THE SPATIAL DISTRIBUTION AND COMPOSITION OF MICROPLASTICS IN PLACENTIA BAY, NEWFOUNDLAND AND LABRADOR

by

© Olivia Dillon

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Abstract

As part of a baseline study for Placentia Bay, one of Newfoundland's prominent fishing regions, I investigated the spatial distribution and characteristics of microplastics. I collected water samples from the surface at nine open-water locations and sediment samples at six locations in the sediment layer. Microplastics were examined under a microscope, and a subset of samples underwent Raman microspectroscopy for chemical identification To tailor methods specifically to the water and sediment samples from Placentia Bay, I conducted method tests. Published methods proved unreliable, as a lack of standardized methodology was evident. Water column samples required an extended digestion time of 24 hours with hydrogen peroxide and acetic acid due to their POC-rich nature. In sediment samples, microplastics were isolated using a sodium tungstate dihydrate solution. . The highest concentrations of microplastics in the water column were identified on the eastern side of the bay, with fibers being the predominant shape and polyethylene as the dominant polymer type. Raman spectroscopy was employed to confirm microplastics and evaluate the reliability of visual identification. 50% of fragments and 21% of fibers were non-plastic, highlighting the limitations of relying solely on visual identification for characterizing microplastics.

Keywords: microplastic distribution, microplastic analysis, Raman microspectroscopy, Placentia Bay, Newfoundland

General Summary

In our research conducted in Placentia Bay, a key fishing region in Newfoundland, we investigated the presence and distribution of microplastics. We collected water samples from the surface at different depths at multiple locations and sediment samples from various locations. Microplastics were isolated via the digestion of organic materials and density separation. Analyzing the microplastics by size, shape type, color, and material type, we observed no notable difference between samples collected at the sea surface interface and in the surface ocean in the water column. Concentrations ranged from 0.08 to 0.46 particles per m^3, with higher levels detected in the bay's eastern side. The most prevalent microplastics in the water were transparent polyethylene fibers. We were not able to confirm the presence of microplastics in the sediment layer, likely due to our methodological approach. This study establishes a baseline understanding of microplastic distribution in Placentia Bay.

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Co-Authorship Statement

The research outlined in this thesis, including the design and organization of field sampling, sample collection and analysis, methodological tests, and data interpretation was conducted by Olivia Dillon under the supervision of Dr. Uta Passow. Assistance with microplastic analysis was conducted by Tatiana Zaliznyak. All thesis chapters were written by Olivia Dillon.

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Chapter 1 – General Introduction

1.1 Characterization of Microplastics

Microplastics, a term coined by Prof. Thompson in 2004, are small plastic particles below five millimeters (Thompson *et al.,* 2004). There are two types of microplastics found globally: primary and secondary (Figure 1-1). Primary microplastics are deliberately produced as microbeads or nurdles. Secondary microplastics are generated from the degradation of larger plastic items through natural processes such as wave action, wind abrasion, and ultraviolet radiation from the sun, causing physical fragmentation and chemical weathering (Andrade *et al.,* 2019). Characterizing microplastics involves assessing their physical and chemical properties, including size, type, shape, color, density, and polymer composition. Size categories range from one to five millimeters while the types can be classified as fibers, fragments, films, and microbeads (Andrady, 2017). Microplastics can exhibit various colors, from opaque to bright, with a range of densities depending on their polymer composition, with polyethylene (PE), polypropylene (PP), and polystyrene (PS) being the most prevalent polymers identified (Hong *et al.,* 2017).

Microbeads (Figure 1-2d), as primary microplastics, refer to minute, usually spherical plastic particles that were previously incorporated into numerous personal care and cosmetic products for their exfoliating and abrasive properties (Tanaka & Takada, 2016). Conversely, nurdles are small pre-production plastic pellets used as raw materials in the manufacturing of various plastic goods that escape into the environment during production, transport, or storage (Tunnell *et al.,* 2020). Microfibers (Figure 1-2b), a secondary microplastic, consist of minuscule synthetic polymer strands derived from textiles, garments, fishing gear, and industrial sources. Fragments (Figure 1-2c), another type of secondary microplastic, result from the breakdown of larger plastic items like bottles, packaging materials, industrial components, and various consumer products. Their irregular shapes, often characterized by jagged edges and uneven surfaces, differentiate them from

other microplastic forms (Yu *et al.,* 2021). Films (Figure 1-2a), a further class of secondary microplastics, manifest as thin layers or sheets of synthetic polymers widely used in packaging, manufacturing, and numerous industrial applications. Films are typically associated with single-use plastic products such as shopping bags, packaging materials, disposable items, and agricultural films (An *et al.,* 2020).

1.2 Sources and Pathways

The advent of the first synthetic plastic in 1907 by Leo Baekeland, signified the dawn of a new era (Mercelis, 2020). The widespread use of plastic is driven by its durability, cost-effectiveness, and ease of manufacturing (De-la-Torre *et al.,* 2021). Over the past five decades, the demand and production of plastic have experienced an exponential surge, leading to a global annual production volume of approximately 280 megatons (mt) (Qi *et al.,* 2020). Consequently, an estimated 20 Mt of plastic waste is introduced into the world's oceans each year and is expected to triple in magnitude by 2030 (Borrelle *et al.,* 2020). A total of 79% of these plastics are presumed to be distributed across landfills, natural environments, and ecosystems (Geyer *et al.,* 2017). Although the primary origins of plastic pollution are predominantly terrestrial, approximately 20% of the global presence of ocean plastic contamination is attributed to activities conducted in marine environments, including aquaculture, offshore mining, and fisheries (Niaounakis, 2017). The emergence of marine plastic pollution, including the pervasive issue of microplastics, has profoundly reshaped the environmental landscape, suggesting it is one of the most pressing global environmental challenges confronting contemporary society (United Nations Environment Programme, 2014).

The small size, durability, and buoyancy of most microplastics enable their infiltration into various environmental compartments, including aquatic systems, soil, and the atmosphere, through both natural processes and anthropogenic activities (Ballent *et al.,* 2012 & Cole *et al.,* 2011). River systems serve as critical conduits for the transportation of microplastics from terrestrial environments to the oceans, as they carry and deposit plastic waste accumulated along their banks and tributaries (Alfonso *et al.,* 2021; D'Avignon *et al.,* 2021). The rapid flow and reach of major rivers, such as the Yangtze in China and the Ganges in India are both ranked among the top 20 polluting rivers globally, contributing to the distribution of microplastics across oceanic regions (Lebreton *et al.,* 2017; Li *et al.,* 2022; Napper *et al.,* 2021). Atmospheric transport plays a role in the dissemination of microplastics, whereby airborne microplastics can be carried over long distances before settling into marine environments through precipitation or atmospheric fallout (Shao *et al.,* 2022). Estuaries also serve as conduits for the transport of microplastic to marine ecosystems (Browne *et al.,* 2011).

The inadvertent discharge of plastic materials from cargo ships during transport contributes to plastic waste, especially in regions with high marine traffic when dumping or shipping accidents occur (Claessens *et al.,* 2011; Higgins & Turner, 2023; Mistri *et al.,* 2020; Wan *et al.,* 2022). Fishing nets, lines, and other fishing gear can degrade over time and release synthetic fibers into the water (Saturno, 2020). Improper disposal of fishing equipment (including ropes, lines, and floats) and the accidental loss of fishing gear, such as ghost nets, perpetuates the issue by continuously releasing microplastics into the water column (Gilman *et al.,* 2021). Washing machines contribute through the shedding of fibers from clothing during the washing process. These fibers, too miniscule to be filtered out by wastewater treatment plants, are discharged into the rivers and oceans (Cesa *et al.,* 2017). Additionally, litter situated near beaches and harbors is

also a source of plastic waste as it can be carried by wind, rain, or runoff directly into the surrounding water bodies (Neto *et al.,* 2019; Kaviarasan *et al.,* 2022; Tun *et al.,* 2022).

Once in marine waters, the distribution of microplastics is influenced by different processes. In undisturbed settings, most microplastics exhibit buoyancy in seawater, consequently staying afloat on the water's surface (Issac & Kandasubramanian, 2021). However, these microplastic particles have multiple pathways leading them to the ocean floor. Microplastics may become ensnared in marine snow, which can include the fecal matter of marine organisms, inducing a downward trajectory due to their density transporting them to the seafloor (Long *et al.,* 2015). In another scenario, when deceased animals sink to the seafloor, the microplastics they have ingested accompany them, facilitating their downward transport (Karlsson *et al.,* 2017).

1.3 Impacts on Marine Organisms

The proliferation of microplastics in marine environments poses a considerable and escalating threat to the intricate balance of aquatic ecosystems and the well-being of diverse marine organisms. The ingestion of microplastics has been observed across numerous marine species, with documented cases in over 300 marine species, spanning from invertebrates and small fish to cetaceans (Bergmann *et al.,* 2015; Dawson *et al.,* 2018; Panti *et al.,* 2019).

The ingestion of these particles poses multifaceted risks. Physical damage due to the sharp edges of certain microplastics has led to internal injuries, blockages in digestive systems, and bleeding in a range of marine organisms including fish, seabirds, and whales (Wright *et al.,* 2013). Microplastics also act as carriers for harmful substances. During their lifecycle, microplastics can absorb and accumulate chemical additives, toxic compounds, persistent organic pollutants (POPs), and heavy metals (Engler, 2012; Rani-Broges *et al.,* 2021). Upon ingestion, microplastics serve

as chemical vectors, releasing accumulated toxic elements into the muscle tissue of the organism (Andrady, 2017; Brennecke *et al.,* 2016).

The bioaccumulation and sorption of hydrophobic organic chemicals to these minute plastic particles have been demonstrated to induce adverse reactions in humans and aquatic wildlife, such as metabolic dysfunction, declining feeding behavior, and inhibited larval growth and development (Gigault *et al.,* 2021; Li *et al.,* 2021; Setälä *et al.,* 2014; Ziccardi *et al.,* 2016; Zolotova *et al.,* 2022).

1.4 Fragmentation and Weathering

In the dynamic marine environment, the weathering of microplastics stands as a prominent force reshaping their physical characteristics. Under the influence of sunlight, saltwater, and mechanical stress, microplastics change both color and size (Liu *et al.,* 2019). UV radiation, triggers the gradual degradation of chemical bonds, resulting in a shift from the vibrant hues of freshly released particles of blue, red, and green to subdued tones such as transparent and brown owing to photooxidation and chemical breakdown (Veerasingam *et al.,* 2016). Concurrently, the incessant mechanical action of waves and currents, compounded by surface abrasion, fosters the fragmentation of larger plastic entities into microplastics, perpetuating their dispersion (Duan *et al.,* 2021).

1.5 Current Methods in Microplastic Research

To capture microplastics dispersed throughout the water column, researchers commonly employ a range of nets, including neuston nets, plankton nets, manta trawls, catamarans, and bongo nets. Mesh sizes typically range from 100 to 500 μm, the most common being 300 μm, as smaller

mesh sizes tend to become clogged with plankton and other biological particles (Mai *et al.,* 2018). To obtain particles smaller than 300 µm, pumps are often used to collect microplastics (Enders *et al.,* 2015). The duration of the trawl times varies based on the research objectives in time and distance (Razeghi *et al.,* 2021; Stock *et al.,* 2019). Assessing microplastics in sediment samples entails distinct methodologies, involving direct shoveling, box corers for insights into sediment layers, or van Veen grabs to collect surface sediment samples (Hidalgo-Ruz *et al.,* 2012). Microplastic abundance is commonly measured in "particles per m^3," and "particles per m^2," (Mai *et al.,* 2018).

Post-collection, microplastics can be separated from biological material through various methods, often involving an initial step of digesting the biological components in chemical solutions such as acetic acid (HAc), hydrochloride (HCl), hydrogen peroxide (H_2O_2), potassium hydroxide (KOH), and ammonia (NH_3). These chemical solutions are meant to digest the sample matrix without compromising the microplastics (Nuelle *et al.,* 2014; Reineccius *et al.,* 2021). Subsequently, microplastics can be further isolated through density flotations and size fractioning via filtration or sieving (Mai *et al.,* 2018; Rocha-Santos & Duarte, 2014).

Filters such as glass fiber filters, polycarbonate filters, silver filters, gold filters, and aluminum filters are used for sample collection, with the selection based on the Microspectroscopy type (Medina Faull *et al.,* 2021; Oßmann *et al.,* 2017; Zhu *et al.,* 2020). Each filter possesses distinct fluorescence properties that improve or impede analysis. Once dried in an oven, the quantification of microplastics begins. Initially, visual identification under a dissection microscope (typically a stereomicroscope) allows for the differentiation and categorization of microplastics based on their shapes, sizes, and colors. For the categorization of microplastics by their chemical composition, Fourier-transform infrared spectroscopy (FTIR), Raman microspectroscopy, nuclear magnetic

resonance (NMR), and scanning electron microspectroscopy (SEM) techniques provide detailed insights into the chemical composition, structure, and surface characteristics of the microplastics (Harrison *et al.,* 2011; Peez *et al.,* 2021; Van Cauwenberghe *et al.,* 2013; Wang *et al.,* 2017). While some researchers bypass visual identification and proceed directly to imaging techniques like FTIR or Raman, this detailed analysis is time-consuming. To conserve resources, many researchers opt to combine visual identification with comprehensive spectroscopy.

During the sampling process, researchers exercise caution to minimize potential sources of contamination, opting for plastic-free materials such as glass and metal. Measures are also taken to mitigate the impact of airborne plastic fibers originating from clothing and sampling equipment. Typically, researchers wear clothing made of natural fibers and collect blank controls such as placing a blank petri dish with ultrapure Milli-Q water nearby to capture floating plastic particles, indicating potential contaminants during sample analysis.

1.6 Research Gaps

The current research on microplastics faces several gaps and challenges, allowing for areas of improvement. A fundamental issue is the absence of a universally acknowledged and standardized operating procedure, requiring the development of uniform protocols for the collection, extraction, and polymer identification of microplastics across diverse environments. The establishment of standardized protocols is essential because the current variability in methods hampers comparability and undermines credibility among different studies (Mai *et al.,* 2018).

The limited accessibility of materials, the high cost of specialized instruments, and the need for highly skilled individuals required for the identification of the chemical composition of microplastics present further challenges. The lack of widespread access to these sophisticated

analytical tools impedes the investigation of the diverse characteristics and sources of microplastics.

Nanoplastics, typically measuring below 100 μm in size, originate from post-consumer waste and the fragmentation of larger microplastics (Andrady, 2011). Due to their diminutive size, they can infiltrate numerous areas within ecosystems and organisms. One study found nanoplastics penetrating the blood-brain barrier and inducing oxidative stress in marine invertebrates (Prust *et al.,* 2020). The sampling, isolation, and identification of nanoplastics pose even greater challenges compared to their larger counterpart, microplastics, as methods are still in the early stages of development. The study of nanoplastics demands attention, given the knowledge gap of their behavior, fate, and environmental consequences (Cunningham *et al.,* 2023).

Research initiatives are needed to explore the long-term effects of microplastic exposure and ingestion on biodiversity, food webs, and ecosystem functioning (Akdogan & Guven, 2019). Such initiatives aim to elucidate the intricate relationships between microplastics and various ecological components and assess their impact. Recent discoveries highlight the role of microplastics as significant carriers for microorganisms, facilitating the formation of fully developed biofilms on these synthetic substrates (Yang *et al.,* 2020). These developing biofilms might display variations in microbial composition compared to the natural, free-living, or particle-associated microbial populations found in the surrounding water.

1.7 Research Goal and Thesis Structure

The main goal of my thesis is to provide a baseline understanding of the characteristics and spatial distribution of microplastics located in Placentia Bay, Newfoundland. This is encapsulated within a two data chapters that contribute toward the study.

Chapter 2 presents a detailed overview of the quantitative and qualitative assessments of microplastic contamination in Placentia Bay, shedding light on concentration levels, polymer compositions, and physical characteristics. This chapter provides a critical analysis and interpretation of the implications of microplastic distribution in Placentia Bay, elucidating the broader environmental significance and potential impact on marine ecosystems and human activities. The results of this chapter is intended to establish a foundation for the future, enabling us to track changes as the expansion of aquaculture in Placentia Bay is underway.

Chapter 3 encompasses the method development of the analysis of microplastics in this study, including the methodological tests that were examined. This chapter describes and discusses the detailed findings and insights gleaned from the quality control and method development process. This chapter is essential because previously published methods were not effective for these specific samples given the low concentration of microplastics compared to POC concentration.

In Chapter 4, the results will be summarized and future outlooks will be presented. This chapter aims to offer broader perspectives and identifying potential areas for future research.

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1.9 Figures

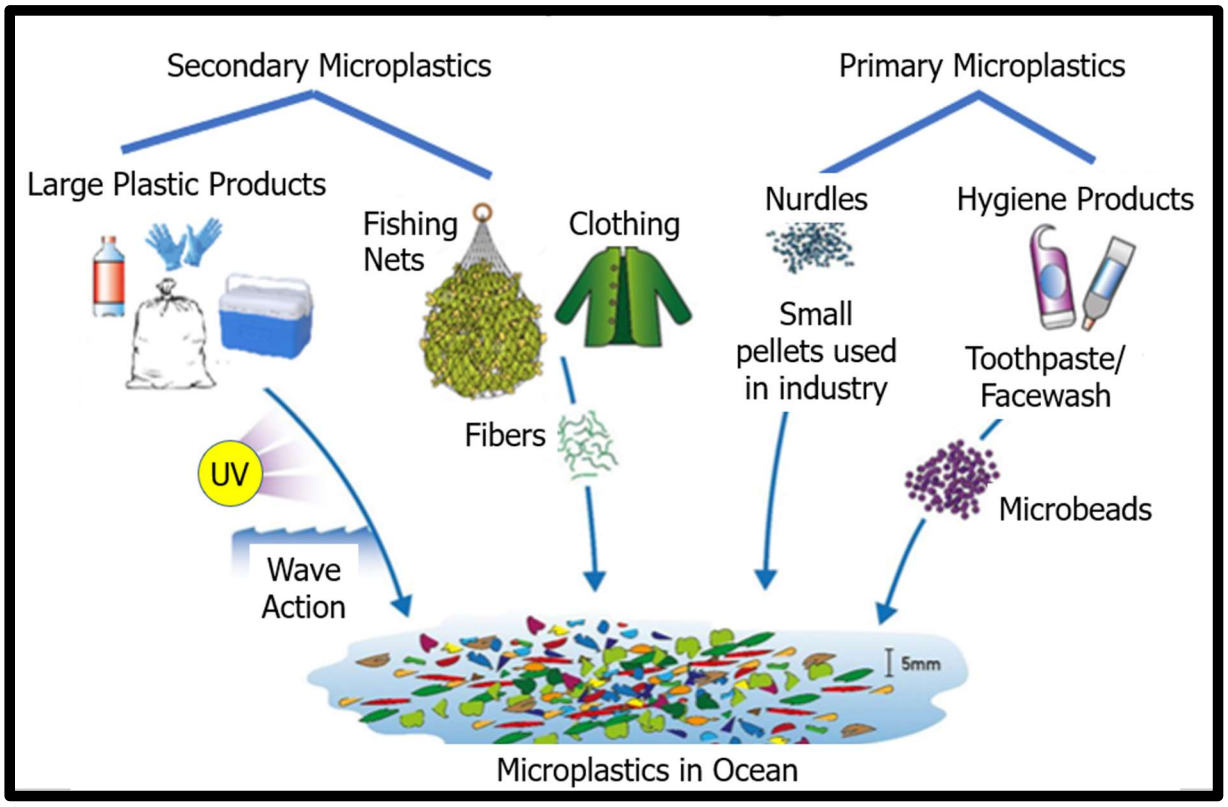

Figure 1-1. Various sources of primary and secondary microplastics in the ocean adapted from NERC Science of Environment (2022).

