites that had particles was 3–18 (Fig. 6B), and the range in average microfiber length for sites that had microfibers was 0.22–3.88 mm, with

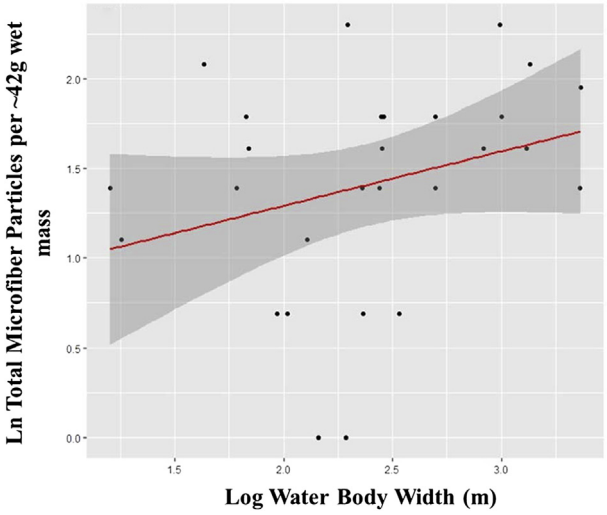

Fig. 3. Total microfiber particles per ~42.5 g of oyster tissue [ln(x+1) transformed] as a function of water body width (R^2 = 0.0833; p = 0.137). The 95% confidence interval is shown in gray.

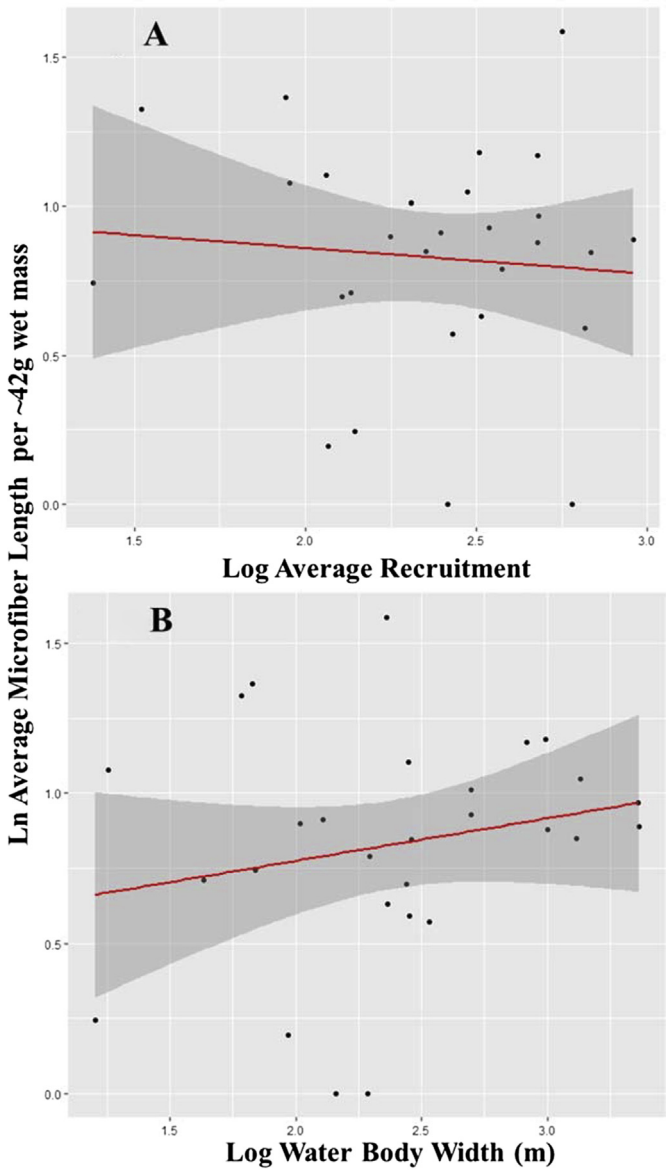

Fig. 4. Average microfiber length [ln(x+1) transformed] as a function of **(A)** log average oyster recruitment (R^2 = 0.0075; p = 0.662) and **(B)** log water body width (R^2 = 0.0456; p = 0.275). The 95% confidence interval is shown in gray.

larger water body width sites having greater values (Table 2, Fig. 6C). For sites that had microfragments, the average microfragment length ranged from 0.14-1.17 mm, with sites with larger fetch having greater values (Table 2, Fig. 6D).

