SOURCES, FATE, AND BIOLOGICAL IMPACTS OF MICROPLASTIC DEBRIS IN THE DELAWARE ESTUARY

by

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ABSTRACT

The goal of this project was to determine the sources, distribution, composition, and biologic impact of microplastic debris within the Delaware Estuarine system. Specifically, this project (1) provides the methodology to quantify mismanaged plastic debris entering the coastal Delaware Bay from terrestrial sources through watershed raster modeling; (2) quantifies and characterizes microplastic debris throughout the water-column in the Estuarine Turbidity Maximum; and (3) seeks to determine the biological impact plastic has to the estuary through body burden analysis of ecologically important species and physiological impacts to important zooplankton species.

I developed a geospatial hydrologic model quantifying mismanaged plastic debris based on land-use characteristics to determine areas of high plastic output along the coastal Delaware Bay watersheds. This model, along with past observations, aids in interpreting plastic concentrations throughout the Delaware Bay with a focus on previously recorded high plastic zones. The model will be constructed through aggregation of population density, industrial, commercial, and institutional (ICI) land-use, as well as municipal and mismanaged waste data. Utilizing reported United States litter statistics I calculate that an additional 1.8% of municipal solid waste is released into the environment as plastic litter. Given land-use characteristics I find that residential land-use litter between 6×10^{-7} tons m^{-2} to 0.001 tons m^{-2} given population density. Additionally, ICI wastes 6×10^{-6} tons m^{-2}. Using these spatial statistics, a modeling case study of the St. Jones River Watershed find that 2.7 tons of plastic is

exported to the Delaware Bay annually by the St. Jones River watershed. This model was used to determine that northern Delaware watershed had the potential to contribute more plastic litter into the environment than southern Delaware watersheds.

Marine microplastics are a pervasive pollutant, with estuaries considered a sink for plastic debris. Due to dynamic physical influences in an estuarine system, residence times of particulate debris – including plastic material – increase, thus aggregating material at higher rates than the open ocean. The goal of Chapter 3 was to quantify, characterize, and contextualize microplastic debris in the Delaware Bay's Estuarine Turbidity Maximum (ETM) and the surrounding region. In the Delaware Estuary it was previously found that the ETM region accumulates microplastics at higher concentrations relative to the remainder of the bay. Previous research in this region has focused on the characterization of surface material. In this study, I characterize the morphology, size, and polymer identities of microplastic material throughout the water column within the Delaware Bay ETM region. Microplastics were found at all stations throughout the sampling region, with average ETM concentrations of 1.4 MP/m^3, 0.5 MP/m^3, and 0.8 MP/m^3 found at the surface (0.5m), mid-water (7m), and bottom (12m) depths, respectively. Fragments were the most abundant plastic type, followed by fibers, across all depths, whereas beads were found more commonly at depth. μFTIR analysis suggests fragments were predominately polyethylene, fibers were commonly polyester or rayon (a semi-synthetic material), and beads were primarily polystyrene. These analyses establish that microplastics are not simply a surface water feature and provide a comprehensive understanding of common microplastic characteristics. In doing so, we further contextualize the microplastic problem in the Delaware Estuary.

Plastic concentrations in the water column were then related to the occurrence of plastic material within estuarine fish and invertebrate digestive tracts and gills, with a focus on the economically important blue crab, *Callinectes sapidus*. The aim of Chapter 4 is to investigate whether such plastic aggregations correlate with plastic body burdens in estuarine organisms, such as the Atlantic Blue Crab (*Callinectes sapidus*) and other abundant and ecologically important species. Estuarine organisms were sampled across 4 stations within the Delaware Bay throughout the spring of 2021, in regions of high and low plastic concentrations. Additionally, monthly mature female blue crab stomach samples were collected throughout 2021 and summer of 2023, along with mature male blue crabs in June of 2024. While 12% of estuarine organisms contained plastic in their stomach or gills, 26% of *C. sapidus* females, and 30% of *C. sapidus* males containing plastic. Plastic identified were predominantly fibers with an average of 1-2 pieces found per organism. Seasonal analysis reveals *C. sapidus* collected in the spring (April and May) contained more plastic than other seasons. Plastic body burdens were prevalent across sampling locations and species, with no significant differences observed based on uptake method, age, size, or habitat. These analyses provide insight into spatial patterns of microplastic body burdens within an array of estuarine organisms and *C. sapidus*.

Finally, zooplankton physiological condition in response to environmental plastic was assessed for the mysid shrimp, *Neomysis americana*, a key component to the estuarine food web. Using thermal performance curves for metabolic rate, coupled with O:N ratios as an indicator of metabolic substrate, I tested for seasonal (summer, winter) and location (low and high plastic load) effects on these metrics of physiological performance. The estuarine mysid species, *Neomysis americana,* was

collected from high microplastic and low microplastic locations within the Delaware Bay. Mysids were assessed for their respiration and excretion rate when acutely acclimated in the laboratory to a range of temperatures, representing a multi-stressor scenario. No difference was observed between respiration rates, nitrogen excretion or O:N between locations. Although, an increase in respiration rate and nitrogen excretion rate was observed from winter to summer at the high microplastic location. Specifically, during winter, we see a drastic increase in respiration rate at 10°C, with a high Q_{10} (11.6) between 2°C and 10°C. These results may indicate an initial stress response at acute temperatures of 10°C in the winter as opposed to a larger thermal window in the summer. Furthermore, no metabolic differences were detected at the current concentrations of microplastic in the bay. However, future studies exploring theoretical microplastic exposure limit or ingestion may reveal additional trends.

Overall, this dissertation identifies sources and hotspots of terrestrial plastic waste through land use modeling, the accumulation and identification of microplastic in the Delaware Bay, and highlights potential ecological implications. This work lays the foundation for targeted mitigation and management strategies through identifying body burdens of estuarine species, metabolic effects on zooplankton, and identifying regional plastic litter aggregations. Ultimately, this work serves to address the pervasive issue of plastic pollution and its threat to ecosystem health.

Chapter 1

INTRODUCTION

1.1 Microplastics in the Marine Environment: Sources and Fate

Marine plastic debris is a pervasive pollutant of global concern. By nature, plastic is extremely durable, with residence times in the marine environment dependent on composition and manufacturing (Meng et al., 2020). Microplastic (MP) is defined as plastic material <5mm in diameter and can be the product of larger plastic degradation or directly manufactured at these sizes (Andrady, 2017). MP directly produced at sizes less than 5mm, are termed primary microplastic, and are generally used in personal care products as exfoliants, sand blasting media, or pellets used in the fabrication of products (Browne et al., 2007). The degradation of plastic is attributed to UV photodegradation and frictional weathering due to wave action or abrasion from sediment particles, these MP are termed secondary microplastic (Barnes et al., 2009). However, MP are not the smallest fraction of plastic, as MP can further deteriorate into nanoplastics (<1μm) (Hartmann et al., 2019, Lambert and Wagner, 2016) with Besseling et al., 2018, estimating the fragmentation of spherical microplastics have the potential to generate > 10^{14} times the number of nanoplastics.

MP material is among the most abundant form of plastic debris in the world's oceans (Arthur, Baker, and Bamford 2009), with estimates of more than 260,000 tones floating in the world's oceans in 2014 (Eriksen et al., 2014), a number that has most likely increased in recent years, as plastic production continues to increase. MP debris has been quantified in almost every marine environment, occurring from open ocean

(Cozar et al., 2014, Pan et al., 2022, Courtene-Jones et al., 2022), coastal regions (Steer, 2017, Sutton et al., 2016, Nakakuni et al., 2024), estuaries (Cohen et al., 2019, Bailey et al., 2021, Roscher et al., 2021, and Pan et al., 2021) to coastal and deep-sea sediments (Zhang et al., 2020, Xu et al., 2020, Cunningham et al., 2020). As production of plastic material is economically cheap and extremely important, the global dependence on plastic material has increased (Barrowclough and Birkbeck, 2022).

In 2016 alone, the United States is estimated to have produced 42.0 metric tons (Mt) of plastic waste, with the largest annual per capita plastic waste generation of any country (Law et al, 2020). Estimates of plastic waste in the ecosystem show that 2% of all plastic waste can be categorized as mismanaged (Jambeck et al. 2015). Originating from land, mismanaged MP's infiltrate ecosystems through flooding and surface run-off into coastal waterways and estuaries (Jambeck et al. 2015, Schmidt et al., 2017). Geospatial modeling allows for the identification of sources and hotspot detections of MP infiltrating the environment. In a study by Lebreton et al., 2017 estimates between 1.15 and 2.41 million tonnes of plastic waste enters the ocean from rivers annually. This analysis was completed through assessments of waste management, population density, and hydrologic information. Similarly, Meijer et al., 2021, reports more than 1,000 rivers account for 80% of global annual plastic emissions based on plastic waste, land use, wind, and precipitation. Kawecki and Nowack, 2020, spatially resolved plastic emissions of commonly used polymers finding most emissions in regions of human activity. Ultimately, geospatial analysis has aided in determining large scale plastic emission approximations, leaving gaps in state-wide watershed

scales based on land-use and population density, particularly regarding the export of debris to estuarine systems.

Estuarine systems serve as a sink for terrestrial plastic debris (Gray et al., 2018). Several studies have found elevated microplastic concentrations in estuarine environments relative to the open ocean (Bikker et al., 2020, Cohen et al., 2019, Sutton et al., 2016, Roscher et al., 2021, Yonkos et al., 2014, Defontaine et al., 2020). As plastics are lightweight, they are easily transported by winds and currents (Law, 2017). Additionally, plastic can disperse horizontally and vertically throughout the water column due to wind stress, surface heat fluxes, mixing of salt and fresh water, and biofouling of material (Kukulka et al., 2012, Kukulka et al., 2016, Vermeiren et al., 2016). As such, once MPs are introduced into the coastal estuarine system, distributions can be spatially and temporally variable (Zhang, 2017).

The global occurrence of MPs is highly documented, yet less is known about the mechanisms that influence accumulation or the impacts these accumulation zones have on marine biota. It is hypothesized that plastic waste accumulates based on shape, size, and density of the material. Thus, understanding the polymer composition is important for understanding the mechanisms contributing to plastic accumulation. A previous study in the Delaware Bay (Cohen et al., 2019) found that plastic debris is found throughout the bay and its tributaries. Accumulation of plastic was dependent on size, and characterization of plastic type (i.e., fragment, fiber, bead). Their study also showed polyethylene was the most common fragment in Delaware Bay, and polypropylene the most common fiber. However, only surface microplastic was examined, and spectroscopic analysis was limited to larger microplastic particles

(~500 μm and larger) and only a small fraction of collected particles were analyzed for polymer composition.

1.2 Microplastics and Biota: Interactions and Biological Impacts

Microplastics pose a multitude of threats to marine organisms as expressed by laboratory studies (Cole et al., 2013, Coppock et al., 2019, Browne et al., 2008). An organism's exposure can range from little to no detriment where microplastic pass through the organism's gastrointestinal tract with little to no quantifiable impact on the organism (Jovanović, 2017), to a reduction in feeding rates (Cole et al., 2013, Steer, 2017) and cellular damage due oxidative stress from microplastic ingestion (Lu et al., 2016). Additionally, microplastic material has been found to translocation within the body (blood, brain or tissues) as shown by translocation of MP from the gut to circulatory system in mussel species *Mytilus edulis* (Browne et al., 2008) and gut to hepatopancreas in fiddler crabs *Uca rapax* (Brennecke et al., 2015). Microplastic can leach chemicals further compounding the hazard microplastics pose on organisms. MP can be manufactured with hazardous monomers, additives, and chemical byproducts (Lithner et al., 2011) or sorb harmful chemicals from the marine environment (Rochman et al., 2013) causing bioaccumulation and liver toxicity in fish species *Oryzias latipes* (Rochman et al., 2013). Plastic can also attach itself to the organism due to static electricity which can increase drag on the organism and increase the energy expenditure needed for activity and locomotion (Cole et al., 2013). This can decrease the organism's ability to escape predation as well as capture prey as the organism is not as stable and uses more energy to move. Additionally, exposure may increase energy expenditure due to the cost of handling microplastic as found for crab zoeae (Boettcher, 2024).

As seen in previous studies impacts such as, delayed or inhibited larval development (Boettcher, 2024, Oliviero et al., 2019), damaged eggs or offspring defects (Sussarellu et al., 2016), decreased energy reserves (Wright et al., 2013) are all observed impacts microplastics have had on organisms. These impacts indicate microplastics acting as stressors on an organism where their energy budgets must be reprioritized to minimize damage due to stress (Sokolova, 2013). When exposed to stressors, abiotic or biotic forcing factor resulting in a shift of one or more biologic process (Kültz, 2020) where energy compensation occurs in the form of energy for reproduction in adult organisms, or growth and development in larval organisms (Sokolova, 2021).

Often, laboratory studies use microplastic concentrations orders-of-magnitude higher than environmental conditions (Lenz et al., 2016), as implications of ingestion are difficult to quantify in a natural setting. Thus, under current conditions, many biological impacts are not environmentally relevant. Understanding how microplastics interact with organisms in real-world environments is critical to addressing this gap. Gastrointestinal dissections can indicate the rate at which microplastic exposure is occurring in the environment, organismal affinity for plastic, method of plastic ingestion, and assess the risks posed to the organisms given an increasing plastic loading to the environment (Egbeocha et al., 2018). Plastic loading to the environment is set to increase over time, as plastic presence in an organism's GI tract can be indicative of susceptibility to further microplastic exposure in the future (Vendel et al., 2017).

1.3 Dissertation Objectives and Structure

The principal objectives I set out to complete were as follows: (1) develop a methodology to determine litter quantities (tons) produced within a drainage basin; (2) quantify and characterize microplastic debris within the Delaware Estuary Estuarine Turbidity Maximum (ETM) region; (3) investigate the body burdens of microplastic in estuarine organisms; and (4) determine the metabolic impacts of microplastic and temperature on an important zooplankton species.

Overall, this dissertation is composed of four manuscripts in preparation (Chapter 2, Chapter 3, Chapter 4, and Chapter 5) and a collection of concluding remarks (Chapter 6). Chapter 2 produces a methodology to determine litter production based on municipal solid waste. Utilizing the methodology from Chapter 2, the manuscript will contain analyses of spatially derived litter statistics, quantify the loading of litter from coastal watersheds to the Delaware Bay. This methodology will help explain and contextualize the patterns observed in Chapter 3, which quantifies and characterizes the microplastic concentrations and polymer types observed within the Delaware ETM region. To understand the impacts of microplastic in the ecosystem, body burden of estuarine organisms from low and high regions of microplastic in the bay are analyzed in Chapter 4. This chapter places special emphasis on the body burdens of microplastic in blue crabs *Callinectes sapidus.* Finally, Chapter 5 explores the physiological impacts of environmental microplastic exposure on a key zooplankton species *Neomysis americana* through a range of temperatures. Lastly, Chapter 6 summarizes and contextualizes the results from my analyses and provides recommendations for future work.

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Chapter 2

METHODS FOR TERRESTRIAL PLASTIC LITTER MODELING WITHIN A DRAINAGE BASIN: CASE STUDY FOR THE DELAWARE BAY

2.1 Introduction

The presence of plastic in the global environment has sparked interest in the sources and magnitude of which plastic litter enters coastal waterways. According to the U.S. Environmental Protection Agency (EPA, 2019), only 9.2% of plastic waste is recycled, while 75.4% is disposed of in landfills, and 15.3% is incinerated. In a well-maintained landfill there is little risk of plastic leaching, however plastic can enter the environment through littering within major municipalities (e.g. roadways, rivers, parks) (Hoellein and Rochman, 2021). Assuming that 2% of all plastic waste is mismanaged (Jambeck et al. 2015), much of this mismanaged plastic waste ends up in the environment. It is estimated that between 1.15 and 2.41 million metric tonnes of plastic is exported from coastal river systems into the open ocean every year (Lebreton et al., 2017).

The majority of the global population lives at or below 100 m of sea level, with much of plastic debris in coastal waterways assumed to be proportional to the amount of mismanaged waste (Small and Nicholls, 2003). Consequently, coastal and estuarine systems serve as a sink for terrestrial plastics entering the marine system (Gray et al., 2018), with rivers facilitating the transport of inland material to the coastal regions (Schmidt et al., 2017). Concentrations of material deposited into the coastal waterways depend on many factors, including the management and categorization of the natural

environment (land use) (Kawecki and Nowack, 2019) and the regional hydrologic forcings. Land use contributes to higher occurrences of mismanaged plastic debris through the region's socio-economic status, or presence of industrial manufacturing (Geyer et al., 2017). Furthermore, previous studies report an increase in per-capita income and population is strongly correlated to solid waste production (Law et al., 2020). For example, in the Chesapeake Bay it was found that population density was significantly correlated with microplastic in the estuary (Yonkos et al., 2014). By integrating regional population and socio-economic data, we can create a plastic waste production rate as demonstrated by Lebreton et al., (2017).

The Delaware coastal watersheds provides a populous region of which plastic waste on coastal beaches have been monitored from 2008-present (DNREC, 2021) and previous bay-wide studies have shown that plastic is infiltrating into the bay and its tributaries (Cohen et al., 2019). Thus, the Delaware coastal watersheds are an ideal site to project plastic accumulation zones through solid waste data, socio-economic information, and environmental forcings.

Geospatial analysis in ArcGIS PRO provides a platform to integrate population density, land use, and hydrology to best identify plastic pollution pathways and hotspots. Utilizing the Hydrology toolset within the Spatial Analysist toolbox, the flow direction and accumulation of surface water flow can be extracted from a digital elevation model (DEM) (Ye and Djokic, 1999). These outputs can be used to delineate streams and watershed boundaries. More importantly, these tools enable the modeling of plastic over the landscape and quantifies the total expected export of debris within a given stream or watershed. This approach provides insights for identifying hot spot regions for intervention and aid in mitigation strategies.

The objective of this study is to develop a methodological workflow to estimate the magnitude of plastic pollution entering the Delaware Estuary based on human activity and land use spatial analysis. We hypothesize that watersheds with elevated plastic litter will correspond to elevated bay plastic concentrations.

2.2 Methods

2.2.1 Data Collection and Sources

Data collected for this model include population, land cover and land use, flowlines (streams) and watershed boundaries, municipal solid waste data, and continental United States litter characteristics and statistics. Data collection focused on the year 2019, as it was the primary year of microplastic data collection and for which the other datasets sources were available. Population data was collected from the U.S. Census Bureau, American Community Survey 5-year estimates (2019). Land-use and land-cover data were collected from Delaware First Map feature layer Delaware Landuse/Landcover 2017. The USGS National Hydrography Dataset (NHD) provided flowlines (HU-8 subregion streams) and watershed boundaries (WBD) (HU-2 subregion). 30-meter Digital Elevation Models, covering the study region of Delaware, were collected from the USGS 3DEP LidarExplorer. Litter and municipal solid waste characteristics were collected from the Keep America Beautiful 2020 National Litter Study Summary Report, 2021 and the Delaware Solid Waste Authority (DSWA) Municipal Solid Waste Authority Statewide Waste Characterization Study, FY 2016 and Annual Reports, respectively.

2.2.2 Methodology for Estimating Litter in Delaware

To estimate the annual quantity of plastic litter in Delaware, we applied a methodology combining municipal solid waste data with road mileage-based litter estimates. Using the Keep America Beautiful (KAB) study, which reports an average of 2,857 pieces of litter per road mile across the continental United States, and the finding that 38.6% of this litter is plastic, we calculated the number of plastic litter pieces per road mile to be 1,099. This estimate was then multiplied by the total road mileage in Delaware (5,776 miles, according to the Delaware Department of Transportation) to determine the total number of plastic litter pieces generated annually in the state. Using total tons of plastic litter compiled from the Department of Natural Resources and Environmental Control (DNREC) coastal clean-up surveys from 2008 - present, we developed a linear regression model to relate the weight of plastic waste (tons) to the number of plastic waste pieces (Figure 2.1), allowing us to estimate the weight of plastic waste based on the quantity of pieces. This value was then divided by the 2019 total municipal solid waste to calculate the percentage of plastic litter produced annually relative to the total municipal solid waste.

2.2.3 Methodology for Assessing Institutional, Commercial, and Industrial Litter

The DSWA Solid Waste Characterization Study, 2016 grouped institutional, commercial, and industrial (ICI) land uses into one category (Appendix A1). Therefore, these LULC classes were grouped within ArcGIS PRO V3.1.3 and ICI polygon areas were summed to calculate the total area (m^2) of ICI land-uses in Delaware. A percentage of total waste contribution of ICI sources was determined from the DSWA Solid Waste Characterization Study, 2016 and was applied to the total litter (tons) in Delaware assuming that the same proportion of waste generated by

ICI sources is also littered. Finally, by dividing the total area of ICI land use to the ICI contribution of litter, we can determine the generated litter per square meter of ICI land-use.

2.2.4 Methodology for Assessing Residential Litter

Residential land uses were grouped into one category within ArcGIS PRO V3.1.3. Similar to ICI contribution to plastic litter, residential contribution to plastic litter was determined from the DSWA Solid Waste Characterization Study, 2016 and was applied to the total litter (tons) in Delaware. Additionally, a per capita litter rate was determined by dividing the residential litter contribution by the population of Delaware. In ArcGIS PRO, population block groups were intersected with residential land use data to calculate the total residential area (m^2) for each block group. Plastic litter per square meter of residential land use was spatially derived by multiplying the population of each block group by the per capita plastic litter value, then dividing by the total residential land area within each block group.

2.2.5 Analytical Framework for Waste Potential and Hotspot Detection

The Saint Jones Watershed was chosen as a representative watershed for a case study of waste potential in this dissertation (Figure 2.2C). To identify trends in plastic waste flow and detect hotspots of plastic litter, a 100-meter buffer in ArcGIS PRO was created around each river and stream within the Delaware Bay coastal watersheds. This buffer was then intersected with residential and ICI land use vector layers to spatially calculate plastic debris within the buffer region and creating a “litter plastic” layer. The litter plastic layer was then subsequently rasterized into 25-meter grid cells generating a plastic waste raster grid. Flow direction of the Saint Jones watershed was

delineated based on the 30-meter DEM from USGS. Using this flow direction raster and the rasterized litter plastic layer as inputs, Flow Accumulation tool in ArcGIS PRO was performed to determine the loading of plastic litter along the Saint Jones River and entering the bay at the mouth of the river (Figure 2.4B).

