

## Preliminary Analysis of Identifying Microplastic Polymers Using FTIR Spectroscopy from Surface Waters in the Pacific Northwest

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<b>Major:</b>	Biomedical Sciences
<b>Abstract:</b>	<p>Microplastics (MP) are synthetic polymers &lt; 5 mm in length. They have been found in a range of aquatic environments and inside the organs of human and aquatic species. There have been a number of studies surveying waterways for MP presence and abundance using microscopic analysis, however there is limited research findings on MP polymer identification. For this project, surface water samples were collected from 5 sites in the Pacific Northwest, extraction was done using wet peroxide oxidation, and Fourier Transformed Infrared (FTIR) spectroscopy was used to identify the polymers. Microplastics were found at all 5 sites, with the dominant polymer being polyethylene (&gt; 65%), fragment was the most common morphology (&gt; 74%), black was the most common color (<math>\geq</math> 33%), and the most common lengths were below 1 mm (&gt;50%). Understanding the MP composition in surface waters can help direct policies related to reduction of plastic pollution in aquatic environments. Furthermore, knowing the chemical composition of polymers can help researchers understand how MPs influence the physiology and ecology of biota. Future research should investigate if spatiotemporal variables influence the results.</p>
<b>Key Words:</b>	Microplastics, FTIR, Surface waters, Pacific Northwest, Pollution, marine, contamination, microparticles, spectroscopy, Salish Sea, Puget Sound, polyethylene
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This research project was done as my undergraduate thesis at the University of Washington Tacoma's School of Interdisciplinary Arts and Sciences. As a student research assistant to Professor Julie Masura, I had the opportunity to be the first to conduct preliminary research identifying microplastic polymers found in surface water samples using Fourier Transformed Infrared spectroscopy. The archived samples I used for analysis were solids extracted from surface water samples by former UWT students Kaleigh Faulhaber and Madison Drescher. The motive for the study was to create a methodology that students in the Microplastics Research Lab at UWT could use following the extraction of microplastics from sediments and water samples.