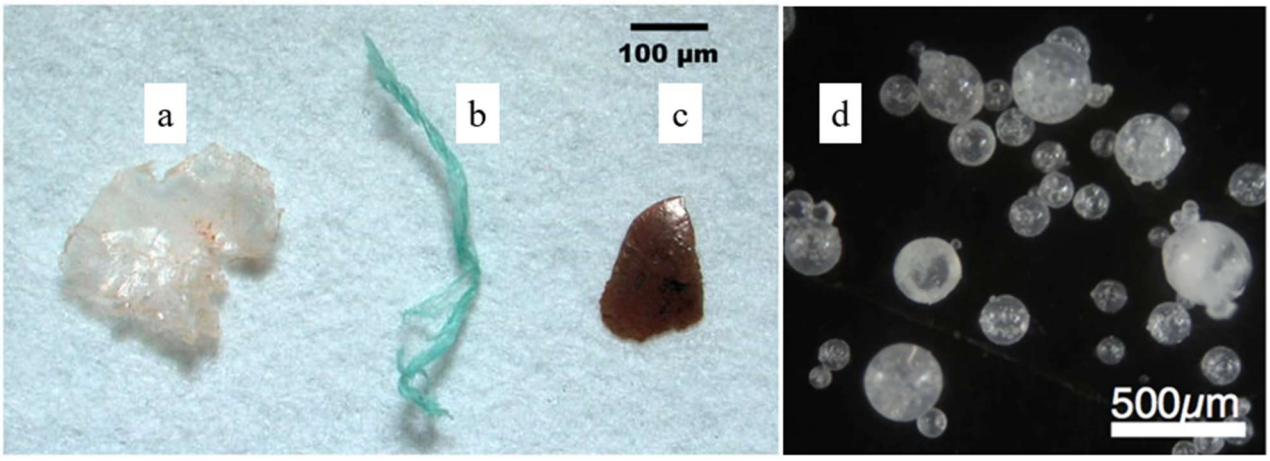

Figure 1-2. Various types of microplastics categorized as films (a), fibers (b), and fragments (c); from Cheang *et al.* (2018). Microbeads and nurdles (d); from Tanaka & Takada, (2016).

Chapter 2 – Spatial Distribution and Composition of Microplastics in Placentia Bay, Newfoundland

2.1 Abstract

I determined the spatial distribution and characteristics of microplastics in one of Newfoundland's most prominent fishing regions, Placentia Bay as part of a baseline study for the area. Water samples were collected from the surface using neuston (0 m-0.5 m depth) and plankton (1 m-6 m depth) nets (>0.3 mm) at nine open-water locations. Sediment samples were collected using a van Veen grab at six locations in the sediment layer. Water samples were treated with a solution that contained hydrogen peroxide and acetic acid. Density separation using sodium tungstate dihydrate was performed to isolate microplastics from the sediment samples. Microplastics were counted and classified by size, shape, and color using a microscope. Polymers were identified on a subset of samples via Raman microspectroscopy in the water column. There was no significant difference between microplastics collected with the neuston and plankton nets. Microplastic concentrations in the water ranged between 0.08±0.00 particles per m^3 and 0.46±0.10 particles per m^3. The highest concentrations were found in the eastern side of the bay. Fibers were the most common shape, polyethylene was the dominant polymer type, and transparent was the most common color of microplastics in the water column.

Keywords: density separation, digestion, neuston net, plankton net, microplastic analysis, Placentia Bay

2.2. Introduction

Placentia Bay, located in the North Atlantic region along the south east coastline of Newfoundland and Labrador, has historically functioned as a repository for various industrial, military, and urban waste activities (Khan, 2003). It offers year-round access for shipping as it is ice-free, sustains an active oil and gas sector, facilitates commercial and recreational fishing industry, supports the growth of aquaculture, and serves as a vital component in an interprovincial ferry network (Government of Canada, 2018). The bay remains a focal point for aquaculture, with ongoing expansions under Newfoundland's "The Way Forward" policy, alongside the persistent legacy of crude oil spills from the now inactive Come By Chance oil refinery (Maxwell & Filgueira, 2020). Despite the attention given to various anthropogenic impacts, there remains a notable gap in understanding the microplastic pollution in Placentia Bay. This study, funded by the Department of Fisheries and Ocean Canada (DFO), aims to address this knowledge gap.

As part of the National Oceans Protection Plan, the Department of Fisheries and Oceans (DFO) has initiated the Coastal Environmental Baseline Program, which includes an examination of six vital coastal ecosystems across Canada, including Placentia Bay. The implementation of a "baseline" study enables DFO to establish a reference point for future assessments, facilitating the detection of significant changes in the area over time. This master's thesis concentrates on identifying and quantifying microplastics in both the water column and the sediments of Placentia Bay. This thesis aims to fill the existing knowledge gap concerning microplastics focusing on the quantity, types, shapes, sizes, colors, and polymer compositions of the microplastics and emphasizes the importance of understanding the characteristics and spatial distribution of microplastics. Assessing microplastic concentrations in the surface sediment layer, surface near

ocean (1 m-6 m), and sea surface layer (0 m-0.5 m) represents a crucial step in evaluating the extent of microplastic pollution in Placentia Bay.

Several hypotheses guided this research. One expectation is that Placentia Bay's microplastic concentrations would mirror those observed in other bays with similar anthropogenic activities, particularly in the northern and eastern regions influenced by human settlements, industrial facilities, and fishing activities. The study further anticipated that river inputs and nearby industrial operations would serve as primary sources of microplastic contamination, with heightened levels expected along the coastline and near beaches due to recreational and commercial activities. Additionally, the study expected higher microplastic concentrations in the sediment compared to the water column, emphasizing the importance of understanding vertical distribution and long-term persistence.

The study hypothesized a wide variety of polymers as there are numerous types of plastic materials in contemporary society. It was hypothesized that the predominant microplastic type within Placentia Bay would be fibers as fishing gear could be an important source (Liboiron *et al.,* 2019).

2.3. Materials and Methods

2.3.1. Study Area and Sampling

Between July and October 2021, net tow samples were collected at nine stations in Placentia Bay, Newfoundland. At each station, particles >0.3 mm were collected at 0 m to 0.5 m depth with a 0.5 x 1 m neuston net, and at 1 m-6 m depth with a 0.75 m diameter plankton net (Figure 2-1 and Table 2-1). Stations were chosen to provide an optimum spatial coverage of the Bay. The EXO system (YSI EXO2 probe) was used to measure temperature, salinity, and

Chlorophyll a (based on fluorescence) at the surface (1-6 m) (Table 2-1), with data collected per second. At stations where the EXO system was not deployed, a thermometer and refractometer were used for temperature and salinity. Subsamples for particulate organic carbon (POC) analyses were collected from select net tows (Table 2-1). There was no temporal component of this study and sampling times, for logistical reasons, varied widely.

The nets (0.3 mm mesh size) were deployed at 20 m behind the Royal Breeze vessel to minimize any mixing from the vessel's propeller. Each tow lasted an average of one hour at a speed of 2.5 knots. The total filtered volume (Table 2-1) was measured with a flowmeter (General Oceanics, Inc 2030R). Samples were stored for three months in 1.9 L glass mason jars at 4 °C until analysis, and processed for each net separately. Subsamples for POC determination were processed immediately after the arrival at the laboratory after each field trip.

Sediment samples were collected in September 2021 from six locations at depths from 6 m- 256 m depth throughout Placentia Bay. These sample stations were selected in collaboration with the ongoing benthic field sampling study conducted by the Department of Fisheries and Oceans Canada focusing on areas with a soft bottom (Figure 2-1 and Table 2-1). Sediment samples were collected using a van Veen grab with a volume of 24 L (WildCo 1775-A10). Using a metal spoon, samples roughly 10 cm deep were carefully mixed to create a homogenous composition and transferred to 1.9 L glass mason jars. Samples were stored in a refrigerator at 4 °C until analysis for four months.

2.3.2. Sample Preparation

2.3.2.1. Method of Net Sample Analysis

Samples collected with nets were processed according to a modified version of Reineccius *et al.* (2021) (Figure 2-2). In brief, samples were sieved through a 0.3 mm stainless steel mesh and rinsed with Ultrapure Milli-Q water to remove the salt (not shown in Figure 2-2). Next, samples were freeze-dried for three to five days to remove all remaining moisture. Plastic particles larger than 5 mm were isolated manually using forceps into scintillation vials, and then the dried sample was digested in hydrogen peroxide (H_2O_2 30%) for 24 hours at 50 °C. Next, an equivalent volume of acetic acid (HAc 24%) was added and incubated for 12 hours at room temperature. The sample was rinsed through a 0.3 mm stainless steel sieve to remove residual organic matter (Masura et al., 2015). The remaining particles were filtered under low vacuum (<200 mg Hg) onto a 10 µm pore size 47 mm diameter polycarbonate filter (Whatman Cyclopore 10418450) using vacuum filtration. Due to the high number of microplastics per sample, multiple filters were prepared per station (between one and eleven). Each filter was stored in a glass petri dish for later enumeration and identification by light microscopy and Raman-Microspectroscopy.

2.3.2.2. Method of Sediment Sample Analysis

One kg of wet sediment was weighed and placed into a pre-cleaned glass 4 L beaker. 3 L of sodium tungstate dihydrate solution (Na_2WO_4 $2H_2O$, density: 1.6 g cm^{-3}) was added to the glass beaker in a 3:1 ratio, as recommended by Pagter et al., 2018. The sodium tungstate solution was prepared by mixing 1800 grams of dried sodium tungstate dihydrate powder with 3 L of ultrapure Milli-Q water to achieve a density of 1.6 g cm^{-3}. The mixture of the tungstate solution and wet sediment was stirred with a metal spoon for three minutes, covered with aluminum foil, and sat at

room temperature for 24 hours. After the settling period, 250 mL of the supernatant was pipetted onto stacked stainless steel sieves of 0.3 mm and 0.075 mm and rinsed. Materials collected were filtered under low vacuum (<200 mg Hg) onto a 10 µm pore size 47 mm diameter polycarbonate filter (Whatman Cyclopore 10418450). Ultrapure Milli-Q water was used to rinse the glass pipette to collect any residual particles. This density separation method was repeated three times per sample to ensure that all microplastics were collected (Akkajit *et al.,* 2021) (Figure 2-3).

2.3.2.3. Particulate Organic Carbon

Three replicates of two mL subsamples of each net tow were isolated using a vacuum pump filtration system onto a combusted 0.7 µm pore size 25 mm diameter glass fiber filter (GF/F, Whatman 1825-025). Filters were dried in an oven for 24 hours at 60° C. Filters were transferred to a desiccator with acid fume for 24 hours to remove inorganic carbon and placed in a drying oven for 24 hours at 60° C. Filters were wrapped in foil discs and shaped into a pellet form. Analysis was conducted with a CHN analyzer PerkinElmer, 2400 Series II CHNS/O at Memorial University of Newfoundland (Passow *et al.,* 2001).

2.3.3. Determination of Microplastic Abundance and their Characterization

2.3.3.1. Visual Determination of Microplastics Abundance and Characteristics

The filters with isolated microplastics was placed on top of a 1 mm square grid paper to categorize particles by size, type, and color under a stereomicroscope (Wild Heerbrugg 256530 magnification 75x). The type was categorized as fragments or fibers. Fragments were identified by a length-to-width ratio ≤3 and sized by area (mm^2). Fibers were identified by a length-to-width ratio of >3 and were sized by length (mm) (Vianello et al., 2019). Tangled fibers, which made up

roughly 10-20% were carefully separated using dissecting tweezers and individually counted and measured. Tangled and individual fibers were counted in the same category.

Visually identified particles underwent prodding to inspect rigidity using dissecting forceps. Those particles that broke apart upon probing were excluded from the plastic count. Conversely, particles that retained shape after probing were considered plastic. Particles displaying bright, non-earth tone colors were categorized as potential microplastics.

Fragments were classified into four size classes based on area: 0.3 mm^2 to <0.6 mm^2, 0.6 mm^2 to <1.2 mm^2, 1.2 mm^2 to <2.4 mm^2, 2.4 mm^2 to <4.8 mm^2, and >4.8 mm^2 and fibers in four size classes based on length: 0.3 mm to <0.6 mm, 0.6 mm to <1.2 mm, 1.2 mm to <2.4 mm, 2.4 mm to <4.8 mm, and >4.8 mm. Fragments and fibers from sediments were classified into six size classes: 0.075 mm^2 to <0.15 mm^2, 0.15 mm^2 to <0.3 mm^2, 0.3 mm^2 to <0.6 mm^2, 0.6 mm^2 to <1.2 mm^2, 1.2 mm^2 to <2.4 mm^2, 2.4 mm^2 to <4.8 mm^2, and >4.8 mm^2 for fragments and 0.075 mm to <0.15 mm, 0.15 mm to <0.3 mm, 0.3 mm to <0.6 mm, 0.6 mm to <1.2 mm, 1.2 mm to <2.4 mm, 2.4 mm to <4.8 mm, and >4.800 mm for fibers. Fragments and fiber colors were classified into eight categories: transparent, black, green, white, blue, red, yellow, and orange. Transparency was assessed based on the ease of visibility of the grid paper behind the microplastic in question. In each category the average number and standard deviation is given.

2.3.3.2. Raman Microspectroscopy

The composition of microplastics visually identified and counted were confirmed using Raman Microspectroscopy at the Nano-Raman Image Laboratory (NARMIL) at the School of Marine and Atmospheric Sciences, Stony Brook University. Suspected microplastic particles were measured using the Renishaw® inVia™ confocal Raman microspectrophotometer configured with

a modified upright Leica® DM2700™ fluorescence microscope. A He-Ne laser (633 nm wavelength) or Diode laser (785 nm wavelength) was used as the excitation light source. Spectra were acquired using a 1200 line/mm diffractive grating. In the case of the He-Ne laser, the diffractive grating was centered at 1200 cm^{-1} in the static mode and allowed 217–2050 cm^{-1} wavenumber coverage with 1.8 cm^{-1} spectral resolution. For the Diode 785 nm laser, the range of wavenumbers covered between 200 cm^{-1} to 2000 cm^{-1} in continuous mode with 1.1 cm^{-1} spectral resolution (or the grating was centered at 1250 cm^{-1} in the static mode) and allowed for 672–1767 cm^{-1} wavenumber coverage with 1.1 cm^{-1} spectral resolution.

Each particle's laser power and exposure time were individually selected to achieve the best possible results. Laser power ranged from 1.01 ± 0.08 mW (10% of nominal laser power) to 9.66 ± 2.8 mW (100% nominal laser power) at the sample using 50x objective for He-Ne laser and from 0.55 ± 0.05 mW (10% of nominal laser power) to 17.85 ± 3.12 mW (100% nominal laser power) at the sample using 50x objective for Diode 785 laser.

Spectra were minimally processed using Renishaw's® Wire 5.1™ software to remove the universal baseline fluorescence by polynomial fitting. Raman spectra were plotted with the Python Matplotlib library and the NARMIL internal reference library was used to match particle spectra to determine their chemical composition. Correctly identifying the particle as plastic was achieved by matching the Raman shift of the characteristic peaks to the particular plastic of interest. Particles were categorized as plastic, non plastic, or dyes using the Raman spectra results.

Representative filters with diverse particle types were selected for analysis. A preliminary assessment of the likely chemical composition was recorded for each particle during Raman analysis. For example, polypropylene exhibits a distinct peak at 841 cm^{-1} and 809 cm^{-1} allowing experienced researchers to identify it as such immediately (Wang et al., 2023). The final detailed

analysis of the Raman spectra was later compared with this preliminary assessment. Of the 54 particles where both a preliminary assessment and a final Raman identification exist, the preliminary assessment identified the chemical composition of the particles correctly in 88% of the particles. For 74 particles only a preliminary assessment existed, as time constraints did not allow a detailed spectral analysis. In those cases, the preliminary assessment was assumed to correctly identify the particle's chemical composition.

2.3.3.3. Calculation of Microplastic Abundance

The abundance of microplastics was adjusted based on findings from Raman analysis of particles, acknowledging the limitations associated with solely visually identifying microplastics. Particles with identified matches were documented for their type, color, and chemical composition, categorized into non-plastic, dyes, and plastic. With this information, a correction percentage of 79% plastic was applied to all fibers and 50% for all fragments in the water column. To gain insight into possible concentrations of microplastic in the sediments, we assumed the general correction of 65% from the water column (an average of 79% for fibers and 50% for fragments).

For a more in-depth understanding of the correction techniques, Chapter 3 provides detailed insights into the process, which involves combining visual identification and Raman Microspectroscopy to accurately calculate the abundance of microplastics in Placentia Bay.

2.4. Quality Control

The accuracy and confidence in the concentration and chemical composition of microplastics in Placentia Bay are assessed by considering various errors. To avoid contamination, all cotton or natural fiber clothes were attempted to be worn during field and lab work. Glass or

metal materials were used during sample collection, sample storage, sample processing and counting, and composition analysis. Materials used in the field and the laboratory were rinsed with Ultrapure Milli-Q water before use. Nets were rinsed with *in situ* seawater between each sampling station to avoid contamination. Glass Petri dishes with Ultrapure Milli-Q water were left out as blanks during all laboratory procedures conducted under a fume hood. Blanks were counted and analyzed with Raman Microspectroscopy and found to contain only cotton, implying that no significant polymer contamination took place in the lab.

The flowmeter measures the volume of water filtered through the net, but errors are included during the deployment and retrieval of the net. We assume an error of 10% for the flowmeter. The statistical counting error was estimated using the table provided by Lund et al., (1958). This error is dependent on the number of particles counted. The accuracy of each count varies indirectly with the square root of the number counted. As the count is higher, the accuracy also increases. On average, 403±341 particles were counted per station with an average of 1727±574 m^3 of seawater filtered per station. The average counting error was 12%. Therefore, the combined error considering the fieldwork error and counting error was 16%.

It is important to note that the concentration of microplastic captured by the neuston net may be an underestimation. The filtered volume was calculated based on the whole area of the net and the flowmeter reading, thus ignoring the area of the neuston net that was above the sea surface. The full area was considered to take the impact of wave action during the sampling process into account. The net's position remained >50% below the water surface at all times, activating the flowmeter. At times, it submerged to 80% or 90% below the water line, complicating the accurate measurement of the volume of water passing through the net. Thus the assumed water filtered by

the neuston net is an overestimate, resulting in an underestimate of microplastic concentrations in samples from this net. We estimate microplastic concentrations to be underestimated by up to 30%.

2.5. Results: Microplastic Distribution in Placentia Bay

Here we present the first study of microplastic concentration at and near the surface of Placentia Bay.

2.5.1. Water Column

2.5.1.1. Context Data

Particulate Organic Carbon (POC) in net samples collected for microplastic analysis ranged from 0.03±0.00 - 0.26±0.22 µmol L^{-1}, with a C: N ratio of 4.64±0.26 - 5.29±0.01 µm L^{-1}. The Chlorophyll-a concentration in surface waters ranged from 1.13±0.26 - 1.46±0.23 µg L^{-1} (Table 2-2). The surface temperature was between 15 to 18 °C.