2.3 Results and Discussion

We find that 1,099 pieces of plastic litter are found per road mile in the continental United States and by applying the total road miles (5,776 mi) in Delaware (Delaware Department of Transportation), we calculate that 6,373,092 pieces of plastic litter are polluted along Delaware roadways every year. Using the linear regression model, this equates to an estimated 21,654 tons of plastic litter per year. Thus, we find that an additional 1.8% plastic litter along roadways is produced annually relative to the total municipal solid waste (Figure 2.3). This percentage is similar to Jambeck et al., 2015's 2% although more appropriate for the spatial scale of the Delaware Bay. Based on the annual roadway estimates, about 0.01 tons of plastic litter is produced per capita in Delaware. Considering population density, calculated plastic litter per square meter of residential land uses ranged from 6×10^{-7} tons m^{-2} to 0.001 tons m^{-2} across Delaware. Additionally, ICI contributes 6×10^{-6} tons of plastic litter per square meter.

At current, this model integrates residential and ICI land use to create plastic waste production potential throughout the Delaware coastal watersheds. A modeling case study of the St. Jones River watershed suggests 2.7 tons of plastic litter is exported from the St. Jones River into the Delaware Bay annually (Figure 2.4). St. Jones River plastic concentrations pieces m^{-3} – averaged from the summer of 2019 – reveal elevated concentrations (130.5 pieces m^{-3}) at a fork in the river downstream of

Dover, Delaware, comparatively higher than the river concentrations just above Dover (89.9 pieces m^{-3}). High concentrations were also observed close to a region of high population density and ICI (unpublished data) (Figure 2.4A). These river plastic concentrations are similar to Yuan et al. (2021) who found increased concentrations with proximity to urban or industrial land use. This model result is due to flow accumulation summing all contributing pixels downstream.

The geospatial model was then applied to all watersheds in Delaware, revealing elevated plastic litter production potential in the northern watersheds compared to southern (Figure 2.5). This pattern is similar to bay-wide surface microplastic sampling in spring and summer of 2017 (Cohen et al., 2019), where more microplastic was found in the upper bay compared to lower bay (Figure 2.5A). A linear model was used to investigate the relationship between surface microplastic and plastic litter production potential, however no significance was found (Figure 2.6A). Microplastic aggregates based on a multitude of factors including wind, landscape characteristics, mixing, bathymetry, and organism-plastic interactions (Vermeiren et al., 2016), thus these results prompt further field validation to assess the modeled plastic loading and where it may be accumulating in the environment. Additionally, the plastic litter production for each watershed was compared to DNREC Coastal Cleanup data from 2019, where citizen scientists quantified and characterized litter along the Delaware coastline (Figure 2.5A). No relationship was found between modeled plastic litter production and DNREC Coastal Cleanup surveys normalized for sampling effort (Figure 2.6B). These results indicate further validation is needed for this model as a variety of limitations may be influencing these results.

Road miles were used as the best approximation of littered plastic in the state of Delaware according to the KAB 2021 study. This approach assumes an even distribution of litter per road mile across the state, without accounting for the potential difference in rural and urban roads. Similarly, the spatial contribution of plastic litter from ICI and residential land use is based on the assumption that ICI litter is evenly distributed across all ICI locations and categories as well as residential housing units generate the same amount of waste annually per population density. However, this assumption does not account for the variability of waste generation across ICI categories or residential areas.

Socio-economic status (Capps et al., 2016), as well as land use and land cover characteristics such as construction (Kawecki and Nowack, 2020), can contribute to elevated plastic waste production and pollution. A modified river buffer, taking into consideration surrounding land use, would increase the robustness of the model. Additionally, tourism, a major industry in Delaware, was not considered for this study. In 2022, Delaware experienced 9.8 million overnight visitors contributing $4.5 billion to the state's gross domestic product (GDP) in 2022 (Value of Tourism, 2022). As tourism can be a significant source of marine pollution (Loizidou et al., 2018 and Cordova et al., 2021), this factor is important to consider in future modeling. Future iterations of this model will address these limitations, allowing for the model to be reproduced throughout the United States. Expanding the range of the model throughout the extent of the Delaware River drainage basin, including Philadelphia, Pennsylvania, and coastal New Jersey, will allow for a more precise indication of loading of plastic waste into the Delaware river and ultimately the Delaware Bay. This broader geographic scope will better account for the diverse sources and pathways of

plastic pollution. Ultimately, this model allows for the quantification of plastic litter based on population and land use, providing a tool for identifying river systems to target for mitigation strategies.

2.4 Figures

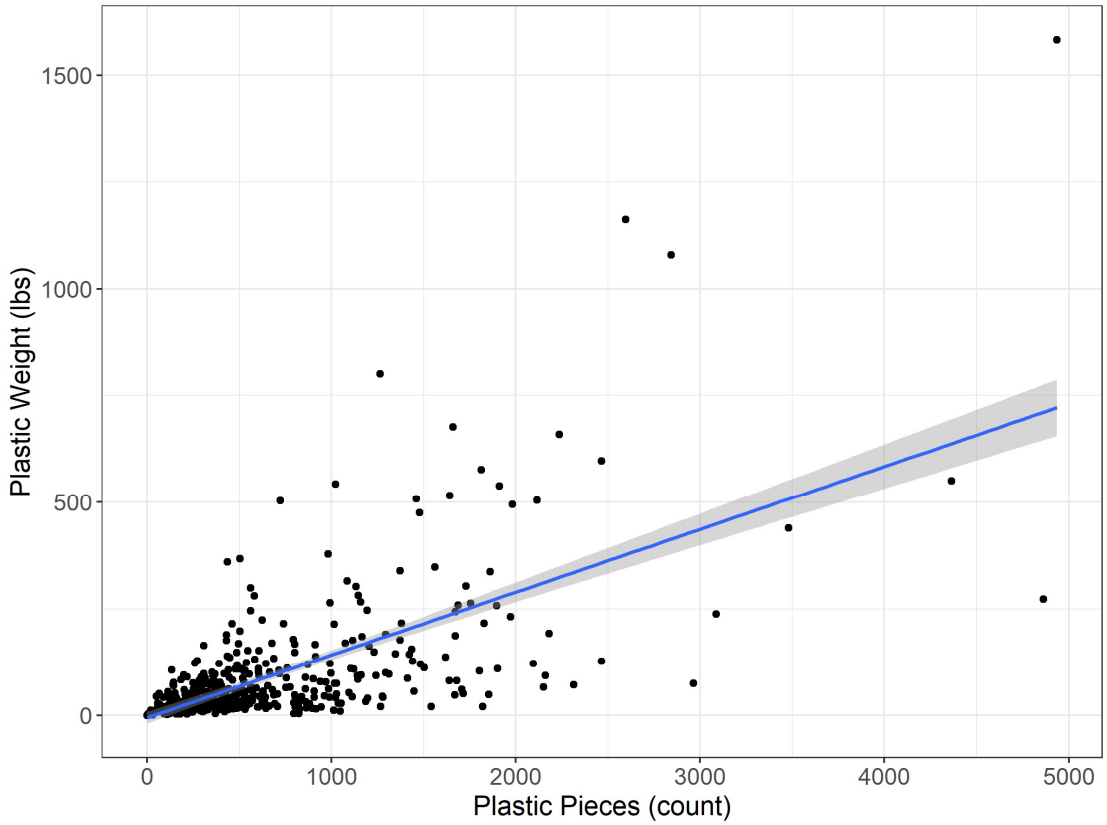

Figure 2.1: Linear regression of plastic pieces (count) to the plastic weight (pounds, lbs). Data collected from DNREC Coastal Cleanup data 2008 – 2019 (P = <2.2e-16, n = 492, Plastic weight = 0.147*Plastic Pieces – 5.07).

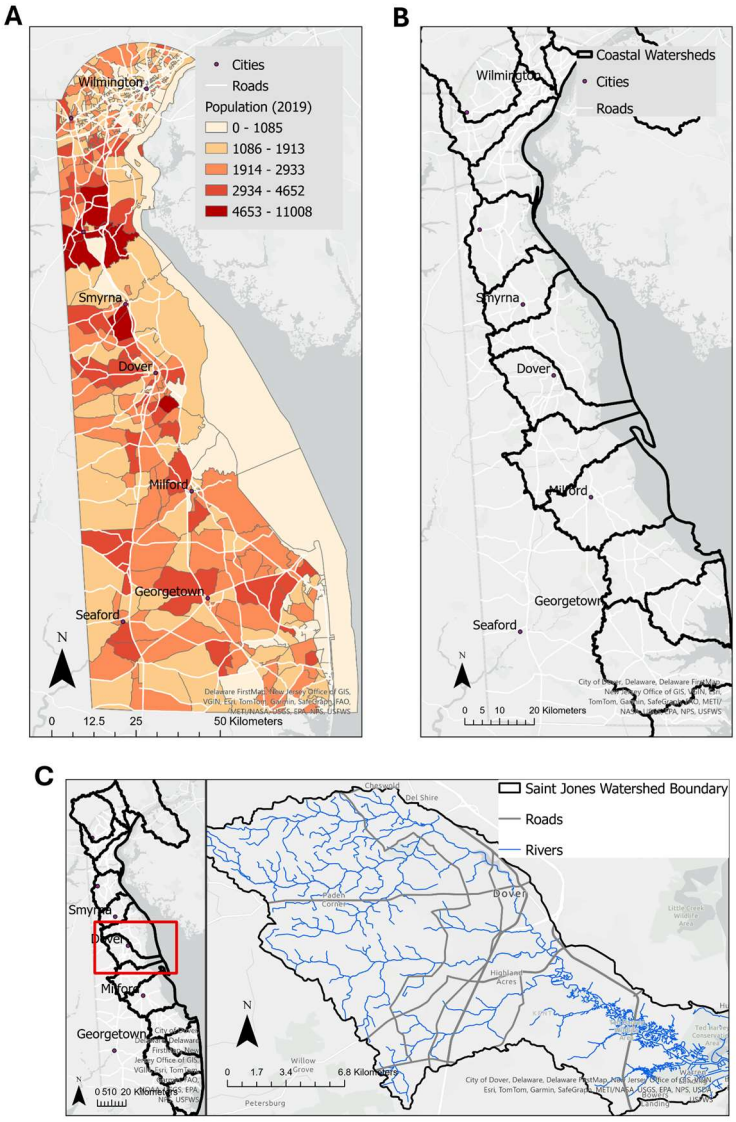

Figure 2.2: Study site plots A) Population by U.S. Census groups in gradients of red, with Delaware roads (white) B) Delaware coastal watershed boundaries with roads (white) C) Map of the Saint Jones Watershed with roads (grey) and river lines (blue).

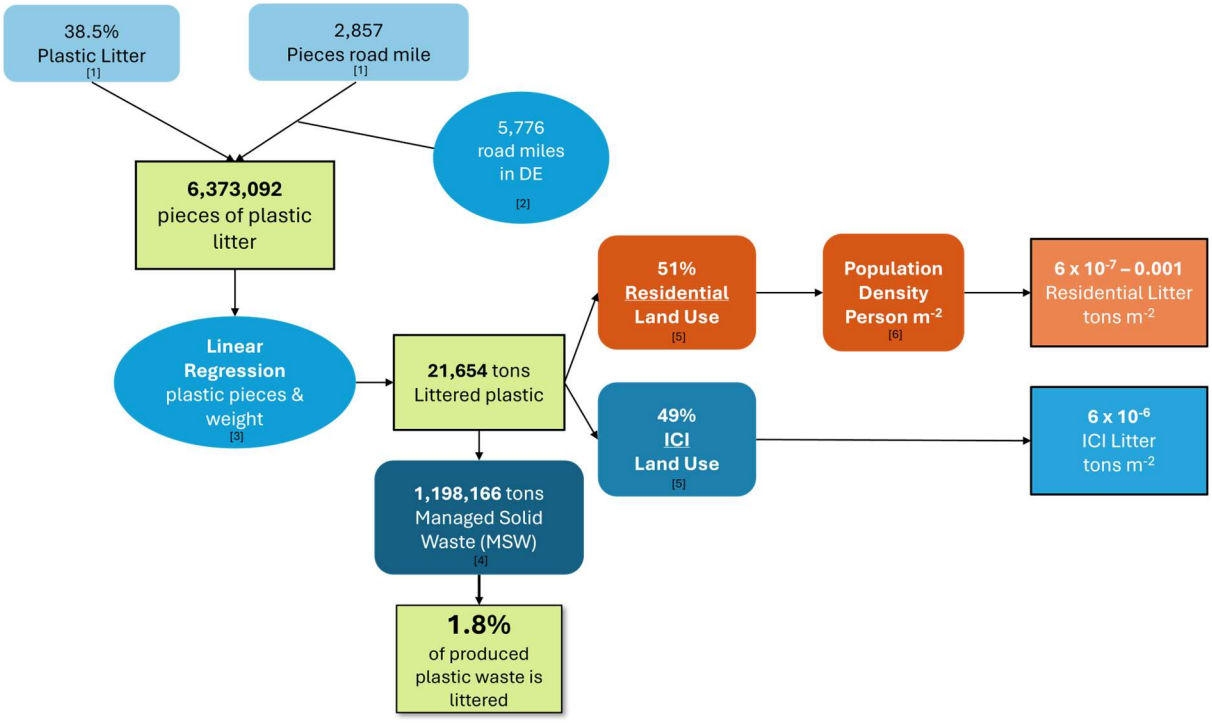

Figure 2.3: Flow diagram of plastic litter model inputs and outputs (Outlined Boxes).[1] Keep America Beautiful, 2021, [2] U.S. Department of Transportation, [3] DNREC Coastal Cleanup, [4] Delaware Solid Waste Authority Annual Report 2020, [5] Delaware Solid Waste Authority, Solid Waste Characterization 2017, [6] U.S. Census Bureau.

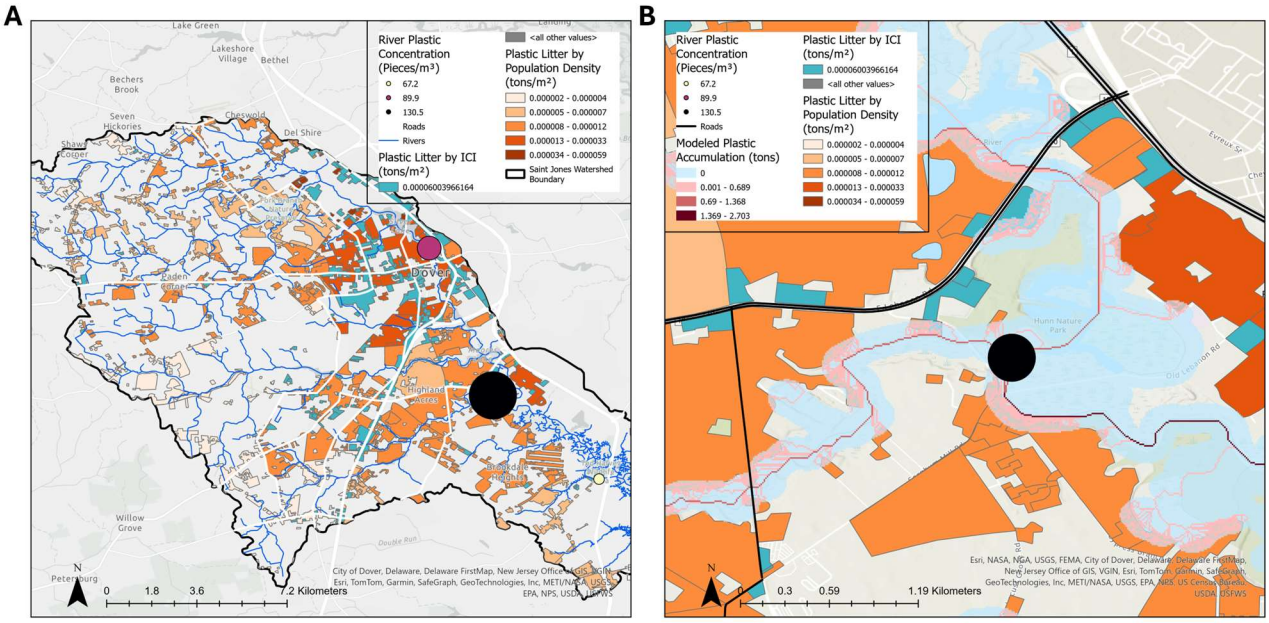

Figure 2.4: Map of the St. Jones Watershed revealing spatial distribution of land uses and river microplastic concentrations. Calculated litter statistics are shown by litter by population density (tons m^{-2}) polygons shown in a gradient of light orange to dark orange, litter by industrial, commercial, and institutional (ICI) land uses in teal, and river plastic concentrations (microplastic pieces m^{-3}) in bubbles of yellow, purple, and black. A) shows the entirety of the Saint Jones Watershed Boundary, B) zoomed in to the sampled microplastic location south of Dover, Delaware. The blue region indicates the 100 meter buffer around the rivers, gradient of pink to dark purple pixels indicate the model predicted plastic loading (tons) for the river branches.

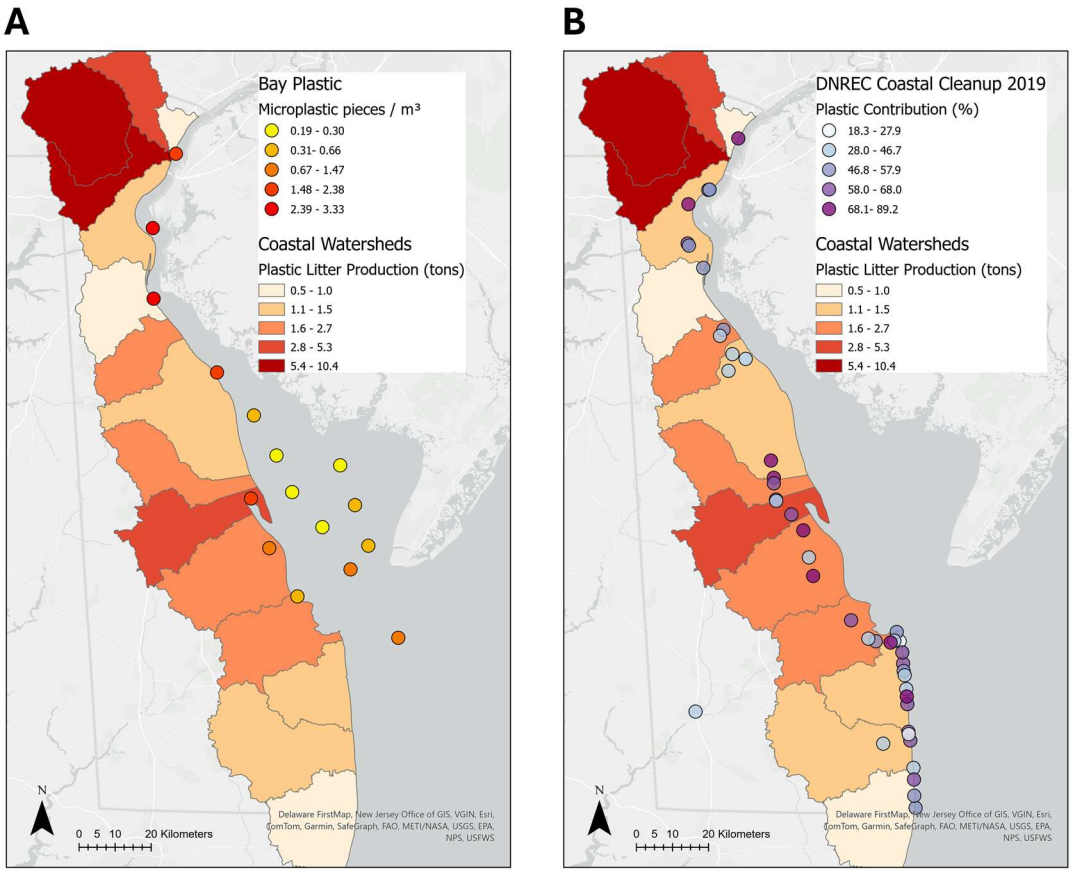

Figure 2.5: A) Delaware coastal watersheds gradient filled with model output of plastic litter production potential (tons) compared to bay surface microplastic concentrations (microplastic pieces m^{-3}). B) Delaware coastal watersheds gradient filled with model output of plastic litter production potential (tons) compared to 2019 DNREC coastal clean up survey data normalized to the number of people and plastic contribution per station.

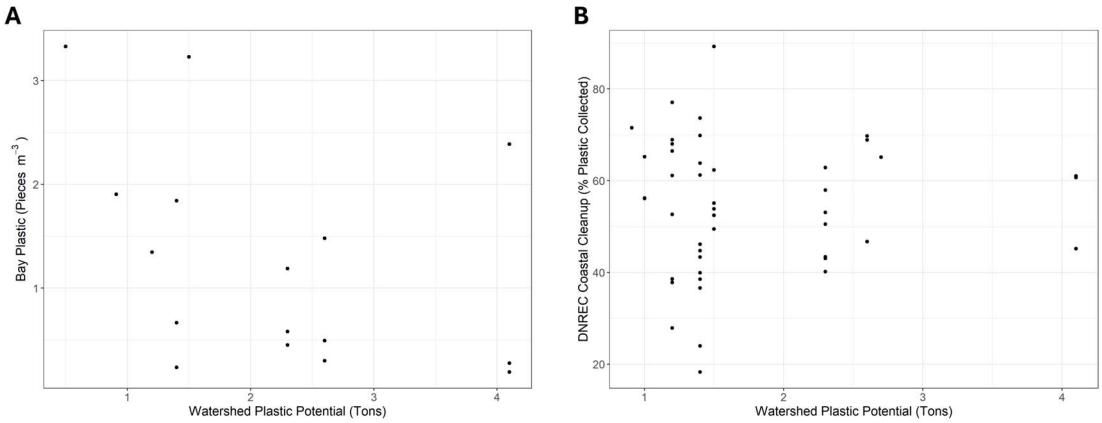

Figure 2.6: Linear Regression of A) Watershed plastic potential (tons) and Surface bay microplastic (Pieces m-3). No significant relationship (P = 0.1307, Linear Regression, Bay Plastic (Pieces m^{-3}) ~ Watershed Plastic Potential (tons). B) Watershed plastic potential (tons) and DNREC coastal clean up plastic percentages. No significant relationship was found (P = 0.8511, Linear Regression, DNREC Coastal Cleanup (% Plastic Collected) ~ Watershed Plastic Potential (tons)).