4. Discussion

Overall, microplastic concentration within oysters in St. Catherines estuary was low compared to other similar studies assessing plastic concentration in estuarine organisms (Davidson and Dudas, 2016; Li et al., 2018; Su et al., 2018; Waite et al., 2018). Oysters from our study area contained an average of 0.724 microplastic particles per adult oyster (Table 1). Following a similar protocol for microplastic extraction, Waite et al. (2018) working in a microtidal estuary located along the east coast of Florida, Mosquito Lagoon, found between 7 to 23 microplastic pieces per oyster (Waite et al., 2018), and a study sampling the Middle-Lower Yangtze River Basin in South China found between 1 to 7 particles per oyster (Li et al., 2018). Both studies found a positive

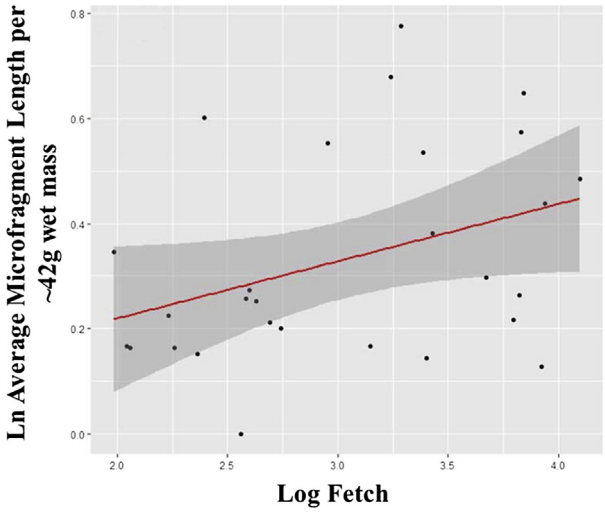

Fig. 5. Average microfragment length [ln(x + 1) transformed] as a function of log fetch (R^2 = 0.134; p = 0.055). The 95% confidence interval is shown in gray.

relationship between microplastic content in the water column and plastic concentration within oysters, as well as increased plastic concentration from samples collected near more urbanized areas. Because our study area is located in a rural watershed, there may be lower ambient plastic concentrations in the water column compared to more developed and urbanized coastal areas. Also, estuarine systems located near river mouths receive additional microplastic inputs from freshwater systems (Horton et al., 2017). St. Catherines estuary is not located near any freshwater rivers, which could also contribute to the relatively low microplastic concentrations observed in our study.

Oysters also likely efficiently expel or purge microplastic particles, a process that may be aided in an environment that is not saturated in plastics. Even in the Waite et al. (2018) study where higher microplastic loads were found in oysters, the concentrations were still far below the levels found in the water column. Their water samples averaged 23.1 microplastic pieces per L, while adult oysters, which can filter multiple liters of water per hour (Ehrich and Harris, 2015), averaged 16.5 microplastic pieces. Eastern oysters (*Crassostrea virginica*) are active suspension-feeders that filter large quantities of fine organic and inorganic particulate matter and excrete it, a process referred to as biodeposition (Nelson et al., 2004). During this process, oysters bind unwanted particulates into pseudo-feces that sink into the sediments preventing the re-suspension of particles back into the water-column (Haven and Morales-Alamo, 1972). Through this process of biodeposition, oyster reefs may play a role in removing plastic particles suspended in the water column and increasing microplastic concentrations in sediments, making reefs potential "hotspots" for microplastic accumulation. Furthermore, oysters might be efficient at purging microplastics from their own tissue, resulting in the low, but relatively consistent residual levels of microplastics that we observed in this study.

Microfragments (50.2%) and microfibers (49.3%) were the predominant microplastic particle types observed. Similar studies focused on microplastic quantification, report microfibers as the predominant particle type (Davidson and Dudas, 2016; Li et al., 2018; Su et al., 2018; Waite et al., 2018). For example, Su et al. (2018) examined microplastics in freshwater Asian clams (*Corbicula fluminea*), water, and sediment from 21 river and estuary sites and reported that microfibers comprised 60–100% of particles found in clams across all sampling sites. Microfibers and microfragments may be more common in filter feeders like clams and oysters because those particle types are more common in the water column, or because they may be harder to expel. In contrast, microbeads, which were < 0.5% of the microplastics found, tend to be smoother and more spherical, and it has been suggested that this allows for easier passage through the digestive system, resulting in faster egestion rates compared to fibers and fragments of varying form and roughness (Sussarellu et al., 2016). The reduced surface area of a bead for a given mass could also make them less buoyant, and therefore less abundant in the water column (Waite et al., 2018).