2.5.1.2. Microplastic Concentration

Microplastics >0.3 mm were present in the surface water of all stations, independent of the net type used. The concentrations of microplastics from the mean of both nets ranged between 0.08±0.00 particles per m^3 and 0.46±0.10 particles per m^3 with a median concentration of 0.19 particles per m^3 (average of 0.24±0.13 particles per m^{-3}) (Figure 2-4). The stations with the highest concentrations were S1, S5, S7, and S8, with concentrations from 0.32±0.10 particles per m^3 to 0.46±0.10 particles per m^3. At stations S2, S3, S6, and S9 we found microplastic concentrations between 0.11±0.01 particles per m^3 and 0.19±0.08 particles per m^3, and the lowest concentrations were observed at S4, with 0.08±0.00 particles per m^3.

2.5.1.3. Microplastic Type, Color, and Size

Fibers dominated microplastic particles at all stations from the mean of both nets, contributing from 77% to 96% at each station (Figure 2-5). The remaining 8% to 23% of microplastic particles consisted of fragments with none or negligible sheets, microbeads, or nurdles.

Microplastics from the mean of both nets at all stations were categorized into eight various colors (Figure 2-6) and 80% of all microplastics collected in the study area were transparent. Other microplastic particles were blue (8%), black (4%), white (3%), red (2%), yellow (2%), orange (1%), and green (<1%).

Particles were compiled in size classes to interpret the fragmentation process during weathering in Placentia Bay (Figure 2-7). The value for each size class was obtained by calculating the mean values of both nets for fragments and fibers for each size class at all stations. A total of 447 fragments were found in the size range of 0.3 to <0.6 mm^2, 347 in the size range of 0.6 to <1.2 mm^2, 172 in the size range of 1.2 to <2.4 mm^2, 109 in the size range of 2.4 to <4.8 mm^2, 44 in the size range of >4.8 mm^2. 1566 fibers were found in the size range of 0.3 to <0.6 mm, 2413 in the size range of 0.6 to <1.2 mm, 1220 in the size range of 1.2 to <2.4 mm, 1048 in the size range of 2.4 to <4.8 mm, and 618 in the size range of >4.8 mm.

2.5.1.4. Polymer Composition of Microplastics

There were varying polymer compositions for each shape type and color from samples of both nets at all stations analyzed with Raman Microspectroscopy (Table 2-3). The polymer composition of transparent fragments tested (3) was 67% polypropylene and 33% polystyrene. The analyzed fragments for black (2), white (13), blue (3), red (1), and yellow (2) were all 100%

polyethylene. The polymer composition of transparent fibers analyzed (30) was 3% polyester, 50% polyethylene, 40% polypropylene, and 7% polystyrene. Black fibers analyzed (6) were 17% polyester, 33% polyethylene, and 50% polypropylene. Green (2) and yellow (4) fibers analyzed were 100% polypropylene. White fibers analyzed (9) were 11% polyethylene and 89% polypropylene. Blue fibers analyzed (8) were 25% polyethylene and 75% polypropylene. Red fibers analyzed (3) 67% polyester and 33% polypropylene. Orange fibers analyzed (1) were 100% polyethylene.

Overall, polymer composition was dominated by polyethylene (48%), followed by polypropylene (44%), polyester (5%), and polystyrene (3%) (Figure 2-8). The polymer composition of all fragments was dominated by polyethylene (88%), followed by polypropylene (8%), and polystyrene (4%) (Figure 2-9). The polymer composition of all fibers was dominated by polypropylene (57%), followed by polyethylene (33%), polyester (7%), and polystyrene (3%) (Figure 2-10).

2.5.2. Sediments

In sediment samples, a total of 1681 particles were counted using visual identification, consisting of 618 fragments and 1063 fibers of seven colors (Table 2-4). The higher quantity of fibers compared to fragments mirrors the results of the water column samples.

Raman Microspectroscopy spectra were collected on six fibers and one fragment, although we attempted to obtain spectra from 33 fibers and five fragments. The low success rate of capturing usable spectra from fibers in sediments may in part be due to the small diameter of these fibers compared to many of those found in the water. The fragments and fibers for which we did capture spectra were identified as cotton (Table 2-5). The historical use of natural fibers for fishing ropes

and nets, including vegetable fibers, cotton, and hemp, could have contributed to the low concentration of microplastic fibers in the sediments of Placentia Bay (Proskurowski *et al.,* 2011). The absence of confirmed microplastic fibers, along with the presence of cotton fibers, may indicate a delayed use of plastic fishing lines, resulting in fewer synthetic fibers in the sediment.

However, microplastics were likely collected for this study although their presence could not be confirmed via Raman Microspectroscopy, due to the difficulty in capturing spectra and time constraints.

Using mentioned assumption, microplastic concentrations in the sediments of Placentia Bay ranged between 136 particles per kg to 677 particles per kg, as depicted in the distribution patterns across the bay (Figure 2-11). Contrary to our initial hypothesis, the highest concentrations were observed at station O, situated centrally within the bay, while the lowest concentrations were found at the inner (E) and outer (S) stations of the bay. Our findings revealed slightly higher microplastic concentrations in Placentia Bay sediments compared to a study conducted on Belgian marine sediments at different locations on the continental shelf using similar methods, where sediment microplastic levels ranged from 49 particles per kg to 360 particles per kg (Claessens *et al.,* 2011). The Belgian study sampled locations near heavily populated coastal harbors, including areas with naval, commercial, fishing, and pleasure boat traffic, extending up to 21 km offshore in the North Sea. The higher concentration observed in our study might be associated with the reported high levels of fishing activities or with the uncertainty of our estimates (Liboiron *et al.,* 2019).

Microplastic fibers and fragments exhibited variation across all stations (Figure 2-12). Fibers account for 43% to 88% and fragments account for 12% to 57% of the total microplastics. The higher occurrence of fragments in comparison to fibers in the sediment samples compared to

the water column may suggest the preferential sinking of denser microplastics in Placentia Bay. This observation aligns with the prevalent use of higher-density plastic types, e.g. polyvinyl chloride (1.47 g cm^{-3}), in the manufacturing of items prone to become microplastic fragments (Hanvey *et al.,* 2017). Alternatively, transport pathways, e.g. within marine snow, may favor transportation of fragments.

2.6. Discussion of Microplastics in Placentia Bay

2.6.1 Total Concentrations Compared to Other Bays

The microplastic concentrations in the water column in Placentia Bay ranged from 0.08 ± 0.00 particles per m^3 to 0.46 ± 0.10 particles per m^3. To provide context, we compared these concentrations with those of various other bays, where sample collection and analytical approaches (e.g. size range) were similar to this study. The average population density for Newfoundland and Labrador is 1.4 persons per square kilometer, but the population is concentrated along the coastline (Newfoundland and Labrador, 2023). Placentia Bay has a surface area of 1,398 km^2 (Placentia Bay, 2023).

Chesapeake Bay, located along the East coast of North America, exhibited concentrations ranging from 0.009 particles per m^3 to 0.715 particles per m^3, which was slightly larger than the range found in Placentia Bay (Bikker *et al.,* 2020). Notably, Chesapeake Bay is ten times larger at 11,600 km^2 and the population density ranges from 46 persons per square kilometer to 1018 persons per square kilometer (Maryland State Archives, 2023; Open Data Network, 2018).

In contrast, the Bay of Biscay, situated to the east in the North Atlantic Ocean, showed lower concentrations, ranging from 0.00098 particles per m^3 to 0.35 particles per m^3 (Mendoza et al., 2020). The Bay of Biscay is significantly larger in size than Placentia Bay or Chesapeake Bay

with a surface area of 223,000 km^2. The population density of the Bay of Biscay is 299 people per square kilometer (Borja *et al.,* 2019).

In Tokyo Bay concentrations of microplastics ranged from 0.90 particles per m^3 to 17.75 particles per m^3 (Nakano et al., 2020). Tokyo Bay, located in the Philippine Sea, has a similar surface area as Placentia Bay at 1,500 km^2 and the population density is high, with 4,400 persons per square kilometer (Demographia, 2016; The Editors of Encyclopaedia Britannica, 2009).

A study sampling microplastics in multiple locations throughout the North Atlantic Ocean, which Placentia Bay is part of, found concentrations ranging from 0.000012 particles per m^3 to 543 particles per m^3 (Lusher *et al.,* 2014).

Regions with high population densities and extensive industrial activities such as Tokyo, the Gulf of Mexico, and the Baltic Sea, typically exhibit high concentrations of microplastics in surface waters and sediments due to extensive plastic waste generation and discharge (Di Mauro *et al.,* 2017; Esiukova *et al.,* 2019; Nakano *et al.,* 2020). In contrast, in Placentia Bay, where population density is low and few industrial sites exist, a lower degree of microplastic pollution would be expected. Indeed, concentration in Placentia Bay was appreciably lower than in the similarly sized, but densely populated Tokyo Bay.

2.6.2. Reasons for the Observed Distribution Patterns

To elucidate the distribution patterns characterized by microplastic concentrations extending from the inner bay toward the outer bay, a comprehensive mapping of potential microplastic sources was conducted (Figure 2-13). This mapping specifically considered river inputs and potential land-based anthropogenic sources, encompassing dump sites, communities,

and industrial centers, all of which could potentially contribute to microplastic pollution in Placentia Bay.

Notably, stations displaying both low and high microplastic concentrations in the eastern outer bay (S1 and S2) were found near a land-based anthropogenic activity center. In contrast, stations in the western outer bay featuring lower concentrations (S3 and S4) were not located near either anthropogenic activities or river inputs. Stations in the northwestern area with moderate to low concentrations (S5 and S6) were distant from anthropogenic activities but were exposed to river inputs. Conversely, a station in the northeastern part of the bay exhibiting high microplastic concentrations (S7) was situated near multiple land-based anthropogenic activities and river inputs. The stations with high microplastic concentrations, positioned in the central-eastern area of the bay (S8), was in proximity to an anthropogenic activity area but not close to any river inputs. A station in the eastern section of the outer bay featuring low microplastic concentrations (S9) was situated near both anthropogenic activities and river inputs.

Other factors are important in determining the distribution of microplastics within Placentia Bay as it is characterized by its diverse bottom topography as well as the presence of several islands centrally located in the inner Bay. This area in particular exhibits variations in microplastic concentrations throughout its different regions (Ma *et al.,* 2012). The head of the bay, with its deep channels and proximity to land-based anthropogenic activity, shows higher microplastic concentrations. This pattern aligns with the distribution of cod eggs observed in the same area, where eggs tend to concentrate in the inner and eastern parts of the bay (Bradbury *et al.,* 2000). Just as the eggs stay in these regions, it appears that microplastic also tends to persist in the bay's inner and eastern parts due to local circulation patterns influenced by the island.

The mean ocean circulation in Placentia Bay in summer generally follows a cyclonic (counter clockwise) pattern (Ma *et al.,* 2012). Within the inner bay, currents tend towards the eastern shore, influencing the transport and distribution of microplastics (Figure 2-13). The circulation pattern may help explain the observed higher microplastic concentrations in the eastern Bay area and the lower concentrations in the western region.

Specifically, the roughly cyclonic circulation pattern provides a potential justification for the microplastic concentration disparity between sampling stations S8 and S9. S8, located north of various river inputs and anthropogenic activities, exhibits a notably higher concentration of microplastics. Conversely, S9, situated near similar sources but positioned differently within the circulation pattern, displays lower concentrations. The geographical placement of station S9 is in the more exposed area of the Bay, potentially influenced by exchange with the North Atlantic Ocean, contrasts with S8's location (Figure 2-14).

Generally, the islands in the inner bay may provide shelter, possibly resulting in a longer residence time within this inner section of the Bay. Such a difference in residence time may explain the generally higher MP concentrations found inside the Bay, compared to concentrations at stations located in the more exposed outer Bay.

As the primary aim of this study was to obtain an initial assessment of baseline concentrations in Placentia Bay, the study did not consider tidal impact as sample collection occurred during different tidal stages. Tides in Placentia Bay are significant, with high tide often 2 meters above low tide (Tides Chart, 2023). Stations S2, S4, S6, S7, and S9 were sampled during high tide. Stations S3 and S5 were sampled during low tide, while S1 and S8 were sampled during mid-tide. Sample collection at varying tidal stages may lead to variations in the concentration of microplastic collected at different stations, as the movement of water during tides may influence

the spatial distribution of these particles. The observed differences between microplastic concentrations determined at roughly the same location, collected one directly after the other with each type of the net, do indicate high variability on small spatial and temporal scales as the average variability of each net tow was 17%.

2.6.3. Possible Sources of Microplastics

Industrial centers, dump sites, and shoreline communities serve as major entry points for microplastics into the bay through diverse pathways such as direct discharge, runoff from dump sites, sewage and storm water drainage systems, wind transport, and river inputs. These anthropogenic activities constitute potential sources of plastic pollution in the environment, primarily stemming from domestic and industrial activities (Cole *et al.,* 2011). Plastics can also enter oceans directly due to improper management of maritime, aquaculture, and fishing waste, such as abandoned fishing gear, accidental cargo loss, and illegal dumping (Lenz *et al.,* 2015). Given, the ongoing expansion of aquaculture in Placentia Bay, conducting a baseline study of this kind becomes increasingly important.

While rivers are one of the primary sources of microplastics in areas such as the Los Angeles and San Gabriel river watersheds, our study in Placentia Bay did not consistently show higher concentrations near river inputs (Moore, 2008). For instance, S7, located in the inner Bay near a river input and anthropogenic activity, exhibited a high microplastic concentration. However, S9, in the eastern mid-section near multiple river inputs and anthropogenic activity, such as, industrial centers, dump sites, and shoreline communities, had a lower concentration of microplastics. In contrast, S4, in the center-western region of the bay, far from river inputs and anthropogenic activity, had the lowest concentration.

The existing data suggests that while anthropogenic activity is often linked to higher microplastic levels (Dai *et al.,* 2018), this relationship was not universally consistent in our study. Likely microplastic distribution in Placentia Bay is driven by several interacting factors, including proximity of stations that are potential sources, water currents, and residence time. A much more comprehensive survey would be needed to tease apart the different factors, but another potential factor could be shoreline accumulation of plastic debris.

2.6.4. Linking Shoreline Plastic Litter to Microplastic Concentrations

A study conducted by Liboiron *et al.,* (2019) presented a comprehensive regional analysis of plastic debris pollution in Newfoundland and Labrador. This report compiled data covering various aspects of plastic pollution, including entanglement, ingestion, litter, nest incorporation, sediment, shoreline studies, and surface water analysis. In the context of Placentia Bay, only shoreline studies were conducted as part of their research.

Among the shoreline locations studied in Newfoundland and Labrador, Arnold's Cove stood out with the highest concentration of plastic shoreline litter consisting of fishing gear, plastic foam, and fragments. This significant finding led Liboiron *et al.,* (2019) to characterize Arnold's Cove as a major entry point for plastics into the marine environment. Arnold's Cove is located in the inner bay near sampling station S7 where the highest concentration of microplastics was discovered in our study, suggesting that microplastic concentrations at S7 were heavily influenced by plastic litter accumulating at Arnold's Cove shoreline. The presence of plastic litter in Arnold's Cove has also likely contributed to the elevated concentration of microplastics observed at all stations in the inner bay of Placentia Bay. These findings closely mirror the results of other field

studies assessing plastic pollution along shorelines, e.g. Australia, Japan, and the United Kingdom (Browne et al., 2011).

2.6.5. Polymer Composition of Microplastics

Our hypothesis concerning the polymer composition of microplastics found in Placentia Bay encompassed a wide range of polymers commonly used in various anthropogenic activities, such as industry, fishing, and domestic practices. However, our findings revealed a limited diversity of polymer types, with only four types of polymers detected in Placentia Bay, e.g. polyester (PES), polyethylene (PE), polypropylene (PP), and polystyrene (PS).

The polymer types dominating in this study align with the most commonly produced plastics worldwide. Polyethylene (PE) and polypropylene (PP) are the most produced polymers for fragments (Geyer *et al.,* 2017). The widespread presence of these polymers in the marine environment can be attributed to their extensive use and durability in various applications. For instance, PE is used in supermarket bags, drink bottles, tubes, pipes, and microwave packaging, while PP is found in bottle caps, drinking straws, appliances, car parts, yogurt containers, and fishing components (Link *et al.,* 2019; Nakano *et al.,* 2020; Li, 2016). It is thus astonishing that fragments collected in Placentia Bay consisted of 88% of PE and PP contributed only 8% to microplastic fragments. About 4% of fragments (Fig 2-14) consisted of polystyrene (PS) which is a popular material in disposable containers, food packaging, and the construction industry (Maharana *et al.,* 2007).

Fibers consisted of 90% PE and PP, each contributing about equal amounts (Fig 2-14), with the rest made up of PES and PS. PES is a widely used polymer for textiles and clothing and typically enters the environment through wastewater, primarily from washing machines (Geyer *et*

al., 2017; Napper & Thompson, 2016). The low concentrations of PES and PS in Placentia Bay may be attributed to the region's relatively low population density, as existing research often demonstrates a connection between population density and the prevalence of these polymers (Browne et al., 2011). A key finding of this analysis is the low prevalence of polyester and polystyrene, common polymers used in the everyday household, and the dominance of PE in fragments, and PE and PP for fibers.

Nylon (PA), PE, PP, and polypropylene-polyethylene blend (PP-PE) are common polymer types used in the fishing industry (Saturno, 2020). It is noteworthy that this study did not detect the presence of PA microplastics, despite their widespread use in fishing gear.

2.6.6. Fiber Dominance

Our hypothesis suggested that fibers would be the predominant type of microplastics in the marine environment, as microplastic fibers are recognized as a major global marine pollutant (Mishra *et al.,* 2019). Given that Placentia Bay is heavily associated with the fishing industry, our study aimed to investigate microplastic fibers in the area to shed light on this anthropogenic source of pollution (Liboiron *et al.,* 2019).

Our research revealed that 95% of the microplastics in the water of Placentia Bay are fibers. This outcome corroborates studies conducted on other shorelines in Newfoundland and Labrador, where over 50% of microplastic was identified as fibers (Liboiron *et al.,* 2019; Lindeque *et al.,* 2020). In the Bay of Biscay and along the coastal waters of Maine and British Columbia at least 75% of microplastics were identified as fibers, highlighting a pattern in those regions (Barrows *et al.,* 2017; Desforges *et al.,* 2014; Mendoza *et al.,* 2020).

The low concentration of polyester fibers, a common fiber type in clothes, rules out clothing and textiles as a major source of fibers in Placentia Bay. Fishing operations are well-documented sources of fibrous microplastics due to the persistent nature of materials like ropes and nets (Saturno, 2020). The prevalence of fibers in our samples is consistent with the idea (or may suggest) that fishing-related activities contribute significantly to microplastic pollution in the region.

In other studies, microplastic fibers are categorized into two sections: fibers and filaments (Walls et al., 2022). Fibers, characterized as thin, elongated pieces of plastic that are flexible, typically originate from textiles and fabrics. Whereas filaments, which are generally stiffer and more rigid than fibers, stem from fishing equipment or maritime activities. Our study grouped fibers and filaments into one category. Making this distinction for future studies could offer additional insights into understanding the microplastic sources.

