The Quantification of Microplastics in Intertidal Sediments in the Bay of Fundy, Canada

by

Colin Forsythe BSc.

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Supervisor: Dr. Peter Ross

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COMMITTEE APPROVAL

The members of Colin Forsythe's Thesis Committee certify that they have read the thesis titled The Quantification of Microplastics in Intertidal Sediments in the Bay of Fundy, Canada and recommend that it be accepted as fulfilling the thesis requirements for the Degree of Masters of Science:

Dr. Peter Ross [signature on file]

Dr. Matt Dodd [signature on file]

Final approval and acceptance of this thesis is contingent upon submission of the final copy of the thesis to Royal Roads University. The thesis supervisor confirms to have read this thesis and recommends that it be accepted as fulfilling the thesis requirements:

Dr. Peter Ross [signature on file]

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Abstract

The Bay of Fundy in Atlantic Canada is noted for the world's highest tides, heights of which reach 15 meters (Percy, 1996; Thurston, 2011). We characterized microplastic concentrations and distribution in this region. Sediment samples were collected from 15 intertidal sites along New Brunswick's southwestern coast. Sites were selected based on variations in tidal range (7m to 15m), exposure to prevailing winds, and proximity to urban centres. Microplastics were widespread and detected in all samples. Microplastics averaged 268 pieces per sample of 1kg (+/- SD) and total microplastic composition consisted of fibers (89%), fragments (8%) and microbeads (2%). ANOVA results indicated a significant difference between concentrations at sheltered vs. exposed sites and no significant difference between quantities at high or low tide delineations or across tidal ranges. While a larger sample size would strengthen the ability to explore factors influencing microplastics in the Bay of Fundy, the widespread detection of microplastics in our study indicate that there are no sites in the Bay of Fundy that are immune to this growing marine pollution problem.

Table of Contents

Table of Contents Continued

List of Figures

List of Figures Continued

List of Tables

List of Tables Continued

List of Tables Continued

List of Appendices

Appendix II: Summary of microplastic site data

Appendix III: Summary of microplastic composition data

Appendix IV: Photos of enumerated microplastic

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Quantification of Microplastics in the Bay of Fundy

Introduction

Microplastics in the Marine Environment

The creation and subsequent mass production of plastics in the 20th century has led to increasing amounts of this material being distributed into marine ecosystems on a global scale (Andrady, 2011; Cole et al., 2011; GESAMP, 2015; and Mathalon., & Hill, 2014). Further, the long-term durability of this class of material allows it to persist in marine environments potentially for centuries (GESAMP, 2015; Mathalon., & Hill, 2014; Hopewell, Dvorak, & Kosior, 2009; Thompson et al., 2004). The long-term durability and abundance of the material have created appropriate conditions for plastics to become one of the most substantial and problematic components of marine related debris (Andrady, 2011; Auta, Emenike & Fauziah, 2017; Cole et al., 2011; GESAMP, 2015; UNEP, 2005). Previous research has identified several types of plastic that form the majority of plastic pollution in the aquatic environment. These include polyethylene, polypropylene, polystyrene, acrylic, nylon, polyvinyl chloride (PVC), and polyester (Browne, Dissanayake, Galloway, Lowe & Thompson, 2008). These plastics are used in a wide variety of consumer products, including packaging, clothing, cosmetics, commercial fishing gear and aquaculture applications. Many of these plastics are created with single-use intent and once deposited in the marine environment start to degrade and fragment through mechanisms of wave, tide and solar action breaking them down into microscopic particles (Andrady, 2011; Auta et al., 2017; Cole et al., 2011).

The microscopic particles are termed microplastics; although their definition varies, they can generally be defined as polymer debris that is composed of a variety of tiny plastic beads, fibers and fragments that are < 5mm in size (Andrady, 2011; Boucher & Friot, 2017; Cole et al., 2011; GESAMP, 2015; Mathalon & Hill, 2014). Microplastics can be further classified to recognize their source as either a primary or secondary microplastic (Auta et al., 2017; Boucher & Friot, 2017; Cole et al., 2011) Primary microplastics are plastic polymers released into the environment at a microscopic size. They can consist of microbeads, pellets, fibers and nurdles. Primary microplastics can include microbeads in exfoliating facial-cleansers, hand cleansers, toothpaste and other cosmetics products as discussed by Auta et al. (2017); Boucher & Friot, (2017); Browne et al. (2008), and Zitko and Hanlon (1991). Microbeads have also been increasingly used in air-blasting technology as an abrasive medium to remove rust and paint form the hulls of ships and other machinery (Boucher & Friot, 2017; Browne, Galloway, & Thompson, 2007; Derraik, 2002; Gregory, 1996). They can also originate from abrasion of tires and textiles during use, maintenance or manufacturing as discussed by Boucher & Friot, (2017). Lastly, research conducted by Patel, Goyal, Bhadada, Bhatt, and Amin, (2009) indicated that even smaller primary microplastic particles called nanoplastics have increasingly been reported for use as vectors for the introduction of drugs in medical applications.

Secondary microplastics differ from primary in that they are not specifically produced at a microscopic size, but rather comprise tiny plastic fragments that result from the breakdown of larger plastic items such as packaging, rope or fishing gear that is found both on shore and in the ocean (Auta et al., 2017; Boucher & Friot, 2017; Cole et al., 2011; GESAMP, 2015; Thompson et al., 2004). Secondary microplastics form as the degradation of the plastics structural integrity via physical, biological and chemical processes (Auta et al., 2017; Browne et al., 2007; GESAMP, 2015). This degradation results in the fragmentation over time of the plastic debris into microplastic particles (Auta et al., 2017; Browne et al., 2007; GESAMP, 2015). Microplastics can enter the marine environment via several mechanisms including direct use in marine applications such as aquaculture and fishing or from land based sources such as municipal outfalls, accidental release and poor waste management practices. Several studies have indicated the mechanisms for the formation of secondary microplastics (Andrady, 2011; Auta et al., 2017; Barnes, Galgani, Thompson, & Barlaz, 2009; Browne et al., 2007; GESAMP, 2015; Moore, 2008). These studies indicate that over prolonged periods the continuous exposure to sunlight, particularly on beaches where ultraviolet (UV) radiation oxidizes the polymer, brittle the structure making the plastics susceptible to breakages by wave, wind and tidal action in an ongoing process (Andrady, 2011; Auta et al., 2017; Barnes et al., 2009; Browne et al., 2007; GESAMP, 2015; Moore, 2008). The widespread distribution of microplastics in the marine environment presents the opportunity for these plastics to be ingested by marine biota.

Ingestion of Microplastics by Marine Organisms

The small size of primary and secondary microplastic particles provide and opportunities for them to be accidently or selectively ingested by marine biota and several studies have indicated the potential for marine biota to ingest plastic when present in the environment. Studies conducted in the laboratory by Browne et al. (2008) on the ingestion of plastics in blue mussels (*Mytilus edulis*) and Thompson et al. (2004) who investigated plastic ingestion in amphipods, lungworms and barnacles. Further, Desforges, Galbraith, & Ross, (2015) conducted field investigations on the ingestion of microplastics in zooplankton from the Northeast Pacific Ocean. These studies provide further evidence that marine detrivores; filter and suspension feeders will ingest microplastics when present in their environment.

Many other marine species have also been documented ingesting microplastics, for instance a paper by Lusher, McHugh, and Thompson (2012) tested 10 species of fish from the English Channel for microplastics. Of the 504 fish that were examined plastics were found in the gastrointestinal tracts of 36.5% (Lusher et al., 2012). They reported that the 'average number of pieces per fish was 1.90 ± 0.10'(Lusher et al., 2012). The researchers found a total of '351 pieces of plastic', which were identified using FT-IR Spectroscopy and determined that rayon (57.8%) and polyamide (35.6%) were the most common plastics found in samples species. Their research also showed that all of the pelagic and demersal fish species investigated had ingested plastic and that there was 'no significant difference between the abundance of plastic ingested by pelagic and demersal fish' (Lusher et al., 2012). This indicated that the ingestion of small amounts of microplastics in a range of fish species have different feeding habitats appeared to be common (Lusher et al., 2012).

A recent study by Rochman et al., (2015), which investigated microplastics in the digestive tracts of fish and bivalves sold for human consumption at markets in the United States and Indonesia, found that that microplastic debris was present in 28% of individual fish sampled and in 55% of all species sampled from Indonesian markets (Rochman et al., 2015). The results from the United States indicated that microplastics were found in 25% of individual fish sampled and 67% of all species sampled (Rochman et al., 2015). The microplastic debris was present in 33% of individual bivalves sampled (Rochman et al., 2015). The authors note, "all of the anthropogenic debris recovered from fish in Indonesia was plastic" (Rochman et al., 2015). They also noted that anthropogenic debris extracted from fish sampled for US markets were primarily fibers and not specifically identified as all plastic debris (Rochman et al., 2015). This paper indicates the presence of microplastics entering the human food chain and shows that the potential negative impacts of microplastics are not restricted to marine species and environments.

Another study investigated microplastic ingestion by seabirds (van Franeker et al., 2011). In this study conducted between 2003 and 2007 the 'abundance of plastics in stomachs of northern fulmars from the North Sea' was investigated (van Franeker et al., 2011). The researchers determined that '95% of 1295 fulmars sampled in the North Sea had plastic in their stomachs' with an average of '35 pieces weighing 0.31 g' (van Franeker et al., 2011). The plastic content in seabirds was weighed against the 'Oslo/Paris Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) Ecological Quality Objective for marine litter', which describes the Ecologically acceptable level for marine debris in the North Sea marine ecosystem (van

Franeker et al., 2011). The resulted showed that the EcoQO acceptable level of 0.1 g of plastic was 'exceeded by 58% of birds, with regional variations ranging from 48 to 78%' (van Franeker et al., 2011).

One of the concerns raised in regards to the ingestion of microplastic particles is that it increases the opportunity for these organisms to become potentially contaminated by the chemicals absorbed by the microplastic particles. Some papers have indicated that this process might result in additives from the plastic leaching into the marine environment and biota (Auta et al., 2017; GESAMP, 2015; Talsness, Andrade, Kuriyama, Taylor, & vom Saal, 2009). Recent research has shown that microplastics present in the marine environment not only leach chemicals into marine environments they can also accumulate a variety of persistent organic pollutants including PCBs, DDTs and PAHs (Rios, Moore, & Jones, 2007). A review of this research by Teuten et al., (2009) has shown that microplastics can accumulate contaminants such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), petroleum hydrocarbons and organochlorine pesticides as examples. Further, Tanaka, Takada,Yamashita, Mizukawa, Fukuwaka and Watanuki (2013) investigated polybrominated diphenyl ethers (PBDEs) in abdominal adipose of short-tailed shearwaters, (*Puffinus tenuirostris*) which were collected in the North Pacific Ocean. Their results show that 3 of the 12 birds sampled had higher-brominated congeners in their abdominal adipose. The study noted that these are the same chemical compounds, which were present in plastic found in the stomachs of 3 of the birds. These chemical compounds are not present in pelagic fish the short-tailed shearwaters natural prey. The findings of this study suggest that plastic-derived chemicals from ingested plastics can transfer to the tissues of marine-based organisms.