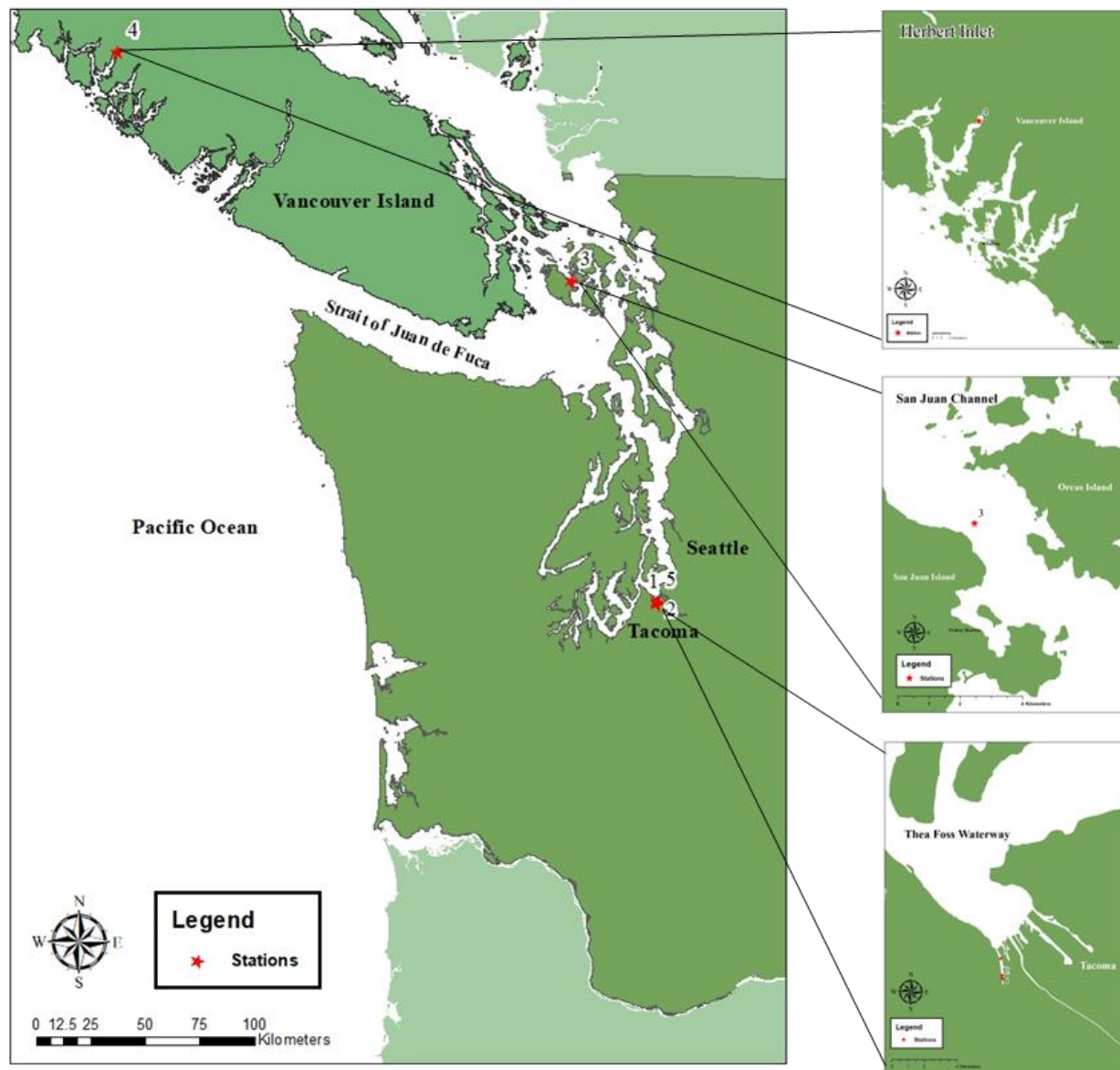
## 1.0 INTRODUCTION

At least 14 million tons of plastic are projected to end up in the ocean each year (IUCN 2021). Typically, plastics enter aquatic environments from land-based sources, however, they can be directly deposited into waterways from marine vessels. There are two types of microplastic pollutants, primary and secondary microplastics. Primary plastics are synthetic polymers, such as plastic pellets, that have been synthesized for industry use. Meanwhile, secondary plastics are the byproduct of primary plastics that have been broken down into smaller fragments due to natural weathering after entering marine environments (Zhang et al. 2020). Secondary plastics do not have to be fragmented in water to be considered secondary plastics. They can also be fragmented due to mechanical abrasion on land and eventually end up in local waterways. Examples of this include tire or clothing abrasion. Microplastics (MP) in the ocean can originate from primary or secondary sources and are identified as synthetic polymers that are < 5 mm in length (NOAA 2023). The transportation of primary or secondary plastics from land sources into the ocean can be due to wind, stormwater runoff, or direct pollution from anthropogenic activities near waterways. Researchers highlight the importance that rivers have on the transportation of plastics from land to the ocean (Wang et al. 2021; He et al. 2020; Blettler et al. 2018).

Microplastics have been found in all aquatic ecosystems. Research studies have found evidence of ingested MPs inside marine biota (Jung et al. 2018; Lefebvre et al. 2019) as well as human organs (Jenner et al. 2022; Ragusa et al. 2021). In vivo experiments have shown that MPs negatively influence the physiology of organisms (Banaei et al. 2022; An et al. 2021). Moreover, trophic transfer has been previously found to be a threat to ecological interactions of marine organisms across different regions of the water column (Nelms et al. 2018; Hasegawa & Nakaoka 2021; Varg et al. 2022; Hasan et al. 2023).

Much of the research in the Pacific Northwest focuses on quantifying the concentration of MPs in different regions of waterways and sediment samples as well as comparing how spatial and temporal factors influence the MP concentrations (Mahoney 2017; Hall 2021; Harris et al. 2022; Moats 2019). However, minimal research studies investigate the polymer composition present in their samples. Identifying the types of polymers that compose the microplastic pollution in the ocean is vital because it can inform us of the effect MPs will have on these ecosystems. For example, polymers of lighter mass may tend to be located on the surface of the water column, and this may signify that marine life that is endemic to the surface of the water column may be the most affected by such polymers.

