

Quantifying microplastics in water and sediment along river-marsh transects in the Choptank River

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Highlights

- Microplastic was quantified in water and sediment of the Choptank River.
- Water column microplastic concentrations were higher in the marshes flanking the river than the channel.
- Sedimentary microplastic concentrations were higher at subtidal than intertidal sites.
- Microplastic size distributions are similar between the water column and sediment but show morphological grouping.
- We explore the relationship between river discharge and plastic abundance.

Microplastics (MPs, <5 mm) are pervasive contaminants in aquatic environments. This study quantified MP abundance, distribution, size, and morphology in water and sediment along river-marsh transects of the Choptank River, a major tributary of the Chesapeake Bay. We compare differences in microplastic concentrations due to locations of transects, positions across the river, and seasonality. We also examine patterns in particle sizes due to the potential importance of this characteristic for microplastic transport and fate. In the water column, abundance of microplastics was higher in the marshes flanking the river than the deeper channel at all transects and in all seasons. In the sediment, abundance is higher at subtidal than intertidal sites. Size distributions are comparable between the water and sediment, but show distinct patterns based on particle morphology. Our results agree with expectations based on what is understood about the trapping capacity of salt marsh vegetation and sedimentary depositional tendencies at subtidal sites, improving knowledge of microplastic transport and fate in an estuarine tributary by quantifying abundance and characterizing morphology based on size and shape.

Keywords: microplastics, Chesapeake Bay, salt marsh

1. Introduction

Understanding the transport and fate of microplastics may yield a better understanding of how this suite of pollutants moves through and affects the environment. To begin addressing this formidable challenge, there must be a better understanding of abundance and distribution of these pollutants in different environments such as marine sediment, terrestrial ecosystems including soils, estuaries, and coastal areas supporting aquacultural activities (Kukkola et al. 2022; Baho et al. 2021; Bikker et al. 2020; Hantoro et al. 2019). Plastic is exposed to different environmental forces as it is altered chemically and physically, including disintegration into smaller pieces. Monitoring a diversity of environments (such as the water column and sediment) for microplastic presence and abundance through time will strengthen our knowledge of where plastic tends to accumulate. Hydrodynamic features in an estuary play an important role in structuring distribution patterns of microplastics and sedimentary particles in general—specifically, river discharge, land use patterns, precipitation and wind events, interactions with organisms, and river bathymetry will interact to yield the observed distribution of microplastics. In addition, the characterization of size and shape (Zhang et al. 2017; Li et al. 2022) will help determine how these factors affect movement from source to sink. There is likely a complex interaction between hydrodynamic forces and MPs as they travel through the environment; it is necessary to consider how the properties of a given particle (changes in size, shape, density, surface area and charge, and the amalgam of accreted matter with which it is associated) will be altered as it encounters various environmental forces. The sources of plastic likely to contribute to detection in a given sampling effort should also be considered, whether proximate or much farther away. It is likely that size and shape tend to influence how far a given particle is likely to travel, for example fibers (as compared to fragments or particles) have been shown to move farther away from their source (Engdahl 2018; Lloret et al. 2021).

There has been a recent shift in microplastics research from the open ocean to include terrestrial and freshwater ecosystems (Rochman et al. 2018). Estuaries are a unique environment in which to study microplastics because they may receive input from the land via precipitation or runoff, atmospheric deposition, and the ocean by way of tidal cycles. One of the few studies to examine plastics in the Chesapeake Bay, Bikker et al. (2020) quantified microplastic pollution in the surface layer throughout the Chesapeake Ba, identifying the proportions of different morphologies and plastic types. In contrast with other publications (Wang et al. 2020; Yonkos et al. 2014), this study was unable to correlate population density and land use with microplastic abundance due to complicating hydrological processes within the Bay. In addition, though they recognized wastewater treatment plants (WWTPs) as an important plastic source, the numerous WWTPs in the Chesapeake Bay watershed prevented attributing specific land origins of plastic found in the water. However, as expected, increased microplastic presence was related to heavily populated urban areas.

While other studies have quantified microplastics in different estuaries (e.g., Zhao et al. 2015; Bakir et al. 2014; Ragoobur et al. 2023), data on microplastic abundance on the eastern shore of the Chesapeake Bay is currently limited. A data map published by the NOAA National Centers for Environmental Information shows multiple data points in the Chesapeake Bay are from a single study from Barrows et al. (2018), highlighting the need for further study in this area. Though there are other studies taking place in the Chesapeake Bay (e.g., Yonkos et al.

2014; Bikker et al. 2020), our study sites in the Choptank River provide a targeted, higher resolution sampling effort in different seasons in a rural area on the eastern shore of the Chesapeake Bay. In contrast, Barrows et al. (2018) generated a global dataset to understand large-scale plastic pollution patterns such as coastal areas and the open ocean.