2.6.7 Microplastic Weathering

Our hypothesis anticipated that transparent, blue, and green microplastics would dominate in Placentia Bay, considering the impact of the weathering processes and the common use of green and blue in fishing nets within the marine industry. However, the dominant color observed in this study was transparent. Approximately 83% of the microplastics were transparent, aligning with the findings off the coast of Maine and in Tokyo Bay, where over 80% of microplastics were also transparent (Barrows *et al.,* 2017; Nakano *et al.,* 2020). This prevalence of clear particles can be attributed to the leaching of color during plastic weathering and aging (Gewert *et al.,* 2015). The high percentage of transparent microplastic suggests that most particles were heavily weathered and makes source identification based on coloring more difficult.

Additionally, our hypothesis predicted an exponential increase in microplastic numbers with decreasing size, consistent with the continuous fragmentation process (Hidalgo-Ruz *et al.,* 2012). The significant concentration of fragments in the smallest measured size range in this study supports this expectation and substantiates the idea that most particles were heavily weathered. The small diameter of each fiber compared to their length could have allowed them to escape through the net, explaining the relatively small number of particles in the smallest size class compared to the next size class as a methodological artifact.

These findings are consistent with previous studies that have reported higher concentrations of plastics in smaller-size classes (Enders *et al.,* 2015; Isobe *et al.,* 2017; Pan *et al.,* 2019). In summary, the dominance of transparent microplastics and small particles highlights the significant impact of weathering and fragmentation for microplastics in Placentia Bay.

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2.8. Tables

Table 2-1: Sample Collection Information. N/A: Not Applicable, N/D: Not Determined

Site	Sample Type	Latitude (N)	Longitude (W)	Sample Date	Volume Filtered (m^3)	Surface Temperature (°C)	Surface Salinity	Chlorophyll a* ($\mu g\ L^{-1}$) n=31	Ocean Depth (m)
1	Plankton Net	47.117441	54.187436	Jul-16-2021	1144	N/D	N/D	N/D	66
1	Neuston Net	47.117441	54.187436	Jul-16-2021	2809	N/D	N/D	N/D	66
2	Plankton Net	47.130381	54.292036	Jul-16-2021	1390	N/D	N/D	N/D	236
2	Neuston Net	47.130381	54.292036	Jul-16-2021	2545	N/D	N/D	N/D	236
3	Plankton Net	47.322983	54.288838	Jul-25-2021	1406	N/D	35	N/D	195
3	Neuston Net	47.322983	54.288838	Jul-25-2021	2347	N/D	35	N/D	195
4	Plankton Net	47.416538	54.382690	Jul-25-2021	13302	18	29	N/D	45
4	Neuston Net	47.416538	54.382690	Jul-25-2021	2233	18	37	N/D	45
5	Plankton Net	47.616233	54.282010	Sept-23-2021	1120	16	34	N/D	138
5	Neuston Net	47.616233	54.282010	Sept-23-2021	1904	16	34	N/D	138
6	Plankton Net	47.678799	54.189166	Sept-23-2021	1192	16	34	1.26±0.29	46
6	Neuston Net	47.678799	54.189166	Sept-23-2021	1778	16	34	1.26±0.29	46
7	Plankton Net	47.743746	54.098672	Sept-23-2021	1235	15	34	1.43±0.26	133
7	Neuston Net	47.743746	54.098672	Sept-23-2021	2100	15	34	1.43±0.26	133
8	Plankton Net	47.575858	53.999740	Sept-22-2021	1477	15	35	1.46±0.23	157
8	Neuston Net	47.575858	53.999740	Sept-22-2021	2693	15	35	1.46±0.23	157
9	Plankton Net	47.350709	54.045149	Sept-22-2021	1449	15	35	1.13±0.26	129
9	Neuston Net	47.350709	54.045149	Sept-22-2021	974	15	35	1.57	129
Head	Van Veen Grab	47.75328	54.23472	Sept-29-2021	N/A	N/A	N/A	N/A	26
West	Van Veen Grab	47.64558	54.26754	Sept-29-2021	N/A	N/A	N/A	N/A	153
East	Van Veen Grab	47.74767	54.06439	Sept-30-2021	N/A	N/A	N/A	N/A	158
Central	Van Veen Grab	47.57921	54.1269	Sept-30-2021	N/A	N/A	N/A	N/A	6
Outer	Van Veen Grab	47.18	54.37683	Oct-1-2021	N/A	N/A	N/A	N/A	242
Shelf	Van Veen Grab	46.79598	54.79602	Oct-2-2021	N/A	N/A	N/A	N/A	256

Table 2-2. Water column properties at all stations in Placentia Bay.

Site	Ocean Depth (m)	Temperature (°C)	Salinity n=2	Chlorophyll a* (µg L^{-1}) n=31	POC (µmol L^{-1}) n=3	C:N ratio n=3
1	66	N/D	N/D	N/D	N/D	N/D
2	236	N/D	N/D	N/D	N/D	N/D
3	195	N/D	35±0.00	N/D	N/D	N/D
4	45	18	33±4	N/D	N/D	N/D
5	138	16	34±0.00	N/D	0.04±0.02	4.64±0.26
6	46	16	34±0.00	1.26±0.29	0.26±0.22	5.25±0.30
7	133	15	34±0.00	1.43±0.26	0.22±0.10	4.74±0.15
8	157	15	35±0.00	1.46±0.23	0.05±0.03	5.09±0.14
9	129	15	35±0.00	1.13±0.26	0.03±0.00	5.29±0.01
Head	26	N/A	N/A	N/A	N/A	N/A
West	153	N/A	N/A	N/A	N/A	N/A
East	158	N/A	N/A	N/A	N/A	N/A
Central	6	N/A	N/A	N/A	N/A	N/A
Outer	242	N/A	N/A	N/A	N/A	N/A
Shelf	256	N/A	N/A	N/A	N/A	N/A

Table 2-3. The plastic polymer composition of microplastics by the Raman analysis technique from both nets at all stations.

Color	Microplastic Type	N Analyzed	% Polyester	% Polyethylene	% Polypropylene	% Polystyrene
Transparent	Fragment	3	0%	0%	67%	33%
	Fiber	36	3%	50%	40%	7%
Black	Fragment	9	0%	100%	0%	0%
	Fiber	8	17%	33%	50%	0%
Green	Fragment	2	0%	0%	0%	0%
	Fiber	3	0%	0%	100%	0%
White	Fragment	17	0%	100%	0%	0%
	Fiber	11	0%	11%	89%	0%
Blue	Fragment	13	0%	100%	0%	0%
	Fiber	13	0%	25%	75%	0%
Red	Fragment	1	0%	100%	0%	0%
	Fiber	3	67%	0%	33%	0%
Yellow	Fragment	3	0%	100%	0%	0%
	Fiber	5	0%	0%	100%	0%
Orange	Fiber	1	0%	100%	0%	0%

Table 2-4. Microplastic concentrations (particles per kg) identified via visual identification from the sediment samples at all stations in Placentia Bay.

Color	Microplastic Type	Shelf (S) (particles per kg)	East (E) (particles per kg)	Head (H) (particles per kg)	West (W) (particles per kg)	Central (C) (particles per kg)	Outer (O) (particles per kg)
Transparent	Fragment	1	5	9	9	7	20
	Fiber	42	52	87	122	120	222
Black	Fragment	5	1	5	1	2	20
	Fiber	17	21	26	33	107	33
Green	Fragment	0	0	0	1	0	0
	Fiber	0	0	0	0	0	0
White	Fragment	0	3	1	3	4	16
	Fiber	0	0	0	0	1	2
Blue	Fragment	1	0	1	5	0	1
	Fiber	4	19	24	36	28	15
Red	Fragment	1	2	0	1	2	0
	Fiber	1	0	11	6	11	2
Brown	Fragment	31	2	83	36	37	302
	Fiber	1	2	14	3	0	1

Table 2-5. Non plastic and dye particles identified by Raman Microspectroscopy from all sediment samples in Placentia Bay (n=6).

Color	Particle Type	N Analyzed	% Non Plastic	% Dye	Non Plastic Raman Identification
Transparent	Fragment	1	100%	0%	Cotton
	Fiber	3	100%	0%	Cotton
Blue	Fiber	3	100%	0%	Cotton

2.9. Figures

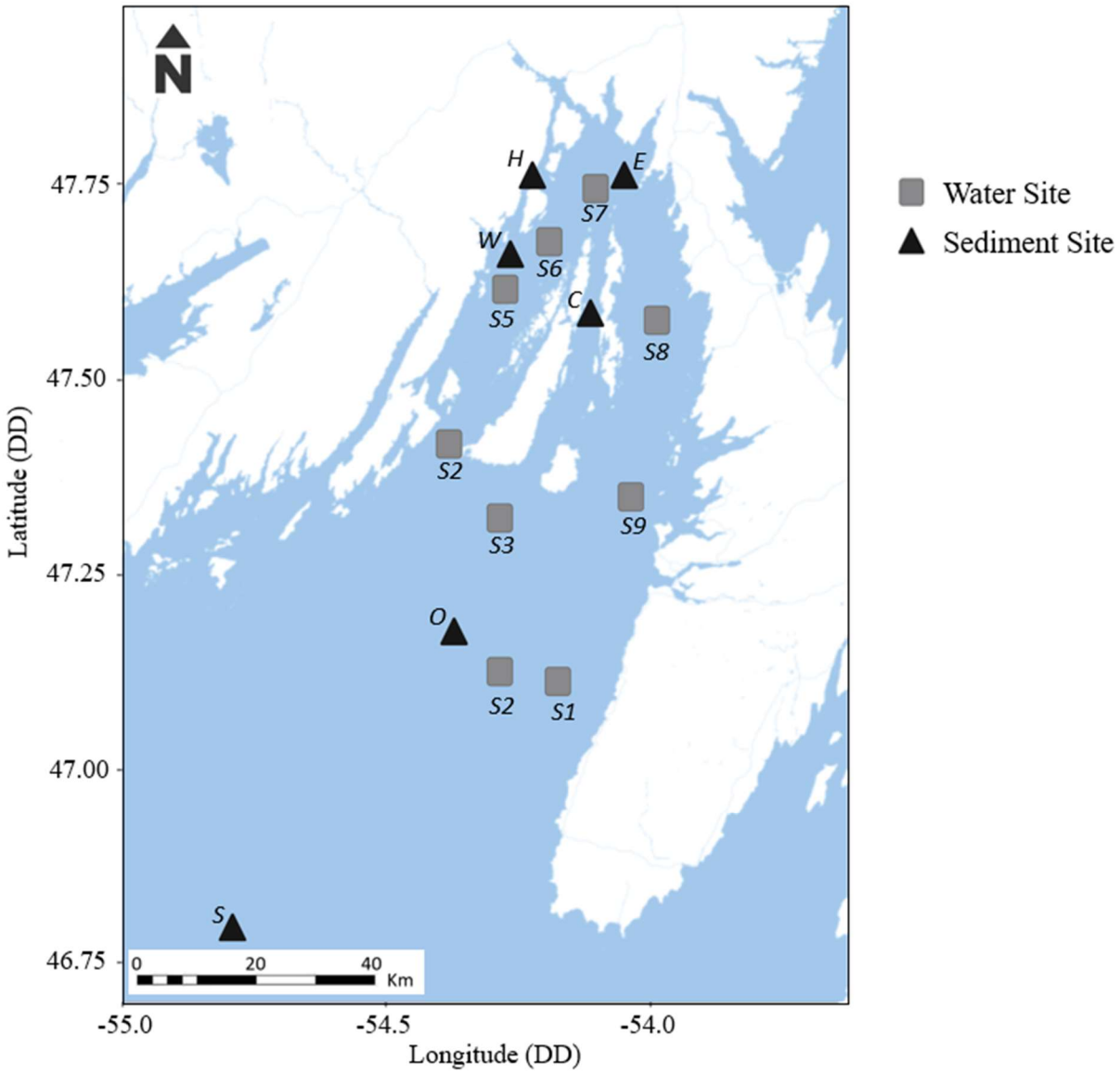

Figure 2-1: Sampling stations for the water column and sediment layer located in Placentia Bay, Newfoundland.

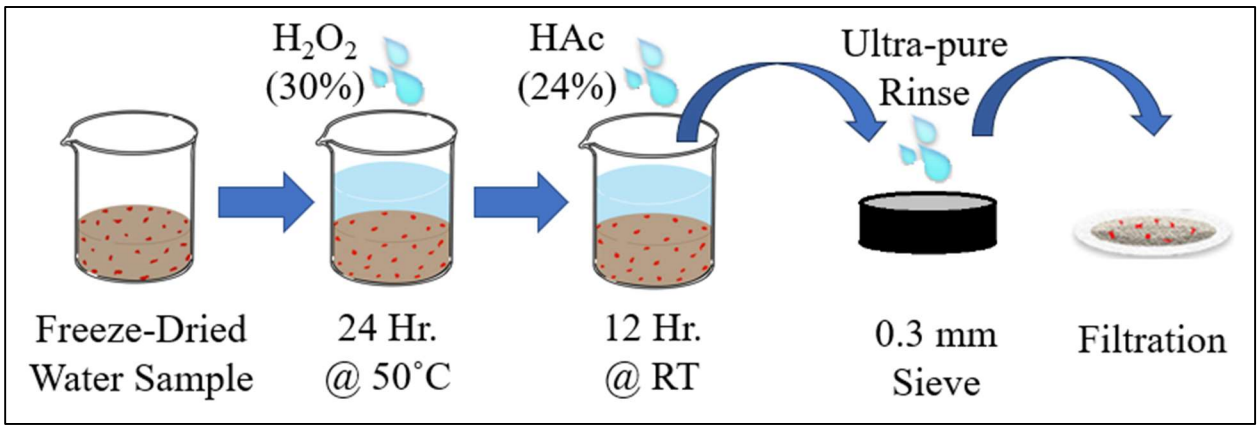

Figure 2-2. Digestion and microplastic isolation for water samples. RT= Room Temperature

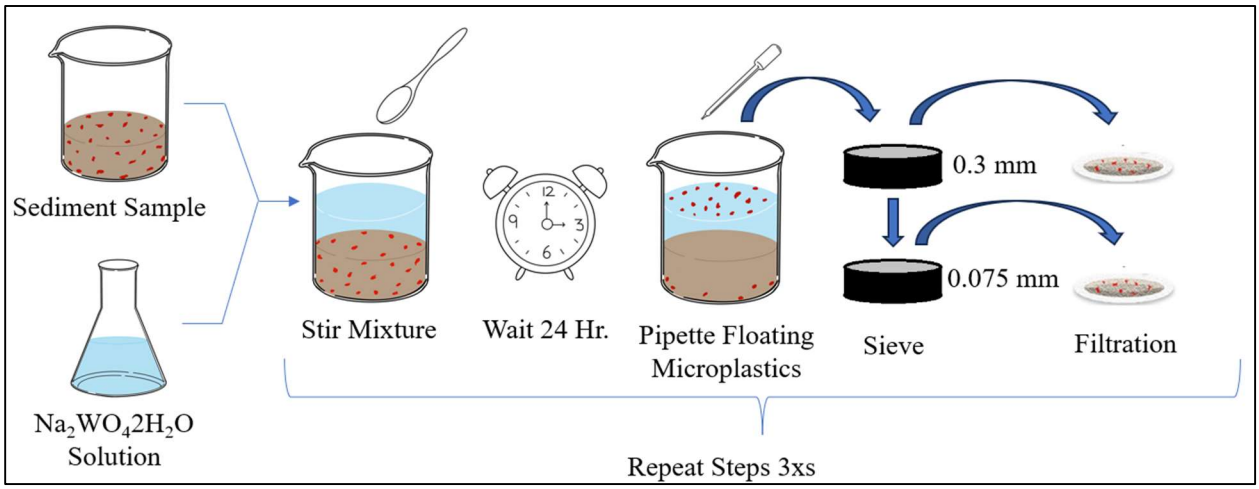

Figure 2-3. Scheme of microplastic isolation procedure for sediment samples.

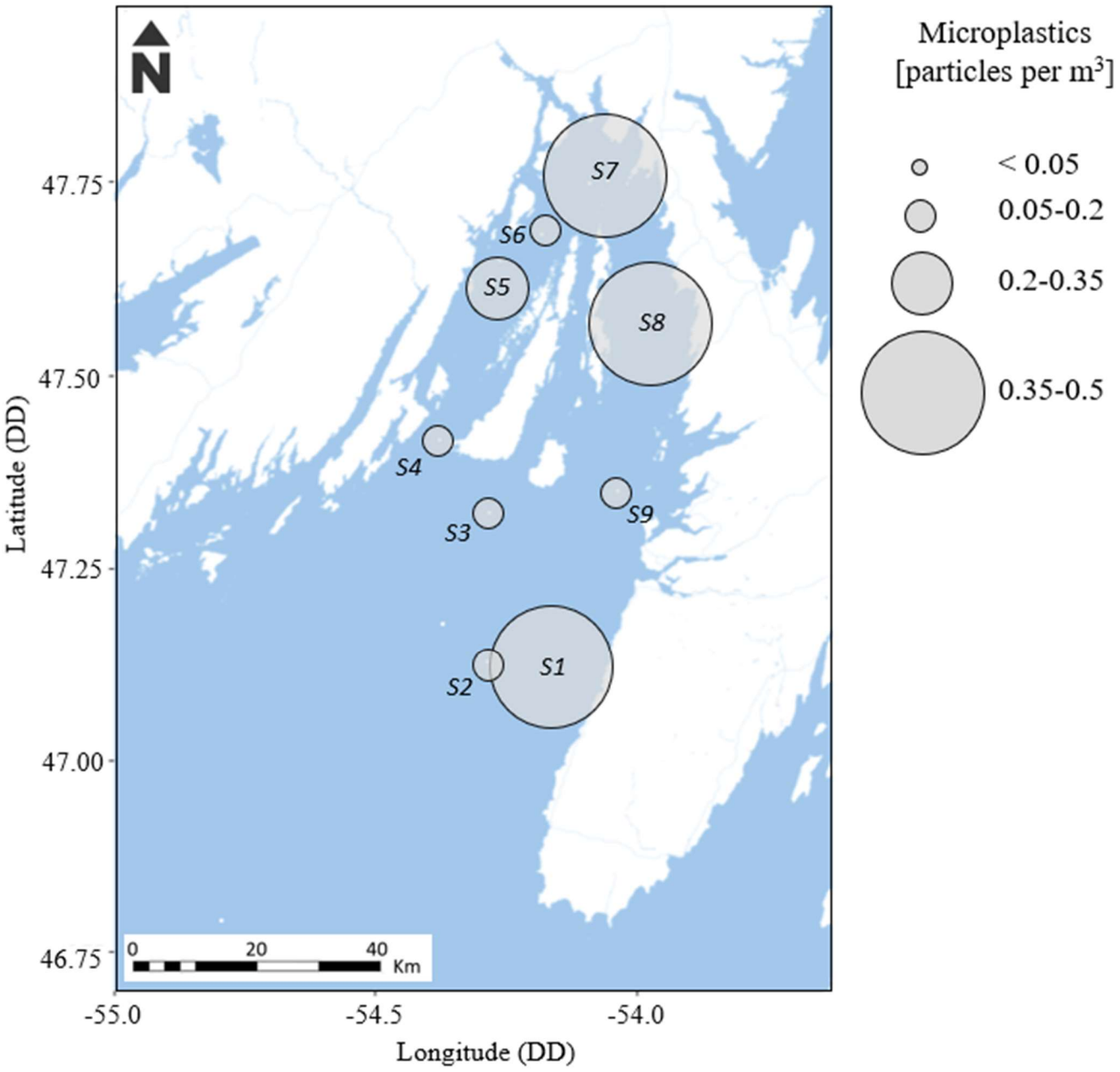

Figure 2-4. Microplastic concentrations (particles per m^3) calculated from the mean from both nets at all stations in Placentia Bay.