In this research study, randomly selected archived microplastic environmental samples at the University of Washington Tacoma were obtained for analysis and taken from 5 sites in the Pacific Northwest in 2015 (figure 1). The purpose of this study was to analyze the extracted solids using Fourier-Transformed Infrared spectroscopy (FTIR) to determine the polymer composition. The hypothesis was that the polymers most abundant in the samples will be single-use plastic polymers such as polyethylene and polypropylene. This hypothesis is supported by previous research findings in the Pacific Northwest that have shown evidence of polyethylene polymers being most abundant (Harris et. al 2022). Additionally, statistics of plastic production



**Figure 1. Overview of field collection sites in the Pacific Northwest (PNW). Five total samples were obtained and analyzed from surface waters of the PNW. Station 4, the furthest northern site, was in Vancouver Island (VI), British Columbia, Canada.**

from 2015, the year samples from this project were obtained, further support the hypothesis. In 2015, packaging accounted for the dominant primary plastic production, with 146 million tons of plastic packaging produced globally (OWID 2022). Plastic polymers typically used for packaging are made from polyethylene or polypropylene, which accounted for 149 and 68 million tons respectively of plastic polymers produced in 2015 (OWID 2022). The results from this research study could potentially help understand the origin of plastics and in turn influence policies that aim to reduce plastic pollution. In addition, the results could provide a basis for toxicologists in the Pacific Northwest that are doing research on how marine organisms are being impacted physiologically by microplastics.

## 2.0 REVIEW OF LITERATURE

The first synthetic resins were created in the early 1900's, however synthetic polymers did not become widely used until 1976 (PE 2023a). Evidence of MPs in the environment was first noted in 1972 in the Sargasso Sea surface (Carpenter & Smith 1972). Plastic polymers are synthesized and polymerized from naturally occurring organic compounds that are high in hydrocarbons such as cellulose or crude oil. Plastic polymers can be broken down into thermoplastics and thermosets. The important difference between both types of polymers is that thermosets cannot be softened once molded meanwhile thermoplastics can (PE 2023b). This means that thermosets cannot be recycled but can be re-purposed.

### 2.1 MICROPLASTIC ABUNDANCE, DISTRIBUTION, & CHARACTERIZATION

#### 2.1.1 Research in the Salish Sea

Much of the research on microplastic abundance and distribution in the Salish Sea has been conducted by undergraduate students and is unpublished work. However, Harris et al. (2022) published the most recent research article on MPs based in Seattle's Elliot Bay. In Salish Sea-based research, MPs have been found in surface water samples (Mahoney 2017; Hall 2021; Harris et al. 2022; Moats 2019) and beach sediment samples (Eshom-Arzadon 2017). The primary morphology found in three research studies were fibers (Harris et al. 2022; Eshom-Arzadon 2017; Hall 2021). In research by Mahoney 2017, fibers were the second most dominant morphology found, with Styrofoam being the most dominant.

In Elliot Bay, Harris et al.'s (2022) water samples had up to  $6.4 \times 10^{-1}$  microparticles\*L<sup>-1</sup>. In Hall 2021, the concentrations differed by year and location (2017-2018), with an increase observed in 2018 of up to  $1.0 \times 10^1$  plastics\*L<sup>-1</sup> within Puyallup River waterways. Meanwhile in Mahoney's research (2017), they found an average of  $9.2 \times 10^{-5}$  plastics\*L<sup>-1</sup> in the Whidbey Basin and  $3.6 \times 10^{-4}$  plastics\*L<sup>-1</sup> in the Hood Canal. Research by Eshom-Arzadon (2017) quantified concentrations of microplastics from beach sediments using area and found a total of 1,776 microplastics/m<sup>2</sup> across 12 sites, with sediments closer to Seattle being the most concentrated.