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Chapter 3

MICROPLASTIC CHARACTERIZATION AND DISTRIBUTION THROUGHOUT THE DELAWARE ESTUARY TURBIDITY MAXIMUM REGION

3.1 Introduction

The Delaware estuary is a wide salt-wedge estuary known for its distinct freshwater input from the Delaware River and saltwater intrusion from the Atlantic Ocean. The Delaware River is the largest source of freshwater to the estuary with an annual discharge of 330 m^3s^{-1} (Sommerfield and Wong, 2011), with the tidal influence of the Delaware Estuary extending from Trenton, NJ to the mouth of the bay (Sharp 1983). Freshwater outflows tend to hug the Delaware shoreline as it leaves the bay and enters the Atlantic Ocean due to the deflection of water from Earth's rotation (Kukulka et al., 2017). As such, the estuarine exchange flow and salt flux is primarily confined to the main channel of the estuary (Geyer et al., 2020).

Estuarine systems are often densely populated coastal regions that receive freshwater inputs from inland riverine systems. These riverine inputs transport terrestrial plastic waste into coastal waterways (Schmidt et al., 2017). Consequently, estuaries serve as a major sink and intermediate transport pathway for microplastic debris before being flushed into the open ocean or beached on the shoreline (Biltcliff-Ward et al., 2022, López et al., 2021). The distribution of microplastic debris within an estuary can be highly variable, with salinity gradients, vertical density stratification,

tidal cycles, and turbidity maximums affecting the distribution of particulate debris (Vermeiren et al., 2016).

Estuarine Turbidity Maximums play a particularly significant role, creating regions where suspended particulate matter is elevated throughout the water column (Burchard et al., 2018). In an estuary such as the Delaware Bay, the ETM is created where the saltwater intrusion from the Atlantic Ocean reaches its landward limit and meets with the freshwater river discharge of the Delaware River (Sommerfield and Wong, 2011). This process suspends particulate matter from both water masses, forcing them to converge and increase in concentration throughout the water column forming an estuarine turbidity maximum (ETM) (Burchard et al., 2018). ETMs are a highly dynamic feature influenced by freshwater flux and tidal forces; therefore, the exact location of these features can vary spatially and temporally throughout an estuarine system. As ETMs force particles into suspension, it is suspected that material such as plastic will be trapped within these features at greater concentrations relative to its surrounding water (Shiravani et al., 2023, Roscher et al., 2021, Defontaine et al., 2020).

Similar to suspended material, ETM features can congregate biota, indicating these features as potentially important habitats for estuarine organisms. In the St. Lawrence Transition zone in Canada, it was found that the ETM contained high concentrations of zooplankton and larval fishes (Laprise and Dodson, 1994). Phytoplankton concentrations decreased when sampled across the ETM region indicating a loss by grazing activity (Vincent et al., 1996). The ETM has been found to be a grazing ground for zooplankton species such as copepods and mysids (Islam et al., 2005, Winkler et al., 2003). Given the ETM's tendency to aggregate plastic

material, these regions may represent areas of heightened interactions between plastics and zooplankton.

Buoyancy of the plastic material influences its distribution in the water column. Vertical particle concentrations may be distributed due to their characteristic buoyancies and rise velocities, biofouling, and stratification of the water column and hydrodynamic conditions due to tides or wind mixing (Kukulka et al., 2012, Defontaine et al., 2020, Law, 2017, Kaiser et al., 2017). Low-density polymers (polyethylene, polypropylene, polystyrene predicted to be retained in the surface layer as their density is less than sea water 1027 kg m^{-3} (Morét-Ferguson et al., 2010). In contrast, high-density polymers are more likely to be found lower in the water column. In an estuarine system, stratification and mixing are greatly influenced by the tidal cycle with mixing occurring during flood tides and stratification on the ebb (Jay and Musiak, 1994). Strong mixing causes microparticles to aggregate throughout the water column, whereas a stratified system would cause material to be trapped in the deep layer (Jay and Musiak, 1994). Additionally, the ETM may distribute material throughout the water column in contrast to the expected buoyancy distribution.

In the Delaware Estuary the ETM typically arises between Port Mahon and New Castle, Delaware about 90 – 120 km from the bay mouth (Sommerfield and Wong, 2011, Biggs et al., 1983, McSweeney et al., 2016). Previous research in this region has shown that concentrations within the ETM region of the Delaware Bay are nearly double that found in the ambient bay (Cohen et al. 2019) with positively buoyant plastics – fibers and fragments of low density – becoming trapped in the surface layer (Mason et al., 2022, Shiravani et al., 2023, López et al., 2021). These observations are in agreement with modeling results from other estuarine systems

expecting elevated concentrations of microplastic within the ETM relative to the surrounding regions (Roscher et al., 2021, Defontaine et al., 2020).

Surface particle concentrations are commonly studied due to ease of access and global comparisons to environmental datasets, yet targeting only surface material may underestimate the total distribution of microparticles throughout an ecosystem (Kukulka et al., 2012). This study seeks to determine the vertical distribution and characterization of microplastic material within the Delaware Bay ETM region. In previous studies, vertically distributed microplastics have been evaluated in the upper 5 m of the North Atlantic accumulation zone (Reisser et al., 2015) and in a salt-wedge estuary in France (Defontaine et al., 2020). However, comprehensive analyses on vertical particle distributions remain sparsely available with limited evaluation of polymer identities and relative buoyancies. Thus, sampling surface, mid-water, and deep depths of the water column, along with polymer identity characterization, provides a comprehensive understanding of particle trapping patterns throughout the water column and aid in our understanding of particle trapping within estuarine turbidity maximums. I hypothesize that (1) microplastics will aggregate in higher concentrations at the surface throughout the sampling region, (2) microplastics will accumulate in higher concentrations, both at the surface and depth, where hydrography shows the stratified ETM feature relative to more vertically mixed upstream and downstream sample stations, and (3) subsurface microplastics will be dominated by negatively buoyant polymers as compared to surface microplastics.

3.2 Methods

3.2.1 *In-Situ* Sampling

Utilizing UD's *R/V Joanne Daiber,* microplastic material was sampled throughout the tidal cycle on two sampling days in 2019; June 4 and June 6 (Appendix B.1). Microparticles were targeted using a 200µm mesh, 0.5 m^2 area, Tucker Trawl multi-net at 3 depths (surface, mid-water, and deep). Tucker Trawl multi-net was outfitted with a flowmeter on each net opening calculating water volume filtered during sampling (~111.2 m^3 per net). To determine *in-situ* sampling depth, a CTD (RBRconcerto^3) attached to the Tucker Trawl transmitted real-time salinity, temperature and depth data with each sample. Each sampling depth was sampled for 5 minutes per depth, throughout 7 stations daily within the ETM sampling region of the Delaware Bay (Figure 3.1). These stations targeted the region of the estuary where Cohen *et al.* (2019) reported high concentrations of microplastic. Mid-water and deep sampling depths vary based on bathymetry throughout the ETM region, with surface samples targeting 0.5 m in depth, deep samples targeting 1 m above the bottom (average 12 m), and mid-water depths targeting the intermediate depth between surface and depth (average 7 m). The samples were transferred to glass jars and preserved with 4% borax-buffered formaldehyde immediately upon collection and stored for organic material digestion and microplastic quantification.

In addition to microparticle sampling, a CTD cast (Seabird SBE 19) was conducted at each station, targeting temperature (Seabird SBE 19-03), conductivity (Seabird SBE 19-03), and photosynthetically active radiation (PAR) (QSP-2300L) measurements to determine the exact location of the ETM during sampling days.

3.2.2 Microplastic Characterization

In the laboratory, samples were sieved through a 300µm stainless steel sieve and rinsed with deionized water to remove formaldehyde from the samples following methods from Cohen et al. 2019. Sieved samples were then recovered and digested for removal of organic material using Wet Peroxide Oxidation (WPO) following protocol from Masura *et al.* (2015). Following digestion, samples were suspended for a single density separation with a saturated sodium chloride solution (Hidalgo-Ruz *et al*., 2012). Following density separation, samples were collected onto a 200µm Nitex mesh, then covered in tinfoil and set to dry under the fume hood for 24 hours. Suspected microplastic material (hereafter "particles") were identified and enumerated under a stereomicroscope (Olympus CX31) categorized by particle type (i.e., fragment, fiber, bead) and color. Categorized particles were placed on a low-e glass slide (Kevley) and given a unique particle ID. Particles were then imaged using a Canon DSLR camera outfitted onto the stereomicroscope, measured for length and circumscribed area using ImageJ software (version 1.54f) (Schindelin et al. 2012).

To confirm particles as plastic, particles were analyzed for a polymer identity through transflectance using micro Fast-Fourier Transform Infrared (FTIR) spectroscopy (Primpke *et al*., 2017). µFTIR spectra (32 scans at 4 cm^{-1}) were collected using a Spotlight 200i micro-FTIR in reflectance (transflectance) mode between 4000-1000 cm^{-1}. µFTIR spectra were compared against a custom spectral library accepting matches above a 75% search score threshold. Microplastic determination using µFTIR spectroscopy was conducted exclusively on the microparticles collected on June 4, 2019, as time constraints precluded the analysis of samples from June 6, 2019. Thus, presented microplastic analyses of type, polymer, size, and relative buoyancy were conducted on material collected solely on June 4.

3.2.3 Data Analysis

Statistical analysis was performed using R (www.rproject.org). A Pearson's Chi^2 of independence was performed to determine the association between station and relative buoyancy, as all assumptions (categorical variables, independent observations, and mutually exclusive cells) were met. Additionally, Shapiro-Wilk's and Levene's tests were used to test data normality and homogeneity of variance, respectively. One-way ANOVA tests were used to make comparisons of microplastic size and depth within each type category. A Tukey post-hoc test was used when necessary.

3.2.4 Quality Control and Assurance

Preventative measures were used to mitigate plastic contamination during analysis. Bright orange cotton jumpsuits were worn when handling samples at any stage to minimize contamination from clothing. Plastic enumeration was done under a laminar flow hood, and samples were covered with tinfoil or class cover slide when not in use. Metal forceps and glass petri dishes were used for storage to minimize plastic contamination. Control samples of deionized water were put through the same digestion protocols were employed to detect possible microplastic contamination introduced throughout the processing methodology. No microplastic was found within the control samples.

Analysis of polymer identities, rayon versus cotton fibers, were conducted by purchasing 100% cotton (dyed blue and bleached white) and bamboo viscose yarn (dyed blue and bleached white) and creating four reflectance spectra files per yarn material and color, following the protocol above. These spectra were added to the spectral library and used to run a subset of rayon fibers to confirm their identity (Appendix B.1).

3.3 Results and Discussion

3.3.1 Microparticle Distribution

Microparticles – material assumed to be plastic – were found in highest concentrations at the surface, yet microparticles are not solely just a surface feature. Sampling reveals elevated levels of microparticles at depth where surface concentrations are also elevated (Figure 3.2, Table 3.1). Sampling regimes followed the flood tide up the estuary throughout the day on both days (Appendix B.2). June 4 (hereafter, day 1) sampled the mouth of the Delaware River (Station 7) flood tide, reaching high tide when sampling Stations 4 and 5, and sampling at ebb tide at Station 2. June 6 (hereafter, day 2) followed the start of flood tide at Station 6 to high tide at Station 1. Material aggregated in higher concentrations throughout the water column on day 2, which may be caused by mixing due to flood tide (Jay and Musiak, 1994). Although day 2 contained more microparticles overall, Stations 4 and 5 consistently show elevated microparticle concentrations across both sampling days. These elevated concentrations align with the extension of the salt intrusion from the Atlantic Ocean predominantly on day 1 (Figure 3.2A), a feature less evident on day 2 (Figure 3.2B). CTD data was interpreted to better understand the underlying mechanism of microparticle aggregation on day 1.

The estuarine turbidity maximum (ETM) was found between Station 4 and Station 5 on day 1. Calculated density (grams m^{-3}) and salinity (PSU) reveal the transition to fresh water at station 4, indicating the meeting of salt water from the Atlantic Ocean and the freshwater from the Delaware River. Further, a decrease in PAR, indicating a shallower depth of light penetration throughout the water column, was found at stations 4 and 5 (Figure 3.3C). Biggs et al., 1983, finds that the ETM in

the Delaware Bay coincides with higher diffuse attenuation coefficients, indicating more light absorbed or scattered in the water column, and a region of high particulate suspended matter. Thus, this decrease in PAR supports that sample stations 4 and 5 were well mixed as suspended material decreases the ability for light to pass through the water column (Köhler et al., 2018) further supporting the location of the ETM.

The Delaware Bay ETM is created at the extent of the salt tongue where two water masses meet and the frictional forces of the two water masses force particulate material to be suspended in the water column (Burchard et al., 2018, Sommerfield and Wong, 2011). Vertical aggregations of material are expected to be higher in this region as ETMs can cause the resuspension of sedimented material (Vermeiren et al., 2016). This is consistent with my findings at stations 4 and 5 on day 1 as deep samples contained elevated levels of microparticles. However, the surface concentrations of microparticles may be due to a multitude of factors. The convergence of surface plastic could be due to bay widening at this location (Sommerfield and Wong 2011), divergence on ebb tide as the water column constricts to go over the salt tongue, as well as convergence on flood as the water column expands as the current moves upstream of the salt tongue (Burchard et al., 2018). Overall, I find aggregations of microparticles within the ETM to be 2 - 3 times higher relative to the stations surrounding the ETM, consistent with other estuarine microparticle observations (Cohen et al., 2019, Lam et al., 2020, Roscher et al., 2021). As the ETM may explain the concentration pattern I see on day 1, day 2, however, does not seem to follow this reasoning.

3.3.2 Microplastic Characterization

Surface microplastic concentrations varied throughout the sampling region 0.16 – 2.4 plastic m^{-3} (Table 3.1). On average concentrations are similar to other salt-wedge estuarian averages finding between 0 – 3.88 plastic m^{-3} (Defontaine *et al.*, 2020), and Chesapeake Bay microplastic studies finding 0.007 – 1.25 plastic m^{-3} (Bikker *et al.*, 2020). However, these values are greater than Delaware river sampling upriver from our sampling locations and finding 6 x 10^{-5} plastic m^{-3} (Akbari et al., 2024). It is likely that microplastic is accumulated as it moves down river and before entering the Delaware Bay at sample station 7. Additionally, plastic concentrations are lower than previous Delaware estuary research by Cohen *et al.* 2019 finding 0.07 – 4.81 plastic m^{-3} in June of 2017 and the mouth of the Delaware bay and coastal shelf finding 0 - 4.12 plastic m^{-3} (Nitzberg et al., 2024).

The Delaware Estuary ETM functions as a microplastic trap. The sampling region on day 1 contained elevated microplastic concentrations at station 4 and 5 (Table 3.1). Average microplastic concentrations found at the surface are about double that of the deep samples, with mid-water samples the least concentrated (Table 3.1). Additionally, microplastic concentrations were 2 - 3 times higher than the stations surrounding the ETM. However, ETM subsurface sample concentrations were similar to surface concentrations where surface samples contained an average of 2.33 pieces m^{-3} and deep samples contained 1.92 pieces m^{-3}. Elevated subsurface concentrations may be explained by increased mixing at depth at the formation of the ETM allowing for the resuspension of settled material (Burchard *et al.*, 2018). Although concentrations in the surface ETM region are lower than those reported by Cohen *et al.* (2019) (maximum of 4.81 microplastic m^{-3}) in this region, updated methodology using μFTIR spectroscopy reduces the risk of inflated plastic counts due to human

error. I find that microplastic material is overestimated when chemical spectroscopy methods are not conducted. By comparing particle counts to μFTIR plastic counts, it was determined that manually picked particles overestimated confirmed plastic by about 32% (n = 1425 in 2105). Interestingly, in the St. Lawrence Estuary, Kelly, (2024) reported a decrease in microplastic concentrations within the ETM disagreeing with modeling from Vermeiren et al., 2016, Shiravani et al., 2023, and Fontana et al. *in prep*, who suggest elevated plastic aggregation at these features.

In all samples, fragments and fibers were the most common type. Both fragments and fibers were found in similar proportions across depth (Figure 3.4A) yet fibers were more common outside of the ETM region (Stations 2, 3, 7) whereas fragments were more common inside the ETM (Station 4 and 5) (Figure 3.4B). Although found in lower quantities, beads were commonly found deeper (Figure 3.4A). Most fragments were predominantly identified as polyethylene (62%) and polypropylene (34%) with little variability throughout depth (Figure 3.5). Beads are commonly found to be polystyrene (94%) with 6% as polyethylene (Figure 3.5). Fibers are predominantly polyester (33%) as well as a semi-synthetic material rayon (29%), followed by polypropylene (22%) (Figure 3.4). These proportions align with plastic manufacturing as polypropylene, polyethylene and polystyrene are commonly manufactured as non-fibrous materials typically used in packaging whereas polyester (PET) accounts for a large proportion (~70%) of fiber production mostly used in textiles (Geyer *et al.*, 2017). Polyethylene and polypropylene were the dominant fragment polymers and polyester (PET) were dominant fiber polymers from other Delaware Bay and river studies (Cohen et al., 2019, Nitzberg et al., 2024, and Akbari

et al., 2024) as well as studies of the Chesapeake Bay (Bikker et al., 2020) and Weser estuary and German North Sea (Roscher et al., 2021).

Semi-synthetic materials such as Rayon, otherwise known as viscose, as well as cellulose acetate are common materials used in textiles or solid parts as an alternative to plastic. These materials have sparsely been reported in previous literature but have been reported more commonly in ingestion studies (Remy *et al.*, 2015, Lusher *et al.*, 2013). Rayon is typically missing from commonly used spectral libraries, such as OpenSpecy (Cowger et al., 2021), due to the prioritization of synthetic polymers and the novel appearance of rayon reported in the environment.

However, recent studies of the Delaware river report rayon as a significant portion of polymer identity (Akbari et al., 2024) as well as chemically modified cellulose (Roscher et al., 2021). Semi-synthetic materials, much like plastic, are highly manufactured and can contain and accumulate similar harmful chemicals as synthetic plastic (Barrows *et al.*, 2018). Due to the prevalence of Rayon fibers in our samples, they have been included in our analysis as plastic. Although, an 11% decrease in microplastic concentration is seen when semi-synthetic material is removed (Appendix B.3).

Relative buoyancy was determined using the particles' characteristic polymer and assigning a literature derived density value by polymer (Appendix B.4). Density of material can be modified by a multitude of variables including biofouling (Andrady, 2011), weathering and fragmentation (Onink et al., 2022), flocculation (Andersen et al., 2021), and additives such as plasticizers (Koelmans et al., 2016). Given the challenges in determining precise densities for each plastic particle, the term 'relative buoyancy' is employed to characterize the likely behavior of these microplastic

particles based on literature derived density values. Categories of relative buoyancy were determined as neutrally buoyant if the density was between ± 0.01 the density of freshwater and saltwater (0.990 - 1.035 kg/m^3), positively buoyant below 0.989 and negatively buoyant if material was greater than 1.036 kg/m^3. MP types differed in their characteristic buoyancy with beads primarily neutrally buoyant, fragments positively buoyant and fibers negatively buoyant (Figure 3.6A). Interestingly, we find that negatively buoyant material is more common at the surface compared to subsurface samples throughout the sampling region. However, proportions of positively buoyant material aggregate within the ETM (St. 4 & 5) as compared to negatively buoyant material which is found in higher proportions along the upstream and downstream regions St.2 & St.7 (Figure 3.6B). These results are consistent with fibers as the dominant plastic type at stations 2 and 7, and fragments prevalent the ETM (Figure 3.4B). However, modeling results reveal negatively buoyant material down estuary advected to the flanks due to differential advection (Fontana et al., *in prep*). As the ETM creates a strong mixing front, positively buoyant material can be forced to deeper depths in addition to aggregating at the surface. This is due to turbulent mixing effecting the buoyancy of plastic material submerging buoyant material deeper in the water column (Vermeiren et al., 2016, Zhang, 2017).

Larger beads are found at the surface in both length (mm) and area (mm^2). Fragments are longer in length at the surface than subsurface samples with no significant difference in area throughout the water column (Figure 3.7A). Indicating that fragmented material with a longer axis is able to resist sinking due to increased drag whereas more compact material is more likely to be trapped in the lower layers (Onink et al., 2022). Fibers show no significant difference in length but are smaller in

area at depth (Figure 3.7B). Additionally, beads are significantly larger at the surface both by length and area (Figure 3.7A, 3.7B).

3.4 Figures and Tables

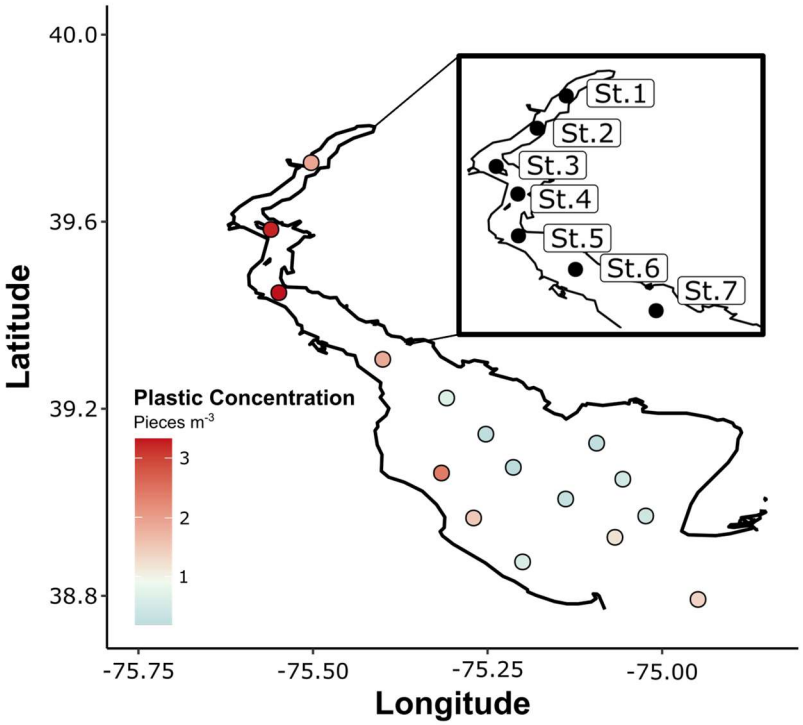

Figure 3.1: 2019 sampling stations (black points) inset with the previously determined microplastic concentrations with a gradient of high concentrations in red and low concentrations in blue. Data from 2017 microplastic concentrations reported in Cohen *et al.* (2019).