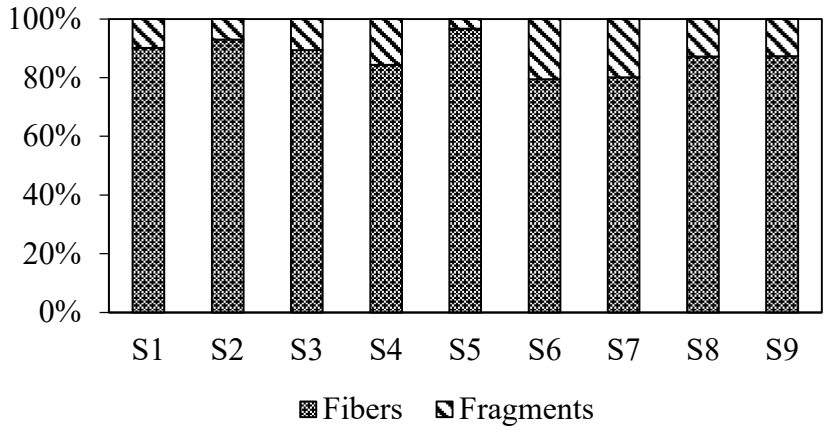

Figure 2-5. Fibers and fragments proportions at each station in the water column from the mean of both nets in Placentia Bay.

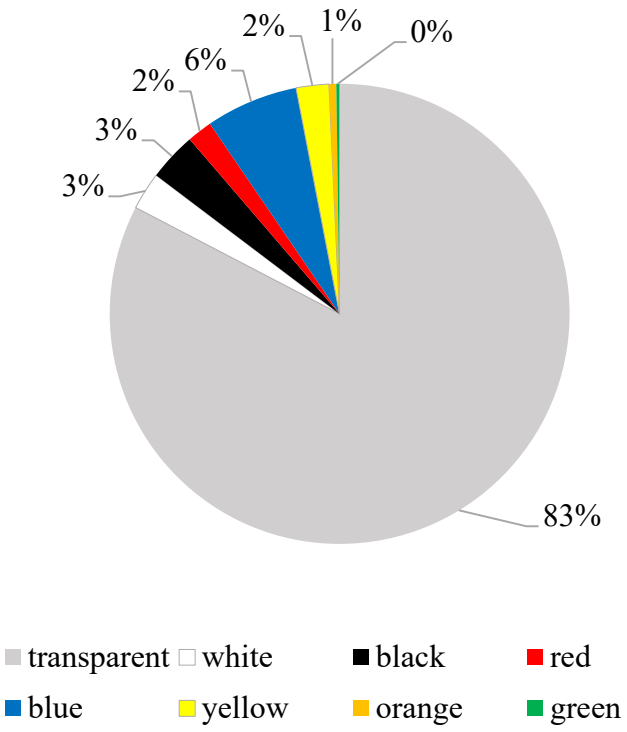

Figure 2-6. Total microplastics categorized into eight colors from the mean of both nets (n=9) at all stations in Placentia Bay.

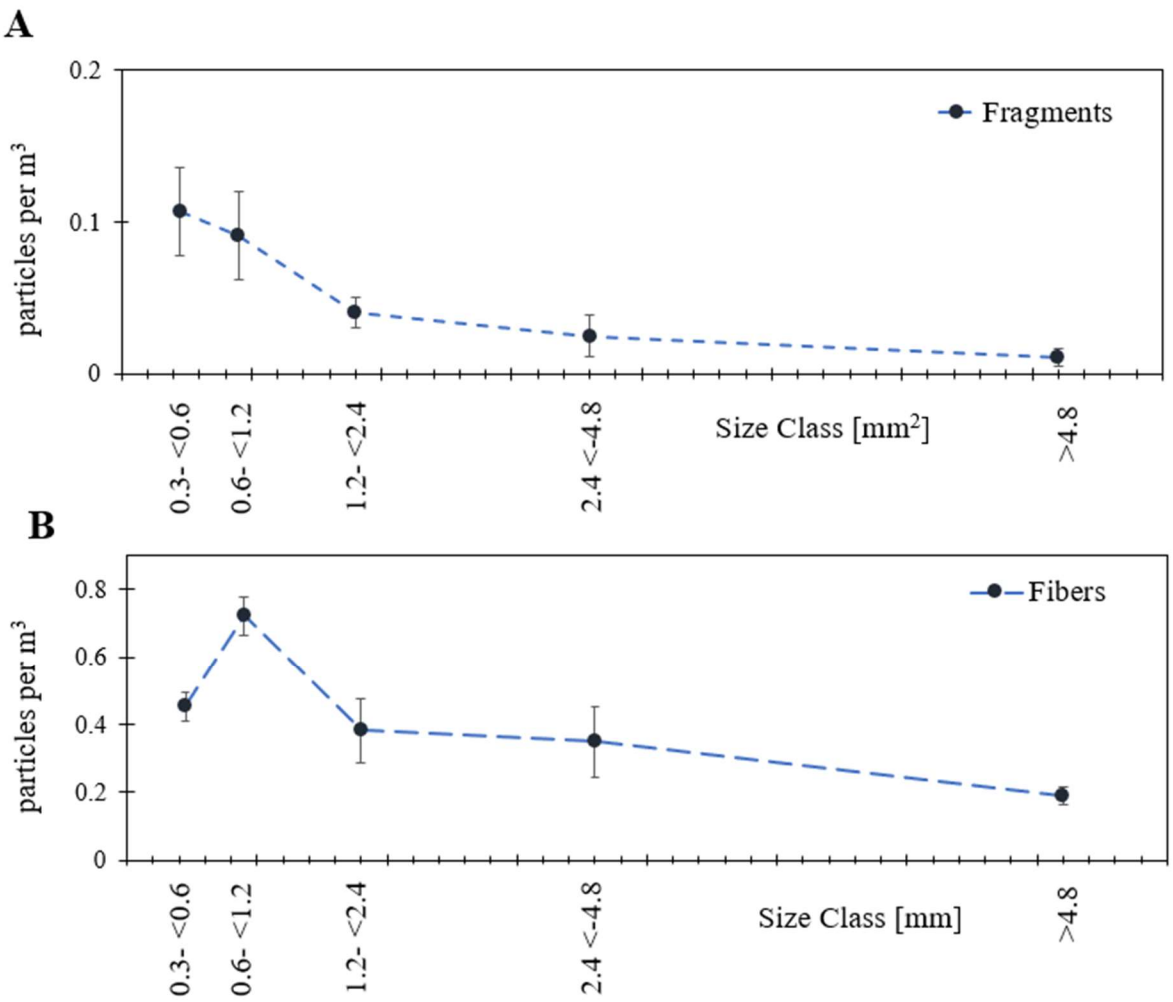

Figure 2-7. Fragments (A) and fibers (B) (particles per m^3) collected in Placentia Bay from the mean of both nets at all stations were categorized into five size classes.

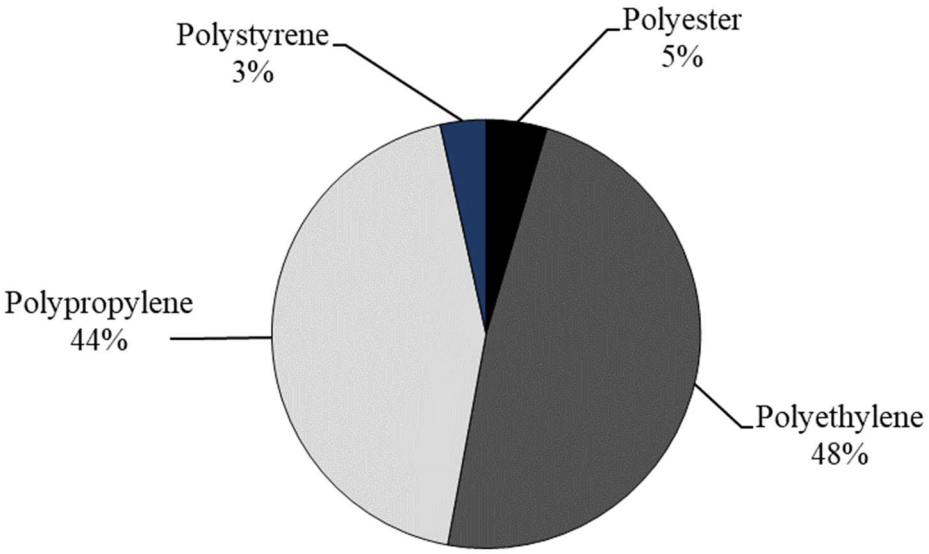

Figure 2-8. The overall polymer composition of microplastics (n=87) analyzed using Raman Microspectroscopy from both nets at all stations.

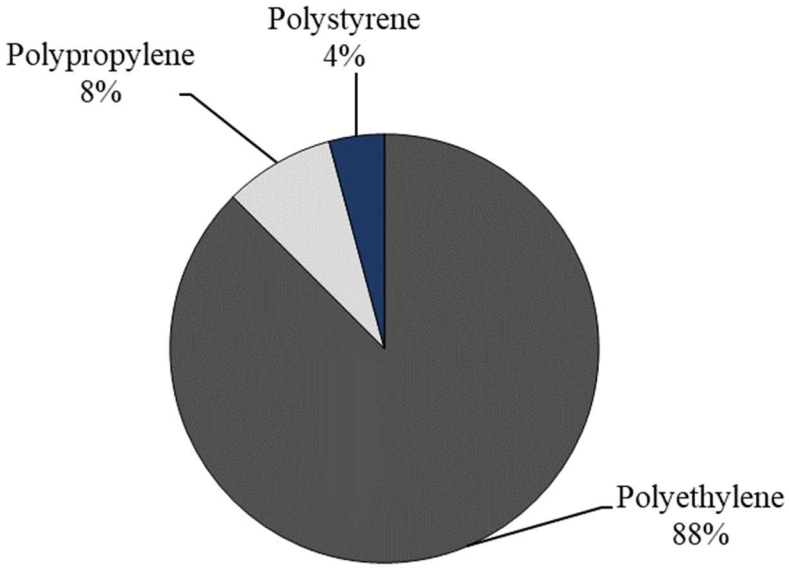

Figure 2-9. The overall polymer composition of fragments (n=24) confirmed by the Raman Microspectroscopy technique from both nets at all stations.

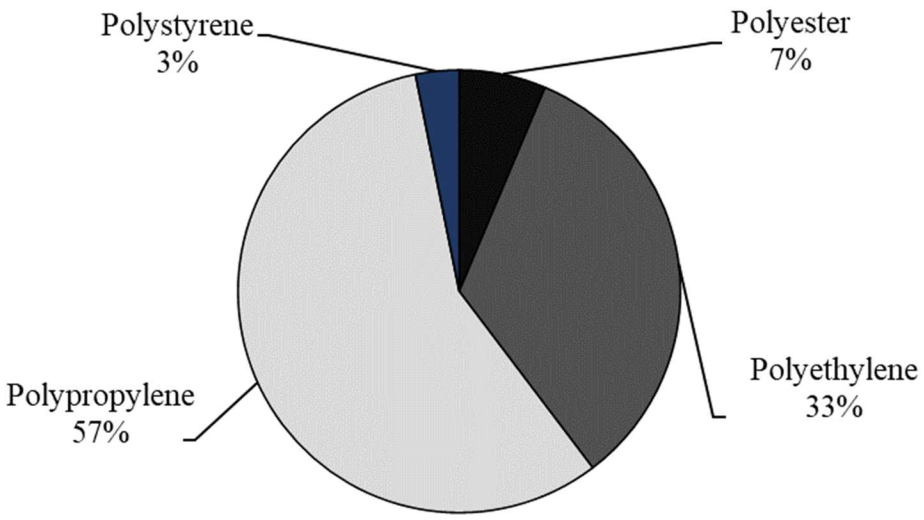

Figure 2-10. The overall polymer composition of fibers (n=63) confirmed by the Raman Microspectroscopy technique from both nets at all stations.

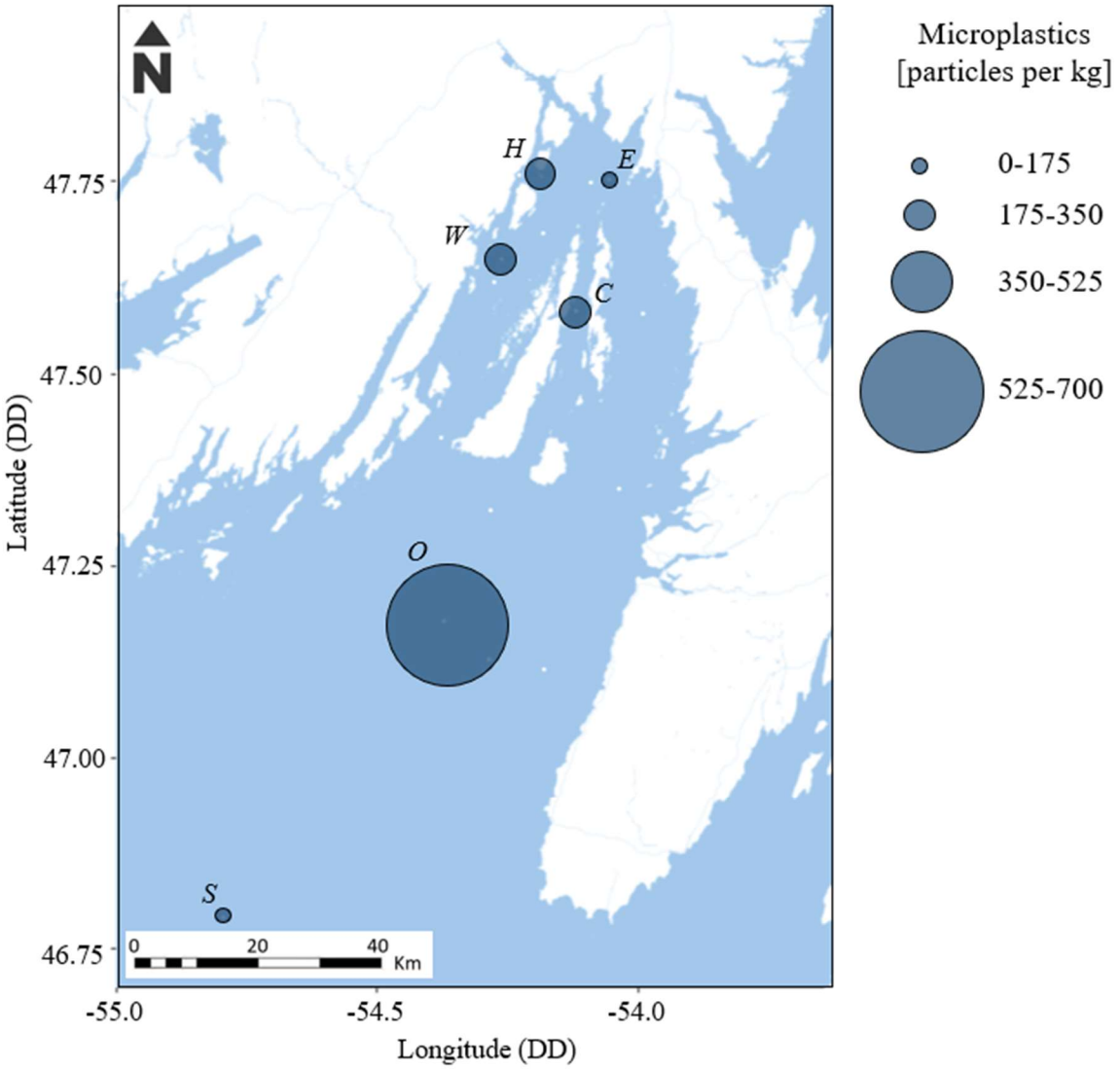

Figure 2-11. Concentration of microplastics in the sediment samples (particles per kg) calculated from the van Veen grab assuming 65% correction from Raman analysis of the water column.

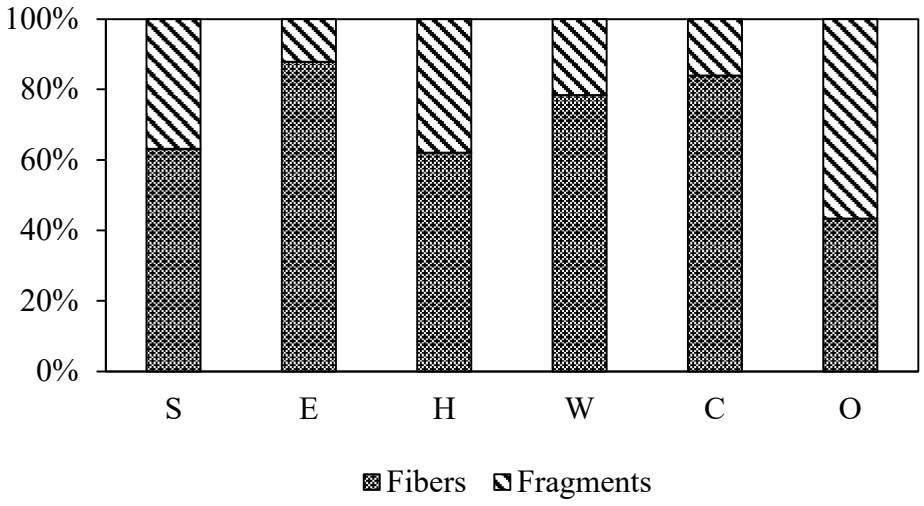

Figure 2-12. The total fibers and fragments proportions throughout each station (n=6) in Placentia Bay sediments.