The factors involved in the distribution of microplastics in water and sediment of a tributary of the Chesapeake Bay (and estuaries in general) include tidal forces, freshwater discharge, wind mixing, turbulence, salinity gradients, location and intensity of the estuarine turbidity maximum (ETM), river bathymetry, precipitation, burrowing organisms, and surrounding land use (Malli et al. 2022). This complex interaction of factors results in observed microplastic abundance and distribution and difficulty isolating a single variable responsible for perceived patterns. In addition, there are multiple sources of microplastic that determine the characteristics of these particles and subsequently their distribution in the environment. For example, some studies report the most prevalent morphology to be fibers, but others, especially in estuarine systems, report fragments and films to be most abundant (Yonkos et al. 2014; Bikker et al. 2020). This variability is likely due to differences in sources from which microplastics are generated and the environment into which they are incorporated. Sources include derelict fishing gear, tires and road wear particles, wastewater effluent (including fibers generated from washing synthetic clothing), shopping bags, food and beverage containers, agricultural films, or paint, among others (An et al. 2020). For this reason, the surrounding land use in the estuarine or riverine watershed is an important factor when characterizing microplastic pollution in these environments. This fact underscores the need for microplastics research to move towards unity in sampling and processing workflows, so that the pollution signatures of different environments can be accurately captured rather than obfuscated by artefacts resulting from idiosyncratic methodologies.

Our study samples across three marsh-river transects along the Choptank River situated within rural surroundings. We explore the effect of multiple variables including seasonality, river position (west marsh, channel, and east marsh), and transect location on microplastic abundance, shape, and size, using two data sets: the water column and sediment. It is important to understand both environmental compartments because each is affected differently by meteorological and hydrological conditions; the water column is likely more influenced by transient conditions or events, while the sediment probably reflects better established patterns of river bathymetry and tidal patterns. In addition, sites were selected due to the presence of intertidal marshes flanking the transects to examine how the presence of vegetation, subtidal and intertidal sites, and location at the river edge and further inland affected microplastic abundance. Salt marshes in general represent an understudied habitat in microplastics research but could potentially act as a sink due to the stabilizing effect and buffering capacity of vegetation and abiotic conditions present in the marsh (Lloret et al. 2021; Ogbuagu et al. 2022; Stead et al. 2020).

We test the hypotheses that position in the river, location of transects, and seasonality influence the abundance, distribution, and characteristics (size and shape) of microplastic particles. Specifically, we expect a greater abundance of microplastics at the middle transect directly downstream from a wastewater treatment plant due to its potential to be a major source of plastic pollution. We expect higher concentrations of microplastics in the shallower marshes flanking the river due to the trapping capacity of vegetation and the lower energy and flux of water in these areas; similarly, we predict a higher abundance of microplastics at subtidal sites due to the depositional tendencies in this type of environment. We expect distinct patterns of morphology in the sediment as compared to the water column due to the effect of MP

characteristics on between-compartment transfer rates (Rosal 2021). For example, fibers have been shown to travel further in the water column before settling to the sediment (Engdahl 2018; Lloret et al. 2021).

2. Materials and Methods

2.1 Sample Sites

Three transects in the Choptank River, a major tributary of the Chesapeake Bay, U.S.A., were selected for sampling (Fig. 1). The Choptank River (so named after the area's indigenous inhabitants) is a major tributary of the Chesapeake Bay with a length of 114 km and is situated within a watershed spanning 1,756 km².

Samples were collected twice in spring, summer, and fall, and once in winter over a period of two years (Table 1). At each station, water temperature and salinity were measured using a YSI ProDO 2030 at 0.5 m depth at the shoal stations, and at 1m intervals to within 1m of the bottom at the channel station (Table 2). Sampling transects were chosen such that the up river transect is located upstream of a wastewater treatment plant, which is situated on the western shore of the river. Transects were chosen so that there were intertidal marshes on both the eastern and western boundaries. Three stations were identified for each transect, West, Channel, and East, all of which were sampled to collect microplastics from both the water and the sediment.

Table 1

Samples were collected at least twice during spring, summer, and fall, and once in winter, spanning a period of two years.

Date	Season	Year
18-May	Spring	2022
2-Jun	Summer	2022
14-Sep	Fall	2022
21-Oct	Fall	2022
6-Nov	Fall	2022
2-Mar	Winter	2023
31-May	Spring	2023
27-Jun	Summer	2023
31-Jul	Summer	2023

Table 2

Seasonally averaged water temperature and salinity during sample collection.

Season	N	Temperature °C	Salinity ppt
		(mean ± SD)	(mean ± SD)
Fall	75	19.5±4.44	6.67±2.00
Spring	47	20.57±0.55	4.40±2.38
Summer	72	26.76±1.70	5.61±1.82
Winter	26	9.07±0.26	5.16±3.14

2.2 Sample Collection, Processing, and Quantification

2.2.1 Sample Collection

Water microplastic samples were collected by hand, vertically deploying a 0.5m diameter plankton net fitted with 63 μm nylon mesh from the side of a 6.4m skiff. The mesh size determined the lower size limit for sampling microplastics. Water samples were collected at 0.5 m in either marsh and at variable depth in the channel depending on the maximum depth so as to sample the entire water column. Volume was calculated as the volume of a cylinder with height as water depth, and the diameter of the plankton net was halved to calculate the radius. The net was retrieved and the sample was collected in a cod end; the net was rinsed with a hand-pump powered spray bottle into the cod end to maximize material collected. The cod end was then rinsed into a glass jar using a spray bottle until a visual check determined it was clean of debris. Field blanks were collected to quantify microplastic contamination during sample collection on the boat. The spray bottle, plankton net, and cod end were all rinsed into a collection jar using 63 μm filtered river water to determine the level of contamination from each of these steps in the collection process. Sediment samples were collected with a van Veen grab sampler and contents were transferred with a metal spatula into a glass sample jar. Sediment was collected at the river edge and inland on the 5/18/2022 sampling trip. Sediment was collected at intertidal and subtidal sites during the following sampling trips: 9/15/2022, 3/2/2023, and 10/3/2023. These sampling efforts were in addition to the core plan of sampling at the three transects and three positions during each sampling trip.