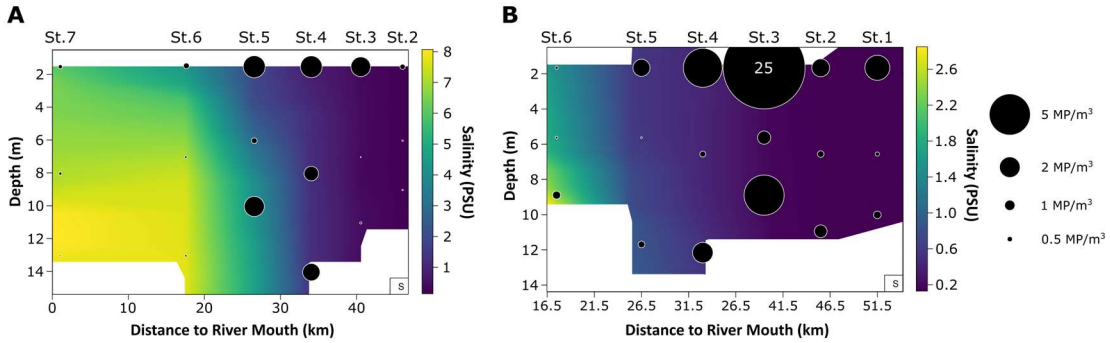

Figure 3.2: Sample region microparticle concentrations (black bubbles) by depth overlayed with the salinity profiles of the Delaware estuarine turbidity maximum region from A) June 4, 2019 and B) June 6, 2019. River mouth is defined as the location where the river widens into the Delaware Bay.

Table 3.1: Microplastic concentration (plastic m^{-3}) by sample station and average depth sampled and average concentration per depth on June 4, 2019.

DEPTH	ST.2	ST.3	ST.4	ST.5	ST.6	ST.7	AVERAGE
SURFACE	0.89	1.98	2.4	2.27	0.71	0.41	1.44
MID	0.18	0.15	1.5	0.6	0.34	0.31	0.78
DEEP	0.18	0.32	1.81	2.04	0.23	0.16	0.51

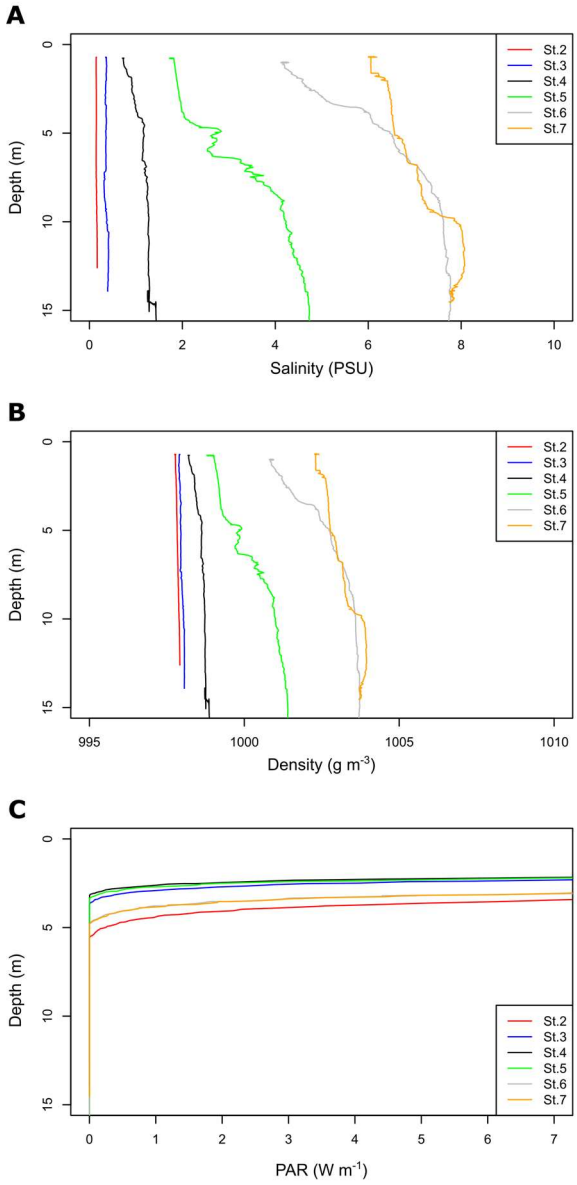

Figure 3.3: CTD water column profiles of A) Salinity (PSU), B) Density (g m-3), and C) Photosynthetically active radiation (PAR) (W m-1). Stations delineated by color; station 2 (red), station 3 (blue), station 4 (black), station 5 (green), station 6 (gray), and station 7 (orange).

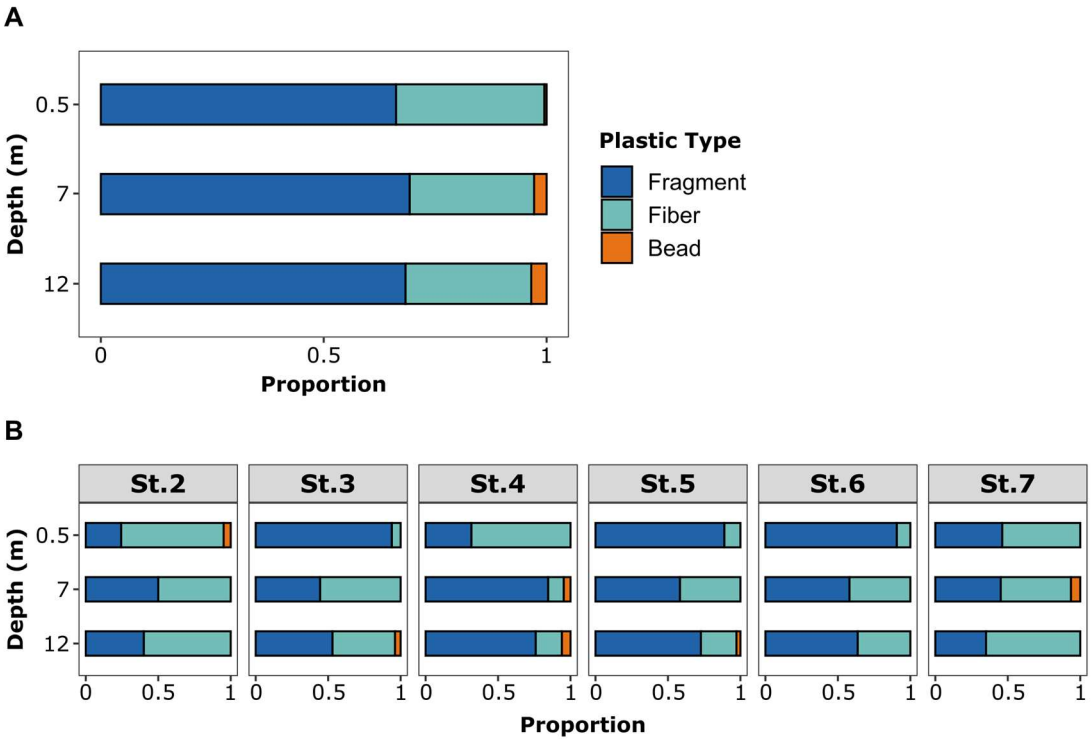

Figure 3.4: Microplastic proportion of type; fragments (blue), fibers (teal), beads (orange) distributed by average depth (m) by A) sampling region and B) station. ETM region located at Station 4 and 5.

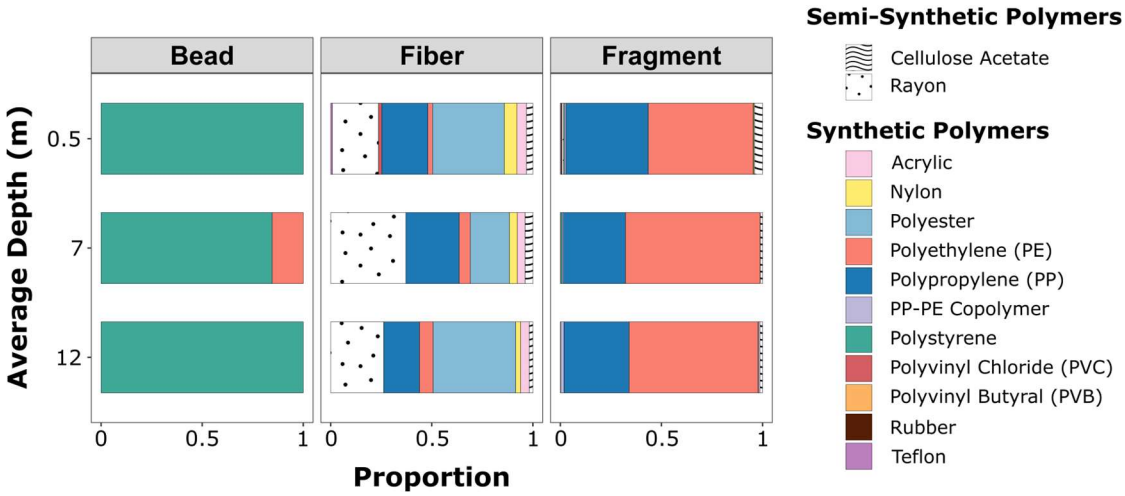

Figure 3.5: Proportion of microplastic polymers identities by average depth (m) and type. Semi-synthetic material denoted by patterned fill.

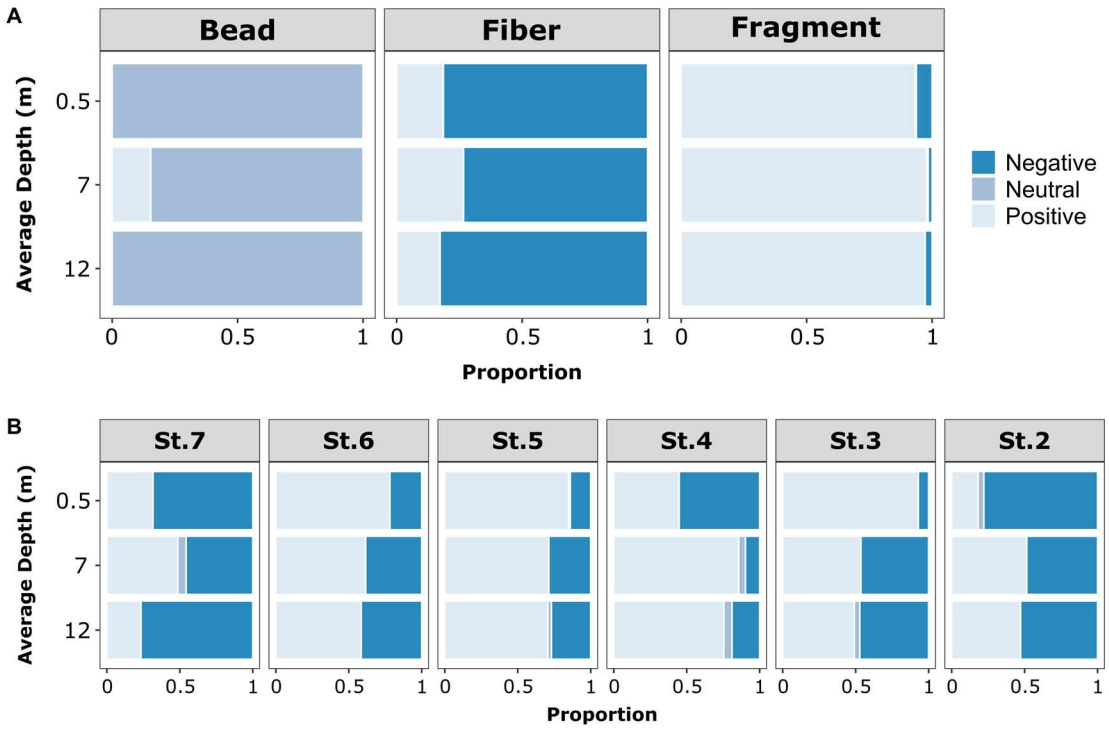

Figure 3.6: Relative buoyancy of positively (light blues), negatively (dark blue) and neutrally buoyant (grey-blue) microplastic material distributed across depth and A) type and B) location along the Delaware estuarine turbidity maximum region. St.7 located at the river mouth with St.2 located furthest upriver. Positively buoyant material is more common inside of the ETM, Stations 4 and 5, where as negatively buoyant material is found at Station 2 and 7 (Pearson's χ^2 test , P = 2.2e-16). Negatively buoyant material is more common at the surface compared to subsurface samples (Pearson's χ^2 test, P = 0.0004835).

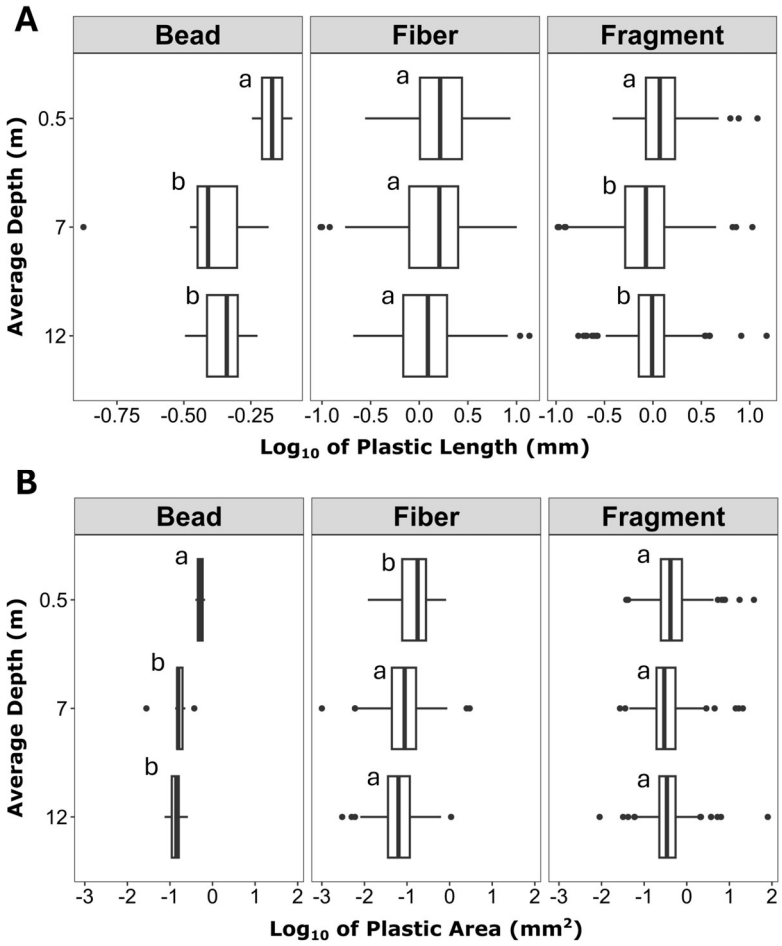

Figure 3.7: Size distribution of microplastics organized by plastic type and compared to average depth (m) A) $\log_{10}$ of length (mm) and B) $\log_{10}$ of area (mm^2). Beads are significantly larger at the surface both by length (1-way ANOVA, Tukey post-hoc, P= 0.00579) and area (1-way ANOVA, Tukey post-hoc, P = 9.59e-08). Fragments are larger at the surface in length (1-way ANOVA, Tukey post-hoc, P = 8.34e-07). Fibers are smaller in area at depth (1-way ANOVA, Tukey post-hoc, P = 0.00116).

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Chapter 4

MICROPLASTIC IN ESTUARINE ORGANISMS OF THE DELAWARE BAY

4.1 Introduction

As plastic waste persists in the environment, understanding the ecological impacts remains of utmost importance. Microplastic interactions with biota are ubiquitous throughout the global environment. Interactions with microplastic may lead to ingestion, entanglement, ecotoxicological effects, tissue translocation, and potentially death (Hale et al., 2020). Here I focus primarily on the passive and active ingestion of microplastic material in estuarine organisms.

Previous studies have reported microplastic ingestion by marine zooplankton; holoplankton (Cole et al., 2013), meroplankton (Boettcher 2024), fish (Parker *et al.*, 2020, Lusher et al., 2013, Egbeocha et al., 2018, Lusher et al., 2016), invertebrates (Hall et al., 2015, Suckling, 2021, Haskell et al. 2024), and megafauna (Besseling *et al.*, 2015) alike. Organisms may ingest microplastic passively through filter feeding as shown by organisms such as baleen whales or bivalves (Besseling *et al.*, 2015, Van Cauwenberghe and Janssen, 2014) respiration through gills by decapods and fish (Watts et al., 2016, Jaafar et al., 2021), or trophic transfer from ingesting prey sources containing microplastic (Lusher et al., 2016, Carbery et al., 2018, Nelms et al., 2018).

Characteristics of microplastics, such as type (shape) and color, may influence ingestion from biota (Hale et al., 2020, Botterell et al., 2020). These characteristics may be selected for on a species level based on food selectivity as evidenced by copepods (Cole et al., 2013), or handling and capture efficiency. Previous laboratory

studies of microplastic ingestion primarily use beads (spheres) and have shown uptake by a number of species (Cole et al., 2013, Watts et al., 2016, Setälä et al., 2014). However, fibers were most commonly ingested in field investigations (Desforges et al., 2015, Steer, 2017). Additionally, abiotic factors such as color, or biotic factors such as microplastic biofouling, can affect the bioavailability of microplastics to organisms. Horie et al., 2024 finds species-specific uptake of colored microplastic particles in different groups of marine and freshwater fish, indicating organisms using vision to selectively ingest microplastic. Biofouling may influence ingestion of microplastic. Many ingestion studies use virgin plastic material, yet in the environment material is likely colonized by a wide variety of microorganisms. Biofouling of microplastics may influence ingestion due to chemical cues released by the microorganisms (Vroom et al., 2017), which may entice organisms such as fish (Savoca et al., 2017) and seabirds (Savoca et al., 2016) to eat them. Fabra et al., 2021, finds increased uptake of microplastic when coated in biofilm than virgin material. Additionally, biofouled material may contain pathogenic viruses and harmful chemicals that can further impact an organism's health when ingested (Hale et al., 2020).

Ingestion of microplastic can also cause material to be translocated to other regions of the organism, harmful chemical leaching, and the blocking of alimentary canals (Hale et al., 2020, Botterell et al., 2020). Previous studies have reported microplastic-induced reduction in feeding rates in a copepod species *Calanus helgolandicus* (Cole et al., 2013), fish larvae (Steer, 2017), and alterations in size, density and sinking rates of salp fecal pellets (Wieczorek et al., 2019). Additionally, microplastic ingestion may cause adverse reactions in energy metabolism, the

antioxidative system and cellular damage (Prokić et al., 2019). If compounded with another stressor such as temperature, organisms may reprioritize their energy budget to keep up with the demands of basal maintenance and sacrificing growth and reproduction (Sokolova, 2013).

Previous studies have shown ingestion across a wide range of species in estuaries close to the Delaware Bay (Parker et al., 2020), yet no study has assessed the body burden of plastic within estuarine species within the Delaware Bay. Thus, the goal of this study is to determine the spatial patterns and body burden of plastic ingestion by estuarine organisms including economically important species such as the Atlantic Blue Crab (*Callinectes sapidus*). We hypothesize that (1) estuarine organisms including *C. sapidus* will contain microplastic body burdens positively correlated with concentration of water column microplastics at the sampling locations; (2) older *C. sapidus*, evidenced by reproductive and carapace conditions, will contain higher body burdens of microplastic than younger crabs; and (3) *C. sapidus* collected in the spring, when water column microplastics are elevated, will likewise contain elevated body burdens. Comprehending the patterns and extent of plastic ingestion on estuarine species will aid in our understanding of microplastic fate and transport throughout estuaries, as well as identify species susceptibility to plastic pollution in the future.

4.2 Methods

4.2.1 Estuarine Organism Collection

Estuarine organisms were collected using an otter trawl across 4 locations in the Delaware Bay (Figure 4.1A). Sample locations were chosen based on previous environmental microplastic sampling (see chapter 3, Cohen et al., 2019). All

organisms recovered from the otter trawls were immediately flash-frozen with cryospray and placed on ice before being placed in a -20°C freezer awaiting dissection. Organisms were later dissected for their gastrointestinal tract including their stomach, and their gills, if present. Invertebrates such as sand shrimp (*Crangon septemspinosa*), long-wristed hermit crabs (*Pagurus longicarpus*), eastern mud snails (*Ilyanassa obsoleta*), and isopods (*Politolana concharum*) were removed from their exoskeleton or shell, whereas the ascidian (*Molgula manhattensis*) were used whole, for analysis. Dissected tissues were then frozen in 20mL scintillation vials at -20°C and stored for later analysis.

Blue crabs were collected over a three-year period from various sources. Mature, non-ovigerous, female blue crabs were collected from a total of 37 stations throughout the Delaware Estuary. Crabs were collected from Delaware Department of Natural Resources and Environmental Control (DNREC) monthly trawl survey using 16- and 30-foot trawls during 2021 (see Hoffmann 2023 for additional details). I obtained additional mature, non-ovigerous female crabs from recreational fishers collected throughout the months of May to September 2023, along with male crabs collected in June of 2024. Female crabs were dissected for their stomachs, with males dissected for both stomach and gills. Samples were stored in 20 mL scintillation vials in a -20°C freezer for later analysis.

4.2.2 Microplastic Body Burden Analysis

Dissected tissues were thawed and then digested using 10% KOH solution in an incubator for 24 hours at 60°C following Lusher et al. 2017. Digested tissues were then filtered onto a 20μm polycarbonate 45mm membrane and rinsed with DI water.

Each filter was then placed into a glass petri dish, covered, and set to dry under a fume hood for 24 hours. Following methodology from Chapter 3, suspected microplastic material (hereafter "particles") were identified and enumerated under a stereomicroscope (Olympus CX31) categorized by particle type (i.e., fragment, fiber, bead) and color. Categorized particles were placed on a low-e glass slide (Kevley) and given a unique particle ID. Particles were then imaged using a Canon DSLR camera outfitted onto the stereomicroscope, measured for length and circumscribed area using ImageJ software (version 1.54f) (Schindelin et al. 2012).

To confirm particles as plastic, particles were analyzed for a polymer identity through transflectance using micro Fast-Fourier Transform Infrared (FTIR) spectroscopy (Primpke *et al*., 2017). µFTIR spectra (32 scans at 4 cm^{-1}) were collected using a Spotlight 200i micro-FTIR in reflectance (transflectance) mode between 4000-1000 cm^{-1}. µFTIR spectra were compared against a custom spectral library accepting matches above a 75% search score threshold.

4.2.3 Quality Control and Assurance

Preventative measures were used to mitigate plastic contamination during analysis. Bright orange cotton jumpsuits were worn when handling samples at all stages to minimize contamination from clothing. Plastic enumeration was done under a laminar flow hood, and samples were covered with tinfoil or class cover slide when not in use. Control samples were employed during the digestion and filtering step with one control per 25 samples per day. Detected microplastic material was removed from the results (n = 1, white fiber).