Further research conducted by Rochman, Hoh, Kurobe & Teh, (2013) has shown that fish, 'exposed to a mixture of polyethylene with chemical pollutants sorbed from the marine environment, bioaccumulate these chemical pollutants and suffer liver toxicity and pathology' Rochman et al., (2013). The researchers also fed Fish virgin polyethylene fragments, which after consumption showed signs of signs of hepatic stress Rochman et al., (2013). It is interesting to note that the researchers indicated less severe signs of stress in fish fed marine polyethylene fragments Rochman et al., (2013). This research provides further information regarding the bioaccumulation of chemicals leached from microplastics and potential associated health effects from ingestion in fish and demonstrates the need to consider the 'complex mixture of the plastic material and their associated chemical pollutants' Rochman et al., (2013). As such recent research has recognized that microplastics represent a growing problem to marine ecosystems and biota via the potential leaching of hazardous chemical into the environment. As many of these microplastics are ultimately deposited in along coastlines and in marine sediments these locations provide an opportunity it further investigate it's role as an anthropogenic pollutant in coastal ecosystems and biota.

Microplastics in Coastal Sediments

There have been numerous studies on microplastics found in sediments of marine environments from around the world (Baztan et al., 2014; Browne et al., (2008), (2010) & (2011); Claessens et al., 2011; Ivar do Sul et al., 2009; Liebezeit and Dubaish, 2012; Mathalon and Hill, 2014; Stolte, Forster, Gerdts, and Schubert, 2015; Tsang et al., 2017). These studies indicate that microplastics are widespread along the world's shorelines and provide an opportunity to gain insight on the type, amount and distribution of microplastics in a given region. For instance, a sediment study conducted by Browne et al., (2011) investigated beach sediments in the northern and southern hemispheres for 18 sites across six continents. The results of this study indicated that microplastics were found in greater amounts in proximity to urban centers and that much of the microplastic debris was present as polyester and acrylic fibers (Browne et al., 2011). The findings for this study suggest that a large portion of the microplastic fibers found in the marine environment originate form wastewater discharge, the result of washing clothes (Browne et al., 2011).

A further study focused in the Canary Islands investigated microplastics present in intertidal sediments around the islands (Baztan et al. 2014). This study sampled intertidal sediment form 125 Canary Island beaches and investigated the concentration of microplastics on these locations. Results indicated that all the sites sampled were highly vulnerable to microplastic pollution (Baztan et al., 2014). Most disturbingly this included sites located in highly protected natural areas (Baztan et al., 2014). Microplastic concentrations on some beaches sampled were reported exceeding '100g of plastic per 1L of sediment' (Baztan et al., 2014). The authors show that even natural areas with the highest level of legal protection are still at risk form plastic contamination. Additionally, the study demonstrates how wide spread microplastics are in the marine environment and the potential value of sampling sediments to provide quantifications of the extent of microplastic contaminants in marine intertidal environments.

Another study that has focused on concentrations of microplastics in sediments is the study conducted by Stolte et al., (2015). This study investigated and evaluated contamination of microplastics on beaches found along the German Baltic coast (Stolte et al., 2015). This study sampled sediments in Baltic estuaries and along the Rostock coast to 'derive possible entry pathways' for microplastic into the marine environment (Stolte et al., 2015). These researchers also monitored 'seasonal variations along the Rostock coast from March to July 2014' (Stolte et al., 2015). Results of this research found that microplastic concentrations for area beaches were '0-7 particles/kg and 2-11 fibres/kg dry sediment' (Stolte et al., 2015). The researchers noted that the largest concentrations of microplastic contamination were 'measured at the Peene outlet into the Baltic Sea and in the North Sea Jade Bay' (Stolte et al., 2015). The researchers concluded that the sources of the high microplastic concentrations in Baltic beach sediments likely originate from municipal outfalls, industrial and commercial activity and tourism (Stolte et al., 2015). The paper further demonstrates the valuable role sediments can play in determining the concentrations of microplastics in marine environments and indicating potential sources. In particular, the confined nature of the Baltic Sea provides some comparison for microplastic research in other confined marine environments like the Bay of Fundy.

Microplastics in the Marine Environment in Atlantic Canada

Although there have been numerous studies on microplastics in sediments in many other locations, there has been very little research conducted on microplastics in the marine environment of Atlantic Canada. One of the few studies conducted on microplastics in this region is the work by Mathalon and Hill (2014). This study quantified microplastic fibers isolated from sediment samples collected from the intertidal zone of three beaches in Halifax Harbour along Nova Scotia's Eastern Shore (Mathalon & Hill, 2014). In addition to sediment, this study also collected samples of polychaete worm fecal casts and blue mussels (*Mytilus edulis*) to determine microplastic content (Mathalon & Hill, 2014). They also compared purchased mussels from an aquaculture site to the mussels collected in the field (Mathalon & Hill, 2014). The findings of this study indicated an average of 20 and 80 microplastic fibers observed in a 10 g sediment subsamples (Mathalon & Hill, 2014). Higher concentrations were observed at the low tide delineation of protected beaches and the high tide delineation of exposed beaches (Mathalon and Hill, 2014). The researchers also found that 'Microplastic concentrations from polychaete fecal casts resembled concentrations quantified from low tide sediments' (Mathalon & Hill, 2014). Additionally, the results of the research found that blue mussels showed 'significantly more microplastics enumerated in farmed mussels compared to wild ones' (Mathalon & Hill, 2014). These findings would appear to be similar with those of other researchers on microplastics in intertidal sediments of marine environments outside of Atlantic Canada. The research conducted by Mathalon and Hill (2014) is significant to research conducted in the Bay of Fundy as it provides a basis for comparison of microplastic quantities found in sediments and biota in the Atlantic Canada region. Additionally, their research may provide an important insight to the potential effects of the Bay's extreme tides the significance of which cannot be understated and may alter the concentration and movement of microplastics in Bay in a manner not seen in other locations. This may potentially elucidate on the role of tides and tidal cycles in the creation and transport of microplastics in the marine ecosystem

Research Questions

The Bay of Fundy in Atlantic Canada is recognized for its uniquely high tides, which are noted as the world's highest (Percy, 1996; Thurston, 2011). These tides can attain as much as 15 meters in height, and are created in part by the Bay's 270km long roughly funnel shape that narrows, and shallows as one approaches its northern end where it splits into Chignecto Bay and Minas Basin (Percy, 1996; Thurston, 2011). This funnel shape, coupled with the gravitational pull from the lunar cycle, causes water in the bay to 'slosh back and forth with a regular rhythm' (Thurston, 2011). This generates a resonance or 'bathtub effect', which in turn is responsible for the amplified tidal effects observed in the Bay (Percy, 1996; Thurston, 2011).

In the upper region of the bay this tidal effect has formed extensive mud flats and salt marshes via the movement of large quantities of fine sediments into coastal areas (Percy, 1996; Thurston, 2011). These ecosystems create an integral part of the Bay's food production system and provide a source of nutrients 'pumped' into the bay by the tides (Thurston, 2011). This tidal motion also generates upwelling in the waters of the mouth of the Bay that concentrate phytoplankton and zooplankton in this region. The result is a nutrient rich ecosystem with a remarkable concentration of marine life (Thurston, 2011). The action of the tides makes the Bay of Fundy a biologically diverse, ecologically significant and thus economically important marine ecosystem (Thurston, 2011).

As fishing, aquaculture and ecotourism are important economic drivers in this region the health of the marine ecosystem is of concern to residents and stakeholders. Though there have been other studies on aquatic pollution in the bay and the potential effects on biota it would appear there are no studies that have investigated the concentration and distribution of microplastics in the Bay. This overall lack of knowledge needs to be investigated to quantify and determine the type and extent of microplastic contamination in this unique marine ecosystem. 250-516-2575

Therefore in this Thesis, I have posed the following questions:

1) *What are the concentrations and types of microplastics present at intertidal sites located along the New Brunswick coast of the Bay of Fundy?*

2) *What role do the Bay's extreme tides play in the variation of microplastic concentration and distribution in intertidal sediments along this coastline?*

Hypothesis

It is hypothesized that sheltered marine environments and those that are in close proximity to population centres will show higher concentration of microplastics in sediments. Intertidal sites further up the bay with amplified tidal effects (higher energy) and further from population centres will show lower concentrations of microplastics in the sediments.

Significance of the Study

This study is significant in that it is the first study to investigate microplastics in the Bay of Fundy. The data and information collected during this research will add to the growing body of knowledge available in regards to microplastics as contaminants in Atlantic Canada's marine ecosystems. In order to collect information of potential significance the study was designed to sample and analyze microplastic pollution for a variety of variables present in the bay. The sampling plan collected sediment from high and low tide delineations at 15 sites spread along the New Brunswick coastline of the bay. Sites were further selected based on variations in tidal range (7 m to 14 m), exposure to prevailing southwesterly winds and proximity to urban centers. This was done to gain insight into which types of locations and transport mechanisms (wind, wave, tide) contribute to the greatest accumulation of microplastics in the sediment. Further this study design was selected to provide information on which types of plastic accumulate the most on area beach and whether the Bay of Fundy tides play a significant role in their distribution and composition on beaches in the region.

One of the significant goals of this research is that the data acquired and subsequent results of this study are intended to create a dialog between local stakeholders, policy makers and researchers on the challenges posed by this type of plastic pollution on the Bay's ecosystem and thus economic drivers. The data acquired could provide a catalyst to assist in the development of future sustainability initiatives and conservation measures focused on a deeper understanding of the role microplastics play as an aquatic contaminant in the Bay of Fundy and other regions in Atlantic Canada.

Methodology

Site Description

All sampling sites were located along the Southwestern coast of New Brunswick in the Bay of Fundy and inspected prior to sampling to ensure the suitability of the site for sample collection. A total of 15 sites were sampled between September 2015 and June 2016 for microplastics, these sites were located in St. Andrews, Back Bay, New River Beach, Saint John Harbour, St. Martins, Alma and Anchorage Provincial Park on Grand Manan respectively (Appendix I). Eight sites in the location of Saint John Harbour were in proximity to urban environments where known point source for municipal outfalls and industrial discharges are located (Appendix I &II). Four sites at Grand Manan, Back Bay, St. Martins and Fundy National Park were also selected for their proximity to the fishing and aquaculture industry (Appendix I &II). Three sites at New River, Anchorage

Provincial Park (Grand Manan) and Fundy National Park served as less urban influenced marine environments in Provincial and National Parks and The Fundy Biosphere Reserve (Appendix I &II). Additionally, the 15 sites were also selected to for their exposure to prevailing winds and wave action as identified in Appendix II. These include high-energy locations where the site experiences intense wave action and low energy locations that were sheltered with lower frequency of wave action and exposure. These locations of varying orientation to prevailing winds, wave action and tidal heights (7m to 14m) have been selected to assist in establishing the type of locations and tidal variation that have the potential to accumulate the greatest concentration of microplastics in intertidal sediments.