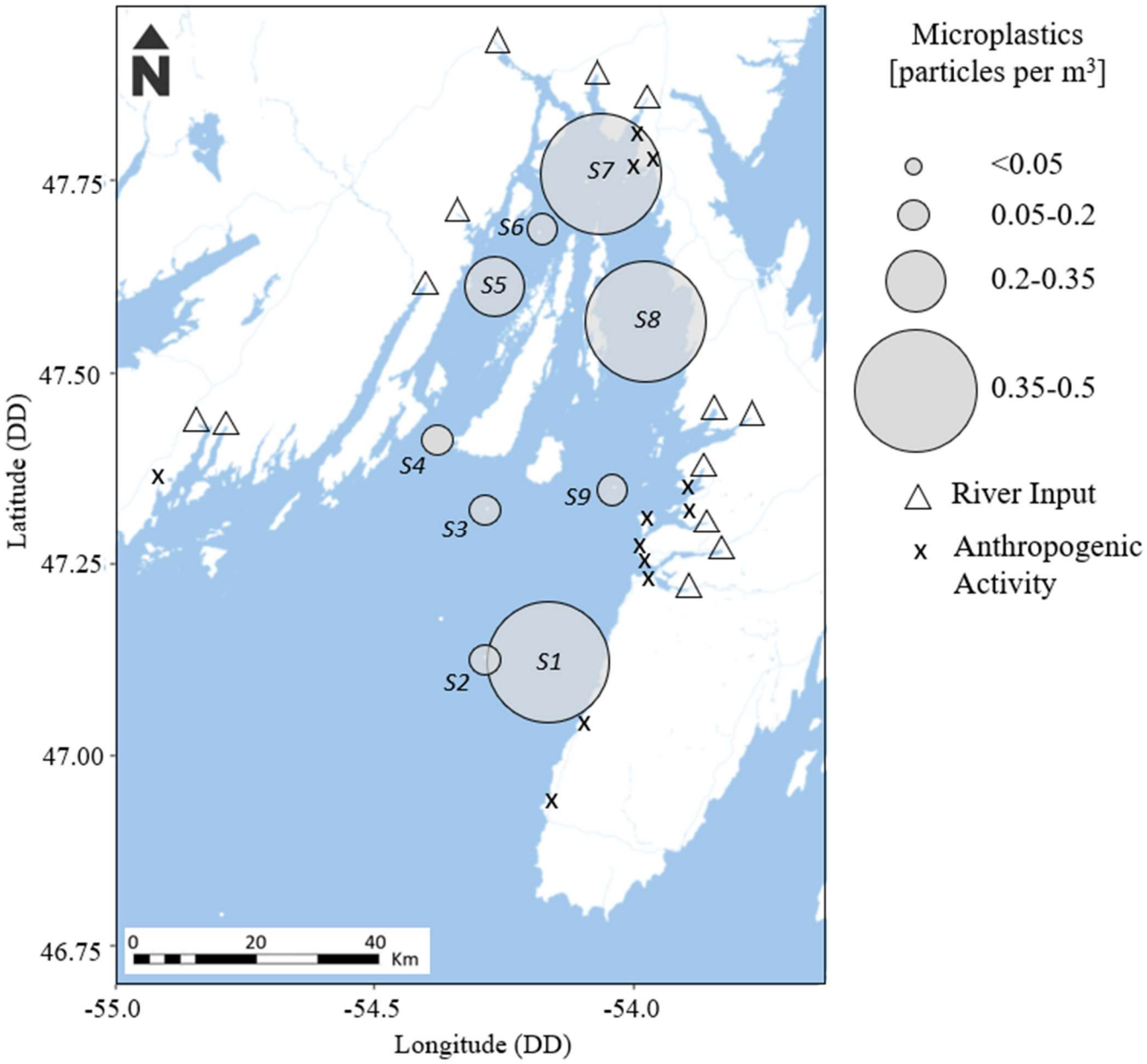

Figure 2-13. Microplastic concentrations (particles per m^3) as the mean of both nets in relation to river inputs and land-based anthropogenic activity, such as dump sites and industrial centers, given by Google (2023) in Placentia Bay.

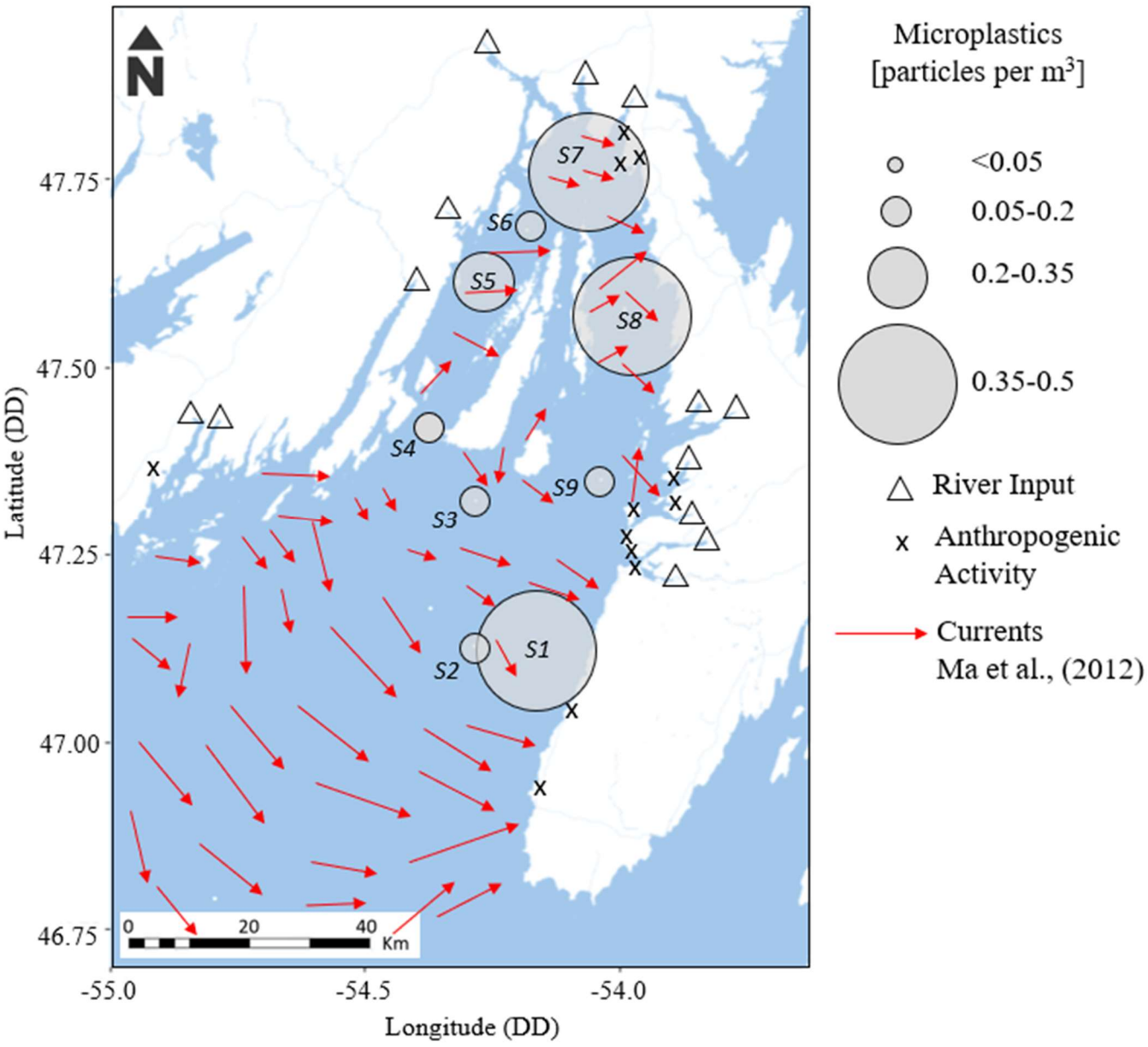

Figure 2-14. Microplastic concentrations (particles per m^3) as the mean of both nets in relation to river inputs, land-based anthropogenic activity given by Google (2023), and currents in Placentia Bay given by Ma *et al.,* (2012).

Chapter 3 – Methodological Considerations

3.1. Abstract

I conducted method tests specific to the water and sediment samples within Placentia Bay, Newfoundland. Published methods when tested did not provide reliable results, and there is a lack of standardized methodology for microplastic research. Our water samples were POC-rich, and standard digestion methods were not sufficient. Samples in the water column required an extended digestion time of 24 hours. An exploration into microplastic separation methods involved a test using a syringe technique inspired by Reinesccius et al., (2021), found ineffective due to the microplastic size in our samples. Sodium tungstate dihydrate (Na2WO4 2H2O) solution was chosen for microplastic isolation in the sediment samples. Microscopical identification of microplastic was confirmed with Raman microspectroscopy to test the reliability of visual identification. Of visually identified particles, 50% fragments and 21% of fibers were non-plastic upon Raman analysis, revealing the limitations of only using visual identification to characterize microplastics. Of visually identified particles, 71% of blue particles had a spectra identical with copper phthalocyanine blue, and 67% of green particles had spectra identifying them as copper phthalocyanine green. Dyes were found to interfere with the Raman spectra and it was impossible to reliable ascertain if these particles were paint chips, plastic or non-plastic.

Keywords: microplastic methodology, microplastic isolation, Raman analysis

3.2. Introduction

In the realm of microplastic research, the absence of a standardized methodology has emerged as a critical challenge, necessitating researchers to meticulously customize their approaches. From the intricacies of sampling methods to the nuances of sample analysis, the variability introduced by this lack of uniformity extends to every facet of the research process. This methodological diversity not only complicates the comparison of results across studies but also hinders the establishment of a cohesive understanding of the prevalence and impact of microplastics in various environments.

Various sample collection methods are employed for the water column, encompassing diverse net types, sample duration times, and mesh sizes, all contingent upon the characteristics of the study (Mai et al., 2018). In the realm of sediment analysis, the collection methods exhibit equal diversity, featuring a spectrum of collection tools and varying quantities of sediment gathered (Hidalgo-Ruz *et al.,* 2012). Microplastics can undergo diverse methodologies for separation from the sample matrix, including digestion, density separation, or size fractioning (Mai et al., 2018; Rocha-Santos & Duarte, 2014). Quantification of microplastics involves visual identification and chemical analysis through imaging techniques such as Fourier-transform infrared spectroscopy (FTIR) and Raman Microspectroscopy.

The process of weathering introduces challenges when attempting to measure the spectra of microplastics using Raman analysis. As microplastics are exposed to environmental factors, their molecular surfaces undergo alterations. These changes can include the degradation of polymer chains, formation of oxidation products, and modifications in surface characteristics (Reineccius *et al.,* 2022). The dynamic nature of weathering-induced alterations in microplastics makes it challenging to establish a consistent and reliable spectral signature. These variations in

molecular composition and structure can lead to shifts in Raman spectra, making it difficult to precisely identify and characterize microplastics in seawater (Fernández-González et al., 2021).

The use of diverse methods at each stage of the process introduces challenges when attempting to compare results across different sampling techniques. This chapter aimed to assess the most effective type of net sampling for collecting microplastics in the water column. The hypothesis was that a higher concentration of microplastics would accumulate in the sea-surface atmosphere layer. Additionally, the chapter sought to test sample preparation methods to separate microplastics from other particulate matter, both organic and inorganic, in water and sediment samples. Furthermore, the evaluation of the reliability of visual identification was a key focus, with the hypothesis that microplastics might be overestimated without the additional step of Raman identification.

The emphasis is on the findings that have surfaced through the method development specific to water and sediment samples obtained from stations within Placentia Bay. This chapter showcases the process leading to the development of our final research methodology and presentation of results in Chapter 2. This study recommends its application to samples exhibiting characteristics akin to the water and sediment samples found in Placentia Bay. The objective of this chapter is to address the current research gap pertaining to the diverse methodological approaches in microplastic research.

3.3. Sample Preparation

3.3.1. Comparison between Neuston and Plankton Nets

First, we investigated the hypothesis that microplastics accumulated at the sea-surface atmosphere interface. This was expected since many types of microplastics are positively buoyant in seawater.

This question was investigated by examining if the neuston net, which samples the sea-surface atmosphere interface (0 m-0.5 m) would consistently collect more microplastics than a plankton net which is deployed just below the surface (1 m-6 m). The concentrations of microplastics did not consistently differ (Figure 3-1). A T-test conducted across all nine stations revealed no significant difference between the two nets (n=9, p=0.77). Nor was a significant difference in microplastic concentration observed (n=9, p=0.67) if the microplastic concentration in the neuston net was assumed to be 30% higher. Thus even assuming the maximal error in the calculations of final microplastic concentration (Chapter 2: 2.4 Quality Control), no systematic differences between nets were observed. Due to this lack of difference, the mean of both nets was used to present the microplastic concentrations across all nine stations in Chapter 2.

3.3.2. Net Sample Analysis

A method by Reineccius *et al.* (2021) suggests digestion with hydrogen peroxide (H_2O_2 30%) for 5 hours at 50 °C following the addition of acetic acid (HAc 24%) for 2 hours at room temperature.

In this work, the organic matter did not digest sufficiently under Reineccius *et al.* (2021) conditions, possibly because the ratio of organic matter to microplastic was much higher in our samples. Digestion experiments were conducted over different periods, specifically 5 hours, 12 hours, and 24 hours with H_2O_2 (30%) at 50 °C to find conditions that resulted in sufficient digestion of organic matter.

To validate the effectiveness of the digestion time, a set of synthetic microplastic samples was created for testing. The synthetic creation of microplastics was sourced from a polyethylene cup and polyester cloth. The success of the digestion was evaluated visually based on the reduction

of organic matter. Additionally, their weights were measured before and after the newly developed digestion process confirming no loss of microplastics.

Digestion experiments revealed that after 5 hours in H_2O_2 (30%) at 50 °C, roughly 30% of the biological material was digested. This percentage increased to roughly 50% after 12 hours and reached around 90% after 24 hours (Figure 3-2). These results indicate that the 24-hour digestion period was efficient in removing the majority of the biological matter and isolating the microplastics. The subsequent digestion effectiveness test revealed that no microplastics were lost during the extended digestion period involving 24 hours of H_2O_2 (30%) and 12 hours of HAc (24%) digestion. Furthermore, the shape and size of the microplastics remained unchanged, as confirmed by a comparison of before and after photographs of individual microplastics.

A subsequent isolation method using a syringe technique with lipid bonding is suggested. This technique uses three cascading plastic syringes coated with lubricating oil. In theory, as the sample matrix is pulled through the syringe, the microplastic would bond to the oil on the inner walls. A detergent solution is used to detach the microplastic from the syringe for isolation (Reineccius *et al.,* 2021).

A test was conducted on the syringe cascade procedure with synthetic microplastics >0.3 mm. We found that the syringe separation did not reliably collect microplastic >0.3 mm in this study. Approximately 19% of the microplastic would not fit through the syringe aperture and was lost. Many of our microplastic particles (>0.3 mm) exceeded the size of the syringe hole. Thus to remove the biological matter remaining after the digestion (15%), the sample was rinsed through a 0.3 mm stainless steel sieve using ultra-pure Milli-Q water.

Initially incorporating an additional density separation step for isolating microplastics in the water samples was considered, but proved unnecessary, because the remaining biological

material (after digestion) passed through the sieve and microplastics were successfully collected on the sieve.

3.3.3. Sediment Sample Analysis

A density separation method is frequently used to separate microplastic from sediment particles (Hanvey *et al.,* 2017). Here a sodium tungstate dihydrate (Na_2WO_4 $2H_2O$) solution was used rather than a saturated sodium chloride (NaCl, 1.2 g cm^{-3}) solution because of its greater density, which allows more of the denser microplastics to be collected. Compared to studies using less dense solutions for density separation, the collection efficiency of microplastic should thus be higher.

First, the optimal settling time to efficiently separate microplastic from sediments with the density separation method was examined. Initially, a test was conducted with a settling time of two hours as suggested by Tirkey & Upadhyay (2021), but the sample remained murky, indicating that the sediment hadn't fully settled. Possibly our sediment samples were rich in fine, clay-like particles. To ensure thorough separation, we chose to extend the settling time to a full 24 hours, erring on the side of caution.

Given the relatively large volume of solution (4 L), we modified the method based on Reineccius *et al.,* (2020) by implementing a pipetting system instead of decanting the solution. This adjustment was made to ensure a safe and comprehensive collection of buoyant particles. In anticipation of a high quantity of microplastics, particles were size fractioned through sieves of two sizes to simplify the counting process.

The density separation process was conducted thrice per sample to optimize microplastic collection. By allowing the sample to settle for an extended period in sodium tungstate dihydrate

and stirring during each repetition, microplastic particles were meant to detach from sediment particles and rise to the surface for collection. In two cases, the highest count of visually identified microplastic was recorded after the first extraction, in two more cases after the second extraction, in one case after the third extraction, and once microplastic extracted were the same after the first and last extraction. The application of the three-time density separation approach aligns with the methodology employed in other studies (Hanvey *et al.,* 2017). While the data obtained using this method can be trusted in comparison with similar studies, our results indicate that the collection of microplastics might be an underestimate. This is evidenced by the absence of a decrease in the collection of microplastics over the course of the three separation processes. For improved accuracy, future studies may consider increasing the number of density separation extractions per sample.

The density of plastics varies from 0.98 g cm^{-3} for polyethylene, 1.45 g cm^{-3} for polyethylene terephthalate, 1.16 g cm^{-3} for polyamide-nylon, 0.92 g cm^{-3} for polypropylene, 1.10 g cm^{-3} for polystyrene, 1.31 g cm^{-3} for polyvinyl alcohol, and 1.47 g cm^{-3} for polyvinyl chloride (Andrady, 2017; Duis & Coors, 2016; Hanvey *et al.,* 2017; Hidalgo-Ruz *et al.,* 2012; Moret-Ferguson *et al.,* 2010). Additives can affect density as the density of polypropylene was observed to increase to 1.23 g cm^{-3} with a fiber-reinforcing additive (Pagter *et al.,* 2018). Heavier plastics such as polyvinyl chloride and polyethylene terephthalate would rise more slowly in sodium tungsten dihydrate solution (1.6 g cm^{-3}). Plastics with densities surpassing that of the sodium tungsten dihydrate solution, such as polyvinylidene fluoride (1.73 g cm^{-3}) and polytetrafluoroethylene (2.17 g cm^{-3}), would not be collected using this method (McKeen, 2017; Ramazanov *et al.,* 2018).

3.4 Challenges in the Quantification of Microplastics

Raman microspectroscopy is a slow and tedious method to quantify microplastic, however, visual quantification is challenging and likely fraught with misidentifications, even when done carefully. Here we use a combined approach to determine the errors associated with visual identification and provide insights into the chemical composition of microplastic. The relationship between the visual identification of microplastics and Raman identification was used to correct counts based on visual identification. In total 10730 particles were counted using visual identification consisting of 2041 fragments and 8689 fibers of various colors and sizes (Table 3-1). 1.2% of visually identified microplastic particles were analyzed via Raman spectroscopy to confirm their identity.

Of the 128 particles analyzed via Raman microspectroscopy, 17% were identified as non-plastic (black carbon, sulfur, cotton, biological material), and 15% of the particles were identified as dyes (copper phthalocyanine blue and copper phthalocyanine green) without any further identification. The remaining 65% of the particles were unambiguously identified as microplastic (polyethylene, polypropylene, polyester, and polystyrene) (Table 3-2).

Of the total visual counts, 79% of fibers and 50% of fragments were confirmed plastic. Therefore, visual counts of microplastic fragments were assumed to be overestimated by 50% and visual counts of microplastic fibers were overestimated by 21%. These percentages were used to correct the visual quantification of the total concentration for microplastic analysis in Chapter 2.

The 50% overestimation in fragments is consistent with Lenz et al., 2015 (64%). Misidentifications may have been due to dyes or biological materials resembling plastic. The degrading effect on fragments due to weathering may have also hindered accurate chemical identification. Lenz et al., (2015) indicate higher Raman confirmation success for larger

microplastics compared to smaller ones, possibly contributing to the visual overestimation of fragments in the smaller size class range for our study.

We were unable to apply a correction percentage specific to each size category of microplastics and therefore used the fiber (79%) and fragment (50%) correction across all size classes. This correction aligns with the approach of Lenz et al., 2015.

A special case was considered for black fragments below 1.2 mm^2, which were consistently identified as black carbon by Raman Microspectroscopy. All black fragments below this size were assumed to consist of black carbon and not counted as microplastic. The identification of black carbon in a microplastic sample can lead to two distinct interpretations. Firstly, it might signify the presence of naturally occurring black carbon, which can originate from pyrogenic and biogenic sources such as soot, charred plant materials, or other forms of organic combustion residues (Ramanathan & Carmichael, 2008). In this context, the black carbon could have been generated through natural processes. The black carbon source could also be from dye used in textiles, plastics, and coatings (Lenz et al., 2015). While the detection of black carbon in a microplastic sample suggests the presence of anthropogenic material, further analyses would be required to discern its origin accurately. Black carbon was categorized as a non plastic in this study.