4.3 Results and Discussion

4.3.1 Microplastic Characterization in Estuarine Organisms

A total of 179 organisms from 20 species were collected (Table 4.1). Overall, 12% of estuarine organisms contained plastic. Organisms containing plastic and the average environmental plastic at that location had no significant difference (P = 2.944, Linear Regression, proportion organisms with plastic ~ average surface plastic). Although, the highest proportion of organisms containing plastic, 14% (n = 13 of 94), was found at DE-3. Species exhibiting the highest body burdens include *C. sapidus*, with 12% of individuals affected (n = 16), and *Anchoa mitchilli (*Bay anchovy), with 11% affected (n = 53) (Table 4.1). No plastic body burden was found in Spotted hake (*Urophycis regia*) (n = 21). North Atlantic mesopelagic fishes showed similar proportions of plastic body burdens (11%), with species body burdens ranging from 22% found in *Benthosema glaciale* and 2.8% in *Maurolicus muelleri* (Lusher et al., 2016). Similarly, 9% of fish species in a tropical estuary contained microplastic in their digestive tract with species body burdens ranging from 20% in *Lycengraulis grossidens* and 16% in *Anchoa januaria* to 1% in *Mugil curema* (Vendel et al., 2017).

Fish species (Table 4.1) were analyzed to determine the method of plastic uptake, distinguishing between trapping via gills and ingestion through the gastrointestinal tract (GIT). While plastic counts from gills and GIT do not significantly differ overall (Paired t-test, P = 0.169), of the three fish species containing plastic, all contained plastic in their gills while only *A. mitchilli* also contained plastic in the GIT (Figure 4.1). Passive uptake methods, such as respiration, in fish, may be more prominent than active ingestion. This aligns with findings from Jaafar et al. (2021) where although insignificant, 53% of microplastics occurred in the

gills of finfish. However, due to small sample sizes it is difficult to ascertain the biological and ecological drivers – such as feeding behavior, habitat, or trophic level – that may influence plastic ingestion from this study.

Due to its low trophic level in estuarine and marine food webs, elevated levels of plastic ingestion by *A. mitchilli* could facilitate the transfer of microplastics to higher trophic levels through predation. *Anchoa mitchilli* was the most observed organism with the highest occurrence of plastic body burden of fish species with observations $n > 10$ (Table 4.1). This relatively small planktivorous fish that is found in abundance along coastal bays and estuaries from the Gulf of Maine to Brazil and commonly preyed upon by commercially important species such as weakfish, striped bass, bluefish, and summer flounder (Newberger and Houde, 1994). As such, *A. mitchilli* would be recommended for further studies on trophic transfer of microplastic within the Delaware Bay.

Fibers were the most common polymer type and the only microplastic type found in the gills of organisms. Polymer analysis of fibers reveals a semi-synthetic material rayon as the dominant polymer identity (66.7%) followed by polyester (PET) (19%) (Figure 4.3A). Of the two fragments found, both polymer identities – polyethylene and polypropylene – are consistent findings in other bay-wide microplastic sampling (see Chapter 2) and are of the most common plastic polymers produced (Figure 4.3A). Similarly, rayon and PET were common polymers found by ingested fibers in an urban estuary of Sydney, Australia (Halstead et al., 2018) and fishes in the English Channel (Lusher et al., 2013). Additionally, Remy et al., 2015, found artificial fibers, presumed to be rayon, ingested by amphipod species *Gammarus aequicauda* and *Gammarus fucicola* within the Mediterranean coastal zone.

Organisms in this analysis were neither sexed nor characterized for morphological traits. However, due to the ecological and economic importance of *Callinectes sapidus* in the Mid-Atlantic region, additional analysis was conducted to assess microplastic body burdens in this species.

4.3.2 Microplastic Characterization in *C. sapidus*

Microplastics were found in 26% of female crabs (n = 115 of 442) and 30% of male crabs (n = 9 of 30). These results are higher than *C. sapidus* samples by otter trawl reported in section 4.3.1, yet similar to body burdens of *C. sapidus* reported in the Corpus Christi Bay (25.6%) (Waddell et al., 2020). Among the male crabs that contained microplastics, three had microplastics in their gills, while six had microplastics in their stomachs; no male crab had microplastics in both tissues. For the crabs containing microplastics, each had approximately 1.54 ± 0.85 pieces individual^{-1}, with fibers being the dominant type (96%), followed by fragments (4%). Polymer analysis (Figure 4.3B) revealed that rayon was the most dominant polymer type (78% of fibers), followed by polyester (PET) (15% of fibers). These findings are comparable to the polymer distribution observed throughout the water column in the Delaware Bay (see Chapter 3 results) and are consistent with the distribution of microplastics found in other estuarine organisms presented in section 4.4.1 (Figure 4.3A). Similarly, Waddell et al., 2020 reported 49% of fibers in *C. sapidus* of the Corpus Christi Bay to be rayon and 35% of fibers were polyester. Additionally, these results are consistent with microplastic ingested by fishes reported by Halstead et al., 2018 and Lusher et al., 2013.

4.3.3 Temporal and Spatial Patterns of *C. sapidus* Plastic Ingestion

Spring crabs (those collected in April and May) contained a higher proportion of crabs containing plastic than crabs collected in other months (P = 0.0033, Rayleigh's test of Uniformity) (Figure 4.4). This coincides with the peak river discharge in the spring, which results in higher concentrations of plastic in the Delaware Bay (Cohen et al., 2019). Additionally, during this period, crabs have emerged from overwintering in benthic sediments and consume more food in preparation for the upcoming spawning season where energy demands are heightened due to migration, reproduction and spawning events (Epifanio, 2019). These two factors likely increase the interaction between individual organisms and environmental plastic.

Spatial analysis, however, reveals no clear relationship between location and plastic content (Figure 4.5). A linear regression model was used to examine the relationship between surface plastic concentrations and the proportion of crabs containing plastic in both spring and summer months. The model tested whether crabs collected in areas with higher surface plastic concentrations were more likely to ingest plastic, although no significant relationship was found (Figure 4.5). While species-specific studies directly correlating environmental plastic concentrations to plastic body burdens, Compa et al. (2023) found ingestion of microplastic in *C. sapidus* from the Mediterranean Sea was positively correlated with an increase in sewage pipelines. Further research is needed to establish a direct relationship between environmental plastic and body burdens.

4.3.4 Time since terminal molt and Female *C. sapidus* Plastic Ingestion

Reproductive characteristics of the female blue crabs dissected for this study were examined as part of Hoffman (2023). These characteristics included carapace width (mm), spermatheca condition, ovary stage and size, and the determination of mature and immature females based on their abdomen shape. I used this information to test whether plastic ingestion varied according to these variables in mature females with a range of times since having undergone their terminal molt. No significant correlation was found between crab size and plastic body burden (Figure 4.6). All male crabs collected were above regulation size of 27 cm (5 in) measuring carapace tip to tip. 58 amount of female crabs were fished below regulation size, although Delaware legislation states the minimum size does not apply to female *C. sapidus*, however no female bearing eggs can be collected 7 Del. C. 1953, § 2301, 2302.

Older crabs, determined by partially dirty and dirty carapace conditions (Figure 4.7) show no differences in proportions of crabs containing plastic. The proportion of crabs that had previously brooded was determined by examining advanced-stage ovaries (Stage 4) following Johnson (1980), and reabsorbed spermatheca (Stage 4) according to Wolcott et al. (2005). No difference was observed in the proportion of crabs containing plastic between those that had previously brooded and those that had not (Figure 4.8). Microplastic ingestion has been shown to cause detrimental effects on feeding, egestion, reproduction and survival in copepod *Tigriopus japonicus* (Yu et al., 2020). Sexual maturity, fecundity, and egg quality of Atlantic cod *Gadus morhua* were investigated by Fernández-Míguez et al., 2023 although no significant difference was found. Often unrealistically high concentrations of microplastics are administered in laboratory studies in order to provoke measurable effects, although current naturally occurring concentrations reveal no effect on organisms (Lenz et al., 2016). Although,

as plastic concentrations continue to rise in the environment, increased ingestion may lead to impacts such as decreased feeding capacity, energy reserves, and reproduction potential (Prokić et al., 2019).

4.4 Figures and Tables

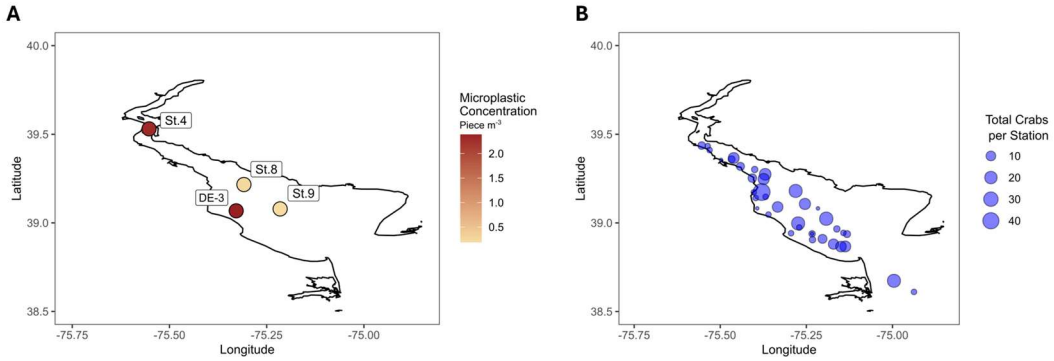


Figure 4.1: Map of sample locations A) estuarine organisms with points of high environmental microplastic concentrations (pieces m-3). High concentrations in red and low concentrations in yellow. B) sample locations and number of crabs collected per station of *Callinectes sapidus.*

Table 4.1: Table of total species collected, total organisms that contained plastic, and proportion of organisms that contained plastic. Data is categorized by classification, primary habitat, feeding strategy (Froese, R. and D. Pauly. Editors. 2024. FishBase). Shaded rows indicate species collected with greater than 2 individuals.

Organism ID	Organisms Containing Microplastic	Total Organisms	Proportion of Plastic	Organism Classification	Primary Habitat	Feeding Strategy
Bay Anchovy (*Anchoa mitchilli*)	6	53	0.11	Fish	Pelagic	Planktivore
Sand Shrimp (*Crangon septemspinosa*)	3	43	0.07	Invertebrate	Benthic	Detritivore, Planktivore
Long-wristed Hermit Crab (*Pagurus longicarpus*)	2	21	0.10	Invertebrate	Benthic	Detritivore
Spotted Hake (*Urophycis regia*)	0	21	0.00	Fish	Pelagic	Piscivore
Blue Crab (*Callinectes sapidus*)	2	16	0.13	Invertebrate	Benthic	Omnivore
Hogchoker (*Trinectes maculatus*)	1	5	0.20	Fish	Demersal	Piscivore, Crustacivore
Northern Pipefish (*Syngnathus fuscus*)	1	2	0.50	Fish	Benthic	Planktivore
White Perch (*Morone americana*)	1	2	0.50	Fish	Pelagic	Omnivore
Mud Crab (*Panopeus herbstii*)	1	2	0.50	Invertebrate	Benthic	Omnivore

Table 4.1: continued

American Eel (*Anguilla rostrata*)	0	2	0.00	Fish	Pelagic	Piscivore
Smallmouth Flounder (*Etropus microstomus*)	0	2	0.00	Fish	Demersal	Planktivore
Lady Crab (*Ovalipes ocellatus*)	0	2	0.00	Invertebrate	Benthic	Detritivore, Carnivorous
Blackcheek Tonguefish (*Symphurus plagiusa*)	0	1	0.00	Fish	Benthic	Omnivore
Lined Seahorse (*Hippocampus erectus*)	0	1	0.00	Fish	Demersal	Planktivore
Menhaden (*Brevoortia tyrannus*)	0	1	0.00	Fish	Pelagic	Herbivore, Planktivore
Silver Hake (*Merluccius bilinearis*)	0	1	0.00	Fish	Benthic	Piscivore, Crustac-ivore
Windowpane flounder (*Scophthalmus aquosus*)	0	1	0.00	Fish	Demersal	Piscivore, Crustaci-vore
Isopod (*Politolana concharum*)	0	1	0.00	Invertebrate	Benthic	Detritivore
Eastern Mud Snail (*Ilyanassa obsoleta*)	0	1	0.00	Invertebrate	Benthic	Detritivore

Table 4.1: Continued

Ascidian (*Molgula manhattensis*)	0	1	0.00	Invertebrate	Benthic	Suspension feeder

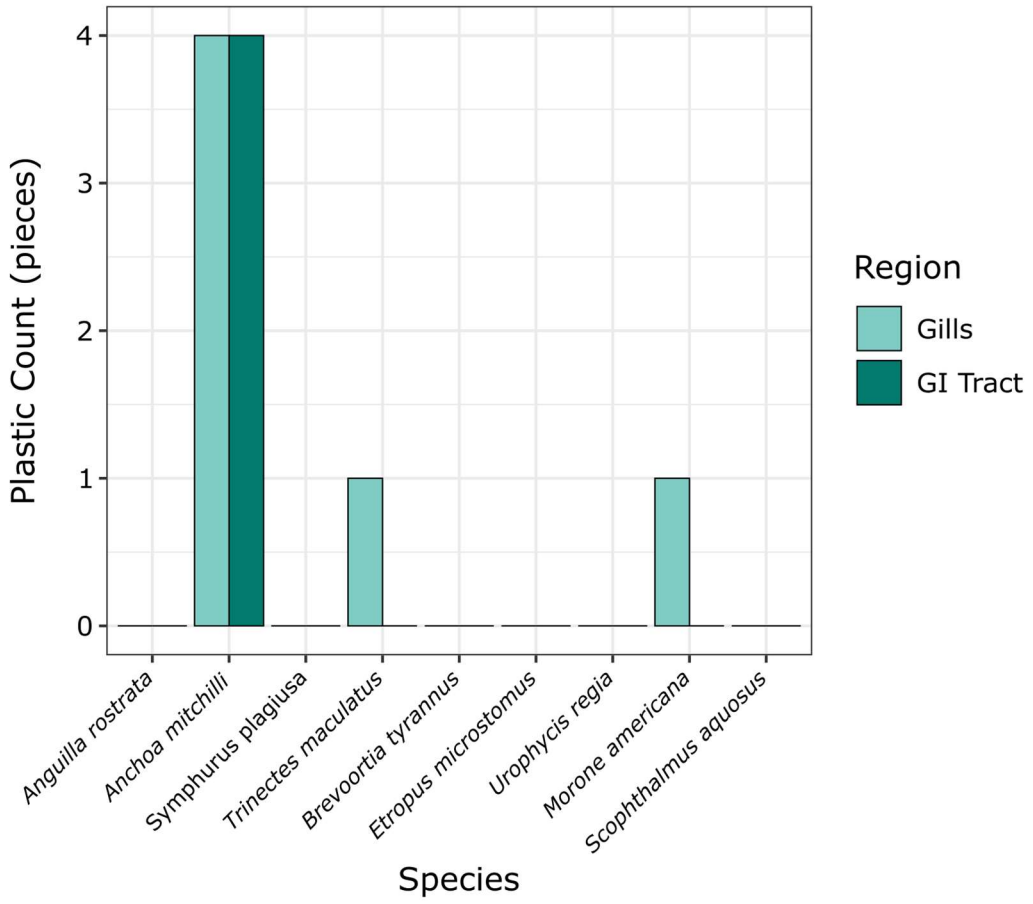


Figure 4.2: Species dissected for gills and gastrointestinal tract compared to plastic count (pieces) found in each body region.

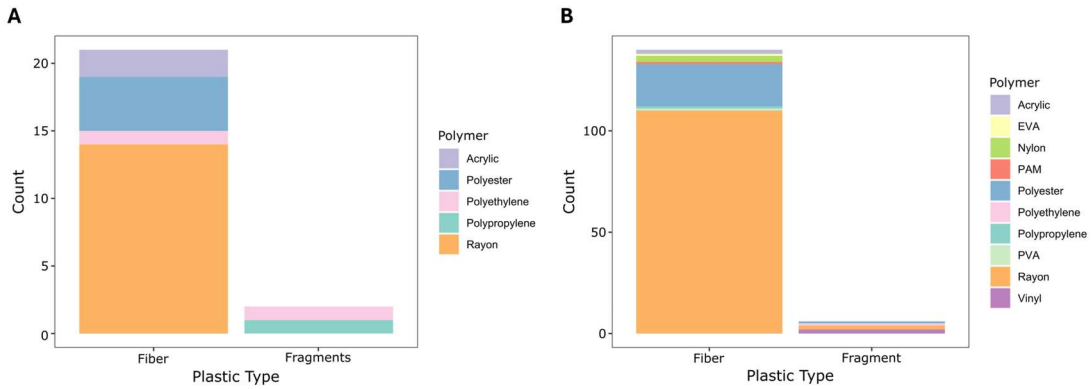


Figure 4.3: Polymer identification of microplastic collected from the stomach and gills of A) Delaware Bay estuarine organisms and B) Delaware Bay blue crabs.

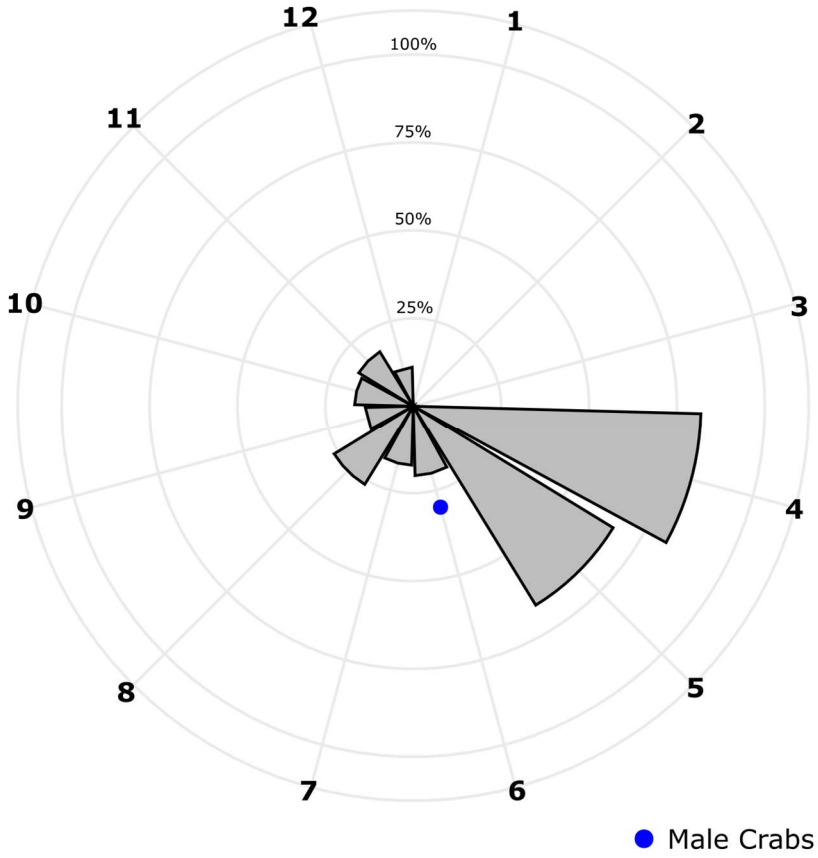
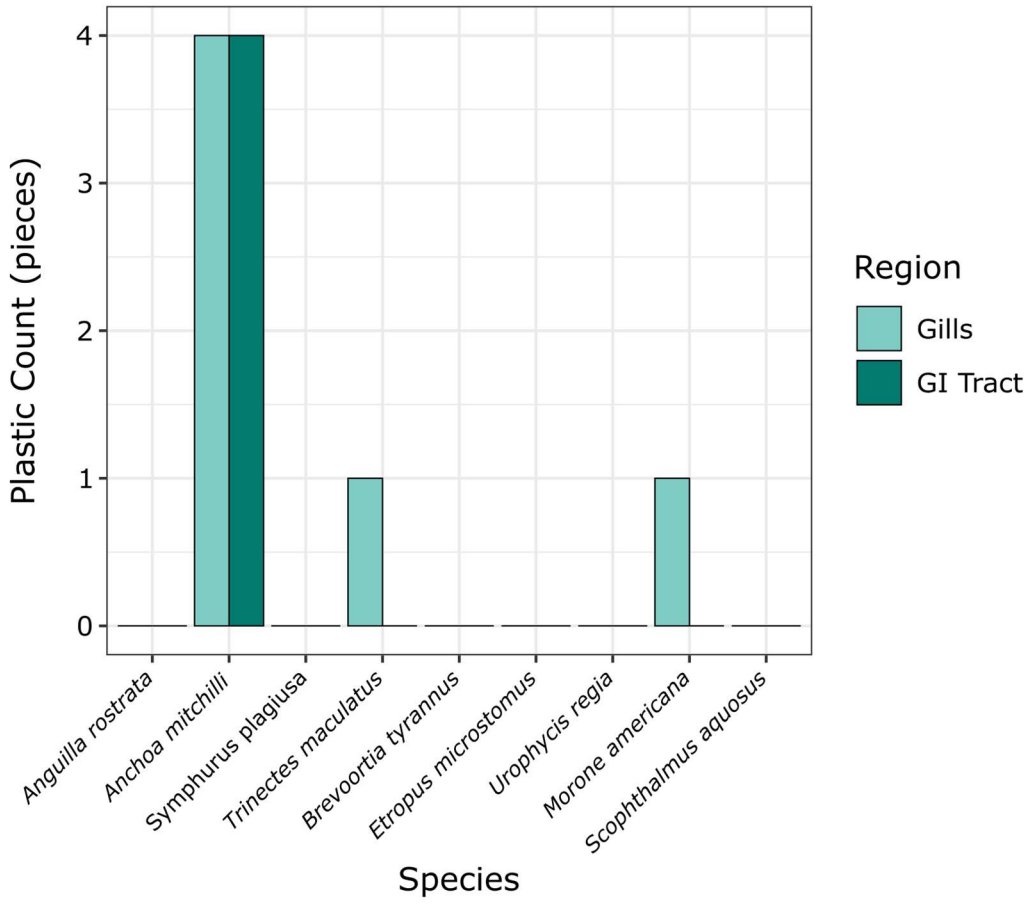


Figure 4.4: Proportion of Delaware Bay *Callinectes sapidus* females (gray bars) and *C. sapidus* male crabs (blue point) containing plastic throughout the months of the year. No crabs were collected January, February and March, April (n = 11), May (n = 33), June (n = 70), July (n = 47), August (n = 72), September (n = 44), October (n = 10), November (n = 22), December (n = 9). April and May crabs contained a higher proportion of crabs containing plastic (P = 0.0033, Rayleigh's test of Uniformity)


Plastic Count (pieces)
4
3
2
1
0
Anguilla rostrata
Anchoa mitchilli
Symphurus plagiusa
Trinectes maculatus
Brevoortia tyrannus
Etropus microstomus
Urophycis regia
Morone americana
Scophthalmus aquosus
Species
Region
Gills
GI Tract

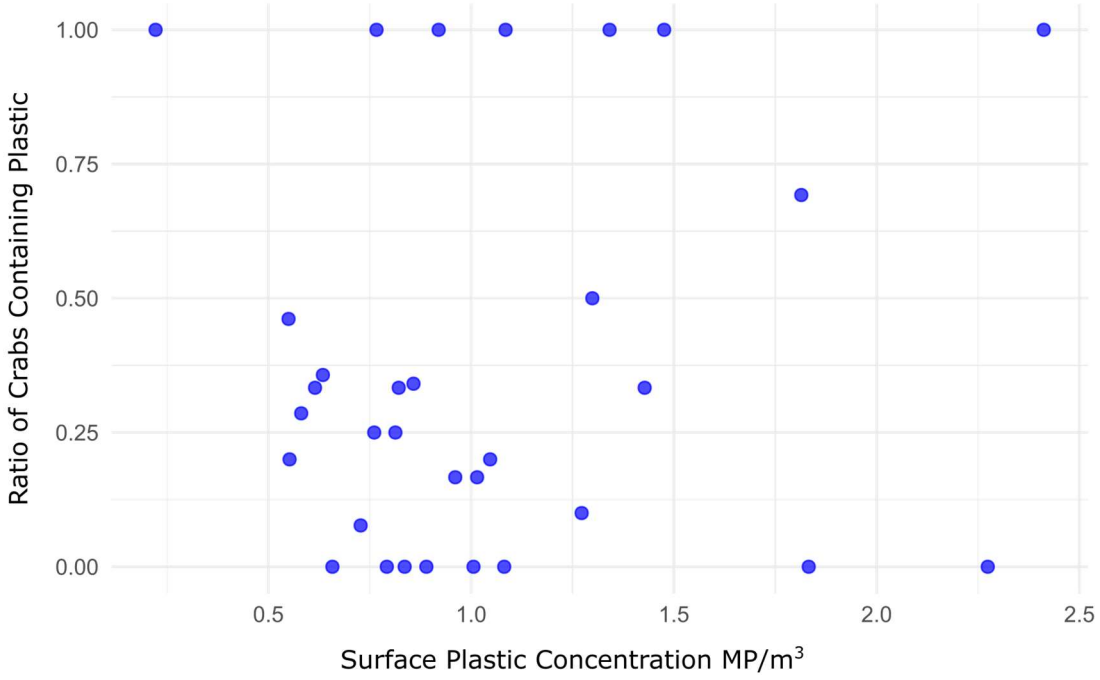


Figure 4.5: Linear regression of the surface plastic concentration (microplastic/ m^3) to the ratio of crabs containing plastic at the same location for spring crabs only. No significant trend was observed (P = 0.5088, Linear Regression ratio ~ surface plastic concentration).