Sample Collection

At each of the 15 sites, sediment samples were collected at the delineation of both high and low tide marks for a total of 30 samples consisting of two from each site. Additionally, duplicate samples taken at Saint Rests and Grand Manan locations and used to evaluate analytical precision and potential contamination of the sample. Sediment samples were collected using a clean metal spoon that was triple rinsed with distilled water at each sample site to minimize potential for contamination from outside sources or other samples. Using a 50 cm quadrat a 500 g wet weight sediment sample was collected between 1 cm and 5 cm depths and placed in sterilized 500 ml glass jars and sealed. The sample jars were labeled with site-specific information and placed in a cooler for transport to dry storage until laboratory analysis.

Laboratory Analysis

Quantification of Microplastics in the Bay of Fundy

All sampling and analysis of intertidal sediments was conducted using sterile techniques to mitigate contamination from other sources of plastics and followed the methodology described by Hidalgo-Ruz et al. (2012) and Mathalon and Hill (2014). As part of the mitigation procedure, cotton clothing of identical colour was worn when performing laboratory work and control samples were taken for both the lab air and distilled water used. A clean Petri dish with filter paper was left exposed in the lab to detect the potential for contamination of sediment samples. Distilled water was filtered using the same procedures as sediment samples again to detect the potential for contamination of sediment samples. All laboratory control samples were enumerated using the same procedures as sediment samples and showed few fibers in the control samples for the lab air (2 fibers) and distilled water (3 fibers). The detection of fibers in control samples led to additional procedures to minimize potential contamination. As such all containers were covered as much as possible at all points of the analysis with glass covers and instruments cleaned and triple rinsed with distilled water after processing each sample to prevent cross contamination.

In the lab, each sediment sample was dried for 48 hours prior to laboratory analysis. A 50 g dry weight subsample of sediment from both high and low tide locations at each site was weighed onto a glass watch glass for a total of 30 samples. Each sample, once weighed, was individually placed in a concentrated saline solution (250 g NaCl/ 1 L distilled H_2O). 100 milliliters of saline solution was used to separate microplastics from sediments via density separation. Using a magnetic stirrer, each 50 g subsample was agitated at high intensity for a 1–2 min interval, followed by a settling time of 3–6 min, depending on the observed clearance rate of the sediment from suspension. Transfer of the supernatant to the vacuum filtration apparatus was conducted via decanting the solution onto a 0.8-µm pore size, 47 mm white non gridded mixed cellulose ester filter for vacuum filtration similar to the method used by Mathalon & Hill (2014). After all the supernatant had been decanted, an additional 100 ml of saline solution was added to each sample, and the procedure repeated to extract any potentially remaining microplastics in the sample. Upon completion of filtration each filter was placed in sterilized and previously sealed Petri dish where the filters were dried at room temperature for 48 hours prior to microscope analysis as supported by Hidalgo-Ruz et al. (2012) and Mathalon and Hill (2014).

To quantify the microplastic content in the filtered sample, visual inspection and enumeration was conducted using a dissecting microscope for plastics ranging from 5 mm to 500 µm starting at 2X and increasing to 40X magnification similar to the method employed by Mathalon and Hill (2014). Each filtered sample was placed under the microscope and examined from left to right starting at the top of the filter and proceeding to the bottom. Plastics were visually distinguished according to the criteria described by Hidalgo-Ruz et al. (2012), with 'no cellular or organic structures visible, fibers should be equally thick throughout their entire length, particles must present clear and homogeneous colors, and if they are transparent or white, they must be examined under high magnification'. Any potential microplastics discovered were further examined with a metal probe and under 40x magnification to provide greater resolution on the material in question. To aid in identification the Marine & Environmental Research Institute's

'Guide To Microplastic Identification' (2016) provided a visual reference to distinguish plastic components form organic material. Enumerated microplastics were recorded and the data presented in (Appendices II, III & IV). The data was then standardized to per kg units to provide easier comparison with other studies.

Lastly, to minimize samples for Fourier Transform Infrared Spectroscopy analysis suspected plastics in enumerated samples were first analyzed with the hot needle test as described elsewhere (De Witte et al., 2014). In this test, a hot needle was placed close to the suspected plastic piece and observed to see if it melted or curled, in which case the item was confirmed as anthropogenic in origin and a sample of the material collected for Fourier Transform Infrared Spectroscopy (FT-IR).

An FT-IR analysis was conducted for each of the high and low tide sample from each of the 15 sites sampled for a total of 30-isolated microplastic samples. A PerkinElmer Incorporated model number L160000A Spectrum Two Fourier Transform Infrared Spectroscopy (FT-IR) unit was employed to further confirm the identity of the examined material. This procedure was done to confirm the presence of plastic in the sample and to determine the types of plastic present at each of intertidal site to elucidate on both the differences and consistencies in the types of plastic present in the Bay of Fundy. This method has previously been used in similar studies to confirm the presence of microplastics and is supported in the methodology review by (Hidalgo-Ruz et al., 2012). In FT-IR analysis a small amount of suspected plastic was placed onto the top of the unit and secured with a clamp. A laser generates IR radiation that was passed through the sample where some of the infrared radiation was absorbed by the sample and the remainder transmitted through (Thermo Nicolet Corporation, 2001). The results of the scan produced an IR spectrum, which represented the molecular absorption and transmission of the material in question (Thermo Nicolet Corporation, 2001). This pattern created a unique molecular fingerprint of the material in question in the sample. The resulting IR spectrum was then cross-referenced with the Nicodom Limited IR polymer spectra database to determine the unknown material in the sample and confirm the presence or absence of plastics in the sample.

Statistical Analysis

Descriptive statistics were carried out on all the data. A Shapiro-Wilk's test was used to test the data for normality and indicated that the null hypothesis that data collected from the enumerated samples was normally distributed. Therefore the null hypothesis could not be rejected for all 15 sites sampled at high and low tide delineations (p = 0.001 and p = 0.032, respectively). Further, Shapiro-Wilk Normality test results also indicated that the data are normally distributed across the outer, mid and inner tidal ranges for the 15 sites sampled in the bay (p = 0.010).

A Student t-test was performed for the parameters of high tide sites vs. low tide sites with the alpha value set at 0.05. The data were then analyzed using a parametric analysis of variance (one-way ANOVA) with the alpha value set at 0.05. One-way ANOVA's were performed for the parameters of low tide sites vs. tidal ranges, high tide sites vs. tidal ranges, total site quantities vs. tidal ranges and, sheltered vs. exposed sites and urban vs. rural sites respectively. The post hoc comparisons for significance utilized a Tukey-HSD test with an alpha value set at 0.05. Microplastic results were also analyzed with a Pearson product-moment correlation to determine the degree of linear relationship amongst sites and tidal ranges. All statistics were calculated using SPSS for Mac version 20.0 and graphs were generated using Macintosh Numbers Ver. 3.6.2 for Mac OS X El Captian Ver.10.11.5.

Results

Intertidal sediments

Intertidal sediment samples collected from 15 sites in the Bay of Fundy were analyzed to elucidate the quantities, distribution and types of microplastic currently present in the Bay. Each of the 50 g subsamples, standardized to 1 kg, analyzed from high and low tide delineation samples from the 15 sites contained microplastics (Figures 1,2,3,4 & Appendix II). The combined and averaged site totals of enumerated microplastics from high and low tide per site-ranged from 40 pieces per 1 kg at Fort LaTour in Saint John Harbour to 870 pieces per 1 kg at Anchorage Provincial Park on Grand Manan Island (Appendix II). The mean number of microplastic enumerated per site was calculated at 268 pieces on average per 1 kg of sediment. The standard deviation of the high and low tide combined 1kg sample was calculated at 208.710 pieces per 1 kg of sediment. The standard error of the high and low tide combined 1kg samples was calculated at +/-53.889 pieces per 1 kg of sediment (Figure 3).

The amount of microplastic pieces enumerated from samples collected at high tide delineations across the 15 sites ranged from 40 pieces per 1 kg at Bay Shore Beach and Fort LaTour to 1320 pieces per 1 kg at Anchorage Provincial Park on Grand Manan Island (Figure 1,4 & Appendix II). The mean amount of microplastic calculated in high tide samples was 321 pieces per 1 kg of sediment. The standard deviation of the high tide 50 g samples was calculated at 331.099 pieces per 1 kg of sediment. The standard error of the high tide 1 kg samples was calculated at +/-85.489 pieces per 1 kg of sediment (Figure 1).

The amount of microplastic pieces enumerated from samples collected at low tide delineations a cross the 15 sites ranged from 40 pieces per 1 kg of sediment at Fundy National Park and Fort LaTour in Saint John Harbour to 660 pieces per 1 kg of sediment at Bay Shore Beach in Saint John Harbour (Figure 2, 4 & Appendix II). The mean amount of microplastic calculated in low tide samples was 215 pieces per 1 kg. The standard deviation of the low tide 1 kg samples was calculated at 179.756 pieces per 1 kg of sediment. The standard error of the low tide 1 kg samples was calculated at +/-46.413 pieces per 1 kg of sediment (Figure 2).

Lastly, an analysis of the microplastic enumerated form the 15 sample sites indicated that the majority of microplastics from these sites were fibers representing 89% of the plastic in the analyzed samples (Figure 5 & Appendix III). Further, microplastics described as angular fragments and microbeads composed 8% and 2% of the plastic in the analyzed samples respectively (Figure 5 & Appendix III). The remaining 1% of microplastics in the analyzed samples was plastic described as irregularly shaped (Figure 5 & Appendix III). Photos of types of enumerated microplastics can be found in Appendix IV.

FT-IR Analysis

Results from the FT-IR analysis did confirm the presence of plastics in all of the samples tested for microplastics in intertidal sediments. It should be noted that the samples from Tin Can Beach, Ft. LaTour, Marsh Creek and Bay Shore Beach did not produce IR spectra that could be crossed referenced with any of the polymers in the database (Table 1). This lack of discernable spectra may be the result of interference generated from potential contamination of the samples from other organic and inorganic material adhered to the polymers surface. In these instances a hot needle test was administered. The results of the hot needle test did determine the samples collected from Tin Can Beach, Ft. LaTour, Marsh Creek and Bay Shore Beach did have microplastic fibers present (Table 1).

The remainder of the sites sampled all produced at least one discernable IR spectrum that could be cross-referenced with the polymers in the database. Specifically, the results for the FT-IR analysis did confirm the presence of plastic at these sites and indicated that polypropylene and polyethylene were both common throughout the Bay of Fundy (Table 1). The results of the FT-IR analysis also showed some differences in types of plastics found in areas of the Bay and are noted for polystyrene and nylon respectively. These IR spectra were only seen in samples tested from the sites in the outer and mid bay region including Grand Manan, St. Andrew and Back Bay in the outer bay and from the samples taken at St. Rest's Beach and Mispec in the mid bay sites (Table 1). Nylon was confirmed by FT-IR for Grand Manan, St. Andrew, Back Bay and St. Rest's Beach and was not seen at any other site (Table 1). Polystyrene was only confirmed by FT-IR for two sites the one on Grand Manan Island and the other at Mispec in Saint John Harbour (Table 1).