2.2.2 Laboratory Processing

Laboratory protocols were modified from the literature (e.g., Yonkos et al. 2014, Prata et al. 2019) and included density separation using hypersaline solution (1.2 g/mL), digestion of organic matter using 30% hydrogen peroxide, vacuum filtration onto a GF/F, and staining with Nile Red. The hypersaline (1.2 g/mL) solution was generated using 1 L of ultrapure, triple filtered, UV sterilized, reverse osmosis (RO) water to which 300 g of table salt was added and stirred with a plastic stir bar until dissolved. This solution was then vacuum filtered through a 0.7 μm glass fiber filter (GF/F) to remove contamination. The density of the solution is greater than the density of commonly used types of plastics, except some PVC, TRWP, and PETE (Table 3), so the density separation should be fairly effective at separating plastic from sediment. Laboratory blanks (Table S2) were generated to quantify contamination by performing every step in the processing workflow without the addition of actual sample. Non-plastic materials were used when possible. All materials were rinsed with tap and DI water before and after use, and all glassware was cleaned with soap and water, and rinsed with DI water.

2.2.2.1 Water Samples

Water samples were poured through a 63 μm sieve and rinsed several times with a wash bottle until the collection jar was clear of debris. Initially, the digestion step was done using a solution of equal parts DI water and 30% hydrogen peroxide, and the sample was allowed to digest overnight. Then, a density separation step with the hypersaline solution was performed

and allowed to settle out for 24 hours, which was followed by vacuum filtration. Due to high organic matter content in some samples, multiple filters were used to reduce the thickness of debris on each filter. There was a change midway through sample processing, and the protocol was revised, such that the remaining water samples were incubated with 30% hydrogen peroxide for three days, after which they were vacuum filtered onto a GF/F. A t-test was performed to compare the difference in microplastic quantification between the two methods, and revealed no significant differences, increasing confidence that there was no systematic bias due to the change in processing ($t=0.63$; $N=7$).

2.2.2.2 Sediment Samples

Sediment samples were analyzed to determine the concentration of microplastics by wet mass using 100g of sediment per sample. In some cases, total sample mass was <100g and thus the whole sample was used. This method involved a density separation followed by an organic matter digestion. Samples were homogenized by stirring and then added to 1 L flasks. For the density separation, 250 mL of the hypersaline solution was added to each flask and agitated for 30 seconds. After allowing the solution to settle for 24 hours, supernatant was removed using a pipette and stored in a covered beaker. 250 mL of fresh salt water solution was added to the sample, stirred, and allowed to settle for 24 hours, after which the second portion of supernatant was removed with a pipette. For the organic matter digestion, both portions were poured through a 63 μm sieve, the contents of the sieve were then rinsed with 30% hydrogen peroxide into a beaker and allowed to digest for 24 hours. The samples were then vacuum filtered onto a glass fiber filter and stained with 0.5 mL of Nile Red at a concentration of 10 μg / mL and stored in the dark until photographed. There may have been a higher concentration of Nile Red used on some samples. Six samples were processed per week.

Table 3
Densities of commonly used plastics.

Plastic	Density (g/mL)
Polypropylene (PP)	0.90-0.91
Low Density Polyethylene (LDPE)	0.92-0.94
High Density Polyethylene (HDPE)	0.95-0.96
Polystyrene (PS)	0.02-1.07
Polyvinyl Chloride (PVC)	1.16-1.45
Polyethylene Terephthalate (PETE)	1.38-1.39
Tire and Road Wear Particles (TRWP)	1.2-1.7

2.2.3 Image Processing

The protocol for digital photography of the samples on the filters was adapted from Bachiller and Fernandes (2011). A Canon T3i was outfitted with a Canon EF-S 18-55mm f/3.5-5.6 II SLR lens, a Tiffen Orange 21 filter, and blue LED ring light with a diameter of 60mm and wavelength of 460. Vertical position of the camera was situated such that the entire filter could be photographed in focus (not blurry). The camera was connected to a laptop and image area was viewed in real time. The blue light was turned on at full intensity, room lights were turned off and the door was closed to darken the surroundings, after which the image was taken.

The raw (.CR2) image files were converted to TIFF format using Photoshop and then opened in ImageJ. The applications MP-VAT and MP-SCALE plugins were downloaded from the website provided in Prata et al. (2020). MP-SCALE calibrates the image size as a reference to enable size classification of particles. The diameter of the filtration apparatus was 38.5 mm and when visible, the area containing the sample was selected using the oval tool and set to this size. Otherwise, the entire filter area was selected and set to 47 mm to reflect the diameter of filters used. Following that, the MP-VAT script was run to quantify microplastic particles on the filter, which yields a file containing the number and characteristics of particles detected (Fig. 2). MP morphology is assigned based on a deviation from circularity, with a value of 1 indicating a perfect circle, and a value of 0 indicating an elongated shape, with the following three labels: particles (0.6 – 1.0), fragments (0.3 – 0.6), or fibers (0.0 – 0.3).

2.2.4 Vegetation Measurement

Vegetation was measured using a quadrat within which stem diameter, stem height, and number of stems were quantified. Measurements were taken at the river edge and approximately two meters inland at different positions along the river. All vegetation measurements were taken during the 5/18/22 sampling trip in the east and west marsh at lower and upper transects. At the lower transect, eleven stems were measured at the river edge and inland, and at the upper transect, four stems were measured at the river edge and inland. Relationships between microplastic abundance and vegetation are reported in supplementary material (Fig. S3).