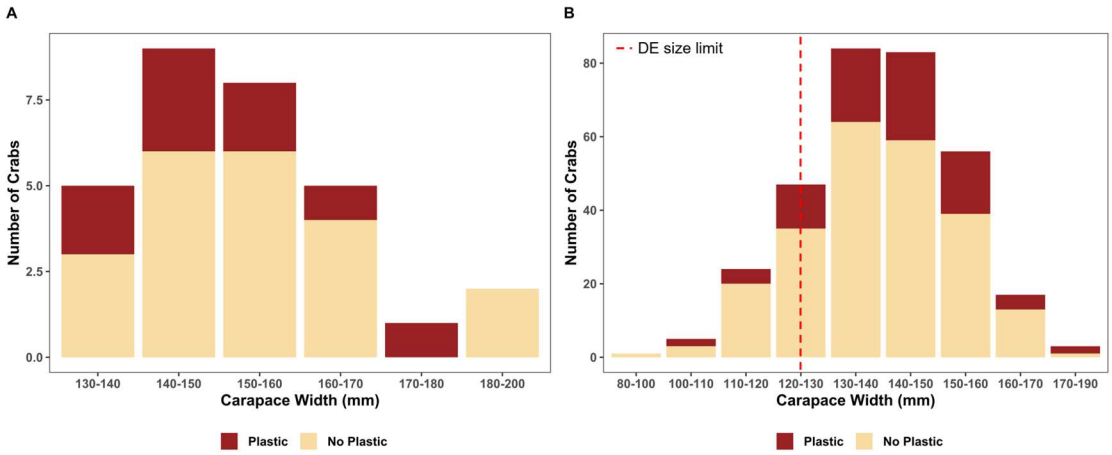


Figure 4.6: Stacked bar plot of binned *Callinectes sapidus* carapace widths (mm) by A) males and B) females with the number of crabs containing plastic in red and number of crabs without plastic in yellow.

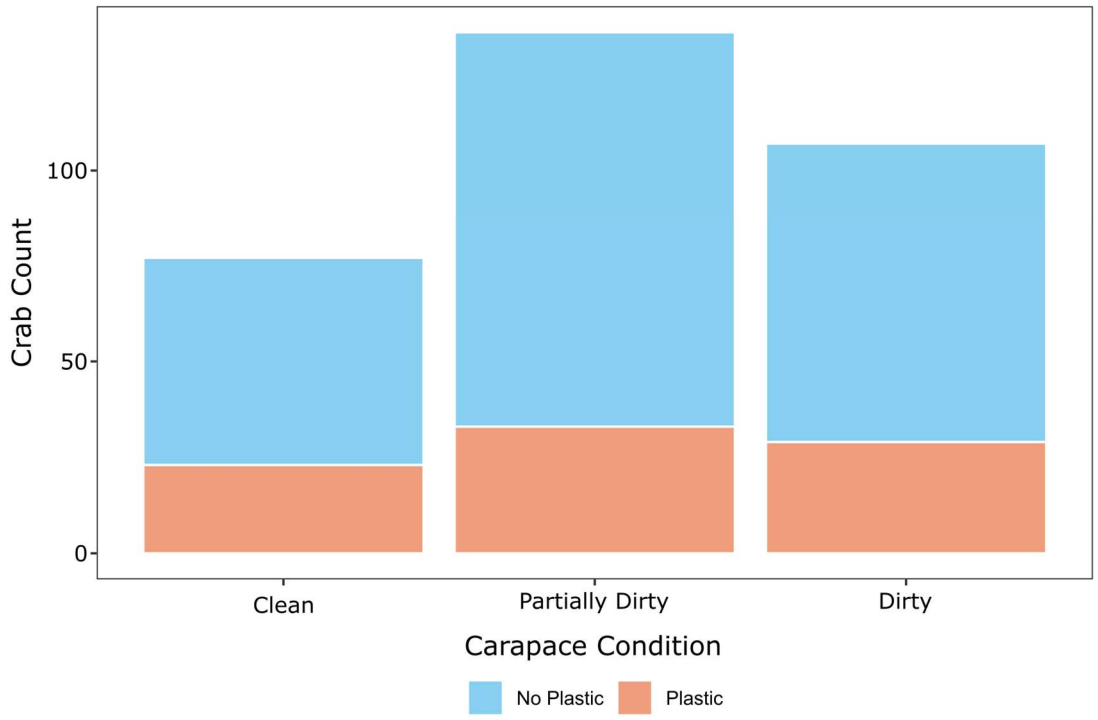


Figure 4.7: Comparison of the number of dirty, partially dirty, and dirty carapace conditions of female *Callinectes sapidus* showing the distribution of crabs containing plastic across the three groups.

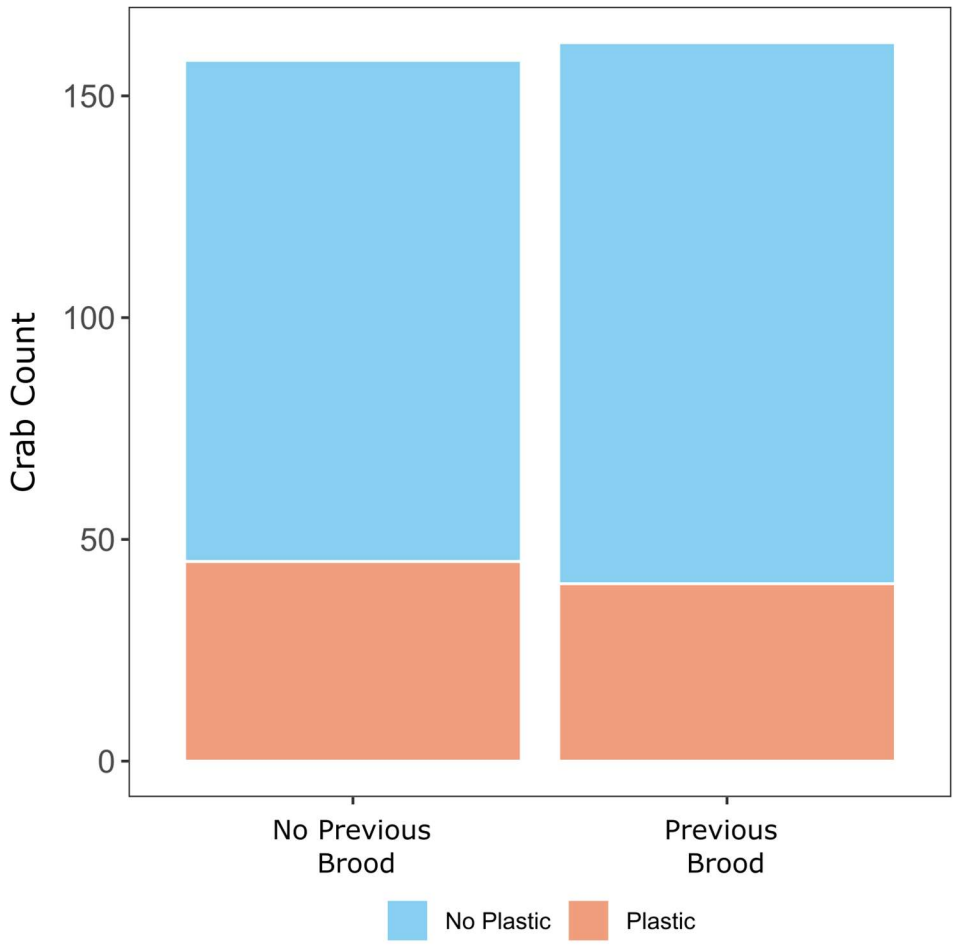


Figure 4.8: Comparison of the number of previously brooded female *Callinectes sapidus* and females without a previous brood, showing the distribution of crabs containing plastic across the two groups.

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Chapter 5

SEASONAL AND REGIONAL IMPACTS ON THERMAL PERFORMANCE OF *NEOMYSIS AMERICANA* UNDER MICROPLASTIC EXPOSURE

5.1 Introduction

Zooplankton are an important grouping of many taxa in the ecosystem, serving as both trophic links to higher trophic levels and recycling nutrients and carbon through the biological pump (Steinberg and Landry, 2017). As zooplankton are not strong swimmers and their inability to effectively swim against tidal currents, physical dynamics in the estuary influence distributions of zooplankton (Mason et al., 2022). As, such zooplankton are hypothesized to aggregate in similar locations to microplastic. In a Canadian estuary, zooplankton are seen in high abundance within the Estuarine Turbidity Maximum (ETM) zone (Winkler et al., 2003). Similarly, Shiravani et al., 2023, Roscher et al., 2021, Defontaine et al., 2020, and Fontana et al. *in prep* have all found elevated concentrations of microplastic within ETM features. These convergent regions may increase the risk of zooplankton interaction with microplastic and invoke a physiological response.

Both ingestion and the exposure to chemical leaching from microplastic pose threats to marine life (Hale et al., 2020). Microplastic is defined as material less than 5 mm in diameter (Baker, Aurther, and Bamford 2009), which are small enough to be ingested by zooplankton. Furthermore, the size of microplastics matches that of zooplankton prey, increasing the likelihood of ingestion (Cole et al., 2013). The comparable size of both microplastics and zooplankton increases the likely hood of

impacts post-ingestion, leading to the obstruction of their alimentary canals, impairing their ability to feed effectively and potentially disrupting their metabolism, growth, and survival (Hale et al., 2020).

As biota interact with microplastics, adverse effects such as oxidative stress and cellular damage along with suppressed metabolism can occur (Prokić *et al.*, 2019). As the environment changes in response to climate change, observing the impact of compounding stressors aids in the understanding physiological response of organisms, and can be applied to an ecological level. One of the greatest stressors can be temperature, as it is well understood that organisms have specific temperature thresholds for physiological function, and these can vary given their life history (Cooney and Gehrs, 1983). A recent study by Richon *et al.* (2024) reveals changes in ocean acidification and sea surface temperature have geographically expanded and intensified since 1955, additional bio-accumulative toxins from microplastic and polychlorinated biphenyls (PCBs) further exacerbate physiological stress on surface zooplankton. Pörtner (2010) explores the idea that oxygen limits the thermal tolerance of organisms (oxygen and capacity limited thermal tolerance), based on energy demands of aerobic organisms. Under stressed conditions (e.g., exposure to foreign objects such as microplastics) aerobic organisms may require more oxygen to keep their body functioning at steady state, in cases of chronic exposure the organism loses its capacity to uptake oxygen efficiently and cannot meet the required energy demands of steady state. Elevated oxygen consumption is correlated with increased metabolic rate, seeking to keep up with energy demands as the body requires. If an organism is under stressed conditions and cannot keep up with energy demands, the organism must

reallocate energy, with reproduction and growth impaired in order to prioritize energy needed for basal maintenance (Sokolova, 2013).

These stress responses can be tested through measuring oxygen consumption, nitrogen excretion and the atomic ratio of oxygen to nitrogen. As oxygen is necessary for cellular respiration, nitrogen, in the form of ammonia, is the primary excretory product of most invertebrate crustaceans. The atomic ratio of oxygen to nitrogen – O:N ratio – reflects the metabolic substrate an organism is metabolizing; lipids, carbohydrates, or protein (Conover and Corner, 1968). The catabolism of lipids increases respiration rate while not as much nitrogen is wasted, thus leading to a larger O:N. On the other hand, protein catabolism increases the amount of nitrogen waste and thus decreases the O:N ratio. When the organism is normally metabolizing carbohydrates ingested through routine feeding, O:N values are around 50-60 (Mayzaud and Conover, 1988). Under starved conditions, O:N decreases dramatically as no ingested food is being catabolized and the organism is surviving off of energy reserves from its own body (Mayzaud and Conover, 1988). Thus, if an organism is catabolizing lipid stores the expected O:N ratio would be around 10-16, whereas if protein is the primary catabolite the expected O:N would fall <7 (Mayzaud and Conover, 1988).

The present work focuses on *Neomysis americana*, an estuarine and coastal species of mysid shrimp found abundantly along the eastern coastline of North America (Pezzack and Corey, 1979). *N. americana* plays an important role in food web dynamics, as it is an opportunistic omnivorous feeder, feeding on detritus and phytoplankton (Pezzack and Corey, 1979) and preyed upon by commercially important species such as striped bass and weakfish (Buchheister and Latour, 2015).

As *N. americana* are strong vertical migrators, they connect the pelagic and benthic parts of the food web, exporting carbon to the benthos while supplying nutrients to the pelagic communities (Jumars, 2007). Additionally, *N. americana* are tidal migrators, using tides to aid in migration patterns. *N. americana* have elevated grazing rates in the St. Lawrence estuarine transition zone as compared to other places in the estuary (Winkler *et al.*, 2003) Additionally, it seems like *N. americana* may be using the estuarine transition zone in Canada as a nursery habitat, as clutch size was positively correlated to particulate organic matter concentrations (Bouchard and Winkler, 2018). As estuarine circulation (such as the formation of an ETM, see chapter 3) also aggregates buoyant material such as plastic, it is possible that ETM formation would increase the likelihood of mysids interacting and risk of ingesting plastic material.

The objective of this chapter is to assess the metabolic response across temperatures for the mysid *N. americana* from high and low microplastic regions of Delaware Bay. It was hypothesized that: (1) mysids collected in the winter will have a smaller thermal window than mysids collected in the summer; (2a) mysids collected from an upper bay location will have been exposed to higher concentrations of environmental microplastic material compared to lower bay mysids; and (2b) Mysids from the high plastic site have a lower thermal optimum and narrower thermal performance curve compared to mysids from the lower plastic site.

5.2 Methods

5.2.1 Plastic Collection and Analysis

To confirm the relative levels of environmental plastic exposure at these stations, water samples were collected for 5 minutes at each location just before

organism collection each day. Sampling included a 0.5m ring net, outfitted with a flow meter to determine the volume (~8.4 m^3 water filtered per sample) of the water filtered and deployed in the same fashion as mysid collection. The samples were transferred to glass jars and preserved with 4% borax-buffered formaldehyde immediately upon collection and stored for organic material digestion and microplastic quantification. While two replicate samples were taken at Woodland Beach during the summer, sampling constraints restricted my ability to collect replicate microplastic samples during winter. This limitation may impact the robustness of seasonal and spatial comparisons.

Water samples were sieved through a 300µm stainless steel sieve and rinsed with deionized water to remove formaldehyde from the samples. Sieved samples were then poured into glass flasks and digested for removal of organic material. A volume of 100mL – 300mL of 10% potassium hydroxide (KOH) was added to the samples, ensuring complete coverage of sample material. Flasks were covered with aluminum foil and digested for 24 hours at 60°C on a shaker table (MaxQ4450 Shaker, Thermo Scientific) set to at 150 rpm. Following digestion, samples were suspended for a single density separation with a saturated sodium chloride solution (Hidalgo-Ruz *et al*., 2012). Following density separation, samples were collected onto a 44µm Nitex mesh, then covered in tinfoil and set to dry under the fume hood for 24 hours. Following methodology from Chapter 3, suspected microplastic material (hereafter "particles") were identified and enumerated under a stereomicroscope (Olympus CX31) categorized by particle type (i.e., fragment, fiber, bead). Categorized particles were placed on a low-e glass slide (Kevley) and given a unique particle ID. Particles were

then imaged using a Canon DSLR camera ensuring a record of particle count prior to μFTIR analysis.

To confirm particles as plastic, particles were analyzed for a polymer identity through transflectance using micro Fast-Fourier Transform Infrared (FTIR) spectroscopy (Primpke *et al*., 2017). μFTIR spectra (32 scans at 4 cm^{-1}) were collected using a Spotlight 200i micro-FTIR in reflectance (transflectance) mode between 4000-1000 cm^{-1}. μFTIR spectra were compared against a custom spectral library accepting matches above a 75% search score threshold.

5.2.2 Mysid Collection

Two Delaware Bay sample locations were selected based on previous Delaware Bay sampling (see chapter 3) and estuarine organism body burdens (see chapter 4). Sample locations were selected based on their likelihood for having high (upper bay) and low (lower bay) microplastic concentrations (Figure 5.1), as well as being habitat for *Neomysis. americana*. During the winter of 2024, *N. americana* were collected from the mouth of the Broadkill River at University of Delaware's Pollution Ecology Laboratory (PEL) (sampled February 11th and 27th), which served as a lower bay, low plastic location. *N. americana* were similarly collected from Woodland Beach, the upper bay, high plastic location (sampled March 5th and 6th). A seasonal summer comparison was conducted at the Woodland Beach station (sampled May 30th and June 2nd) where *N. americana* are abundant in both winter and summer. The mouth of the Broadkill River was sampled only in winter as mysid abundance is lower in summer.

For both locations, N. americana were collected using a 0.5 m ring net with a 200 μm mesh net and cod-end attached. The net was fixed to the dock and deployed

for 20 minutes facing opposite to the direction of the current. Samples were collected at night during slack tide events to increase the chances of encountering zooplankton at the surface. An additional temperature and salinity measurement was taken using a YSI ProDSS Handheld probe during each sampling event (Appendix C.1).

5.2.3 Experimental Setup

Two water baths and an incubator were used to conduct trials of 84 mysids over a two-day period. Each day, 42 mysids were collected, sorted and exposed to three different temperatures for six temperatures per season: winter (2°C, 4°C, 6°C, 8°C, 10°C) and summer (10°C, 14°C, 18°C, 22°C, 25°C, 29°C). Seawater salinities were matched to sampling region salinities (7 PSU and 22 PSU for samples from Woodland Beach and Broadkill River, respectively). Per sampling day 10 to 14 mysids were sorted into three 1000mL glass bottles and placed in circulating water baths set to 2°C, 8°C, 10°C in winter, and 10°C, 14°C, 25°C, 29°C in summer. An additional grouping of mysids was placed in a reach-in incubator set to 4°C and 6°C in winter, and 18°C and 22°C in summer. These temperature ranges were selected based on the average seasonal water temperatures—5°C in winter and 22°C in summer (NOAA Physical Oceanography portal Stations 8551910 and 8557380) —adjusted by ±5°C in winter and ±10°C in summer to account for the greater variability observed in summer temperatures compared to winter. Mysids were acclimated to their experimental temperatures for 24 hours prior to respiration measurements in order to clear gut contents and reduce stress responses when placed in experimental respiration chambers.

5.2.4 Respiration Experimentation

Following the acclimation period, individual mysids were placed in 16mL glass vials filled with 0.45µm-filtered seawater and fitted with a Loligo Systems microplate oxygen sensor spot (#CH10482) on the vial bottom. Vials with organisms were then capped with parafilm and screw top caps, ensuring no bubbles remained in the vial and airtight seal. Vials were then placed on one of two 24 channel microplate oxygen readers (Loligo Systems #OX11900) for 5 minutes to read initial oxygen saturation measurements. Oxygen saturation measurements were collected for each vial every 30 minutes for a total of four hours with vials gently rotated prior to placement on the oxygen reader to oxygen saturation was well mixed. Vials were placed on the sensor for 5 minutes per temperature measuring oxygen every 15 seconds and returned to the temperature baths between subsequent measurements in order to retain the correct experimental temperature. An additional temperature vial filled with filtered water was included in each temperature group to monitor temperature fluctuations. The internal temperature was measured prior to experimental vials being placed on the microplate, and once again after removal. Temperatures fluctuated 1.3°C ± 1.1°C.

Following the experiment, animals were removed, weighed for wet weight, imaged for standard length measurements (rostrum to telson, L in mm) and sexed. All *N. americana* used in this study were males. For consistency, females (n = 1) and gravid females (n = 2) were excluded. They were then flash frozen in liquid nitrogen for archival purposes and stored in a -80°C. The experimental water was frozen at -20°C in 15mL Falcon tubes for later nitrogen excretion analysis.