Student t-test

Microplastics enumerated from sediments collected from high and low tide delineations at each of the 15 sites were compared with each other to investigate any potential significant difference in the amount of microplastic between these delineations. The results of this Student t-test indicate that no significant difference was detected in the amount of microplastic when comparing the low and high tide delineations from the 15 sample sites ($p = 0.282$) (Table 2). This result indicates that the amount of microplastic debris deposited at high and low tide delineations along the New Brunswick Bay of Fundy coastline are not significantly different from one another.

One-Way ANOVA's

A series of One-way ANOVAs were preformed to compare the microplastic results from enumerated samples with different variables present in the Bay of Fundy.

Quantification of Microplastics in the Bay of Fundy

These variables included amounts of microplastic enumerated at high and low tide delineations and across the three tidal ranges found among the 15 sites sampled. Other variables included a comparison of sites with various exposures to prevailing winds and wave activity that were either exposed to shelter and lastly the 15 sites proximity to urban sources of pollution denoted as either urban or rural (Figure 4 & Appendix II).

A series of One-way ANOVA were performed that compared the high, low and combined (high and low) samples to compare the amount of microplastic enumerated a cross the 15 sample sites with three different tidal ranges found in the outer (5 m to 8 m), mid (8 m to 11 m) and inner (11 m to 15 m) Bay of Fundy, respectively (Tables 3,4,5). The results from this series of ANOVA indicated that microplastic enumerated from the high tide delineation a cross the 15 sites showed there was no significant difference detected in microplastic quantities at the high tide delineation at any of the 15 sites when compared to the three tidal ranges tested ($p = 0.149$) (Table 3). Likewise, the microplastic enumerated from the low tide delineations across the same 15 sites and compared to the same three tidal ranges also showed there was also no significant difference in microplastic quantities at any of the low tide delineations for the 15 sites across the three tidal ranges ($p = 0.604$) (Table 4).

Additionally, the enumerated samples high and low tide delineations were combined and an ANOVA was performed on this combined data to compare the total amount of microplastic enumerated from the 15 sites with the three different tidal ranges in the outer, mid and inner Bay of Fundy respectively. This ANOVA of combined data showed that there was still no significant difference detected in total microplastic quantities at the combined low and high tide samples a cross the three tidal ranges (*p* = 0.160) (Table 5). The *P* value indicates that a larger sample size may provide further clarity on the differences between these variables.

An ANOVA was also performed to determine if any significant differences exist in microplastic quantities between urban and rural locations sampled among the 15 sites. It should be noted that a summary of sites classified as urban or rural locations can be found in Appendix II & Figure 4. The results of this ANOVA indicated that no significant difference was detected in the quantity of microplastics between urban and rural locations among the 15 sites sampled (*p* = 0.132) (Table 6). The *P* value indicates that a larger sample size may provide further clarity on the differences between these variables. It should be noted that rural sites did have more enumerated plastics when compared to the urban sites. The types of plastic found at rural sites did vary from there urban counterparts. Urban site recorded a greater diversity in the types of plastic having more angular, round and irregular shaped pieces than the rural sites, which were mostly fibers with fewer round, angular and irregular shaped pieces.

Lastly, an ANOVA was performed to determine if any significant differences exist in microplastic quantities between exposed and sheltered locations sampled among the 15 sites. It should be noted that a summary of sites classified as exposed or sheltered locations can also be found in Appendix II & Figure 4. The results of this ANOVA indicated that the test did show that there was a significant difference in microplastic quantities between exposed and sheltered sites sampled (p = 0.040) (Table 7). These results indicated that the exposed sites sampled among the 15 sites had significantly more microplastics in the intertidal sediments at combined high and low tide delineations than the combined high and low tide delineations at sheltered sites samples (Table 7). It should be noted that exposed sites did have more enumerated plastics when compared to the sheltered sites. Possibly the result of more exposed sites sampled than sheltered ones. The types of plastic found at exposed sites did differ from the sheltered sites sampled. Exposed site recorded a greater diversity in the types of plastic having more angular, round and irregular shaped pieces than the sheltered sites, which were mostly fibers with fewer round and angular shaped pieces. The sheltered sites sampled also recorded no irregular shaped pieces, which were found at the exposed sites.

Pearson Correlations

A Pearson product-moment correlation was conducted to determine the degree of linear relationship between the quantities of microplastics enumerated form the sediment samples with the high and low tide delineations and ranges at the sample sites. The results from this analysis did not indicate a strong correlation between the quantities of microplastic form the samples with the high and low tide delineations at the sites (Table 8).

A Pearson correlation was also performed for samples of enumerated microplastics from the high tide delineation with the three tidal ranges of outer, mid and inner regions of the bay. The results from this analysis indicated that a strong negative correlation exists between the enumerated microplastics from the high tide delineations with the three tidal ranges tested at the 0.01 level (Table 9). The strong negative correlation was seen specifically between the inner bay site high tide delineations with those of the outer and mid bay high tide delineations (Table 9).

A Pearson correlation was performed for samples of enumerated microplastics from the low tide delineation with the three tidal ranges of outer, mid and inner regions of the bay. The results from this analysis indicated that a positive correlation exists between the enumerated microplastics from the low tide delineations with the three tidal ranges tested at the 0.01 level (Table 10). Again the correlation existed between the inner bay sites low tide delineations with outer and mid bay respectively (Table 10).

Finally, a Pearson correlation was also performed for combined samples of enumerated microplastics from high and low tide delineations with the three tidal ranges of outer, mid and inner regions of the bay. The results from this analysis indicated that a strong positive correlation exists between the enumerated microplastics from the combined high and low tide delineations with the three tidal ranges tested at the 0.01 level (Table 11). The correlation existed between the combined inner bay sites with the mid bay sites (Table 11).

Discussion

It was hypothesized that low energy sheltered marine environments and ones in close proximity to urban population centres would show higher concentration of microplastics in sediments when compared to more exposed sites and ones in more remote locations which were expected to have lower quantities of microplastics in their sediments. This is however not the case and the hypothesis was not supported. The results showed no significant difference in microplastics quantities between urban and remote sites sampled.

Mathalon and Hill, (2014) and Stolte et al. (2015) all found higher amounts of plastic in sediments sampled in urban locations in Halifax Harbour and the German Baltic coast compared to sites sampled in our study. This is also supported by Browne et al. (2011) who sampled shorelines at 18 sites globally and whose results also indicate higher amounts of microplastic in sediments from urban locations when compared to the current study from the Bay of Fundy. Many factors may explain these differences. Firstly, sites in the Bay of Fundy were more widely sampled than the study from Nova Scotia that only sampled three sites in Halifax harbour and thus there is no data available to compare rural sites along the Eastern Nova Scotia coastline with those of the Bay of Fundy or Halifax Harbour. Second, tidal variations between the Bay of Fundy and the Eastern Shore of Nova Scotia are significantly different (Thurston, 2011). This may indicate a potential connection between the Bay's tides and the transport of microplastics within it. Additionally, the salinity in the Bay of Fundy is different than that of Halifax Harbour or the Eastern Shore of Nova Scotia. Salinity in the Bay of Fundy is reported by Drinkwater (1987) as being 30.5 to 32.4 parts per thousand (ppt), which is lower than the Eastern Shore of Nova Scotia, which has a salinity of 33.0 to 35.0 ppt (Tang, & Wang, 1996). This small difference in salinity in the waters Bay may change the flotation characteristics of microplastics so that they may deposit sooner than in other locations with higher salinity. Finally, the sources of microplastics between the regions likely explain some of the differences observed. With both the German coastline (Stolte et al., 2015) and Halifax harbour having larger populations in excess of 200,000 people than those of Saint John at 70,063 (Stats Canada, 2012). This is supported by Browne et al. (2010; 2011) who reported that a positive correlation exists between the concentrations of microplastics in coastal areas and human population density from multiple locations surveyed globally. Given this information it is not surprising that the greater population density of the German coast and Halifax Harbour would create favourable conditions for increased amounts of plastic waste to enter marine environments and generate higher microplastics counts in sediments.

Shelter sites sampled along the New Brunswick coastline were hypothesized to have greater quantities of plastics in the sediments. However, results of the sediment analysis show that sites that had Southeastern or Southwestern exposures to prevailing winds and greater wave action had significantly more microplastics in the samples than those of the sites that were sheltered with less exposure to higher wind and wave action. These results differ to those of Mathalon and Hill (2014) who found no significant difference in microplastic quantities between the sheltered and exposed sites sampled. The authors of the study noted that the close proximity of the sites to each other and the Halifax Regional Municipality (HRM) is likely a factor for the similarity in results between the sites. The authors suggested that a wider sampling area with sites further from the HRM and each other might expose statistical differences (Mathalon & Hill, 2014). This suggestion was supported by the results from the Bay of Fundy where sites were widely distributed and showed a significant difference between sheltered and exposed sites. This difference in microplastic quantities between sheltered and exposed locations may be in part due to the prevailing winds during the sampling period which blow from the southwest and would force marine debris on to the exposed shoreline along the New Brunswick coast to be deposited.

Browne et al. (2010) investigated the role of wind in the deposition of microplastic along shorelines at exposed downwind sites. Their results indicated that ‘there was generally a greater abundance of particles of plastic in sites that were downwind’ (Browne et al., 2010). Their results support the findings of this study, which also found greater quantities of plastic at downwind and exposed sites than in sheltered sites not exposed to prevailing southwesterly winds. Findings from the Bay of Fundy differ from those of Liebezeit and Dubaish (2012), who found high microplastic concentrations in tidal mud flats that were in sheltered location protected from prevailing winds. The difference of Liebezeit and Dubaish (2012) with results from the Bay of Fundy may be the result of the extreme tidal action seen in the bay that may help to flush plastics form sheltered locations and transport them into the Bay where wind and wave action deposit them in higher quantities on downwind and exposed sites. Additionally, as Mathalon and Hill (2014) indicate in their study ‘the exposed beach had the highest microplastic concentrations at the high tide line’. And ‘in this high-energy environment, small, relatively low-density plastics remain in suspension until they are stranded at the upper limit of wave action’ (Mathalon & Hill, 2014). This was not seen in the Bay of Fundy and may indicate that scouring by the amplified tides may keep microplastics suspended in the water column for longer durations. The movement of microplastics by wind, wave and tide is supported by several studies including those of Andrady (2011), Baztan, et al. (2014), Browne et al. (2010), Cole et al. (2011), and Stolte et al. (2015).