2.2.5 Statistical Analyses

All statistical analyses were performed in R and Excel. Outliers (see supplemental material - Table S3 & Table S4) were identified using the interquartile range method and removed from further analysis due to the likelihood that they are artifacts. 11 samples were removed from the sediment dataset and four samples were removed from the water dataset. Kruskal-Wallis non-parametric tests were used (due to non-normality of data) to test for statistical significance of differences in microplastic abundance and proportion of fibers due to seasonality, position in the river, and transect location. When significant, Dunn's post-hoc test was performed to determine which groups differed from one another.

3. Results

3.1 Field results

Temperature and salinity (Table 2) were measured during each sampling effort and the patterns were as expected. The temperature was highest in summer and lowest in winter. Salinity decreases upstream, is lowest in the spring, highest in the fall, and intermediate in the summer and winter. Depth of sampling in the river channel varied from 2 m to 5 m and depth of the surrounding marshes varied from 0.5 m to 1 m.

The hydrological context on each sampling date is reported as well (Fig. 3). Sampling took place during average discharge conditions, usually under 2 m³/s and with a maximum of 3.5 m³/s, but sometimes followed high discharge events in the range of 10 – 60 m³/s. There were three extremely high discharge events exceeding 40 m³/s during the sampling timeline, two intermediate discharge events around 30 m³/s, and multiple moderate discharge events ranging from 10 – 15 m³/s. Sample date and seasonally averaged discharge values were obtained from USGS station 1491000 located on the Choptank River near Greensboro, MD (Table S5).

3.2 Laboratory results

3.2.1 Water Samples - Abundance and Particle Types

Mean abundance of plastic in the water column of the channel was below one piece of plastic per L of water (Table 4); mean abundance of plastic in the water column of the marshes flanking the river ranged from two to four pieces of plastic per L of water (Table 4).

We tested the null hypothesis that there was no significant difference in the proportion of fibers to total plastic (Table 5). There was no significant variation in the proportion of fibers due to any of the explanatory variables as determined by Kruskal-Wallis non-parametric statistical test (season ($p=0.5559$), transect ($p=0.7219$), or position ($p=0.7233$)).

We compared plastic concentration in the water across seasons, positions in the river, and location of transects (Fig. 4). Using Kruskal Wallis non-parametric tests we found that the null hypothesis was accepted ($p>0.05$) for all comparisons except for position in the river. Plastic concentrations are highest in the marshes flanking the river ($p=9.131e-09$). There does not appear to be an effect of season on this pattern ($p=0.4975$). The middle transect has a lower mean abundance than either the upper or lower transects, but the difference was not significant ($p=0.7962$).

The relationship between seasonally averaged plastic concentrations and seasonally averaged discharge was reported (Fig. 8); neither was significant.

Table 4

Summary statistics (mean $\pm$ SD) of microplastic concentration (pieces/L) in the water column grouped by transect and position in the river including sample size. Seasonal information is combined and not reported and is not a significant predictor variable.

Transect	Position	Reps	Pieces/L
			(mean $\pm$ SD)
Lower	West	7	2.67 $\pm$ 1.38
	Channel	9	0.212 $\pm$ 0.41
	East	6	2.14 $\pm$ 1.64
Middle	West	8	2.01 $\pm$ 1.51
	Channel	9	0.19 $\pm$ 0.18

Upper	East	8	1.59±1.47
	West	6	2.17±1.82
	Channel	7	0.21±0.23
	East	6	3.37±1.46

Table 5

Summary of proportions of fibers in the water column grouped by transect and position in the river.

Transect	Position	Fiber/Total
Lower	West	0.18
	Channel	0.15
	East	0.2
Middle	West	0.16
	Channel	0.17
	East	0.17
Upper	West	0.16
	Channel	0.16
	East	0.15

3.2.2 Sediment Samples - Abundance and Particle Types

There was below one piece of plastic per g of sediment for all positions, transects, and seasons. We collected four samples at intertidal sites, and six samples at subtidal sites, finding a significant difference in means ($p=0.03$) with a value of 0.16 ± 0.05 at intertidal sites and 1.80 ± 1.31 at subtidal sites (Fig. 6). We collected eight samples at the river edge, and two samples several meters inland from the river edge, and found no significant difference ($p=0.90$) between means (Fig. 6).

There was a significant difference in the proportion of fibers (Table 7) between seasons (Fig. 5), as determined by a Kruskal Wallis non-parametric test ($p=0.0098$). Dunn's post-hoc test shows that fall and spring (unadjusted $p = 0.003$), spring and summer ($p = 0.0101$), and spring and winter ($p=0.0021$) differ from one another. The fiber/total ratio is lower in summer and winter ($p=0.0098$). Dunn's post-hoc test shows that fall and spring (unadjusted $p = 0.003$), spring and summer ($p = 0.0101$), and spring and winter ($p = 0.0021$) differ from one another.

We tested the null hypothesis that microplastic abundance in the sediment did not differ based on position, transect, or season by way of a Kruskal-Wallis non-parametric statistical test (Fig. 5). There was no significant variation in microplastic concentrations at any transect ($p=0.4964$), in any position ($p=0.9627$), or in any season ($p=0.072$) (Table 6). We tested the null hypothesis that there was no variation in microplastic abundance at subtidal as compared to intertidal sites and at the river edge as compared to inland. There was a significantly higher concentration of microplastic at subtidal as compared to intertidal sites ($p=0.03$). There was not a significant difference between the river edge and several meters inland ($p=0.90$).