To determine dry weights a linear regression between wet weight (mg) to dry weight (mg) was created from previously analyzed *N. americana* (n = 145),

unpublished data (Figure 5.2). *N. americana* were removed from experimental chambers, patted dry with Kim-Wipes and placed on a balance for wet weight measurements. Following determination of wet weight organisms were then dried at 60°C for 48 hours and re-weighed to determine dry weight (mg). The regression equation was then used to calculate dry weight of *N. americana* in the current study. Estimated dry weights ranged from 0.098 mg to 1.56 mg in the summer and 0.24 mg to 4.35 mg in the winter, based on a regression of wet weight to dry weight in an unrelated study of *N. americana* within the region.

5.2.5 Nitrogen Excretion and Atomic Oxygen to Nitrogen Ratios

Water samples from the respiration experiment were thawed and analyzed for ammonium content using the Indophenol-blue (IPB) method (Parsons et al., 1984). Following this protocol, 5 mL aliquots of sample water were added to 10mL test tubes and 0.2 mL of 1.1 M phenol, 0.2mL of 0.019 M sodium nitroprusside, and 0.5 mL of oxidizing reagent (0.78 M sodium citrate, 0.25 M sodium hydroxide and 1.5 M sodium hypochlorite) were added. All samples were capped with parafilm and vortexed between each reagent addition. Samples were then covered with a dark cloth and left in the fume hood at room temperature for 1 hour before reading absorbances. Ammonium absorbances were measured at 640 nm in 1cm pathlength cuvettes on an Evolution 201 UV-Visible Spectrophotometer. A standard curve (0 – 100µg/L $NH4^{+}$) was created from Sigma-Aldrich Ammonium Standard #4111610 each day of sample analysis.

Utilizing results from both mass-specific respiration rates and mass-specific nitrogen excretion rates, an atomic O:N was calculated. Respiration rates were first multiplied by the molecular mass of oxygen (32.00 g mol^{-1}, accounting for two oxygen

atoms in one O2 molecule), while nitrogen excretion rates were multiplied by (14.01 g mol^{-1}). The resulting values were then divided by one another to determine the ratio of oxygen consumed to nitrogen excreted.

5.2.6 Data Analysis and Statistical Methods

Statistical analysis was performed using R (www.rproject.org). The effects of temperature on respiration rate between seasons was measured using an analysis of variance (ANOVA) for summer samples and a Kruskal-Wallis Test for winter data. Shapiro-Wilk's and Levene's tests were used to test data normality and homogeneity of variance, respectively. Summer sampling was conducted using a One-way ANOVA as the assumption of normality satisfied for all temperatures except 18°C (P = 0.02891). Therefore, the results of the ANOVA for the summer were considered valid and a pairwise post-hoc Tukey's HSD test were used to identify significant differences in temperature groups. For winter sampling at Woodland Beach, the Shapiro-Wilk's test indicated that the data was not normally distributed in most temperature groups (P < 0.05, 2°C , 4°C , 8°C, 10°C), thus a non-parametric Kruskal-Wallis test was performed as the assumption of normality does not need to be met. A pairwise Wilcoxon test was performed to identify significant differences between temperatures, with p-values adjusted using the Bonferroni correction.

Seasonal nitrogen excretion was conducted using one-way ANOVA for each season, as the assumption of normality satisfied for all temperatures in summer and winter, except 4°C (P = 2.873e-06). A pairwise post-hoc Tukey's HSD test were used to identify significant differences in temperature groups.

Winter O:N statistical tests were conducted using a non-parametric Kruskal-Wallis test as the Shapiro-Wilk's test indicated that the data was not normally

distributed in most temperature groups ($P < 0.05$, 2°C , 4°C , 8°C, 10°C). A pairwise Wilcoxon rank-sum test was performed to identify significant differences between temperatures, with p-values adjusted using the Bonferroni correction. Summer O:N was conducted using a One-way ANOVA as the assumption of normality satisfied for all temperatures ($P > 0.05$, 10°C , 14°C , 18°C, 22°C, 25°C, 29°C). Therefore, the results of the ANOVA for the summer were considered valid and a pairwise post-hoc Tukey's HSD test were used to identify significant differences in temperature groups.

Welch's t-tests were employed investigating differences between overlapping experimental temperatures between seasons (10°C) for each parameter measured (respiration rate, nitrogen excretion, and O:N).

As respiration rates and O:N were not normally distributed by way of Shapiro-Wilk's test, I employed a series of nonparametric statistical tests to measure the interaction between station and temperature. First, Kruskal-Wallis Tests were used to measure the distribution of respiration rate and O:N across temperature and location. Following significant Kruskal-Wallis tests, pairwise Wilcoxson rank-sum tests were performed. To explore interaction effects, I performed a Kruskal-Wallis test to investigate the relationship between temperature and respiration rate and O:N across stations, followed by a pairwise Wilcoxson rank-sum test. A two-way ANOVA and Tukey's HSD test was run on nitrogen excretion data as the assumption of normality was satisfied for all temperatures except Woodland Beach 4°C ($P = 2.873e-06$).

5.2.7 Quality Control and Assurance

During microplastic analysis preventative measures were used to mitigate plastic contamination. Bright orange cotton jumpsuits were worn when handling samples at all stages to minimize contamination from clothing. Plastic enumeration

was done under a laminar flow hood, and samples were covered with tinfoil or class cover slide when not in use. Control samples were employed during the digestion and filtering step, although no microplastic was found on control samples.

5.3 Results and Discussion

5.3.1 Microplastic Concentration

Woodland Beach contained 19.3 microplastic pieces m^{-3} in winter and 3.45 ± 1.31 microplastic pieces m^{-3} in summer. The mouth of the Broadkill River contained considerably lower winter concentrations of microplastic – 0.031 microplastic pieces m^{-3}, with no summer data collected for this study. This distribution of microplastic was expected based on previous bay-wide sampling (Cohen et al., 2019 and Fontana, *in prep*), although winter concentrations were substantially greater at Woodland Beach and lower at Broadkill River than previously sampled. Woodland Beach was sampled on an ebb tide each sampling day as zooplankton and microplastics aggregate at the surface during ebb tides (Fontana *in prep,* Mason et al., 2022). Broadkill River was sampled during spring tide and Woodland Beach was sampled during neap, this is opposite the pattern we would expect during tidal variation (Mason et al. 2022). However, due to the lack of replicates, statistical analysis could not be performed.

Polymer analysis reveals fibers as the most abundant particle type. Fibers (n=15) were commonly polyester (40%) and a semi-synthetic material rayon (33.3%), with fragments (n = 2) being polyethylene and polypropylene, and beads (n = 1) were polystyrene (Figure 5.3). Both chapters 3 and 4 see similar polymer distributions of fibers with high occurrences of rayon and polyester and fragments as polyethylene and

polypropylene. Additionally, these polymer identities are alike other Delaware Bay studies commonly finding polyethylene and polypropylene at the mouth of the Delaware Bay (Nitzberg et al., 2024) and polyester, rayon, polyethylene and polypropylene in the Delaware river (Akbari et al., 2024).

5.3.2 Length and Body Weight Analysis

Seasonal body weight of *N. americana* ranged from 0.5 mg to 7.5 mg wet weight in the summer, whereas winter body weights were larger, ranging from 1.2 mg to 20.9 mg wet weight. Standard body lengths (rostrum to telson, mm) ranged from 5.1 mm to 11.7 mm in the summer and 6.2 mm to 17.04 mm in the winter.

5.3.3 Seasonal Analysis

Summer metabolic rates ranged from 0.003 $\mu gO_2\, min^{-1}$ mg dry $weight^{-1}$ to 0.336 $\mu gO_2\, min^{-1}$ mg dry $weight^{-1}$. Winter metabolic rates ranged from 0.053 $\mu gO_2\, min^{-1}$ mg to 0.275 $\mu gO_2\, min^{-1}$ mg dry $weight^{-1}$. These fall within the range of other reported metabolic rates for *N. americana* (Chapina et al., 2020 and Smith and Hargreaves, 1984), and *Americamysis bahia* (Modlin and Froelich, 1997) at similar sizes and temperature.

As expected, we see a seasonal increase across temperatures with lower metabolic rates in winter and higher rates in the summer at Woodland Beach (One-way ANOVA, P = 3.66e-09) (Figure 5.3A). However, no difference was found between metabolic rates at 10°C seasonally (Welch's Two Sample T-test, P = 0.9001). In winter (2-10°C), *N. americana* show an increase in oxygen consumption rate at 10°C significantly higher than all other temperatures, except 4°C (Kruskal-Wallis Test, P <0.05, Pairwise Wilcoxson Test, P <0.05). Additionally, oxygen consumption

rose between 2°C and 4°C (Pairwise Wilcoxson Test, $P < 0.00149$), however, 4°C was not significantly different than any other winter temperature. Environmental temperatures range from 5°C – 7°C at this time of year (NOAA Physical Oceanography portal Stations 8551910 and 8557380) which may explain why there is a peak around these temperatures (Appendix C.1).

Evaluation of Q_{10} reveals a high Q_{10} value between 2°C and 10°C in the winter (11.6) whereas 2°C and 8°C have a Q_{10} of 3.4 is elevated, however more similar to the expected range for ectothermic animals and closer to the Q_{10} value from *N. americana* of 1.6 between 4°C and 16°C (Clutter and Theilacker, 1971) and *Neomysis integer* between 5°C and 15°C reported as 1.91 at 10 PSU (Roast et al., 1999). The high Q10 value in addition to elevated 10°C respiration rate during winter may indicate that even at this acute exposure to 10°C, organisms are operating at/or near the edge of their thermal tolerance.

Summer metabolic rates also increase between 10°C and 29°C. However, these increases in temperature were insignificant by way of One-way ANOVA ($P = 0.223$). Additionally, the Q_{10} between 10°C and 29°C was determined to be 1.5, considerably lower than winter and slightly lower than the expected range for ectotherms yet is similar to the Q_{10} of 1.6 of *N. americana* between 4°C and 16°C calculated by Clutter and Theilacker, 1971. This result may suggest summer respiration rates are less sensitive to environmental fluctuations in temperature.

Nitrogen excretion was found to be higher than reported values from a similar species *Neomysis rayii* (Jawed, 1969), but within the range for other zooplankton species *Sagita elegans* and *Acartia clausi* (Mayzaud, 1976). Winter nitrogen excretion ranged from 0.001 to 0.006 μgNH_3 mg dry weight^{-1} min^{-1} with one individual

reporting 0.013 μgNH_3 mg dry weight^{-1} min^{-1}. Winter nitrogen excretion steadily increased with temperature (Two-way ANOVA, P = 0.00649) although nitrogen excretion was insignificant between location (Two-way ANOVA, P = 0.164) and the interaction thereof (Two-way ANOVA, (temperature*location), P = 0.392) as shown by Figure 5.4B. Summer nitrogen excretion rates were considerably higher ranging from 0.003 to 0.04 μgNH_3 mg dry weight^{-1} min^{-1}, most likely due to the higher experimental and environmental temperatures experienced in the summer (Clarke and Fraser, 2004) (Figure 5.3B). As such, combined summer nitrogen excretion rates were significantly higher than nitrogen excretion rates found in winter (One-way ANOVA, P = $<2e^{-16}$) (Figure 5.3B). Q_{10} analysis of nitrogen excretion reveals an expected Q10 between 2°C and 10°C of 2.2 in winter, whereas summer Q_{10} between 10°C and 29°C was found to be 1.6. These results were expected, as enzymatic processes involved in metabolism increase as temperature increases (Gillooly et al., 2001), thus as temperatures rise, we expect excretory processes to rise proportionally (Clarke and Fraser, 2004).

Atomic oxygen-to-nitrogen ratios were determined from oxygen consumption and nitrogen excretion data to understand the primary metabolic substrates or compounds being utilized, such as carbohydrates, proteins, or lipids. Summer O:N ranged between 0.17 and 31.3 with winter values ranging from 0.15 to 44.8. O:N are highly driven by respiration rates (Figure 5.4C). These O:N are similar to an estuarine mysid species *Mysidopsis bahia* reported O:N 25 ± 4 of cultured mysids (McKenney and Matthews, 1990). According to O:N ranges for zooplankton reported in Mayzaud and Conover (1988), these O:N values span both protein and lipid catabolism, as higher values indicate lipid catabolism (>20) and lower values (3 - 16) indicate

protein catabolism. Interestingly, the lowest O:N were found at the extreme temperatures tested 2°C, 25°C and 29°C. In conjunction with the Q_{10}, these low O:N at extreme temperatures may be indicative of an initial stress response to these temperatures in *N. americana.*

5.3.4 Location Analysis

Respiration rates between locations were not significantly different from each other (One-way ANOVA, P = 0.0703) (Figure 5.5A), indicating that plastic concentrations are not significantly impacting the metabolism of *N. americana* in the Delaware Bay. *N. americana* collected from the Broadkill River (Figure 5.4A) found a Q_{10} of 8.1 between 2°C and 10°C, similarly elevated compared to Q_{10} at Woodland Beach (11.6). Interestingly, excretion Q_{10} was very high (50.1) between 2°C and 10°C at Broadkill River, dramatically higher than Woodland Beach (2.6). O:N were similar between locations ranging from 0.15 – 34.05 at Woodland Beach, and 0.28 – 44.8 at Broadkill River. These results reveal that location is not a significant factor in *N. americana* metabolics in the Delaware Bay.

5.4 Figures and Tables

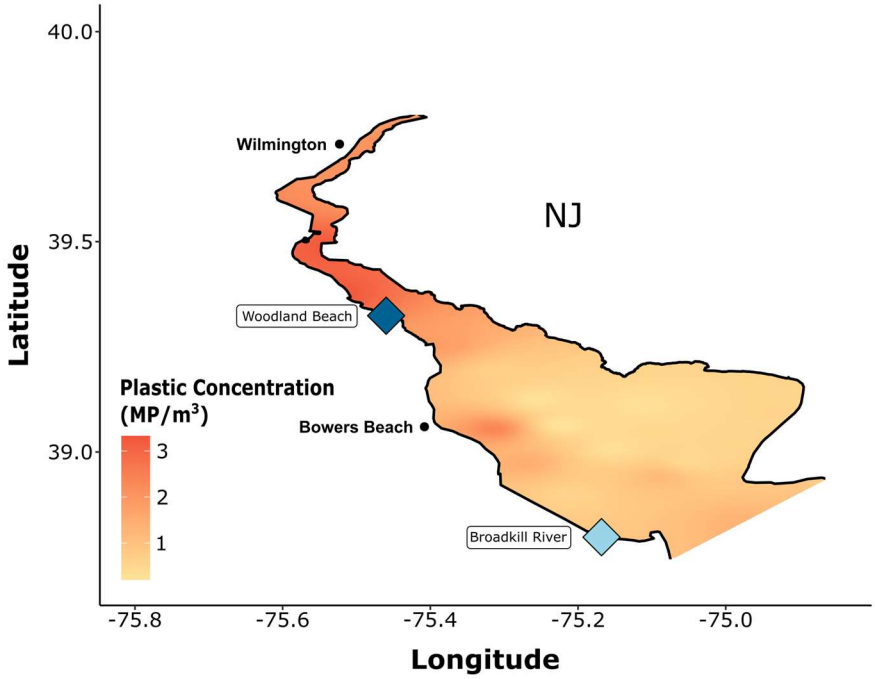

Figure 5.1: Map of the Delaware Bay displaying sampling stations Broadkill River (light blue) and Woodland Beach (dark blue) overlaid on the plastic concentrations microplastic / m^3 (red-high to yellow-low) reported in Cohen *et al.* (2019).

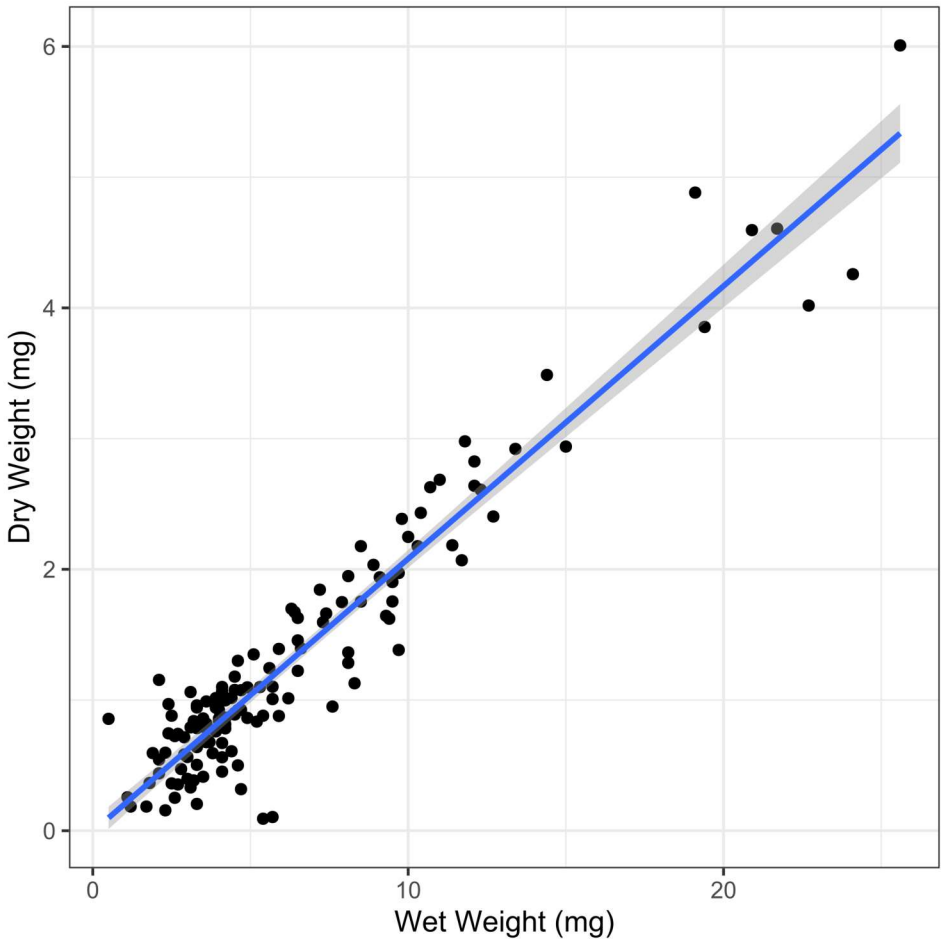

Figure 5.2: Linear regression of *Neomysis americana* wet weight (mg) to dry weight (mg) (Dry weight (mg) = 0.006 - 0.209 * wet weight (mg), n = 145, R^2 = 0.907, *unpublished data*).

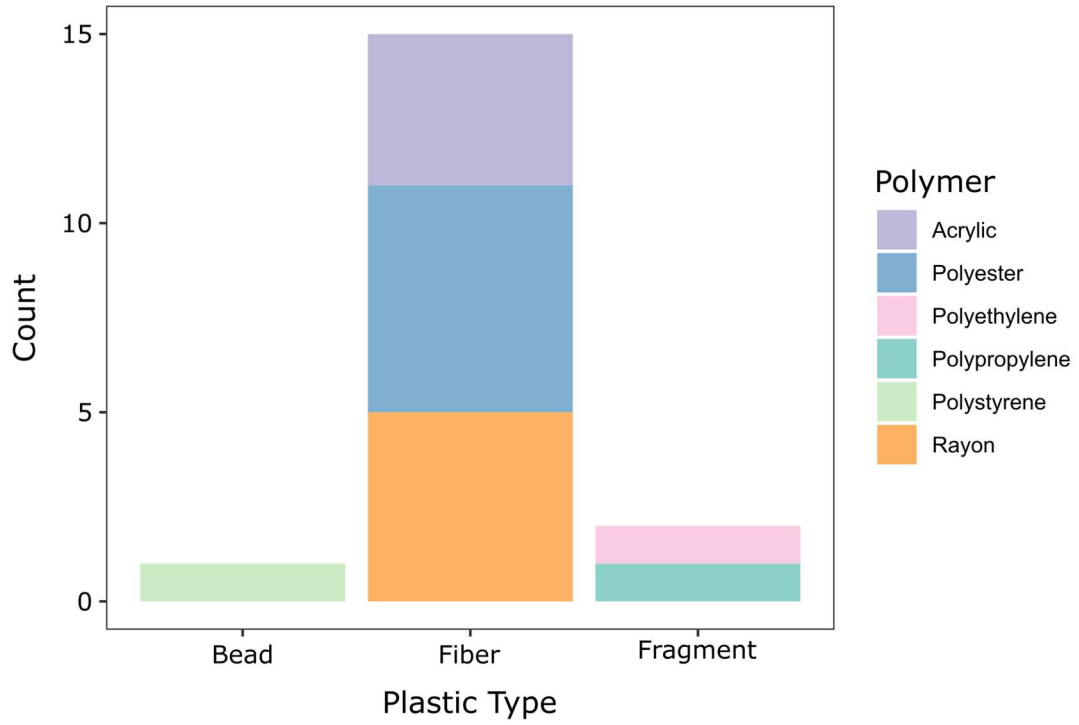

Figure 5.3: Polymer identification of microplastic collected from both the Broadkill River, Delaware and Woodland Beach, Delaware.