Microplastic Composition

The composition of microplastics found along the shoreline of the Bay of Fundy indicated that the majority of the plastic material was fibers followed by angular fragments and round particles respectively. This result is supported by other studies on intertidal sediments. Browne et al., (2010) also reports that 'the majority of fragments of plastic were fibers' at the sites sampled in the Tamar estuary along the channel coast of Cornwall, UK. Further, Hidalgo-Ruz et al. (2012) who conducted a review of microplastic sampling in various media reported that 'most fragments found in subtidal and estuarine sediments were fibers' from the studies review for sampling techniques. Finally, Thompson et al. (2004) reported high concentrations of microplastic fibers in the North Atlantic Ocean. The high amount of fibers in the Bay of Fundy sediments is therefore consistent with the findings of other studies on intertidal sediments from the North Atlantic. As Browne et al. (2010) suggest that this may be due to the density of microplastic fibers in seawater compared to other plastics allowing the fibers to be more readily deposited along shorelines.

The majority of angular and round particles were found at sites exposed to prevailing southwesterly winds and with greater wave action. This again indicates the prominent role that prevailing winds have in the deposition of plastics at down wind locations as highlighted by Browne et al. (2010). Additionally, the heavier particles may be transported to these exposed locations as a result of the wave energy keeping them in suspension for long durations than in more sheltered location where the particles may fall out of suspension sooner with less energy to transport them. The resonance effect of the Bay of Fundy's tides may also contribute to the transport and duration of suspension of plastic particles in the Bay. A further study of the differences in the amount of plastic in the Bays water column in comparison to sediments may shed some light on the role the tides play in microplastic transport in the Bay.

The study by Browne et al. (2010) has reported that wind and plastic density play important roles in the distribution of plastics along the shoreline. This may also partly explain why rural sites did not show a difference from the urban ones in Saint John Harbour. Indeed, the rural location selected on Grand Manan had the highest amount of plastics found in this study with 1740 pieces found in the two 1kg standardized samples. This finding is surprising as the site was located far from urban inputs in a Provincial park and migratory bird sanctuary used by many species. Further, the FT-IR analysis of plastics from this site and others showed it was largely composed of polystyrene, polyethylene, polypropylene and nylon, which are heavily used in packaging and other consumer goods and is also used by both the fishing and aquaculture industries. This finding collaborates with visual inspection of the site taken during sampling where it was noted that a larger amount of debris on the beach was related to uses by these industries. This was not seen in all other locations where macroplastic debris had larger amounts of packaging waste and consumer goods seen on shorelines, which was well demonstrated, at the Mispec site in Saint John Harbour.

Tidal Effects on Microplastic Content

Overall this study found that microplastic quantities between the high and low tide delineations were not significantly different. These results are similar to those of Mathalon & Hill (2014) who also found no significant difference in microplastic concentrations between tidal heights from beaches sampled in Halifax Harbour. However, the authors note that the lack of a difference in concentrations may be the result of the close proximity of the three sites to each other (Mathalon & Hill, 2014). Sites in the Bay of Fundy were spread over 185km of coastline and still showed no significant difference in plastic quantities between the high and low tide delineations. This result is interesting as it was expected that the high tide line where most macroplastic debris accumulates would generate significantly higher amounts of microplastics in the sediment than at low tide locations. This may be the result of the unique tidal phenomenon in the bay that alters how plastics are deposited between tidal delineations. Hidalgo-Ruz et al. (2012) noted that 'in general, microplastics move differently than macroplastics in the sea' and that the 'mechanisms that drive the distribution of microplastics are less well-known'. This result indicates that further research is needed to elucidate the relationship and mechanisms of high and low tide deposition of microplastics in the Bay.

Quantification of Microplastics in the Bay of Fundy

The analysis of microplastics from sites across all three tidal ranges also showed that there was no significant difference in the quantities of microplastics present in intertidal sediments regardless of the tidal delineation or tidal range. This result is also very interesting as it was expected that the greater tidal range seen in the inner bay of Fundy would show significantly less microplastics in the sediments a result of the amplified tidal effects. This result may indicate that other factors such as current, wind and wave action play a more significant role in the distribution of microplastics in intertidal sediments than tidal variation. This notion is supported by Baztan et al. (2014) who reported that 'Floating marine debris is principally carried by wind and ocean currents' to Canary Island beaches. Browne et al. (2010) also investigated wind in the distribution of microplastic on beaches. Their findings 'revealed that wind, size, and density of plastic debris are important factors influencing the spatial distribution of plastic debris'. The unique tides in the bay may therefore assist in transporting microplastics over a wider area by continually churning large sections of the sea floor thereby keeping microplastics suspended in the water column so that wind, current and wave action can readily transport them to exposed downwind sites. Perhaps more sampling sites across the Bays tidal ranges with more exposed and sheltered sample sites that collected data for all seasons to investigate the changing wind patterns at beach locations, a stronger relationship could be obtained between the role of tides, wind and currents in the distributions of microplastics within the Bay of Fundy.

Conclusion

Quantification of Microplastics in the Bay of Fundy

While there is still little known about the concentrations, distribution or effects of microplastics in the Bay of Fundy our findings show that microplastics are widespread along the New Brunswick coastline, in greater quantities on beaches exposed to prevailing southwesterly winds and generally low concentrations when compared to that of other regions, yet of similar composition (Baztan, et al., 2014; Browne et al., 2010; Liebezeit & Dubaish 2012; Mathalon & Hill, 2014; Stolte, et al., 2015). The lack of a significant difference in microplastic quantities at high and low tide delineations and between urban and rural locations (compared to other studies) may reflect the role that the extreme tides have on microplastic transportation. The most interesting question to arise from this study is how significant are the Bay's tides in the transport and deposition of microplastics? The results from this study would indicate that they do potentially impact transport and deposition. However, their significance when compared to other factors such as wind, current or wave action is unknown. I recommend a more comprehensive investigation with larger sample sizes, into microplastics along the New Brunswick coastline to elucidate on the sources, transport and ultimate fate of this material in the Bay.

If this is the case then what effect does the prolonged suspension of these plastic particles have on biota such as filter feeders, fish, whales and seabirds that flock to the Bay? Does this potentially provide a pathway for microplastics to enter local food webs? Certainly more research is needed to elucidate the role of tidal effects and if and how they affect biota to determine what type of relationship if any exists. Additionally, further research should be conducted to determine what kind of threat plastic contamination might be to those whose economic lively hoods are dependent on Bay’s natural resources. Lastly, as this problem is the result of our societies failure to embrace sustainable practices it is this author’s hope that this study will provide a springboard for others and assist in opening dialogs between regulators, scientists, the fishing industry and other stakeholders so that they may work together to find novel solutions to this problem.

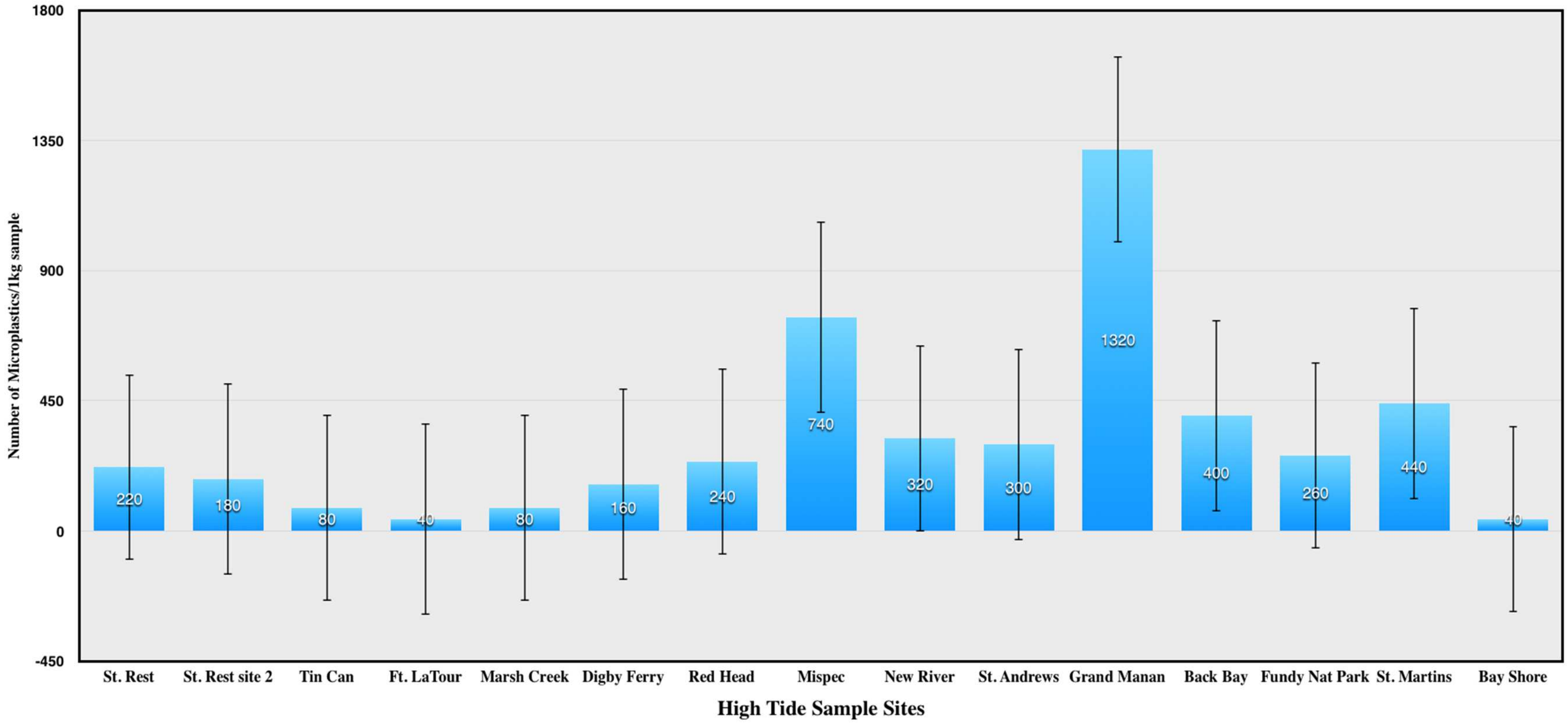

Figure 1: Number of microplastics enumerated form standardized 1 kg samples collected from sediments at high tide delineations from 15 sites along the New Brunswick coastline of the Bay of Fundy. Error bars denoted standard deviation of +/- 331.100 pieces of microplastic per 1 kg of sediment.