There are two possible procedural steps we used that can sometimes inflate microplastic counts in organisms. First, we used a hot needle test to distinguish plastics from organic material present on the filter papers (i.e. shell fragments). As illustrated by Song et al. (2015), a Fourier transform infrared spectroscopy (FT-IR) is a more reliable method to discriminate the amount and chemical types of plastics, because microscope identification with the hot needle test may overestimate microplastic abundance (Löder and Gerdts, 2015; Shim et al., 2017; Song et al., 2015). Second, airborne contamination of microplastics can occur from the use of the (1) ventilation chamber during the oyster shucking process, and (2) fume-hood ventilation during the rinsing and transfer of samples into KOH solution. However, the latter was accounted for by including blanks with each batch of samples, which were largely clean. In all cases, these sources did not seem influential due to the very low levels of microplastics we enumerated compared to other studies.

Water body width was a significant predictor of total microfiber concentration, while microfragment concentration was not predicted by any variable (Table 2). Water body width was nearly a significant predictor of ln average microfiber length, while fetch, a highly correlated variable with water body width, was an important predictor of average microfragment size (Table 2). The difference between models for microfibers and microfragments may suggest that their different physical properties influence their distribution and concentration patterns within the estuary. Alternatively, these two sub-categories of microplastics differ in the specific pathways in which they enter marine environments (Browne et al., 2011; Rochman et al., 2019). For example, Browne et al. (2011) showed that certain polyester and acrylic fibers used in clothing closely resembled those found in coastal sediments that receive sewage discharges, suggesting that sewage effluents represent a primary source of microfibers from clothes washing that are not completely retained during wastewater treatment (Browne et al., 2011). Microfragments typically enter marine environments from secondary pathways via breakdown of macroplastics (e.g., plastic bags) already present in the water column (Browne et al., 2011; Lusher et al., 2014; Rochman et al., 2013).

Water body width and fetch are highly correlated environmental variables. Both may have positive effects on microplastics, but the exact details of their positive associations are unknown. Possibly, wider, more exposed parts of the estuary contain a greater quantity of microplastics than reefs located in smaller constricted areas since water body width controls the volume and velocity of water flow, and thus could affect the concentration of particulates. Water body width through its effects on hydrology could also indirectly affect microplastic concentrations by influencing the size and shape of oyster reefs, which in turn influences the tendencies of the reef to physically entrain particles and the filter feeding efficiency of the resident oysters (Lenihan, 1999; Lenihan et al., 1996). Also, fetch influences how much energy reaches a reef, which determines the volume of water and seston delivered, the size of suspended particles, and flushing potential (Nordstrom and Jackson, 2012). However, ultimately it is important to recognize that no variables or variable combinations explained much of the spatial variation in microplastic abundance in oysters (Table 2, Figs. 2–5). The low influence of predictor variables was likely at least in part due to low overall spatial variation in total microplastic abundance or microplastic length per site throughout our estuarine domain (Fig. 6). It would be informative to examine the strength of these environmental variables in areas with higher microplastic levels to see if their influence increases in areas where there is greater spatial variation to explain.

Given the ubiquity of microplastics in aquatic systems, their presence in a rural Georgia estuary is not surprising. However, the microplastic levels in oysters were low, with low spatial variability