Raman Microspectroscopy suggested that not all bright-colored particles identified visually were confirmed as plastic. 27% of yellow particles were biological material, 71% of blue particles were identified as copper phthalocyanine blue, and 67% of green particles were identified as copper phthalocyanine green. For the latter two, it remains unclear if these were dyed plastic, dyed natural materials, or paint particles. Copper phthalocyanine blue and copper phthalocyanine green are synthetic pigments commonly used as a coating in the manufacturing of paints, inks, plastics, ceramics, textiles, and other materials. These dyes color products such as PVC pipes,

plastic films, and containers. Copper phthalocyanine blue and copper phthalocyanine green collected are associated with plastic particles whose signal interfered with the dye in other studies (Van Cauwenberghe et al., 2013). However, to be conservative with our estimate we assumed they were not plastic. If particles confirmed by Raman as dyes were assumed to be plastic, the accuracy of the visual identification of fibers would increase from 79% to 86% and fragments from 50% to 77%.

To correct our visual counts, we assumed that particles analyzed by Raman were representative of all particles of the same type and color that were counted (Tables 2-3 & 2-7). In Chapter 2: Figure 2-5, microplastic concentrations corrected in this manner are depicted. It's worth noting that colors were not categorized by particle type (fragment or fiber), but rather by the average percent plastic of both types.

There was a presence of biological materials among particles visually identified as plastic, including cellulose wood, biological material, sulfur, and cotton, despite the digestion step. The persistence of these materials might be attributed to their inherent structural properties, suggesting that the digestion process may not have been robust enough to fully disintegrate these materials, potentially owing to their composition or size. A stronger digestion step may however in turn impact the integrity of the microplastic.

3.5. Integration of Results from Visual and Raman Microspectroscopy

In the initial stages of microplastic collection, researchers primarily encountered larger-sized particles, which could be easily observed with the naked eye. However, contemporary microplastic research has advanced to the point where it can analyze much smaller particles that are not visible without the aid of specialized techniques like Raman Microspectroscopy (Zarfl,

2019). This shift reflects the limitations of relying solely on visual identification for microplastic quantification, particularly for particles smaller than 100 μm, as the accuracy of visual identification decreases with decreasing particle size (Lenz *et al.,* 2015).

The technique employed in this study combined two methods 1) stereo microscope visual identification and 2) Raman Microspectroscopy. This hybrid approach proved to be cost-effective and efficient, considering the time constraints associated with Raman Microspectroscopy. Stereo microscope visual identification is an accessible and useful technique but should be paired with another method to confirm the polymer compounds of microplastics.

A limitation exists with the use of visual identification as a first step, as highlighted by Lenz et al., 2015. Although conducting visual identification first and Microspectroscopy second reduces the occurrence of false positives, it cannot address microplastics that may have been overlooked during visual identification, resulting in an unknown false-negative fraction. Conducting Microspectroscopy analysis for every particle in the sample to identify overlooked microplastics would be financially impractical. As a result, a balanced approach is required.

The results of this study emphasize the necessity of integrating Raman identification or a similar Microspectroscopy technique in microplastic research. Removing this analytical component may lead to an overestimation of the total microplastic concentration.

3.6. Literature Cited

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3.7. Tables

Table 3-1. Microplastic concentrations (particles per m^3) identified via visual identification from the neuston and plankton net at all stations in Placentia Bay (n=9).

Site	Fragments (particles per m^3)	Fibers (particles per m^3)
S1 Plankton	0.11	0.93
S1 Neuston	0.05	0.12
S2 Plankton	0.01	0.24
S2 Neuston	0.02	0.09
S3 Plankton	0.03	0.15
S3 Neuston	0.03	0.13
S4 Plankton	0.00	0.01
S4 Neuston	0.04	0.08
S5 Plankton	0.03	0.59
S5 Neuston	0.02	0.30
S6 Plankton	0.03	0.14
S6 Neuston	0.13	0.26
S7 Plankton	0.10	0.30
S7 Neuston	0.19	0.43
S8 Plankton	0.08	0.45
S8 Neuston	0.17	0.65
S9 Plankton	0.02	0.08
S9 Neuston	0.06	0.26

Table 3-2. Non plastic and dye particles identified using Raman Microspectroscopy analysis from both nets at all stations.

Color	Particle Type	N Analyzed	% Non Plastic	% Dye	Non Plastic Raman Identification	Dye Raman Identification
Transparent	Fragment	3	0%	0%	-	-
	Fiber	36	17%	0%	Black Carbon, Biological Material, Cotton	-
Black	Fragment	9	78%	0%	Black Carbon	-
	Fiber	8	25%	0%	Black Carbon, Biological Material	-
Green	Fragment	2	0%	100%	-	Copper Phthalocyanine Green, Copper Phthalocyanine Blue
	Fiber	3	0%	33%	-	Copper Phthalocyanine Green
White	Fragment	17	18%	6%	Biological Material	-
	Fiber	11	18%	0%	Sulfur	-
Blue	Fragment	13	0%	77%	-	Copper Phthalocyanine Green, Copper Phthalocyanine Blue
	Fiber	13	0%	38%	-	Copper Phthalocyanine Blue
Red	Fragment	1	0%	0%	-	-
	Fiber	3	0%	0%	-	-
Yellow	Fragment	3	33%	0%	Biological Material	-
	Fiber	5	20%	0%	Cellulose Wood	-
Orange	Fiber	1	0%	0%	-	-

3.8. Figures

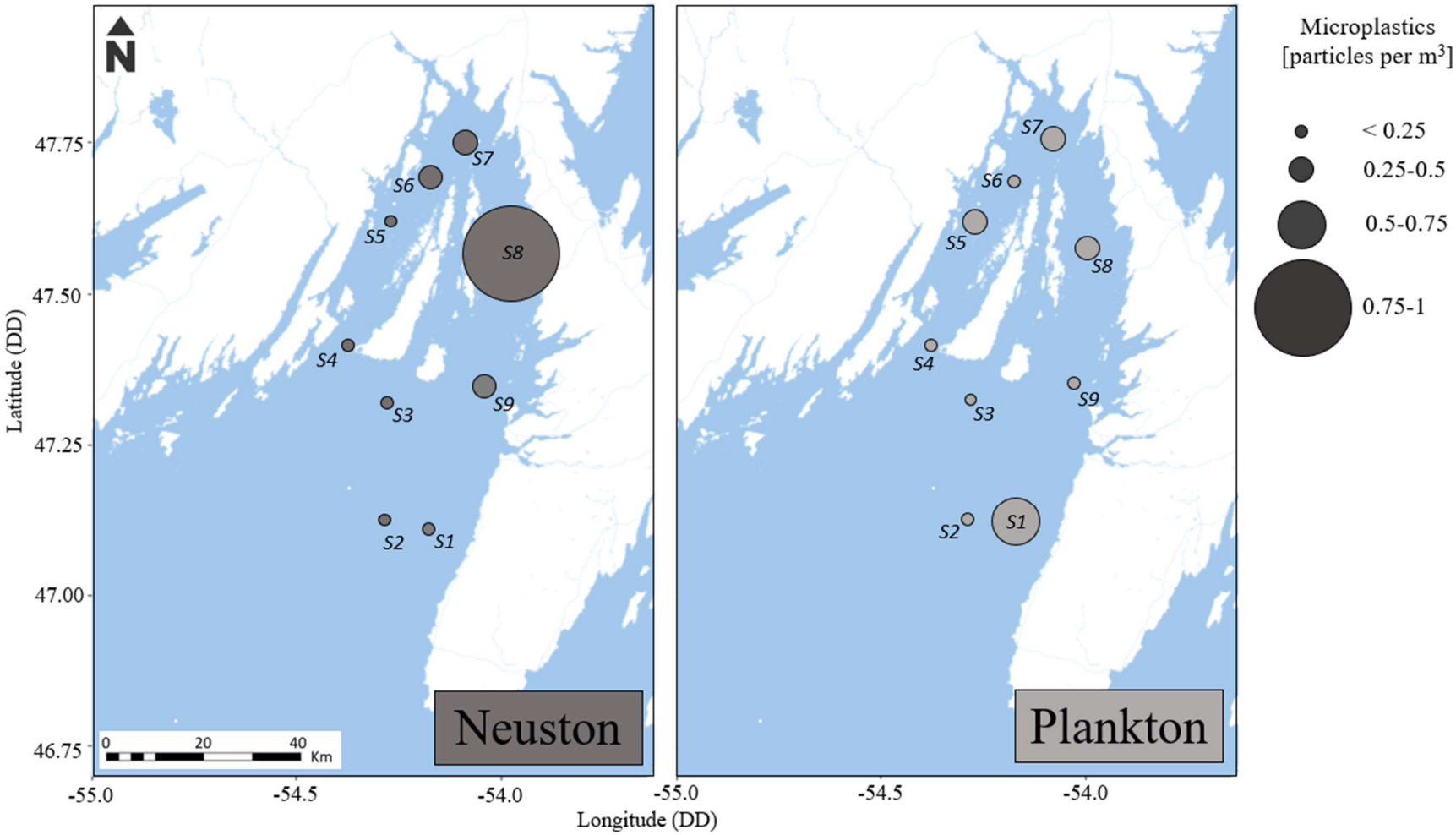

Figure 3-1. The concentration of microplastics (particles per m^3) from the neuston and plankton net at the nine stations in Placentia Bay.

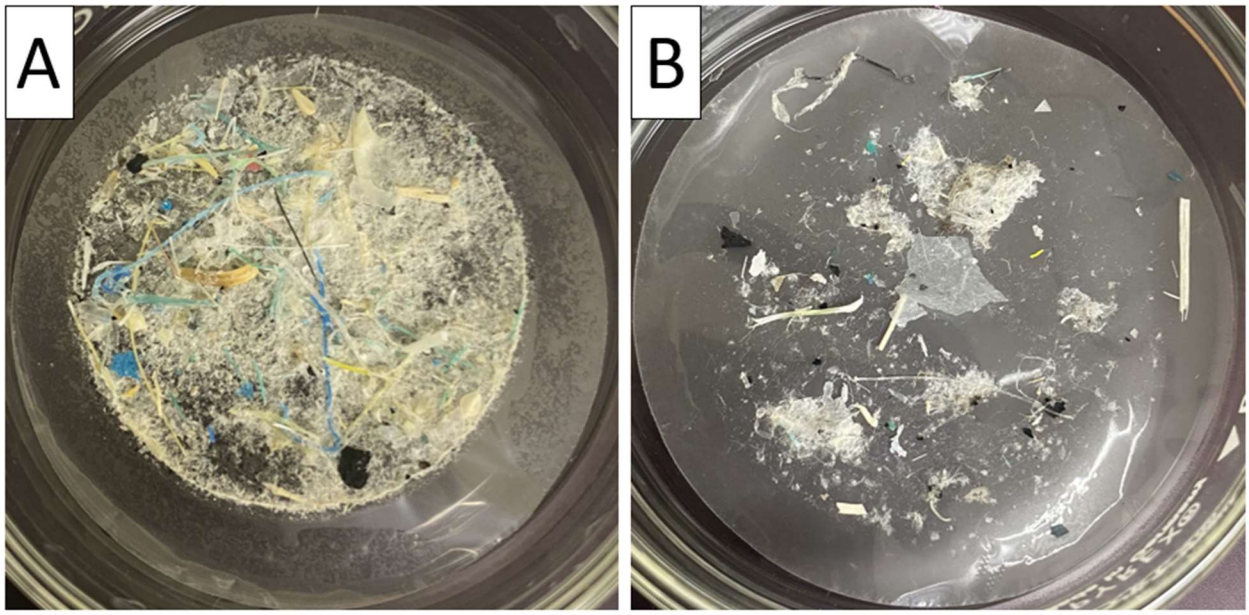

Figure 3-2. Filter A from S7 neuston net was digested for 5 hours. Filter B from S2 neuston net was digested for 24 hours.

Chapter 4 – Conclusions & Outlook

4.1. Background

This thesis contributes insights into microplastic analysis methods and determines the abundance and distribution of microplastics in Placentia Bay. The study delved into the identification of various types, sizes, colors, and polymer compositions of microplastics present in this area. Notably, this thesis stands as the pioneering investigation of microplastics in Placentia Bay as it analyzes both sediment and water samples. Its findings mark the inception of ongoing studies in the region with the Department of Fisheries and Oceans Canada's "baseline" initiative in this area.

4.2. Water Column

This thesis investigated the presence of microplastics at the interface between the sea-surface atmosphere (neuston net) and in the surface ocean (plankton net) at nine stations distributed throughout Placentia Bay.

This research confirmed the presence of microplastics at all stations throughout Placentia Bay and observed higher concentrations on the eastern side of the bay. No significant differences were observed between sampling with the neuston (0 m - 0.5 m) and plankton (1 m - 6 m) net, indicating that microplastics did not accumulate at the water sea-surface interface. Among the microplastics identified, fibers were the predominant type, possibly attributed to intense fishing activity in Placentia Bay. Polyethylene emerged as the dominant type of polymer, consistent with its status as the most commonly produced plastic. Transparent microplastics dominated over colored plastic. Higher quantities of microplastics were found in the smallest size classes compared to larger size categories, indicating microplastic fragmentation.

4.3. Sediment

The abundance of microplastic in the sediment surface layer of Placentia Bay was explored at six different stations. Although visual identification suggested high and variable microplastic concentrations in sediments, the identity of these particles could not be confirmed by Raman Microscopy, potentially due to the small size of the subsamples used for Raman analysis. Natural cotton fibers dominated subsamples measured with Raman. This finding aligns with a study conducted in Mauritius, off the south-eastern coast of Africa, where a cotton polyamide, a blend of both cotton and polymer fibers, comprised 44% of the microplastics found in sediment (Ragoobur *et al.,* 2023). This outcome suggests a possible delayed usage of synthetic polymer materials in fishing gear employed in Placentia Bay.

4.4. Methodological Considerations

Initial testing of methods (e.g. Reineccius *et al.,* 2021) revealed their unsuitability for the specific conditions of the samples from this study. Conventional filtration and isolation techniques proved ineffective in separating microplastics from the biological matrix due to the substantial presence of organic particles and plankton, complicating accurate assessment of their presence—a challenge commonly encountered in samples with similar quantities of organic material (Lusher *et al.,* 2020). To address this issue, a customized approach was adopted involving an extended digestion time (24 hours) followed by the use of a 0.3 mm sieve. This tailored method effectively isolated microplastics from the sample matrix without causing observable harm.

4.4.1 Significance of Fiber Analysis

An essential aspect of this thesis was including careful analysis of fibers. Fibers are often overlooked or ignored due to methodological challenges and contamination risks (Rebelein *et al.,* 2021). In our study, considerable effort was spent to detangle and analyze fibers. Our results emphasize the crucial role of fibers, revealing their dominance and their different polymer composition compared to fragments. Had we neglected fiber analysis, it would have impeded our ability to accurately portray the composition of microplastics in Placentia Bay.

4.5 Future Directions

This thesis acknowledges recognized limitations that necessitate discussion, as this study was a preliminary assessment of microplastic distribution in Placentia Bay. These limitations provide inputs for and contribute to shaping the future trajectory of microplastic research in the marine environment. The goal of this section is to pave the way for subsequent research to improve upon the work conducted in Placentia Bay.

This study focused on microplastics sized >0.3 mm for water samples. For future studies, employing a smaller mesh size could offer further insights, as observed in related studies that reveal significantly increased microplastic concentrations with finer nets (Lindeque et al., 2020).

Follow-up studies in the bay would provide valuable insights into the temporal dynamics of microplastic distribution, offering an understanding of the long-term trends and patterns. Selecting sampling stations near anthropogenic activity, river inputs, and influential currents into account, could offer a nuanced approach to understanding transport and potential sources. Revisiting sampling stations during consistent tidal stages would allow researchers to assess shifts in microplastic concentrations and compositions due to tidal currents. Measurements at river mouths

may help evaluate the importance of river input for microplastic distribution. Additionally, expanding the research scope to include other bays in Newfoundland, particularly those renowned for intensive fishing and aquaculture activities, would enrich our understanding of broader microplastic dynamics in the region.

An initiative to determine microplastic distribution is underway for Fortune Bay/Bay d'Espoir to serve as a subsequent baseline study. The objective is to collect microplastics >20 μm and >300 μm in both the water column and sediment layer. Selected stations will be in proximity to potential sources (sewage, landfills, and aquaculture) and significant physical properties of the area (currents and sills). This study will be conducted in collaboration with the Miawpukek First Nation, leveraging their invaluable knowledge of the land to enhance our understanding of this anthropogenic pollution.

I propose that future studies participate in developing a comprehensive database detailing microplastic characteristics specific to similar regions, encompassing size distribution, shape, color, and polymer composition. Establishing a reference library for future comparative analyses would promote a more consistent and systematic approach to microplastic identification. Such a collaborative effort would enable researchers to effectively assess microplastic prevalence and distribution in the region, contributing to a global understanding of microplastic pollution in marine ecosystems.

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Appendices

Appendix I: The microplastic concentration of the mean of both nets at each station in Placentia Bay categorized by their color and microplastic type. The correction factor of fibers (79%) and fragments (50%) from Raman Microspectroscopy has been applied.

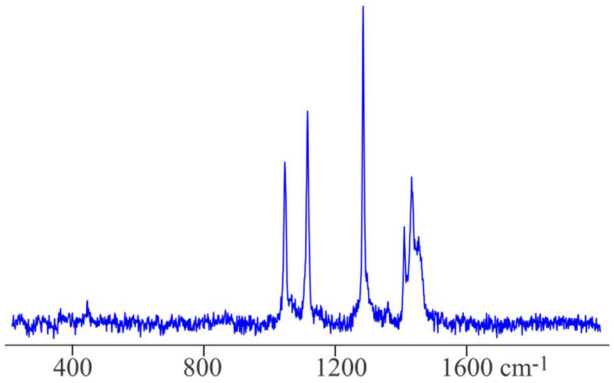
Color	Microplastic Type	S1 (particles per m^3)	S2 (particles per m^3)	S3 (particles per m^3)	S4 (particles per m^3)	S5 (particles per m^3)	S6 (particles per m^3)	S7 (particles per m^3)	S8 (particles per m^3)	S9 (particles per m^3)
Transparen	Fragment	0.02±0.00	0.00±0.00	0.01±0.00	0.01±0.01	0.01±0.00	0.03±0.03	0.06±0.03	0.05±0.03	0.01±0.00
	Fiber	0.22±0.10	0.08±0.04	0.10±0.02	0.06±0.01	0.28±0.04	0.13±0.07	0.18±0.06	0.41±0.21	0.09±0.03
Black	Fragment	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Fiber	0.02±0.02	0.01±0.00	0.00±0.00	0.00±0.00	0.02±0.01	0.01±0.00	0.02±0.01	0.01±0.00	0.00±0.00
Green	Fragment	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Fiber	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
White	Fragment	0.01±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01	0.00±0.00	0.01±0.00	0.00±0.00
	Fiber	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Blue	Fragment	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.00	0.00±0.00	0.00±0.00
	Fiber	0.02±0.01	0.01±0.01	0.01±0.00	0.01±0.00	0.01±0.01	0.01±0.01	0.06±0.03	0.02±0.00	0.01±0.01
Red	Fragment	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Fiber	0.01±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.00	0.00±0.00	0.00±0.00
Yellow	Fragment	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Fiber	0.00±0.00	0.01±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01	0.02±0.02	0.00±0.00	0.01±0.00
Orange	Fragment	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Fiber	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Appendix II: The mean microplastic fiber concentration (particles per m^3) of both nets at each station in Placentia Bay categorized by the size classes (mm). The correction factor of 79% for fibers from Raman Microspectroscopy has been applied.