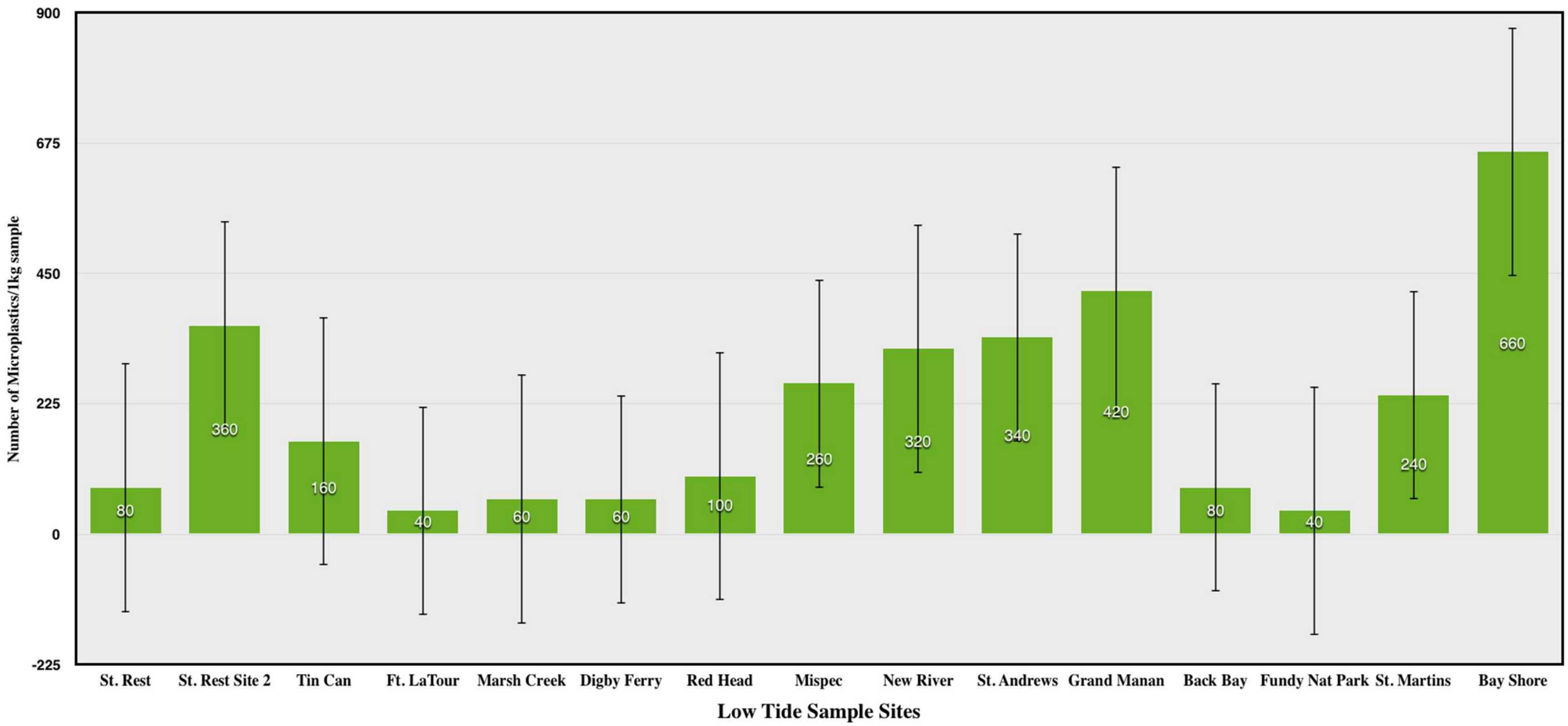

Figure 2: Number of microplastics enumerated form standardized 1 kg samples collected from sediments at Low tide delineations from 15 sites along the New Brunswick coastline of the Bay of Fundy. Error bars denoted standard deviation of +/- 179.756 pieces of microplastic per 1 kg of sediment.

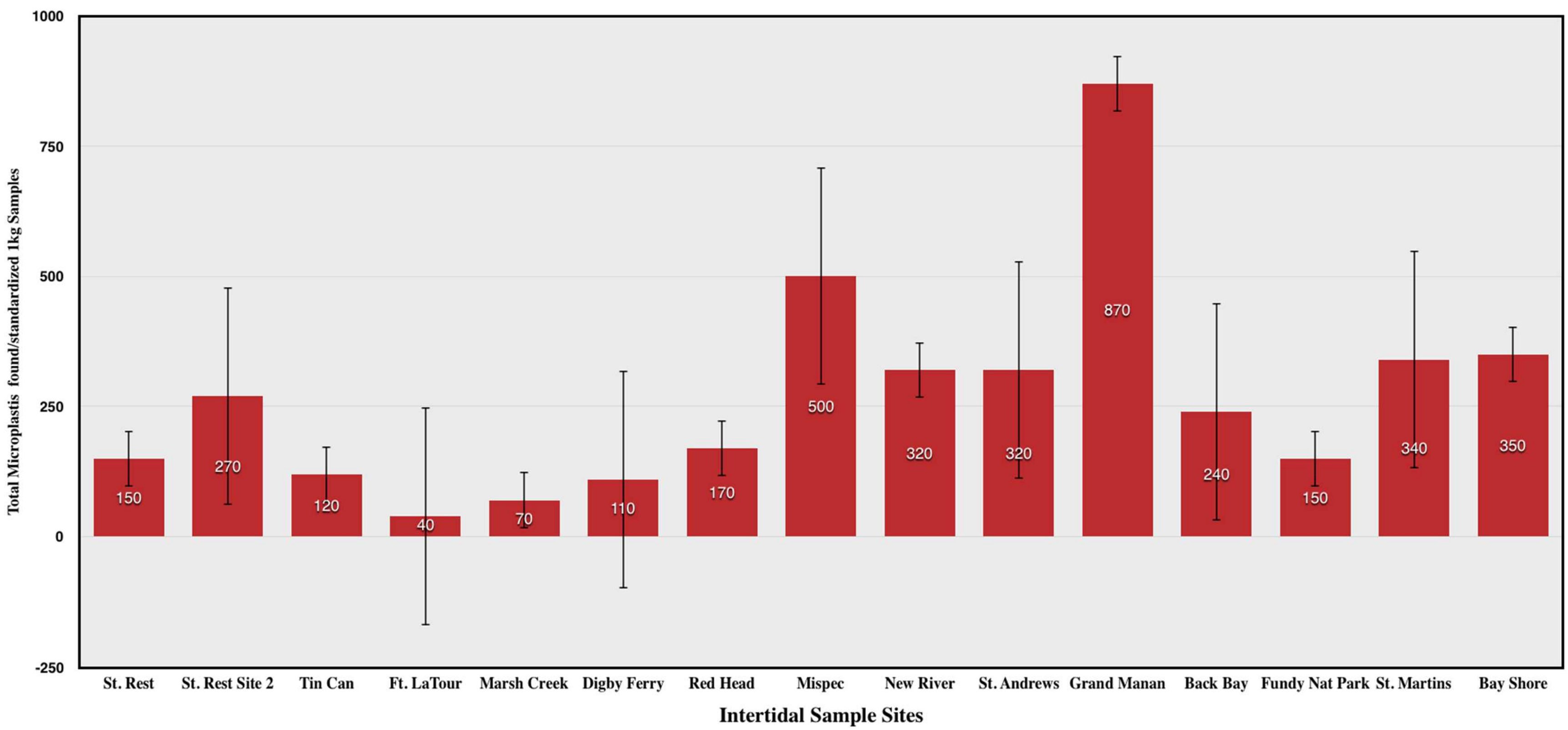

Figure 3: Total number of microplastics enumerated form pooled high and low tide standardized to 1 kg samples collected from intertidal sediments collected at 15 sites along the New Brunswick coastline of the Bay of Fundy. Error bars denoted standard deviation of +/- 208.710 pieces of microplastic per 1 kg of sediment.

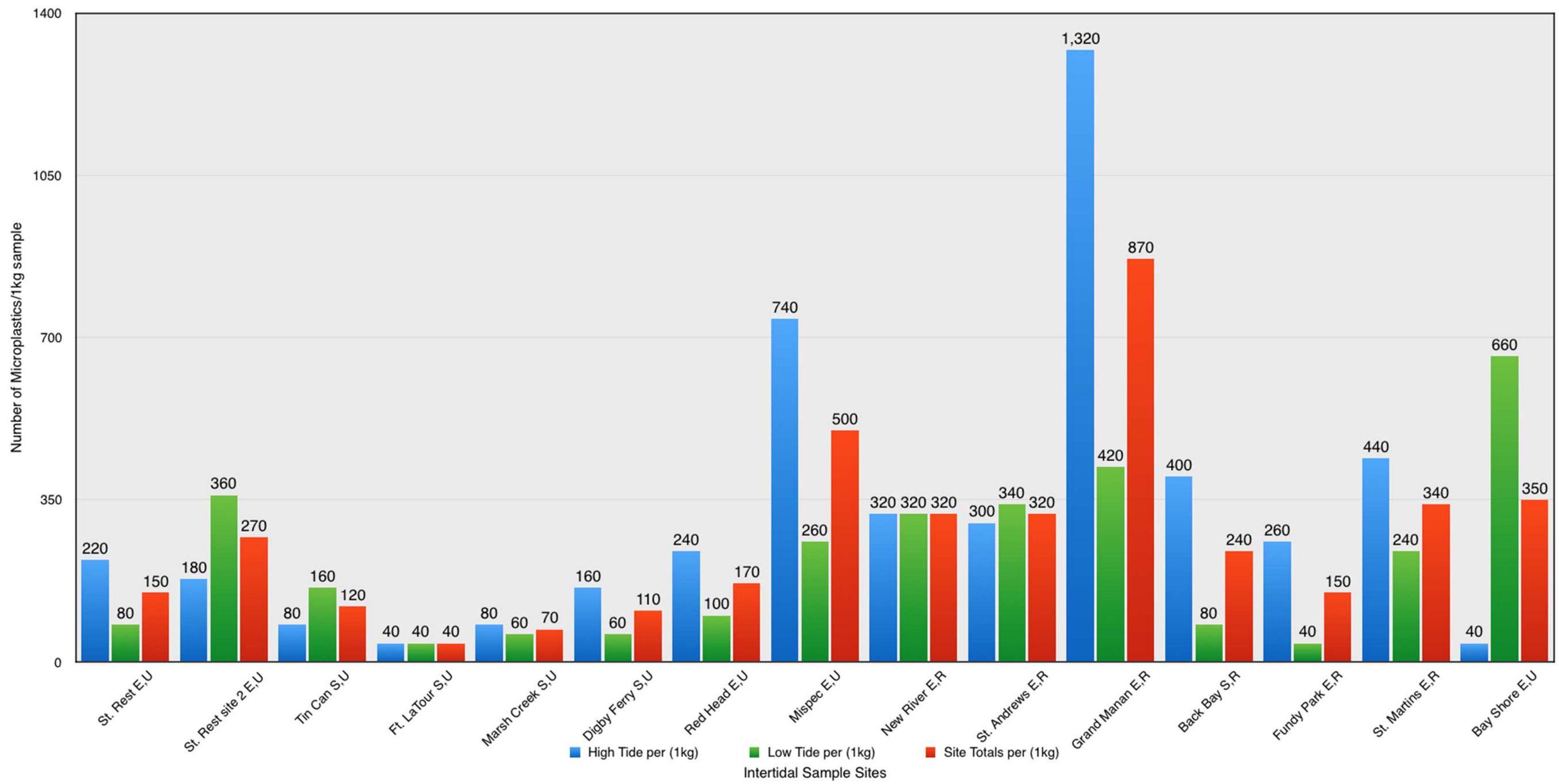

Figure 4: Total number of microplastics enumerated form high tide, low tide and combined site totals standardized to 1 kg samples collected from intertidal sediments collected at 15 sites along the New Brunswick coastline of the Bay of Fundy. Letters after site names denote Exposed (E), Sheltered (S), Urban (U) and Rural (R) site classifications.