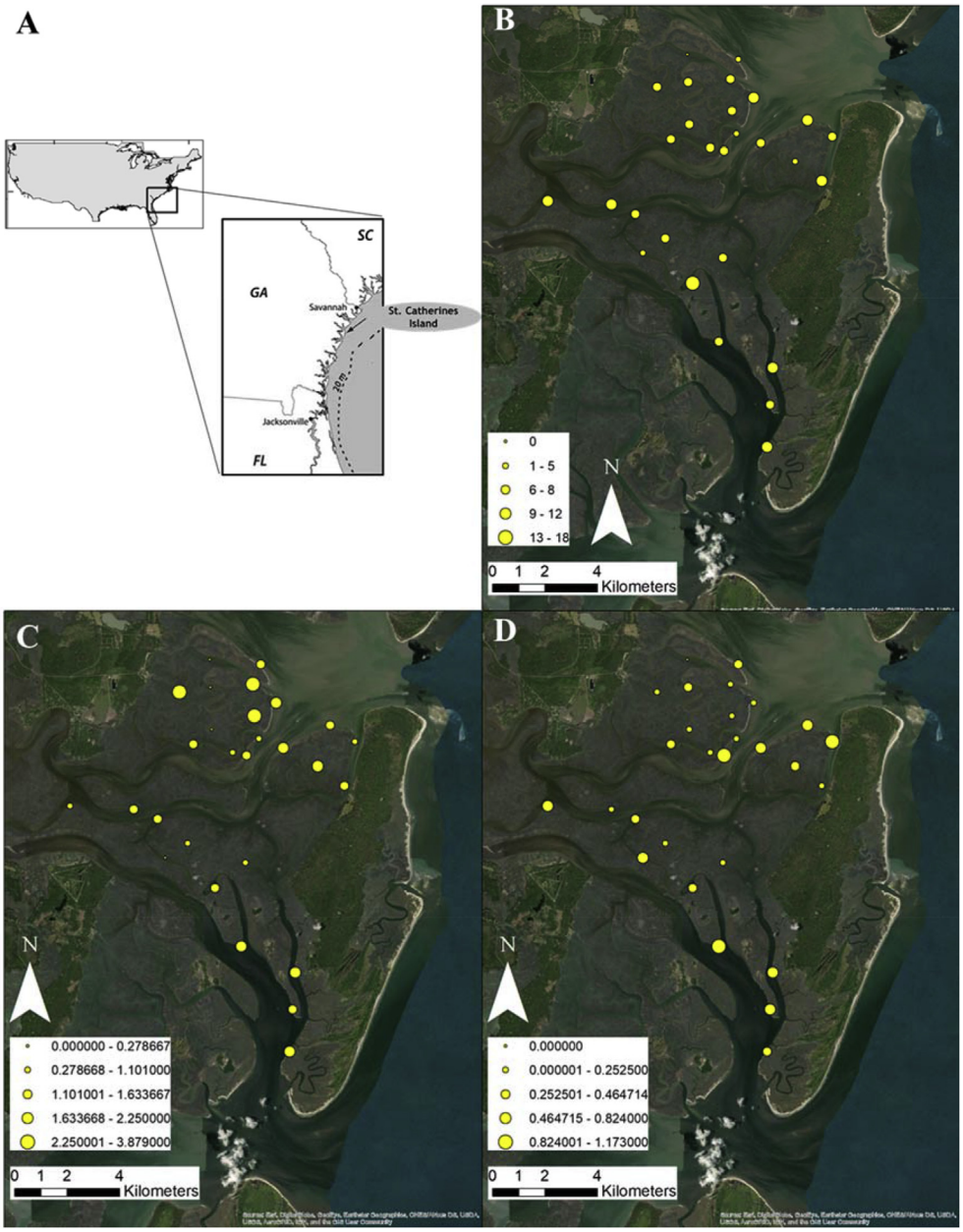

Fig. 6. Oyster reefs sampled in St. Catherines estuary (between St. Catherines and Sapelo Sounds), Georgia **(A)**. The size of yellow dots is scaled proportionately to represent the range of values in ~42.5 g of oyster tissue collected from each site and measured for: **(B)** Total (corrected) abundance of microplastic particles (fibers, fragments, beads); the sizes of circles scale from 0 to 18 particles; **(C)** Average microfiber length (mm); the sizes of circles scale from 0 to 3.88 mm; and **(D)** Average microfragment length (mm); the sizes of circles scale from 0 to 1.17 mm. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

throughout the estuary. The oysters in St. Catherines estuary are likely far removed from the strong physiological effects that have been measured in oysters exposed to sustained high levels of microplastics, such as modified feeding abilities, reduced fecundity, and disrupted reproductive processes, like decreased gamete size, number, and condition (Sussarellu et al., 2016). Studies like ours are important to identify factors driving variation in organismal concentrations of microplastics. Furthermore, our work provides important data to help resolve the growing understanding about the scale of microplastic variation between estuaries, particularly along the continua of rural versus urbanized, and river-influenced versus coastal-dominated systems.

CRediT authorship contribution statement

Clarissa Keisling: Conceptualization, Methodology, Software, Formal analysis, Investigation, Writing - original draft, Visualization. **R. Daniel Harris:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Writing - review and editing, Visualization, Supervision, Project administration, Funding acquisition. **Julie Blaze:** Conceptualization, Methodology, Software, Validation, Investigation, Resources, Data curation, Writing - review and editing, Visualization, Supervision, Project administration. **John Coffin:** Methodology, Software, Writing - review and editing. **James E. Byers:** Conceptualization, Methodology, Software, Validation, Formal analysis, Resources, Data curation, Writing - review and editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2019.110672.

Declarations of interest

None.

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