Seasonally averaged plastic concentrations and seasonally averaged discharge showed only a modest relationship (Fig. 7); sample date averaged discharge and sample date averaged plastic concentration also were not significant.

3.2.3 Size Distributions

Size distributions of particle area show very little distinction between the water column and sediment (Fig. 9). Within morphological classes, particles show the sharpest peak and the most narrow distribution, followed by fragments, and finally fibers have a relatively low peak and a wide distribution in both water and sediment (Fig. 9). Looking at fibers only, the water column has a greater proportion of particles in higher size classes. When distinguishing between transects, the upper transect has a lower proportion of particles in smaller size classes than do the middle or lower transects in the sediment. In the water column, the lower transect has a higher proportion of particles in higher size classes than the middle or upper transect. Size distributions show a majority of MPs in lower size classes, close to our operationally defined lower limit of 63 microns.

3.2.4 River Discharge and Microplastic Concentrations

In order to assess the potential importance of river discharge on observed microplastic concentrations, correlations between river discharge and MP concentrations were generated from the discharge values obtained from USGS station 1491000 located on the Choptank River near Greensboro, MD on each of the sampling dates and seasonally averaged. There is no discernable relationship between discharge values on sample dates and MP concentrations from those dates. Seasonally averaged discharge values and MP concentrations are comparable between the sediment (Fig. 7) and water column (Fig. 8).

Table 6

Summary statistics of sediment samples with intertidal, subtidal, inland, and edge observations removed. Summary statistics (mean $\pm$ SD) of microplastic concentration (pieces/g) in the sediment grouped by transect and position in the river including sample size. Seasonal information is combined and not reported and is not a significant predictor variable.

Pieces/g (mean $\pm$ SD)			
Transect	Positions	Reps	
Lower	West	7	0.24 $\pm$ 0.18
	Channel	7	0.68 $\pm$ 0.66
	East	6	0.60 $\pm$ 0.42
Middle	West	5	0.90 $\pm$ 0.97
	Channel	5	0.48 $\pm$ 0.36
	East	6	0.66 $\pm$ 0.78
Upper	West	7	0.96 $\pm$ 0.96
	Channel	6	0.72 $\pm$ 0.62
	East	6	0.33 $\pm$ 0.17

Table 7

Summary of proportions of fibers in the sediment grouped by transect and position in the river.

Transect	Position	Fiber/Total
Lower	West	0.69
	Channel	0.81
	East	0.51
Middle	West	0.49
	Channel	0.68
	East	0.85
Upper	West	0.8
	Channel	0.77
	East	0.83

4. Discussion

Documenting microplastic abundance, distribution, and morphological characteristics in this environment is a starting point from which more complex information about the implications of plastic pollution can be derived. For example, information about what locations and under which conditions plastic accumulates can be combined with ecological and hydrological knowledge to better understand the functional effects of its presence and mechanisms responsible for its distribution.

4.1 Explanation of results

The automatic quantification program MP-VAT implemented in ImageJ allows for a characterization of shape based on a deviation from circularity, and an approximation of size based on the measured diameter of the filter which provides calibration for the particle quantification and characterization output (Prata et al. 2020). This is important because these characteristics will determine how plastic moves through the environment and interacts with other materials and organisms, including the formation of aggregates that are physically and chemically distinct from plastic particles (de Haan et al. 2019). Interestingly, López et al. (2021) modeled microplastic movement in the Chesapeake Bay and found that particle density, but not size, influenced transport and fate. The study discovered that the vast majority of particles were deposited on land, very few left the Bay, and even fewer remained suspended in the estuarine water column. Our results tend to agree with this distribution pattern, in particular the lower abundance in the river channel as compared to the surrounding marshes, and the higher abundance inland than at the river edge.

The greater abundance of microplastics in water samples collected from the marshes flanking the river (Fig. 4; Table 5) is reasonable considering the greater flux of energy and water through the channel at a given time, which would tend to dilute any particles, including microplastics. Additionally, the trapping capacity of vegetation and resultant dampening of hydrodynamic energy in marsh habitats has the potential to be a sink for sedimentary debris of all kinds (Almeida et al. 2023). Other studies (e.g., Pinheiro et al. 2022; Trusler et al. 2025; Yao et al. 2019) have also found elevated concentrations of microplastics in drier, more heavily vegetated areas of the saltmarsh than the wetter, less colonized areas.

The apparently reduced occurrence of microplastics in the river channel could also be due to plastics being concentrated in a small volume of the total water column, for example in the top

0.5 meters. Other studies have preserved the vertical structure by sampling discrete depths in the water column to better understand the spatial distribution of microplastics. Bagaev et al. (2018) employed this method and found significantly higher concentrations in the top 0.5 meters and near bottom of the water column as compared to intermediate depths. Similarly, Gray et al. (2018) found elevated concentrations of microplastics in the sea surface microlayer. The potential presence of increased MPs in certain vertical positions within the water column reflects the importance of hydrodynamic processes and patterns on distribution structures, including the density of water which can yield distinct layers with the capacity to retain particles and reduce movement. Other studies that employed this technique when sampling sediment have found reduced numbers of microplastics with increasing depth (Li et al. 2020; Uddin et al. 2021). This reflects the increasing use of plastic from past to present and this trend will likely continue as production and use continue unabated. This suggests that aquatic sediments may be a long-term plastic sink and that events (such as intense precipitation or wind, benthic organisms, or human activities) that may disrupt the sediment are not sufficient to resuspend a significant portion of settled MPs.