Most of the Salish Sea research findings had some correlation between high population density and MP concentrations. Research by Harris et al. (2022) showed a decline in MP concentrations related to anthropogenic activity. The research took place during 2019 and 2020, so COVID-19 societal influences were observed. MP concentrations were higher prior to April 2020. During March 2020, a state-wide stay at home order was issued by the Washington state governor (WGJI 2020). Post-April 2020, with low tourism and low anthropogenic activities near the Seattle waterfront, MP concentrations decreased. Meanwhile, research by Mahoney 2017 found no statistical correlation between population density and MP concentration, but instead found a correlation between basin residence time and MP concentration. Research by Moats 2019 had similar findings. Concentrations of MPs were consistently higher in the Hood Canal, a region with low population density, over other field sites with higher population density, suggesting that residence time influences MP concentrations over population density.

There are minimal research publications on plastic polymer identification from the Salish Sea. Harris et al. (2022) found that the most common microparticle overall (any particle,

polymer, or not, < 5 mm) to be cellulose with polyethylene terephthalate (PET) being the most common synthetic microparticle found in Elliot Bay surface water samples.

### 2.1.2 Research outside of the Salish Sea

Fish, water, and sediment samples taken from Vancouver Island, British Columbia were analyzed by Collicut et al. (2019). The researchers found microplastics present in juvenile Chinook salmon tissue, with fibers being the most common morphology. However, they noted that there was no significant difference between MP concentrations in salmon tissue while compared to water. However, sediment samples had variable significant differences in MP concentrations depending on the location they were taken from.

Talbot et al. (2022) collected surface water samples in river sites surrounding Portland, Oregon. The main findings showed that MP concentrations peaked in August and February with polyethylene being the most common polymer found at all sites. The researchers linked MP concentrations to being higher in August due to lower average water velocities, leading to accumulation of MPs. Elevated MP concentrations in February were linked to rainfall that occurred 24 hours prior to collecting data. On the opposite side of the country, Yonkos et al. (2014) found a significant positive correlation between MP concentrations and population density in Chesapeake Bay. Random sampling of microplastics using Raman micro spectroscopy (RMS) found the presence of polyethylene peaks, meaning that polyethylene polymers were present in Yonkos et al.'s (2014) samples. However, no thorough analysis was done on plastic polymer identification. Another research study collected sediments from waterways that connect to the Atlantic Ocean and found the most common morphology were fragments with polyethylene: propylene (PEP) being the most common polymer found (Wilkens et al. 2020).

In the Brisbane River of Australia, sediments have been found to be more concentrated in residential sites with a range of approximately 33 to 45 mg\*kg<sup>-1</sup> whereas industrial areas had approximately 25 mg\*kg<sup>-1</sup>, and commercial sites had approximately 23 mg\*kg<sup>-1</sup> (He et al. 2020). Low concentrations of MPs were found during the dry season and were highest during wet seasons. This is consistent with samples collected in Portland by Talbot et al. (2022). The most common polymers found in Brisbane River sediments overall was polyethylene. Polyethylene being the dominant polymer found in samples was a common theme in research studies that identify MPs using spectroscopy methods (He et al. 2020; Wilkens et al. 2020; Harris et al. 2022).

## 2.2 ENVIRONMENTAL IMPACTS OF MICROPLASTICS

### 2.2.1 Characterization of polymers found in marine animals

One primary concern of MPs is the potential bioaccumulation of plastics in aquatic animals. Several research studies have shown that MPs are indeed ingested by marine life (Jung et al. 2018; Lefebvre et al. 2019; Donohue et al. 2019; Martinelli et al. 2020; Baechler et al. 2020; Collicut et al. 2019). MPs have been found in zooplankton (Kosore et al. 2018) up to large mammals (Donohue et al. 2019). However, the spectrum of how many MPs are required to cause harm to an organism is unclear. MPs have been found in shellfish from the Salish Sea (Baechler et al. 2020; Martinelli et al. 2020). Martinelli et al. (2020) found that out of all microparticles found in oysters collected in the Salish Sea, only about 2% were synthetic polymers, leading the

researchers to suggest that retention of MPs is low in oysters. In both studies, one of the polymers found in random-sampling analysis was polyethylene. However, no thorough analysis of MP polymer identification per sample was done in either study.