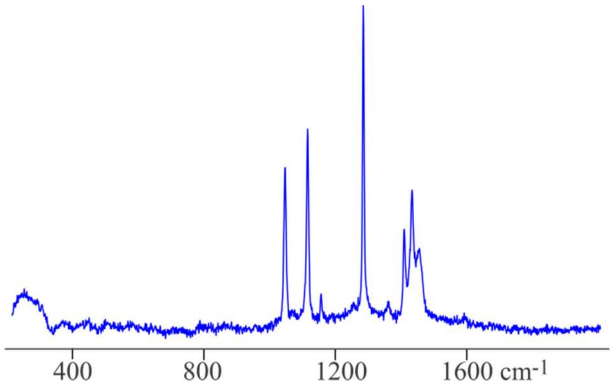
Size Class (mm)	Microplastic Type	S1 (particles per m^3)	S2 (particles per m^3)	S3 (particles per m^3)	S4 (particles per m^3)	S5 (particles per m^3)	S6 (particles per m^3)	S7 (particles per m^3)	S8 (particles per m^3)	S9 (particles per m^3)
0.3- <0.6	Fiber	0.08±0.02	0.02±0.01	0.02±0.00	0.01±0.00	0.06±0.02	0.02±0.01	0.05±0.02	0.14±0.10	0.03±0.00
0.6- <1.2	Fiber	0.08±0.04	0.04±0.01	0.05±0.00	0.02±0.00	0.12±0.01	0.06±0.03	0.09±0.03	0.18±0.10	0.04±0.02
1.2- <2.4	Fiber	0.04±0.03	0.02±0.01	0.01±0.00	0.02±0.00	0.08±0.02	0.03±0.02	0.05±0.02	0.09±0.02	0.01±0.00
2.4- <4.8	Fiber	0.05±0.04	0.02±0.01	0.02±0.00	0.02±0.00	0.05±0.02	0.04±0.02	0.05±0.02	0.04±0.01	0.02±0.01
>4.8	Fiber	0.03±0.02	0.02±0.01	0.01±0.00	0.00±0.00	0.01±0.00	0.02±0.01	0.06±0.04	0.01±0.00	0.03±0.01

Appendix III: The mean microplastic fragment concentration (particles per m^3) of both nets at each station in Placentia Bay categorized by the size classes (mm^2). The correction factor of 50% for fragments from Raman Microspectroscopy has been applied.

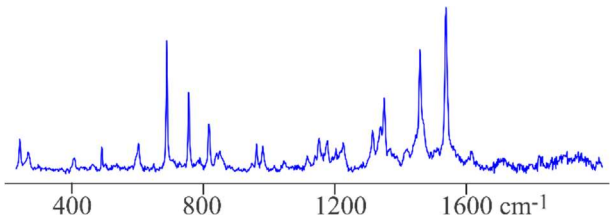
Size Class (mm^2)	Microplastic Type	S1 (particles per m^3)	S2 (particles per m^3)	S3 (particles per m^3)	S4 (particles per m^3)	S5 (particles per m^3)	S6 (particles per m^3)	S7 (particles per m^3)	S8 (particles per m^3)	S9 (particles per m^3)
0.3- <0.6	Fragment	0.01±0.00	0.00±0.00	0.00±0.00	0.01±0.01	0.00±0.00	0.02±0.02	0.03±0.01	0.03±0.02	0.01±0.00
0.6- <1.2	Fragment	0.01±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.00	0.01±0.01	0.02±0.01	0.02±0.01	0.00±0.00
1.2- <2.4	Fragment	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.00	0.01±0.01	0.01±0.00	0.00±0.00
2.4- <4.8	Fragment	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01	0.00±0.00	0.00±0.00
>4.8	Fragment	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00



Appendix IV. Raman Microspectroscopy spectra of red microplastic fragment found at S7 in the water column with a polyethylene match.



Appendix V. Raman Microspectroscopy spectra of transparent microplastic fragment found at S7 in the water column with a polyethylene match.



Appendix VI. Raman Microspectroscopy spectra of blue fiber found at S7 in the water column with a copper phthalocyanine blue match.

Appendix VII. The microplastic count data for each microplastic (MP) type, color, and size class for the neuston net (N) and plankton net (P) at all nine stations in Placentia Bay. The size class units for fragments is mm^2 and the size class for fibers is mm. Counts are not corrected with Raman Microspectroscopy.

		Site	S1	S1	S2	S2	S3	S3	S4	S4	S5	S5	S6	S6	S7	S7	S8	S8	S9	S9
		Net type	P	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N
		Sea water filtered (m^3)	1144	2809	1390	2545	1406	2347	1302	2233	1120	1904	1192	1778	1235	2100	1477	2693	1449	974
MP type	Size class	Color	Microplastic particle count																	
Fragments	0.3- <0.6	transparent	37	35	5	2	19	12	0	25	5	1	4	70	40	107	15	167	7	11
Fragments	0.3- <0.6	white	7	9	0	8	0	0	0	13	3	2	3	21	3	10	6	21	0	6
Fragments	0.3- <0.6	black	16	0	0	1	2	0	0	0	0	0	1	3	5	3	0	0	0	4
Fragments	0.3- <0.6	red	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Fragments	0.3- <0.6	blue	1	4	0	2	1	0	0	1	2	0	5	12	12	26	2	16	4	2
Fragments	0.3- <0.6	yellow	0	0	0	0	0	0	0	0	0	0	0	1	1	4	0	0	0	1
Fragments	0.3- <0.6	orange	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragments	0.3- <0.6	green	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragments	0.6- <1.2	transparent	12	36	3	7	6	19	1	28	7	16	4	43	23	80	35	115	3	8
Fragments	0.6- <1.2	white	9	13	2	3	0	2	0	2	5	6	0	8	2	7	8	27	1	4
Fragments	0.6- <1.2	black	3	2	0	0	0	0	0	5	0	2	1	3	1	4	0	1	0	2
Fragments	0.6- <1.2	red	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0
Fragments	0.6- <1.2	blue	0	1	0	5	2	4	0	1	0	2	2	3	9	9	2	3	1	2
Fragments	0.6- <1.2	yellow	0	1	0	0	0	0	0	0	0	1	0	0	0	3	0	1	0	0
Fragments	0.6- <1.2	orange	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragments	0.6- <1.2	green	0	0	0	1	0	1	0	0	0	0	0	0	3	2	0	0	0	0
Fragments	1.2- <2.4	transparent	11	18	1	6	2	8	1	10	4	5	3	28	6	60	24	44	6	3
Fragments	1.2- <2.4	white	1	3	0	1	0	1	0	0	2	0	0	3	1	2	5	5	2	1
Fragments	1.2- <2.4	black	0	0	0	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0
Fragments	1.2- <2.4	red	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0
Fragments	1.2- <2.4	blue	0	1	1	1	0	0	2	0	1	1	1	1	1	7	2	4	1	0
Fragments	1.2- <2.4	yellow	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Fragments	1.2- <2.4	orange	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragments	1.2- <2.4	green	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Fragments	2.4- <4.8	transparent	8	10	0	8	2	7	0	7	0	3	2	21	3	37	6	21	2	8
Fragments	2.4- <4.8	white	1	6	0	1	0	0	0	3	0	0	0	0	1	3	1	4	0	1
Fragments	2.4- <4.8	black	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1
Fragments	2.4- <4.8	red	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Fragments	2.4- <4.8	blue	0	2	0	0	0	0	0	0	0	0	1	1	1	7	1	4	0	0
Fragments	2.4- <4.8	yellow	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Fragments	2.4- <4.8	orange	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragments	2.4- <4.8	green	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragments	4.8<	transparent	0	1	0	5	1	2	0	1	0	1	1	15	3	9	4	8	0	6
Fragments	4.8<	white	0	1	0	0	0	0	0	0	0	0	0	2	1	1	0	4	0	0
Fragments	4.8<	black	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0
Fragments	4.8<	red	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Fragments	4.8<	blue	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0

		Site	S1	S1	S2	S2	S3	S3	S4	S4	S5	S5	S6	S6	S7	S7	S8	S8	S9	S9
		Net type	P	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N
		Sea water filtered (m^3)	1144	2809	1390	2545	1406	2347	1302	2233	1120	1904	1192	1778	1235	2100	1477	2693	1449	974
MP type	Size class	Color	Microplastic particle count																	
Fragments	4.8<	yellow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1
Fragments	4.8<	orange	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragments	4.8<	green	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fibers	0.3- <0.6	transparent	213	131	62	10	19	90	21	11	135	67	13	40	52	121	113	614	34	44
Fibers	0.3- <0.6	white	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
Fibers	0.3- <0.6	black	35	3	0	1	1	0	0	1	13	2	1	3	1	9	8	5	0	1
Fibers	0.3- <0.6	red	1	0	1	0	0	1	0	0	0	1	0	0	3	2	0	1	0	0
Fibers	0.3- <0.6	blue	14	1	0	5	1	2	0	2	1	0	4	0	17	24	8	13	0	0
Fibers	0.3- <0.6	yellow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fibers	0.3- <0.6	orange	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
Fibers	0.3- <0.6	green	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Fibers	0.6- <1.2	transparent	251	110	107	51	95	115	44	58	227	208	57	165	98	199	169	693	29	92
Fibers	0.6- <1.2	white	2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	2	1	0
Fibers	0.6- <1.2	black	29	2	4	3	4	4	1	0	7	4	4	0	12	10	12	14	1	0
Fibers	0.6- <1.2	red	0	4	0	0	0	1	1	0	0	3	0	0	1	1	2	2	0	0
Fibers	0.6- <1.2	blue	15	1	6	7	0	1	1	3	4	1	6	2	15	45	18	12	1	2
Fibers	0.6- <1.2	yellow	1	0	0	0	0	0	0	0	1	5	0	0	0	1	1	1	0	1
Fibers	0.6- <1.2	orange	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
Fibers	0.6- <1.2	green	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
Fibers	1.2- <2.4	transparent	146	6	67	12	24	22	24	38	120	159	10	81	38	83	161	238	4	9
Fibers	1.2- <2.4	white	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0	6	0	0
Fibers	1.2- <2.4	black	11	0	5	1	1	3	5	0	10	3	4	4	10	9	11	14	0	0
Fibers	1.2- <2.4	red	6	0	1	2	1	0	1	0	0	0	1	1	3	2	6	1	1	0
Fibers	1.2- <2.4	blue	17	2	5	5	2	4	1	1	14	3	4	16	12	31	16	7	4	2
Fibers	1.2- <2.4	yellow	1	0	0	0	0	0	0	1	3	2	0	4	0	7	1	3	0	0
Fibers	1.2- <2.4	orange	0	0	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0	0
Fibers	1.2- <2.4	green	0	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0
Fibers	2.4- <4.8	transparent	148	18	45	18	40	36	28	29	57	94	22	82	41	81	89	77	8	25
Fibers	2.4- <4.8	white	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Fibers	2.4- <4.8	black	27	0	5	6	1	0	6	4	17	2	6	6	5	13	8	2	1	1
Fibers	2.4- <4.8	red	24	0	2	3	0	0	5	2	1	0	4	5	4	1	8	1	0	1
Fibers	2.4- <4.8	blue	22	8	0	4	5	1	8	4	10	5	4	9	12	21	8	8	4	10
Fibers	2.4- <4.8	yellow	2	0	0	17	0	0	0	3	3	0	3	5	2	7	3	1	0	3
Fibers	2.4- <4.8	orange	1	0	0	0	0	0	0	0	13	0	0	0	0	0	1	0	0	0
Fibers	2.4- <4.8	green	0	0	0	0	0	0	0	0	0	0	0	0	5	0	2	0	0	0
Fibers	4.8<	transparent	58	14	4	27	9	7	2	6	5	14	10	13	16	50	16	16	13	28
Fibers	4.8<	white	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	2	0	0
Fibers	4.8<	black	11	1	7	6	3	2	0	0	1	1	3	1	2	6	0	0	3	2
Fibers	4.8<	red	18	0	6	2	2	0	1	0	1	0	1	5	2	6	1	0	0	1
Fibers	4.8<	blue	8	17	2	23	2	6	5	3	3	2	4	7	7	82	2	6	5	15
Fibers	4.8<	yellow	2	7	0	22	0	1	0	3	0	1	3	18	2	72	0	8	6	14
Fibers	4.8<	orange	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Fibers	4.8<	green	0	0	0	0	0	0	0	0	0	0	0	0	2	8	1	0	0	1

Appendix VIII. The microplastic count data for each microplastic (MP) type, color, and size class for the neuston net (N), plankton net (P), and van Veen grab (V). Fragment_size_a is the count of fragments 0.3- <0.6 mm^2 in size per unit volume. Fragment_size_b is the count of fragments 0.6- <1.2 mm^2 in size per unit volume. Fragment_size_c is the count of fragments 1.2- <2.4 mm^2 in size per unit volume. Fragment_size_d is the count of fragments 2.4- <4.8 mm^2 in size per unit volume. Fragment_size_e is the count of fragments >4.8 mm^2 in size per unit volume. Fiber_size_a is the count of fibers 0.3- <0.6 mm in size per unit volume. Fiber_size_b is the count of fibers 0.6- <1.2 mm in size per unit volume. Fiber_size_c is the count of fibers 1.2- <2.4 mm in size per unit volume. Fiber_size_d is the count of fibers 2.4- <4.8 mm in size per unit volume. Fiber_size_e is the count of fibers >4.8 mm in size per unit volume. Units for all particles for the neuston and plankton net are particles per m^3. Units for all particles collected from the van Veen grab are particles per kg. Microplastic values are corrected using Raman Microspectroscopy. This table can be found online at the DFO database catalog for the Placentia Bay baseline project at this provided link: (link to be provided).

Site Name	date	latitude	longitude	Sample Source	volume filtered (m^3)	depth (m)	total concentration	fiber	fragment	fragment size_a	fragment size_b	fragment size_c	fragment size_d	fragment size_e	fiber size_a	fiber size_b	fiber size_c	fiber size_d	fiber size_e	transparent	white	black	red	blue	yellow	orange	green
S1	16-Jul-21	47.117441	-54.187436	P	1144	6	0.69	0.74	0.05	0.03	0.01	0.01	0.01	0.00	0.16	0.19	0.11	0.14	0.06	0.55	0.01	0.07	0.03	0.05	0.00	0.00	0.00
S1	16-Jul-21	47.117441	-54.187436	N	2809	0.5	0.11	0.09	0.03	0.01	0.01	0.01	0.01	0.00	0.03	0.03	0.00	0.01	0.01	0.10	0.01	0.00	0.00	0.01	0.00	0.01	0.00
S2	16-Jul-21	47.130381	-54.292036	P	1390	6	0.17	0.19	0.01	0.00	0.00	0.00	0.00	0.00	0.03	0.06	0.04	0.03	0.01	0.15	0.00	0.01	0.01	0.01	0.00	0.00	0.00
S2	16-Jul-21	47.130381	-54.292036	N	2545	0.5	0.07	0.07	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.01	0.02	0.04	0.00	0.01	0.00	0.01	0.01	0.00	0.00
S3	25-Jul-21	47.322983	-54.288838	P	1406	6	0.12	0.12	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.05	0.01	0.02	0.01	0.11	0.00	0.01	0.00	0.01	0.00	0.00	0.00
S3	25-Jul-21	47.322983	-54.288838	N	2347	0.5	0.10	0.10	0.01	0.00	0.01	0.00	0.00	0.00	0.03	0.04	0.01	0.01	0.00	0.10	0.00	0.00	0.00	0.01	0.00	0.00	0.00
S4	25-Jul-21	47.416538	-54.382690	P	1302	6	0.08	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S4	25-Jul-21	47.416538	-54.382690	N	2233	0.5	0.08	0.06	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.02	0.01	0.01	0.00	0.07	0.01	0.00	0.00	0.00	0.00	0.00	0.00
S5	23-Sep-21	47.616233	-54.282010	P	1120	6	0.42	0.46	0.01	0.01	0.01	0.00	0.00	0.00	0.10	0.15	0.10	0.07	0.01	0.36	0.01	0.03	0.00	0.02	0.00	0.01	0.00
S5	23-Sep-21	47.616233	-54.282010	N	1904	0.5	0.22	0.24	0.01	0.00	0.01	0.00	0.00	0.00	0.03	0.08	0.06	0.04	0.01	0.21	0.00	0.00	0.00	0.01	0.00	0.00	0.00
S6	23-Sep-21	47.678799	-54.189166	P	1192	6	0.11	0.11	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.04	0.01	0.02	0.01	0.08	0.00	0.01	0.00	0.02	0.00	0.00	0.00
S6	23-Sep-21	47.678799	-54.189166	N	1778	0.5	0.27	0.21	0.07	0.04	0.02	0.01	0.01	0.01	0.02	0.07	0.04	0.04	0.02	0.22	0.01	0.01	0.00	0.02	0.01	0.00	0.00
S7	23-Sep-21	47.743746	-54.098672	P	1235	6	0.26	0.23	0.05	0.03	0.02	0.01	0.00	0.00	0.04	0.07	0.04	0.04	0.02	0.18	0.01	0.02	0.01	0.05	0.00	0.00	0.01
S7	23-Sep-21	47.743746	-54.098672	N	2100	0.5	0.41	0.34	0.10	0.05	0.03	0.03	0.02	0.00	0.05	0.09	0.04	0.04	0.08	0.28	0.01	0.02	0.01	0.09	0.03	0.00	0.00
S8	22-Sep-21	47.575858	-53.999740	P	1477	6	0.36	0.36	0.04	0.01	0.02	0.01	0.00	0.00	0.06	0.10	0.09	0.06	0.01	0.30	0.01	0.02	0.01	0.03	0.00	0.00	0.00
S8	22-Sep-21	47.575858	-53.999740	N	2693	0.5	0.56	0.51	0.10	0.05	0.04	0.01	0.01	0.00	0.17	0.19	0.07	0.02	0.01	0.53	0.02	0.01	0.00	0.02	0.00	0.00	0.00
S9	22-Sep-21	47.350709	-54.045149	P	1449	6	0.07	0.06	0.01	0.01	0.00	0.00	0.00	0.00	0.02	0.02	0.00	0.01	0.01	0.05	0.00	0.00	0.00	0.01	0.00	0.00	0.00
S9	22-Sep-21	47.350709	-54.045149	N	974	0.5	0.22	0.20	0.03	0.01	0.01	0.00	0.01	0.01	0.03	0.07	0.01	0.03	0.04	0.17	0.01	0.00	0.00	0.02	0.01	0.00	0.00
H	29-Sep-21	47.75328	-54.23472	V	0	26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
W	29-Sep-21	47.64558	-54.26754	V	0	153	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E	30-Sep-21	47.74767	-54.06439	V	0	158	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C	30-Sep-21	47.57921	-54.12690	V	0	6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
O	1-Nov-21	47.18	-54.37683	V	0	242	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S	1-Nov-21	46.79598	-54.79602	V	0	256	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00