The paucity of microplastics detected at intertidal sites compared to subtidal sites (Fig. 6) could be explained by the higher energy of the intertidal habitat, including sunlight and the movement of water, which could cause photochemical and mechanical abrasion leading to polymer breakdown or movement out of the intertidal zone. It also suggests that the intertidal zone may not be a long-term plastic sink but is instead a transition zone, whereas the subtidal sites are less exposed to forces causing degradation and movement of microplastics and may allow particles to settle and be retained in this area. However, a cautionary note is warranted when interpreting the data from the intertidal and subtidal sediment sampling. Seven samples were obtained from each site, from which three outliers were removed from analysis from the intertidal site, and one from the subtidal site. Further sampling is needed from these locations to determine whether there is a consistent pattern of decreased abundance at intertidal as opposed to subtidal sites.

Higher abundance of microplastics were observed inland compared to the river edge (Fig. 6), though these were not statistically significant. The pattern could be explained by the greater movement of water at the river edge. This higher energy area will be subject to tidal changes, wind-induced water motion, and mechanical forces of water at the river edge. Further inland, plastics could accumulate and maintain their position, because there is less disruption due to the dynamics of the river. Other studies (Pinheiro et al. 2022; Trusler et al. 2025) have reported this finding as well. Future studies should sample these areas to determine if the suggestion of a pattern suggested by our data is a robust signal.

Temperature and salinity (Table 2) will also affect the deposition of microplastics to the sediment, with potentially fewer microplastic particles reaching the benthos if the density of the water is higher. During dry periods, salinity will be higher, and this could explain changes abundance of microplastics in the sediment in different seasons. The patterns of microplastic abundance in the sediment likely reflect longer-term, more stable patterns of precipitation, wind, settling and tidal forces than the patterns of microplastic abundance in the water column which are probably more subject to transient changes.

More frequent, higher intensity flow events during the fall and spring (Fig. 3) coincide with greater abundance of microplastics in the sediment (Fig. 5), and a higher proportion of fibers (Fig. 5). There could be flushing of land-based plastic into the river which settles into the sediment during these two seasons. That this pattern is not observed in the water column could

be explained by the greater, continuous flux of water through the channel so that high flow events may lead to only transiently elevated concentrations of microplastics in the main channel. Sampling directly after high flow events could enable correlation between discharge and microplastic abundance and establishment of a timeframe for how long higher concentrations linger in the water column of the river channel. There also may be greater between-compartment transfer rates (water column to sediment) during the wet season due to the effect of precipitation on mixing the water column and, specifically, increased vertical motion. Intense or more frequent precipitation events could generate the energy necessary for MPs to move from the water column to the sediment. The increased presence of fibers in the sediment during fall and spring also indicates that this morphological category may react differently to hydrodynamic forcing and therefore demonstrate distinct distribution patterns (Engdahl 2018; Lloret et al. 2021), requiring increased energy to induce vertical movement.

Though there was not a direct significance of season on microplastic abundance in the water column or sediment (Fig. 4; Fig. 5), there were relationships between average seasonal discharge and especially frequent high discharge events (Fig. 7; Fig. 8). Microplastics were more abundant in the sediment during fall and spring, both of which, though comparable in mean to summer and winter, had a much greater incidence of high flow events. Also, the correlation between seasonally averaged discharge and sediment microplastic abundance shows that the relevant seasonal variable could be river discharge. The lesser correlation between seasonally averaged discharge and water column microplastics could be attributed to the greater flushing rates and more frequently altered composition of the water column. Seasonal agricultural activities such as planting, tilling, or harvesting could also influence the abundance of microplastics in the river, as activities in the watershed may create microplastic particles.

Though most (e.g., Barrows et al. 2018), but not all (Wang et al. 2020), studies report fibers as the most abundant morphological category, we did not find this to be the case. This could be due to the particulars of our methodologies, for example the use of a plankton net, which could retain fibers so that while they may be present, we were detecting them in quantities less than their true abundance. There were variations in the proportion of fibers, especially in the sediment, which were elevated in relation to high discharge events in spring and fall (Fig. 5). This may indicate that fibers tend to remain buoyant and travel further in the water column from their source rather than settling to the sediment (Engdahl 2018; Lloret et al. 2021).

Kunz et al. (2023) reports a strong positive correlation between urbanization and microplastic concentration, a negative correlation between forest cover and microplastic concentration, and further, a sharp increase in microplastic abundance at the boundary between urban and rural land use surroundings in the Wu River in Taichung, Taiwan. In contrast, Yin et al. (2020) found a greater abundance of microplastic fibers in sediments of East Dongting Lake in rural versus urban surroundings. Rochman et al. (2022) sampled across various locations in North America including the Chesapeake Bay finding microplastics abundances per liter of water at similar levels to our study (generally less than 1 item per liter). In contrast, Yonkos et al. found significantly lower concentrations of plastic per square kilometer than our study. This is likely due to differences in sampling, notably that they sampled only the top 15 cm of the water column and had a significantly higher lower size limit of 0.33 mm as compared to our lower size limit of 63 μm . This could lead to the observed difference between our detected microplastic concentrations. This discrepancy in sampling methods and resulting inability to compare results is characteristic of microplastics research, and standardization of approaches would help allow for such comparisons. These studies highlight the importance of land use patterns in the

surrounding watershed on structuring MP distributions and demonstrate the interaction between sources of plastic pollution on land and the effect that hydrological events and forcings will have on dispersing contaminants throughout the estuarine environment.