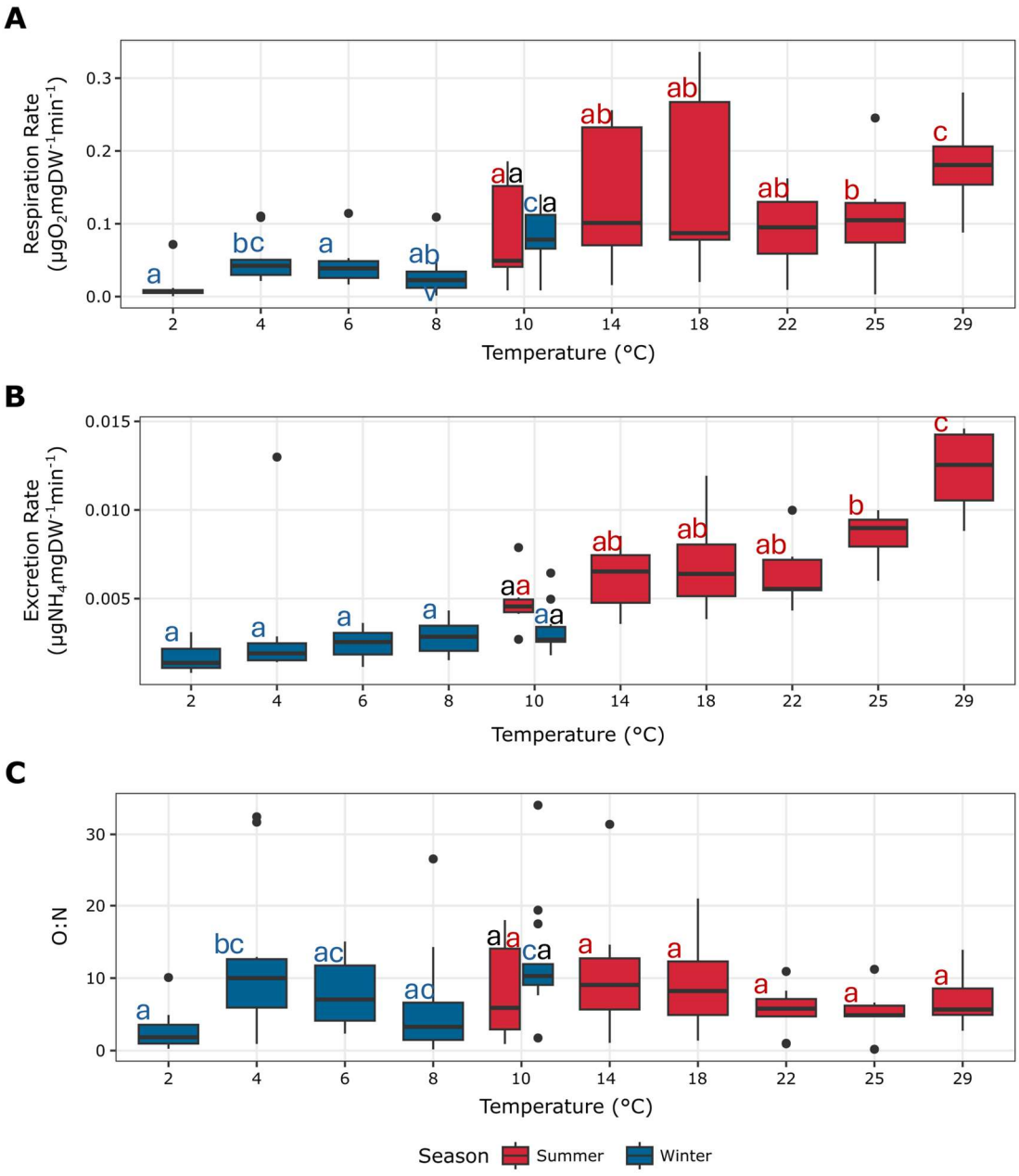
A
Respiration Rate
(μgO2mgDW-1min-1)
0.0
0.1
0.2
0.3
a
bc
a
ab
aa
ca
ab
ab
ab
b
c
2
4
6
8
10
14
18
22
25
29
Temperature (°C)
B
Excretion Rate
(μgNH4mgDW-1min-1)
0.005
0.010
0.015
a
a
a
a
aa
aa
ab
ab
ab
b
c
Temperature (°C)
C
O:N
0
10
20
30
a
bc
ac
ac
aa
ca
a
a
a
a
a
Temperature (°C)
Season
Summer
Winter

Figure 5.4: A) Respiration rates ($\mu gO_2\ min^{-1}$ mg dry weight), statistical significance shown by blue letters (Kruskal-Wallis Test, $P < 0.05$, Pairwise Wilcoxson Test, $P < 0.05$), black letters (Welch's two-sample t-tests), and red letters (One-Way ANOVA, $P < 0.05$, TukeyHSD, $P < 0.05$) (B) nitrogen excretion rates (μgNH_3 mg dry weight min^{-1}) statistical significance shown by blue letters (One-Way ANOVA, $P < 0.05$, TukeyHSD, $P < 0.05$),, black letters (Welch's two-sample t-tests), and red letters (One-Way ANOVA, $P < 0.05$, TukeyHSD, $P < 0.05$), and C) atomic oxygen-to-nitrogen ratios, statistical significance shown by blue letters (Kruskal-Wallis Test, $P < 0.05$, Pairwise Wilcoxson Test, $P < 0.05$), black letters (Welch's two-sample t-tests), and red letters (One-Way ANOVA, $P < 0.05$, TukeyHSD, $P < 0.05$), of *Neomysis americana* between winter (blue) and summer (red) seasons at Woodland Beach, Delaware.

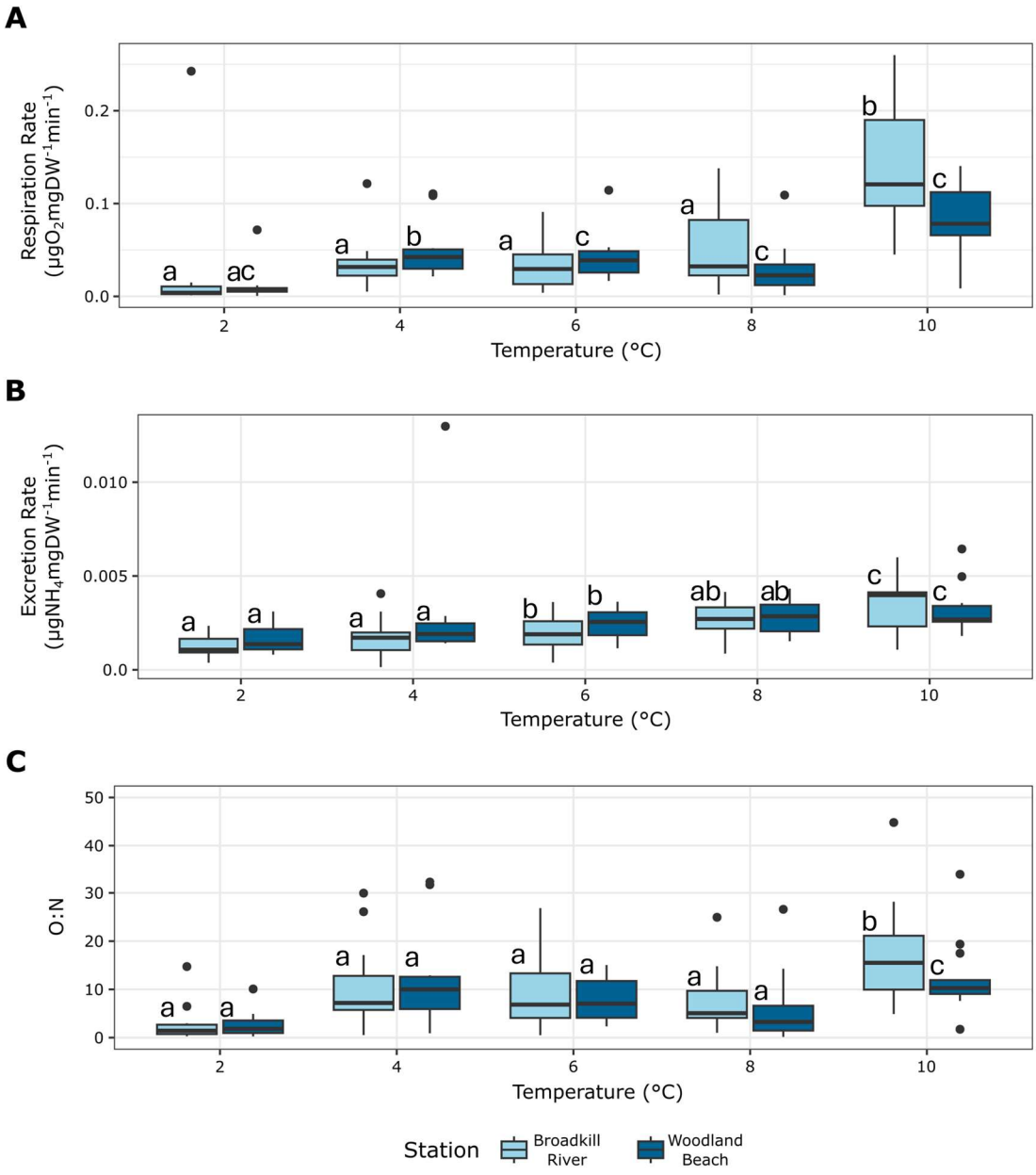
A
Respiration Rate
($\mu gO_2 mgDW^{-1}min^{-1}$)
0.0
0.1
0.2
a
ac
a
b
a
c
a
c
b
c
2
4
6
8
10
Temperature (°C)
B
Excretion Rate
($\mu gNH_4 mgDW^{-1}min^{-1}$)
0.0
0.005
0.010
a
a
a
a
b
b
ab
ab
c
c
2
4
6
8
10
Temperature (°C)
C
O:N
0
10
20
30
40
50
a
a
a
a
a
a
a
a
b
c
2
4
6
8
10
Temperature (°C)
Station
Broadkill River
Woodland Beach

Figure 5.5: A) Respiration rates ($\mu gO_2\ min^{-1}$ mg dry weight) labeled by significance (Kruskal-Wallis Test, $P < 0.05$, Pairwise Wilcoxson Test, $P < 0.05$) B) nitrogen excretion rates (μgNH_3 mg dry weight min^{-1}) labeled by significance (two-way ANOVA, $P < 0.05$, TukeyHSD, $P < 0.05$) and C) atomic oxygen-to-nitrogen ratios labeled by significance (Kruskal-Wallis Test, $P < 0.05$, Pairwise Wilcoxson Test, $P < 0.05$) of *Neomysis americana* between up river location of Woodland Beach, Delaware (dark blue) and lower bay location of Broadkill River, Delaware (light blue). A)

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Chapter 6

CONCLUDING REMARKS

The goal of this dissertation was to determine the sources, distribution, composition, and biologic impact of microplastic debris within the Delaware Estuarine system. This topic was investigated through a combination of field, laboratory and modeling experiments. Here I summarize and contextualize these results and provide recommendations for future research.

Chapter 2 created a methodology to determine the magnitude and hot spots of mismanaged plastic waste in the Delaware Bay coastal watersheds. We find that an additional 1.8% of plastic waste is littered in relation to the total municipal solid waste produced annually. This result is comparable to Jambeck et al. (2015) who reports an average of 2% mismanaged waste throughout the United States. Land-uses such as residential and institutional, governmental, and industrial (ICI) differ in their contribution to plastic waste. We find 0.01 tons of plastic litter is produced annually per capita, with litter per square meter ranging from 6×10^{-7} tons m^{-2} to 0.001 tons m^{-2} in populous regions. Additionally, ICI contributes 6×10^{-6} tons m^{-2} amount of litter per square meter.

A case study of the Saint Jones River geospatial modeling of plastic litter reveals 2.7 tons of litter are exported from the river to the Delaware Bay annually. At current, our methodology includes only a set range of buffer distances around the rivers and streams. This may underestimate the amount of litter entering the waterways, as it does not consider litter production outside of this buffered region.

Future analysis will refine this approach by adjusting the buffer regions based on land cover characteristics. For example, plastic litter may travel farther in urban, developed, and agricultural areas, while its movement may be more restricted in forested regions. Subsequent work should quantify plastic litter given coastal Delaware watersheds identifying regions that contribute to elevated litter rates.

Overall, this study seeks to determine the magnitude of mismanaged plastic waste in the Delaware Bay coastal watersheds. This methodology will provide insights into regions with elevated plastic potential output, and thus, allowing for targeted mitigation strategies within these watersheds to better intercept plastic waste entering waterways.

Following findings from previous studies in the Delaware Bay (Cohen et al, 2019), the estuarine turbidity maximum (ETM) region was sampled in detail June 4, 2019 (Day 1) and again two days later June 6, 2019 (Day 2). Chapter 3 reveals that microplastics are not just a surface water phenomenon and subsurface samples are important to consider when determining the extent of plastic debris in an ecosystem. Microparticles aggregate at depth at sample stations 4 and 5 on Day 1 and Day 2, locations where the ETM is historically located (McSweeney et al., 2016). Analysis of CTD profiles from these sample locations on Day 1 confirm the location of the ETM at stations 4 and 5. Surface microparticles are also elevated at the ETM stations on both days with an anomalistic microparticle concentration of 25 microplastic m^{-3} found at the station 3 on Day 2. These elevated concentrations may be explained by modeling of plastic material (Fontana *in prep*), revealing that tidal influences, channel curvature and bathymetry impact the distribution of material at that location.

Day 1 was followed up with quantification of microplastics through polymer identification using μFTIR spectroscopy and the characterization of size, and relative buoyancy. Polymer analysis reveals fragments predominantly identified as polyethylene (62%) and polypropylene (34%), beads are commonly found to be polystyrene (94%), and fibers are predominantly polyester (PET) (33%) as well as a semi-synthetic material rayon (29%).

The finding of rayon was consistent throughout the water column (Chapter 3, Chapter 5) and was the dominant polymer ingested by *Callinectes sapidus* and *Anchoa mitchilli* sampled over a wide temporal range (2021 - 2024). Only recently has rayon (also known as viscose) been reported in the natural environment, as previously only synthetic polymers have been considered. However, a growing number of studies have reported rayon in water-column as well as making up a significant proportion of ingested fibers by marine biota. Additionally, results from the upper Delaware River report similar proportions of rayon (25-50% in grab samples) to this study (Akbari et al., 2024). Future research would benefit from investigating the biological impacts of rayon ingestion.

Microplastic ingestion by marine and estuarine organisms is an active area of interest and ongoing research. Our study concludes about 12% of estuarine organisms and about 26% of female *C. sapidus* and 30% of male *C. sapidus* contain plastic in the Delaware Bay. Characterization of microplastic directly reflects the characteristics found in water column microplastic throughout the Delaware Bay, with a semi-synthetic material rayon found to be the most common polymer identity. The occurrence of plastic was not found to be significantly correlated with morphological characteristics, spatial distribution, or species. Although morphological characteristics

played an insignificant role in *C. sapidus* body burdens, seasonal analysis found that crabs collected in the spring, when Delaware river discharge is at its peak, contained microplastic more commonly than other seasons.

Plastic ingestion poses a risk to all estuarine and marine organisms, regardless of specific traits or behaviors, as no clear characteristic has been identified that increases or decreases the likelihood of ingestion. Additionally, the clearance rates of plastic—how efficiently organisms expel ingested microplastics—remain poorly understood for most marine species. These rates are critical for understanding the potential for plastic accumulation in individual organisms and, consequently, in the environment.

As it remains difficult to decidedly determine the impacts microplastic have on estuarine organisms, proxies like oxygen consumption, nitrogen excretion and the atomic ratio thereof, are used to quantify the metabolic parameters of an organism. Chapter 5 explores the impacts of environmental microplastic exposure on important zooplankton species *Neomysis americana* throughout a range of temperatures and seasons. *N. americana* were sampled from a northern location within the region of elevated plastic as described in Chapter 2, Woodland Beach, and a low plastic region in the southern Delaware Bay, Broadkill river. No difference in oxygen consumption, nitrogen excretion, or O:N ratio was found between location, thus indicating that environmental plastic was not a contributing factor to the organisms overall metabolism. Seasonally we see an increase in oxygen consumption, nitrogen excretion, and O:N overall. Within seasons, I find that nitrogen excretion increases as temperature increases. However, during winter, oxygen consumption and O:N only significantly increased when acutely exposed to 10°C. Analysis of respiration rate Q_{10}

for respiration rates between 2°C and 10°C reveal an unexpectedly high value of 11.6 whereas Q_{10} for nitrogen excretion rates reveal an expected 2.2. This result may indicate that *N. americana* are operating at/or near the edge of their thermal tolerance when acutely exposed to 10°C in the wintertime. Summer Q_{10} between 10°C and 29°C reveal a value of 1.6, considerably lower than winter, yet similar to Q_{10} calculated for *N. americana* between 4°C and 16°C (Clutter and Theilacker, 1971), suggesting summer respiration rates are less sensitive to environmental fluctuations in temperature.

As this study was primarily conducted on male *N. americana*, investigating the response of females, particularly gravid females, would provide a comprehensive understanding of metabolic responses across the entire population. Additionally, further investigation of the seasonal response, particularly adding a summer season analysis at the Broadkill River sampling station, would elucidate any differences in location based metabolics. Although microplastic concentrations did not influence metabolic rates in this study, it is possible that increased plastic production may impact physiological conditions in the future. Investigating laboratory dosing experiments may help to understand the upper limit of microplastic concentrations that can be handled in the environment if/when that increase occurs.

As biota interact with microplastics, adverse effects such as oxidative damage, increased oxidative stress, and metabolic detriment can occur (Prokić *et al.*, 2019). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced when an organism is under stress. These ROS can lead to oxidative stress within the organism and further decrease an organism's capacity to react to their environment appropriately (Prokić *et al.*, 2019). Enzymes such as catalase (CAT), superoxide

dismutase (SOD), and glutathione reductase (GT) are commonly used as indicators of oxidative stress in organisms. Additionally, the determination of temperature thresholds and stress tolerance of these bioindicator enzymes assist in predicting impacts of climate change on marine and estuarine organisms (Somero, 2020 and Sokolova, 2021). Future research would benefit from focusing on the enzymatic reaction to microplastic interaction, revealing insight in zooplankton response to an increasingly anthropomorphized environment.

Overall, this dissertation reveals the sources, fate, and biological impacts of microplastic within the Delaware Bay. We find that the potential for plastic litter is higher within the northern Delaware watersheds which are similar to elevated bay plastic concentrations. However, bay plastic concentrations may be elevated in this region due to the Estuarine Turbidity Maximum aggregating material. Regional concentrations of plastic did not influence ingestion by biota, indicating that plastic ingestion is largely indiscriminatory. Additionally, at current concentrations, there is little evidence that estuarine microplastic is impacting the physiology of zooplankton. Utilizing this data we can further contextualize the microplastic problem and the pervasive nature of this pollutant. This research contributes to a deeper understanding of the fate of microplastics and their implications for estuarine ecosystems.

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Appendix A

CHAPTER 2 SUPPORTING INFORMATION

A.1 Categorization of Land-Use Characters for Model Application

Table A.1: Land-Use (Delaware Land-Use Land-Cover, 2017, Delaware First Map) categorized for Industrial, commercial and institutional (ICI) and residential categories.

LCLU Category	*Classification*
Retail Sales/Wholesale/professional services	ICI
Mixed urban or built up land	ICI
Industrial	ICI
Other Commercial	ICI
Institutional & Governmental	ICI
Airports	ICI
Mixed single & Multi-family Residential	Residential
Mobile Home park /courts	Residential
Multi family dwellings	Residential
Single family dwellings	Residential

Appendix B

CHAPTER 3 SUPPORTING INFORMATION

B.1 Tidal Times and Height of Sample Stations

Table B.1: Tidal times (EDT) and height (ft) of chapter 3 sample stations throughout the estuarine turbidity maximum region of the Delaware Bay.

Station	Date	Time (EDT)	Tides	Tidal Height (ft)
St.2	4-Jun-19	15:10	Ebb	3.71
St.3	4-Jun-19	14:11	Ebb	5.22
St.4	4-Jun-19	13:22	High	5.99
St.5	4-Jun-19	12:24	High	6.05
St.6	4-Jun-19	11:34	Flood	5.55
St.7	4-Jun-19	10:26	Flood	4.04
St.1	6-Jun-19	14:00	High	5.84
St.2	6-Jun-19	13:27	Max. Flood	5.58
St.3	6-Jun-19	12:34	Flood	4.64
St.4	6-Jun-19	11:48	Flood	3.4
St.5	6-Jun-19	10:58	Flood	1.94
St.6	6-Jun-19	10:10	Min. Flood	0.66

B.2 Polymer Concentrations: Inorganic and Synthetic

Table B.2: Station and depth sampled with inorganic (synthetic and semi-synthetic material) and synthetic material concentrations (microplastic / m^3)

Station	Depth	Inorganic Concentration (MP/m^3)	Plastic Concentration (MP/m^3)
St.2	Surface	0.89	0.50
St.3	Surface	1.98	1.91
St.4	Surface	2.40	2.12
St.5	Surface	2.27	2.14
St.6	Surface	0.71	0.55
St.7	Surface	0.41	0.39
St.2	Mid	0.18	0.14
St.3	Mid	0.15	0.09
St.4	Mid	1.50	1.41
St.5	Mid	0.60	0.55
St.6	Mid	0.34	0.23
St.7	Mid	0.31	0.30
St.2	Deep	0.18	0.13
St.3	Deep	0.32	0.21
St.4	Deep	1.81	1.69
St.5	Deep	2.04	1.96

Table B.2: Continued

St.6	Deep	0.23	0.21
St.7	Deep	0.16	0.14

B.3 Polymer Densities and Associated Relative Buoyancy Assignments

Table B.3: Polymer identities, polymer abbreviations, polymer common names, and semi-synthetic or synthetic categorization or mean density (g/cm^3) and the literature source of density derivation.

Polymer ID	Abbreviation	Common Name	Plastic	Mean Density (g/cm^3)	Density Source Abbv.
Cellulose Acetate	CA	Acetate	Semi-Synthetic	1.31	1
Rayon		Rayon, Viscose	Semi-Synthetic	1.53	2
high-density polyethylene	HDPE	Polyethylene (HDPE)	Plastic	0.957	3,4,5,6
low-density polyethylene	LDPE	Polyethylene (LDPE)	Plastic	0.914	3,5,6,7
Polyamide	PA	Nylon	Plastic	1.1	3,4,6,7,8
Polyester	PET / PETE	Polyester	Plastic	1.63	3,6,7,8,
Polymethyl methacrylate	PMMA	Acrylic	Plastic	1.15	6,7,8
Polypropylene	PP	Polypropylene	Plastic	0.89	3,4,5,6,8
Polystyrene	PS	Polystryene	Plastic	1.03	3,4,5,6,7,8
Polytetrafluoroethylene	PTFE	Teflon (PTFE)	Plastic	2.2	6,7
Polyvinyl chloride	PVC	Polyvinyl chloride (PVC)	Plastic	1.33	4,5,6,7,8
Rubber		Rubber	Plastic	1.1	9

[1]Lewis, R.J., Sr (Ed.). (1997), [2]Vigneswaran et al., 2014, [3]Andrady 2017, [4]Moret-Ferguson et al.,2010, [5]Hanvey et al.,2017, [6]Duis and Coors, 2016, [7]Borges-Ramirez et al., 2020, [8]Hildalgo-Ruz et al., 2012, [9]Material Properties. (n.d.).

Appendix C

CHAPTER 5 SUPPORTING INFORMATION

C.1 Metadata associated with *Neomysis americana* sampling

Table C.1: Temperature (ºC) and Salinity (PSU) measured during *Neomysis americana* sampling at Woodland Beach and Broadkill River sampling stations.

Date	Station	Water Temperature (ºC)	Salinity (PSU)
2/11/2024	Broadkill River	7.5	18
2/27/2024	Broadkill River	6.1	27
3/5/2024	Woodland Beach	7.6	5
5/30/2024	Woodland Beach	22.6	3
6/2/2024	Woodland Beach	23.3	6