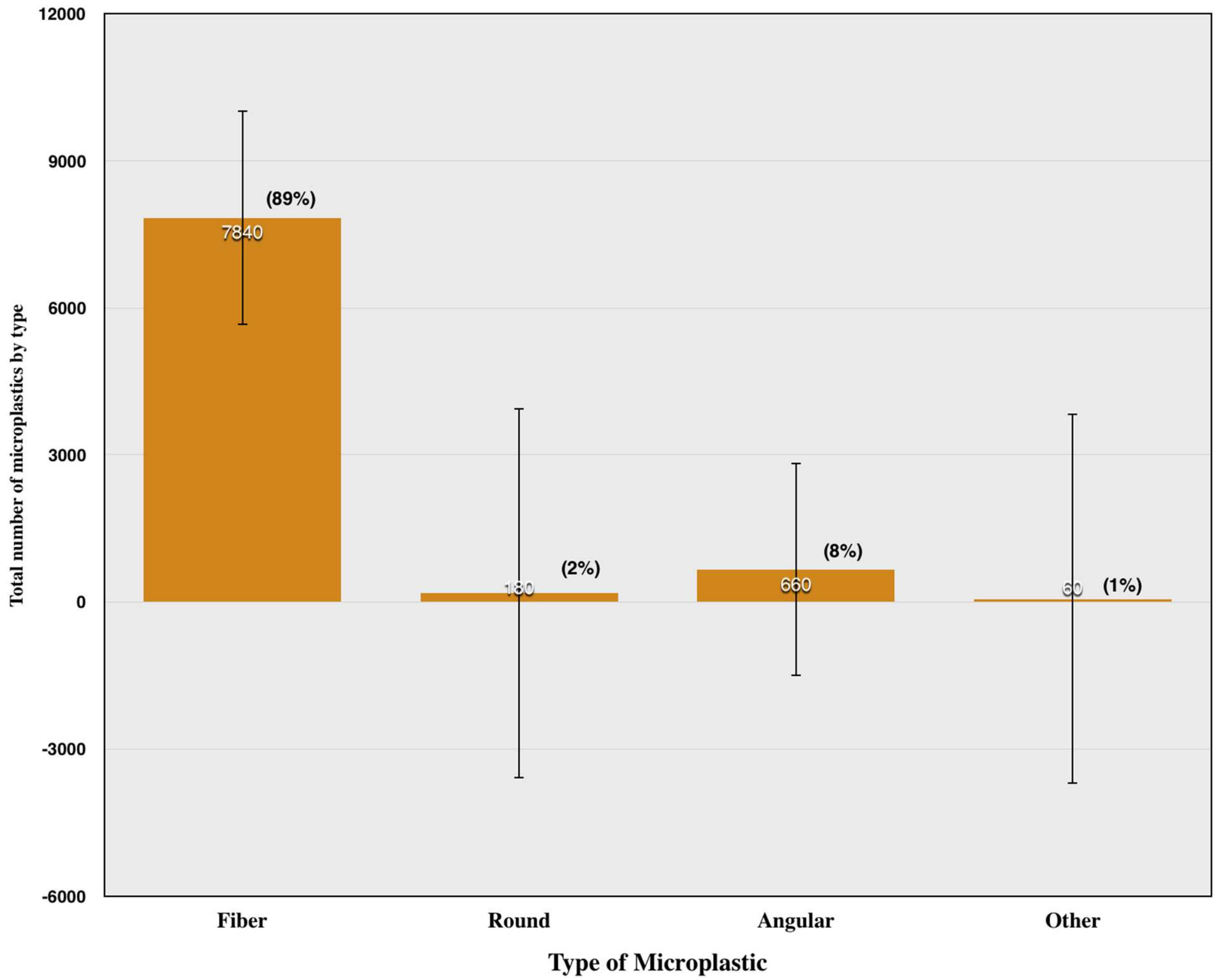

Figure 5: Calculated composition of microplastics enumerated form standardized 1 kg samples collected from sediments at high and low tide delineations from 15 sites along the New Brunswick coastline of the Bay of Fundy. Percentages indicate percent total microplastic composition from all sites. Error bars denoted standard deviation of +/- 3778.902 pieces of microplastic per type.

Table 1: Results from PerkinElmer Spectrum Two FT-IR analysis of microplastics enumerated from sediment samples collected at 15 intertidal sites along the New Brunswick coastline of the Bay of Fundy.

Site	**Type of Material Tested**	**IR Spectra Generated**	**Type of Plastic Indicated**
New River Beach	Fiber	Yes	Polyethylene, Polypropylene,
St. Andrews	Fiber	Yes	Polypropylene, Nylon
Grand Manan	Fiber, Round	Yes	Polystyrene, Polyethylene, polypropylene, nylon
Back Bay	Fiber	Yes	Polyethylene, Polypropylene, Nylon
St. Rest's Beach	Fiber	Yes	Polyethylene, polypropylene, Nylon
St. Rest's Beach Site 2	Fiber	Yes	Polyethylene, Polypropylene
Tin Can Beach	Fiber	Yes	Unknown
Ft. LaTour	Fiber, Angular	Yes	Unknown
Marsh Creek	Fiber, Round	Yes	Unknown
Digby Ferry	Fiber	Yes	Polypropylene
Red Head Beach	Fiber	Yes	Polypropylene
Mispec Beach	Fiber, Angular	Yes	Polystyrene, Polyethylene, Polypropylene
Bay Shore Beach	Fiber, Angular	Yes	Unknown
Fundy National Park	Fiber, Angular	Yes	Polyethylene
St. Martins Beach	Fiber	Yes	Polyethylene, Polypropylene

Table 2: Results from Student T-test on comparison of high vs. low tide quantities of microplastics from 15 intertidal sites along the New Brunswick coastline of the Bay of Fundy.

	df	T	P
High / Low Tide			
Between Groups Within Groups Total	1 28 29	1.096	0.282

Table 3: Results from ANOVA on comparison of outer, mid and inner tidal ranges vs. high tide quantities of microplastics from 15 intertidal sites along the New Brunswick coastline of the Bay of Fundy.

	df	F	P
Tidal Ranges / High Tide			
Between Groups Within Groups Total	2 12 14	2.238	0.149

Table 4: Results from ANOVA on comparison of outer, mid and inner tidal ranges vs. low tide quantities of microplastics from 15 intertidal sites along the New Brunswick coastline of the Bay of Fundy.

	df	F	P
Tidal Ranges / Low Tide			
Between Groups Within Groups Total	2 12 14	0.525	0.604

Table 5: Results from ANOVA on comparison of outer, mid and inner tidal ranges vs. total quantities of microplastics enumerated from high and low delineations collected at 15 intertidal sites along the New Brunswick coastline of the Bay of Fundy. The *P* value indicates that a larger sample size may provide further clarity on the interaction between these variables.

	df	F	P
Tidal Ranges / Total Microplastics			
Between Groups Within Groups Total	2 12 14	2.140	0.160

Table 6: Results from ANOVA comparing microplastics enumerated from urban and rural classified sites from total amounts of plastic per site for intertidal sediment collected along the New Brunswick coastline of the Bay of Fundy. The *P* value indicates that a larger sample size may provide further clarity on the interaction between these variables.

	df	F	P
Urban / Rural sites			
Between Groups Within Groups Total	1 13 14	2.577	0.132

Table 7: Result from ANOVA comparing microplastics enumerated from sheltered and exposed classified sites from total amounts of plastic per site from intertidal sediment collected along the New Brunswick coastline of the Bay of Fundy.

	df	F	P
Sheltered / Exposed sites			
Between Groups Within Groups Total	1 13 14	5.159	0.040

Table 8: Results from Pearson Correlation comparing high vs. low tide quantities of microplastics from 15 intertidal sites along the New Brunswick coastline of the Bay of Fundy.

		High Tide Microplastics	**Low Tide Microplastics**
High Tide Microplastics	**Pearson Correlation**	1	0.271
	Sig. (2-tailed)		0.328
	N	15	15
Low Tide Microplastics	**Pearson Correlation**	0.271	1
	Sig. (2-tailed)	0.328	
	N	15	15

Table 9: Results from Pearson Correlation comparing outer, mid and inner tidal ranges vs. high tide quantities of microplastics enumerated from 15 intertidal sites along the New Brunswick coastline of the Bay of Fundy.

		Outer Bay	**Mid Bay**	**Upper Bay**
Outer Bay High Tide Microplastics	**Pearson Correlation**	1	-0.469	-1.000^{**}
	Sig. (2-tailed)		0.531	.
	N	4	4	2
Mid Bay High Tide Microplastics	**Pearson Correlation**	-0.469	1	-1.000^{**}
	Sig. (2-tailed)	0.531		.
	N	4	9	2
Upper Bay High Tide Microplastics	**Pearson Correlation**	-1.000^{**}	-1.000^{**}	1
	Sig. (2-tailed)	.	.	
	N	2	2	2

***. Correlation is significant at the 0.01 level (2-tailed).*

Table 10: Results from Pearson Correlation comparing outer, mid and inner tidal ranges vs. low tide quantities of microplastics enumerated from 15 intertidal sites along the New Brunswick coastline of the Bay of Fundy.

		Outer Bay	Mid Bay	Upper Bay
Outer Bay Low Tide Microplastics	**Pearson Correlation**	1	0.524	1.000**
	Sig. (2-tailed)		0.476	.
	N	4	4	2
Mid Bay Low Tide Microplastics	**Pearson Correlation**	0.524	1	1.000**
	Sig. (2-tailed)	0.476		.
	N	4	9	2
Upper Bay Low Tide Microplastics	**Pearson Correlation**	1.000**	1.000**	1
	Sig. (2-tailed)	.	.	
	N	2	2	2

***. Correlation is significant at the 0.01 level (2-tailed).*

Table 11: Results from Pearson Correlation comparing outer, mid and inner tidal ranges vs. pooled quantities of microplastics enumerated from high and low tide delineations collected at 15 intertidal sites along the New Brunswick coastline of the Bay of Fundy.