Size is an important measurement in that it will affect the interaction of the microplastic with other particles and affect its movement through the environment. The comparability in two metrics of size measurement between water and sediment could suggest this is not the key variable in determining where and in what type of environment the microplastic is found during a given sampling effort. Other factors such as density or time in the environment could be more important but were not measured in this study.

4.2 Implications and broader impacts

Understanding degradation mechanisms and the products they yield is important in describing the fate and transport of microplastics because as the properties of plastic change, the way they interact with other substances and organisms is subsequently altered. For example, the production of different functional groups following UV exposure or bond cleavage via hydrolysis can cause adsorption of nutrients or heavy metals, for example (Nguyen et al. 2023). This has implications for the leaching and transfer of possibly hazardous materials or organisms and thus affects human and ecosystem health. This highlights the complexity of plastic pollution in that it may indirectly foster the occurrence and prevalence of other issues such as proliferation of harmful organisms or other types of contamination.

In general, the deposition and retention of microplastics in sediment is favored by low-energy conditions (Ghani et al. 2013). However, episodic high flow events may mobilize plastic particles and flush them into the river where they may settle when the energy dissipates. This highlights the need to distinguish between discrete high energy events as compared to a continuously higher energy environment. The former, based on our findings relating higher discharge events in spring and fall to higher abundance of microplastics in the sediment during these seasons (Gupta et al. 2021), appear to contribute to accumulation of microplastics in the sediment. The latter appears to counter this effect, as demonstrated by our findings of lesser microplastic abundance in the river channel, at intertidal sites, and at the river edge as compared to inland. In general, there is a strong relationship between hydrodynamic energy and the accumulation of particles, which is a finding shared by other studies conducted in estuarine environments (Jiwarungrueangkul et al. 2021).

Vertical wind-induced mixing is an important physical process to consider because the Bay is relatively shallow—this could lead to reduced microplastic abundance found in the surface layer and thus this study (Bikker et al. 2020) must be complemented with others that sample the entire water column, as well as the sediment in surrounding marshes and in the benthos. Surface microlayer, surface, water column, benthos, sediment surface microlayer, core preserving vertical structure of sediment should be sampled and monitored over time and through different flow regimes, as well as after discrete meteorological events with high wind speed and large amounts of precipitation (Range et al. 2023).

The interaction between marine tides and freshwater discharge leads to the formation of the estuarine turbidity maximum (Liu et al. 2023), an important structure when considering the transport and fate of microplastics. Settled plastic particles may become resuspended in this

highly turbulent area where flocculation and aggregation, with organic matter and other sedimentary materials, could occur, changing the shape, size, and density of the particles. Alternatively, advective transport downstream could be disrupted by the frictional boundary between freshwater and saltwater and reduce further transport out of the region. The ETM will be affected by changes in tidal amplitude and river discharge, moving upstream or downstream depending on the relative strength of these forces which will in turn influence the movement of microplastics.

Rising sea level and associated tidal amplitude is likely to affect transport, distribution, and fate of microplastics in several ways (Chen et al. 2024). One is that sites previously submerged only during high tides or high flow events are likely to be underwater during average conditions. This could result in complete submersion of vegetation in the salt marshes of, for example, the Chesapeake Bay. Plant communities are likely to be affected by associated increase in salinity (due to saltwater intrusion) and water levels (Ge et al. 2015). Due to the particle trapping capacity of vegetation, this could alter the storage of microplastics in the marsh if plant communities decline or their associated morphologies (diameter, stem density, root configuration) are altered. Saltwater intrusion into the marsh could also disrupt sediment (Zhang et al. 2024), resuspend microplastic particles that had settled and potentially redistribute them to another area of the estuary, marsh, or out to sea (Yin et al. 2022).

There is likely an interaction between multiple sources of plastic and transport mechanisms that lead to the distribution of microplastics. This presents a complication when trying to attribute observations to a single variable. The challenge exists in understanding the mechanisms underlying the patterns we observe. Trends could be obscured by intermittent high flow events, increased river discharge, intense precipitation or wind, significant changes in tidal amplitude, or pulses of plastic leakage into the environment.

Contamination of different environmental compartments means that multiple habitats and types of organisms will be exposed to microplastics with consequences for their health, physiological function, and reproduction (Lima et al. 2014; Rebelein et al. 2021). Ambient microplastics concentration may correlate with presence in organisms (Cai et al. 2023). Even within the life cycle of one organism, there could be multiple points at which contact occurs, for example in oysters with a free-swimming larval stage and benthic adult stage; this type of organism could encounter both suspended and buried microplastics at different points in time (Bringer et al. 2021). Another biological community likely to be especially affected by rising levels of microplastic contamination are submerged aquatic vegetation (SAV) species; the physical trapping effects of these ecosystems and dampening of wave energy promotes particle deposition and suggests a potentially high vulnerability and exposure to this particular stressor (Peller et al. 2021).

Despite the variation in physical and chemical characteristics of microplastics (e.g., density, shape, presence of additives), it may be informative to consider their transport and fate within the paradigm for natural sedimentary particles (Paduani 2020; Harris 2020). A possible complication from consideration of microplastics as natural sedimentary particles is their mostly unknown degradation process and into what types of components they disintegrate. That is, the snapshot captured at the time of sampling may become an unreliable explanation as time goes on and degradation proceeds. Which types of byproducts will accumulate as contamination levels rise and which will exist only transiently represents a critical knowledge gap. Studies that leverage seasonal or yearly sampling and characterize size, shape, and chemical composition are

addressing the question of how microplastic pollution changes over time in different ecosystems (Covernton et al. 2019; Range et al. 2023).