Research studies outside of the Salish Sea have also found and characterized microplastics ingested by marine animals. In Hawaii, the polymer that was found to be most ingested by pelagic Pacific Sea turtles was low density polyethylene (LDPE) (Jung et al. 2018). Another study from the Northwest Mediterranean Sea found that only 23% of the small pelagic fish sampled (n=168) had ingested MPs. The most common polymer identified was polyethylene terephthalate (PET) (Lefebvre et al. 2019).

### 2.2.2 Potential physiological impacts of microplastics

Research on the health impacts of MP ingestion is limited to controlled laboratory settings. For humans and other animals, the primary source of ingestion of MPs is through the gastrointestinal tract (Yuan et al. 2022). This can be through food or water uptake. In section 2.2.1, the presence of MPs in aquatic animals was discussed, particularly shellfish, commonly consumed by humans and other animals. MPs have also been found in public water fountains (Shruti et al. 2020) and water bottles (Zuccarello et al. 2019). Most recently, a third exposure pathway was found to be through inhalation. Jenner et al. (2022) found evidence of MPs in human lung tissue, with fibers being the most common morphology. Polypropylene and polyethylene terephthalate were the most abundant MPs in the lung tissue sampled. In 2021, research by Ragusa et al. found microplastics in human placenta for the first time, with 3 out of 12 MPs being identified as polypropylene. The researchers noted that identification of the other polymers was not possible.

Goodman et al. (2021) conducted the first research study on the impact polystyrene-MPs (PS-MP) have on alveolar cell lines. The researchers found that PS-MP reduces cell proliferation, however, there was no significant evidence of cell viability decreasing with polystyrene exposure. However, there were changes in cell morphology after 72-hour exposure of PS-MPs. With the subject of MPs being relatively new, research on the physiological impact MPs have on the human body is very low.

Most of the research on the physiological impacts that come with MP exposure is conducted on non-human animals. A controlled research study by Banaei et al. (2022) using common carps (*Cyprinus carpio*) found that high density polyethylene (HDPE) ended up in the liver after ingestion. The exposure of HDPE at different concentrations was found to have significant changes in gene expression of *Mt2* and *Ces2*. Another research study by An et al. (2021) found PS-MPs to impact the reproductive system of female rats. Specifically, granulosa cells were found to have high levels of reactive oxygen species (ROS) after PS-MP exposure. High ROS species are highly reactive and can interfere with basic cell functions.

### 2.2.3 Ecological impacts and how they vary by polymer chemical properties

Knowing the chemical composition of microplastic polymers can help identify the stratification of MPs in the water column and in turn, the potential ecological impacts. Density is an important variable that can come from MP identification, because the density can tell us where the polymers will end up and how they can be transported. For example, an item like a

PVC pipe (high in density) can sink down the water column meanwhile a plastic bag made of polyethylene (low in density) will typically float for most of its lifetime, making its way to the ocean floor after a length of time.

The density of the materials can tell us what the transportation routes of polymers are during initial exposure to marine environments. Stratification of MPs in the water column is important because different organisms live in different zones of the water column which can lead researchers to infer what species will be affected the most by certain MPs. In Jung et al. (2018), they found that polyethylene (PE) and polypropylene (PP) were the polymers that were most ingested by pelagic phase Pacific Sea turtles. Their findings also suggest that species of turtles that feed at shallower depths were more likely to ingest low density PP and PE. Polymers for single use plastics like PE and PP have been the most found polymers in research studies mentioned in this literature review. However, there is minimal understanding on the differences between MPs ingested by biota that are endemic to certain regions of the water column and how foraging ecology influences the ingestion of MPs. More specifically, it is unknown if single use plastics are most found in marine animals due to their high buoyancy capacity or if it is because they are the most abundant in marine environments. As mentioned in section 1.0, single-use plastic materials are the most produced and used. What is currently known is that MPs have a potential to be transferred via trophic levels (Nelms et al. 2018; Hasegawa & Nakaoka 2021; Varg et al. 2022; Hasan et al. 2023). To add on, chemical composition of polymers and its role in ecology may be further complicated by sediment entrapment of MPs.