		Outer Bay	Mid Bay	Upper Bay
Outer Bay Total Microplastics	**Pearson Correlation**	1	-0.064	.[a]
	Sig. (2-tailed)		0.936	.
	N	4	4	2
Mid Bay Total Microplastics	**Pearson Correlation**	-0.064	1	1.000**
	Sig. (2-tailed)	0.936		.
	N	4	9	2
Upper Bay Total Microplastics	**Pearson Correlation**	.[a]	1.000**	1
	Sig. (2-tailed)	.	.	
	N	2	2	2

***. Correlation is significant at the 0.01 level (2-tailed). a. Cannot be computed as at least one of the variables is constant.*

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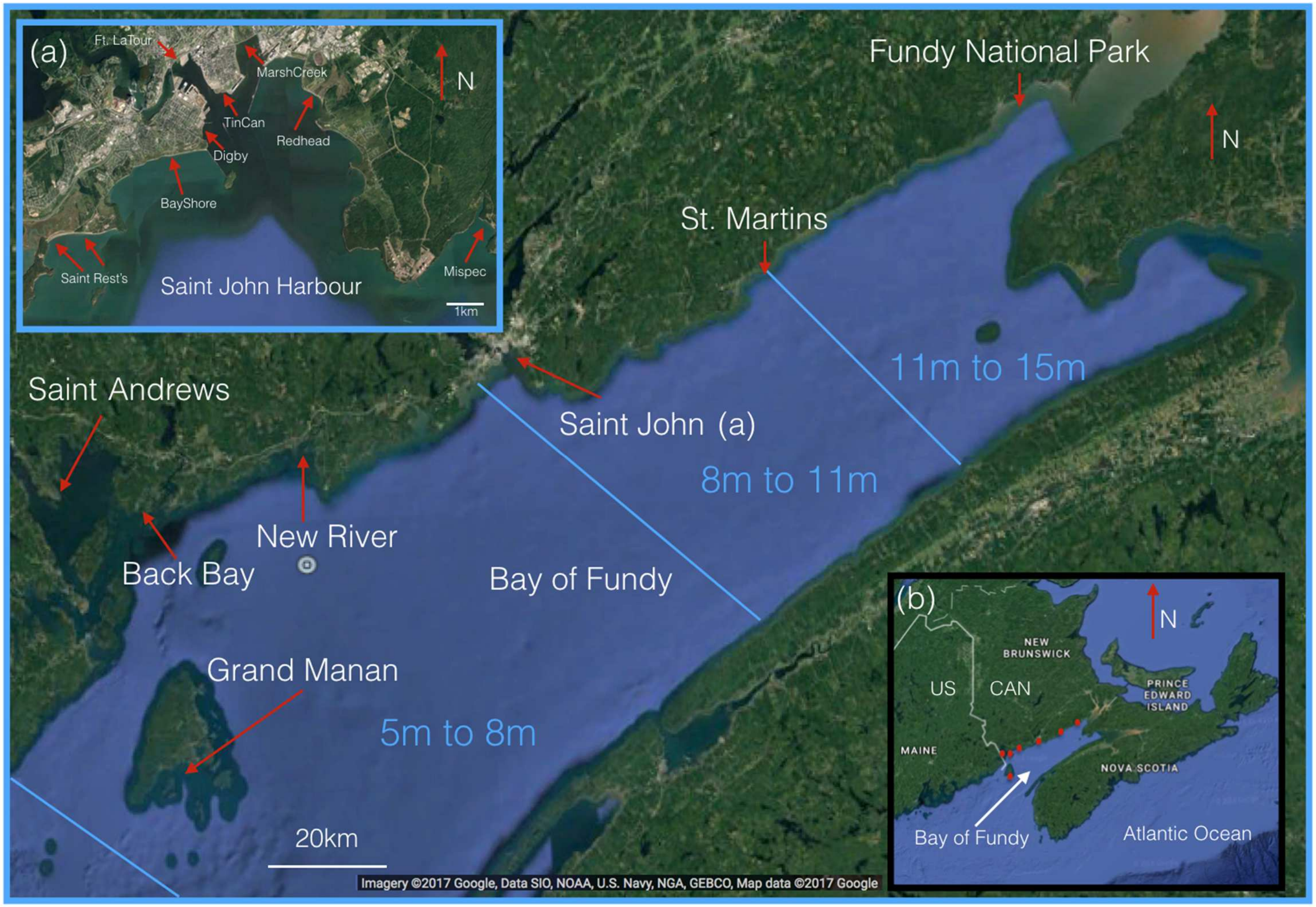
Appendix I. Map of microplastic sampling locations along the Southwestern Coast of New Brunswick in the Bay of Fundy.



Map 1: Indicating the locations of the 15 intertidal sampling sites for microplastics in the outer, mid and inner tidal ranges in the Bay of Fundy found along the Southwestern coast of New Brunswick in Atlantic Canada. (a) Enlarged map indicating locations of sampling sites in Saint John Harbour. (b) Insert highlighting sampling sites and location of Bay of Fundy. Please note that each site was sampled at high and low tide delineations.

Appendix II: Summary of microplastic site data

Chart 1: Summary of results for microplastics sampled from sediments collected at 15 intertidal sites along the New Brunswick coast of the Bay of Fundy. Enumerated results standardized to 1kg.

	Site	GPS Coordinates (DDM)	Site Exposure	Rural /Urban	Enumerated microplastics High Tide (1kg)	Enumerated microplastics Low Tide (1kg)	Total Enumerated microplastics per site (2kg)
Outer Bay	New River Beach	45^{0}08.105’N, 66^{0}31.923’W	Exposed	Rural	320	320	640
	St. Andrews	45^{0}04.124’N, 67^{0}02.309’W	Exposed	Rural	300	340	640
	Grand Manan	44^{0}39.568’N, 66^{0}47.937’W	Exposed	Rural	1320	420	1740
	Back Bay	45^{0}02.504’N, 66^{0}53.304’W	Sheltered	Rural	400	80	480
Mid Bay	St. Rest’s Beach	45^{0}13.411’N, 66^{0}07.608’W	Exposed	Urban	200	80	300
	St. Rest’s Beach Site 2	45^{0}13.527’N, 66^{0}07.065’W	Exposed	Urban	180	360	468
	Tin Can Beach	45^{0}15.781’N, 66^{0}03.253’W	Sheltered	Urban	80	160	240

Chart 1: (Continued) Summary of results for microplastics sampled from sediments collected at 15 intertidal sites along the New Brunswick coast of the Bay of Fundy. Enumerated results standardized to 1kg.

	Site	GPS Coordinates (DDM)	Site Exposure	Rural /Urban	Enumerated microplastics High Tide	Enumerated microplastics Low Tide	Total Enumerated microplastics per site
Mid Bay	Ft. LaTour	$45^0$16.359'N, $66^0$04.313'W	Sheltered	Urban	40	40	80
	Marsh Creek	$45^0$16.582'N, $66^0$02.819'W	Sheltered	Urban	80	60	140
	Digby Ferry	$45^0$15.193'N, $66^0$03.836'W	Sheltered	Urban	160	60	220
	Red Head Beach	$45^0$15.602'N, $66^0$00.966'W	Exposed	Urban	240	100	340
	Mispec Beach	$45^0$13.254'N, $66^0$56.977'W	Exposed	Urban	740	260	1000
	Bay Shore Beach	$45^0$15.757'N, $65^0$04.621'W	Exposed	Urban	40	660	700
Inner Bay	Fundy National Park	$45^0$35.917'N, $64^0$56.794'W	Exposed	Rural	260	40	300
	St. Martins Beach	$45^0$21.503'N, $65^0$31.551'W	Exposed	Rural	440	240	680

Appendix III: Summary of microplastic composition data

Chart 2: Summary of results for composition of microplastics sampled from sediments collected at 15 intertidal sites along the New Brunswick coast of the Bay of Fundy standardized to 1kg.

Site	Fiber		Round		Angular		Irregular	
	High	**Low**	**High**	**Low**	**High**	**Low**	**High**	**Low**
New River Beach	320	320	0	0	0	0	0	0
St. Andrews	280	320	0	0	20	0	0	0
Grand Manan	1180	400	140	0	0	0	0	20
Back Bay	380	80	20	0	0	0	0	0
St. Rest's Beach	220	800	0	0	0	20	0	20
St. Rest's Beach site 2	160	360	0	0	0	0	0	0
Tin Can Beach	80	160	0	0	0	0	0	0
Ft. LaTour	40	20	0	0	0	20	0	0
Marsh Creek	60	40	0	20	0	0	0	0
Digby Ferry	160	60	0	0	0	0	0	0
Red Head Beach	240	100	0	0	0	0	0	0
Mispec Beach	440	240	0	0	300	0	0	20
Bay Shore Beach	40	380	0	0	0	280	0	0
Fundy National Park	240	40	0	0	20	0	0	0
St. Martins Beach	440	240	0	0	0	0	0	0
Total	**4280**	**3560**	**160**	**20**	**340**	**320**	**0**	**60**

Appendix IV: Photos of enumerated microplastic

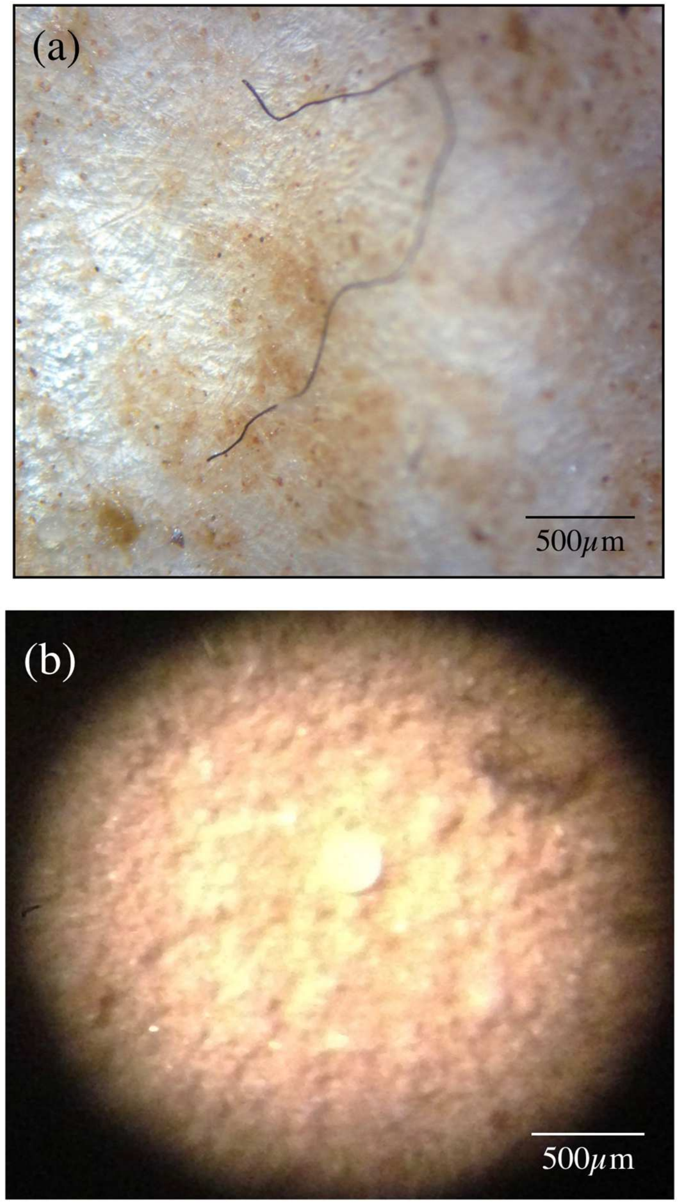


Photo 1: (a) Microplastic fiber extracted form sediment sample collected at St. Andrews, New Brunswick Canada. (b) Microbead extracted form sediment sample collected from Saint John Harbour, New Brunswick Canada.