4.3 Research Limitations and Future Prospects

The removal of outliers from statistical analysis whereby samples with MP counts above the 75 percentile threshold were removed should be considered carefully. There were only four samples in the water dataset that exceeded this value, all of which were from the marsh sites. However, in the sediment dataset, 12 samples were identified and removed, three of which were from the intertidal site. This raises concerns about the validity of these values and whether they represent true data points or are artefacts. Further targeted sampling at these sites is warranted.

It is not possible from our sampling scheme and data to determine from where the detected MPs originated. Without identifying the chemical signature of specific polymers, we are limited to broadly categorizing identified particles as being plastic. It is also impractical to speculate on the sources of detected particles, whether recently flushed into the river from a precipitation event, deposited from the atmosphere, resuspended from the sediment, or carried in from a tidal cycle; this information is beyond the scope of the present study but represents a future critical research need.

Another limitation of the present study is reliance on a nearby USGS station for river discharge measurements. Future studies in the area should include direct monitoring of hydrodynamic conditions including discharge values throughout the sampling timeline, characterizing precipitation events, and relating these variables to MP abundance. Specifically, levels of MPs directly following precipitation events should be carefully measured so as to establish timelines of flushing from land into the water column and subsequent settling to the sediment or movement out of the sampling window.

In order to more precisely discern distribution patterns, future sampling should maintain vertical structure of water and sediment. This will enable characterization of specific layers within which MPs tend to accumulate, including the surface microlayer, surface layer, discrete depths, near-bottom, and benthos. Intact sediment cores should be obtained to better understand distribution patterns of plastic pollution through time.

5. Conclusions and future work directions

Our study is the first to quantify microplastic pollution in the Choptank River from both water and sediment and across seasons, contributing to understanding of this contaminant within a major tributary of the Chesapeake Bay and provides critical data that contributes to the literature examining the role of estuaries and salt marshes in the transport and fate of microplastics through this environment. Results demonstrate lower microplastic abundance in the river channel water column than the shallower marshes flanking the channel. In the sediment, there is higher microplastic abundance in subtidal than intertidal sites, and a lower proportion of fibers in summer and winter. It is unclear if the lower proportion of fibers in the summer and winter is consistent in estuarine environments or even the entire Chesapeake Bay. It is likely highly linked to surrounding land use and seasonal patterns of activities on land including agricultural activities such as planting and harvesting. This study is one of few to examine microplastic abundance in different seasons and therefore this should be repeated in future studies to determine the consistency of variation in plastic morphology during different seasons.

From our results, it is possible to better target high probability areas of plastic accumulation, as well as apply changes to sampling methods. For example, maintaining vertical structure of water by sampling at discrete depths to better resolve the spatial distribution of plastic in the water column. Maintaining vertical structure of sediment is also warranted and could potentially be used to identify differences in timeframes of plastic accumulation in different areas of the river (e.g., if plastics are found at variable depths of sediment, it could indicate differences in the time at which plastic accumulation began in that area and contribute to understanding major sources of plastic). Our finding of less plastic in the river channel could be complemented by testing whether there is uniformly less plastic in the channel or is instead concentrated in a smaller volume of area and resultantly diluted by sampling a larger volume of water without attention to potential heterogeneity in vertical distribution. In addition, our results demonstrate higher plastic abundance in subtidal sites, and slightly inland. Future work should target these areas to confirm these results which agree with what is understood about the physical characteristics of depositional areas in estuarine systems, including that lower energy states allow the settling of particles. The principle finding and one that contributes to research on the transport and fate of microplastics in estuarine systems is that there is a strong relationship between hydrodynamic variables and accumulation of plastic, which is a finding shared by other studies in estuaries. Future work could include strengthening the understanding between specific variables that vary seasonally including precipitation patterns and high discharge events to better resolve the relationships between hydrodynamic variables and microplastic abundance and distribution. Also, classification of plastic type may enable identification of major sources of plastic pollution in this environment. Retention of all our samples will allow us to analyze the types of plastic present without requiring further sampling efforts.

Therefore, our results support the concept that plastics, like other sedimentary particles, accumulate in lower energy areas of the estuary. Likewise, seasonal differences in hydrodynamic energy and land use activities and patterns likely contribute to variation in plastic abundance and proportions of different morphologies, the latter due to the contribution of particle morphology to its transport dynamics, movement, and interaction with other particles.

Credit authorship contribution statement

- **Conceptualization** – JJP, WN
- **Data curation** – KMB, JJP, WN
- **Formal analysis** – KMB, JJP, WN
- **Funding acquisition** – JJP, WN
- **Investigation**
- **Methodology** – KMB, JJP, WN
- **Project administration** – JJP, WN
- **Resources**
- **Software**
- **Supervision** – JJP, WN
- **Validation**
- **Visualization** – KMB, JJP, WN
- **Writing – original draft** – KMB, JJP, WN
- **Writing – review and editing** – KMB, JJP, WN

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.

Data availability

Data will be submitted to the NOAA National Centers for Environmental Information and is available upon request.

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Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work the author(s) used ChatGPT in order to contribute (30%) towards assembling the graphical abstract sketch - data included is from this study. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

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