This literature review highlighted some of the latest findings on microplastic abundance and distribution patterns, the physiological impacts, and the potential ecological impacts. Research on microplastic abundance and distribution is the most common type of study conducted in the field. However, a big gap in research based in the Salish Sea and other regions is identifying the polymers by their name using FTIR, Raman micro spectroscopy (RMS), or other spectroscopy instruments. Identifying the MPs found in our waterways is important because by knowing the source of the pollutant, we can limit production or use of the source. Moreover, knowing polymer composition by water column regions can tell us about the stratification patterns, and in turn, how ecological impacts may arise. The research on the health impacts of MP ingestion is likely the most important missing piece on the microplastic issue and one that the public is likely the most curious about. As presented in section 2.2.2, all the research on MP effects on physiology is limited to laboratory settings and no true evidence of MPs negatively influencing human health has been found outside of a laboratory setting conducted on cell lines or animal models. More importantly, research on how long MPs stay in an animal's body after ingestion and if it is long enough to cause systematic physiological damage is currently unknown. With the topic of microplastics in aquatic ecosystems still being relatively new, progress in the field is to be expected in the upcoming years.

### **3.0 METHODS**

#### **3.1 LOCATION OF STUDY**

The field sites for this research study were in the Salish Sea and British Columbia's Clayoquot Sound located on the west coast of Vancouver Island (figure 1). Three of the samples were taken from Thea Foss Waterway, an industrial channel near Commencement Bay in

Tacoma, WA within Tacoma Tide Flats. The flats have been historically industrialized and are high in population density in comparison to the other 2 sites where samples were obtained from (figure 2). As of 2020, Tacoma's population density is 1,703.53 people per km<sup>2</sup> (USCB 2022a). One sample was taken from the San Juan channel, adjacent the San Juan Islands (figure 3). San Juan County is made up of an archipelago low in population density with 39.5 people per km<sup>2</sup> residing in the county as of 2020 (USCB 2022b). The final region where one sample was obtained from was in the Herbert Inlet which is in a region that is a protected biosphere (figure 4). Herbert Inlet makes up part of Clayoquot Sound and is closest to Tofino, a town with a population density of 238.3 people per km<sup>2</sup> (Statistics Canada 2022a) and Ucluelet, a town with a population density of 318.8 people per km<sup>2</sup> (Statistics Canada 2022b).

The origin of all 3 sites in which the water samples were obtained are part of the Pacific Northwest. Therefore, the geography is homogenous with temperate rainforests, mountains, and coastlines in which many flora, fauna, and aquatic biota reside in. However, Anthropocene activity that may influence results varies by location, with the site in Tacoma being the most populated and industrialized. The site numbers for all figures were based on when the samples were collected. See table 1 for details of when and where each sample was collected.

### 3.2 FIELD SAMPLING AND EXTRACTION

For this project, microparticles will be the term used to refer to all solids extracted from the water samples whether they are synthetic plastic polymers, plastic additives, or non-polymer microparticles. For convenience, polymers will be the term used when discussing microplastic polymers. Non-polymer microparticles are solids with spectrums that do not indicate they are of synthetic plastic polymer origin or derive from synthetic polymer origin in the form of plastic additives. All microparticles scanned were obtained from polymer extractions in previous research studies at the Microplastics Monitoring Laboratory at the University of Washington Tacoma. A total of 5 vials were randomly selected for FTIR spectroscopy analysis.

Samples for this project were collected from surface waters of the Pacific Northwest using a manta net. Refer to Table 1 for coordinates and dates of sample collection. Contents from the net were sieved between 0.33 and 5 mm and stored in a glass jar at 4°C until processed in the lab. Extraction of suspected polymers was done using NOAA's water sample extraction protocol (Masura et al. 2015). The suspended solids were sieved once more in the lab and then placed in a

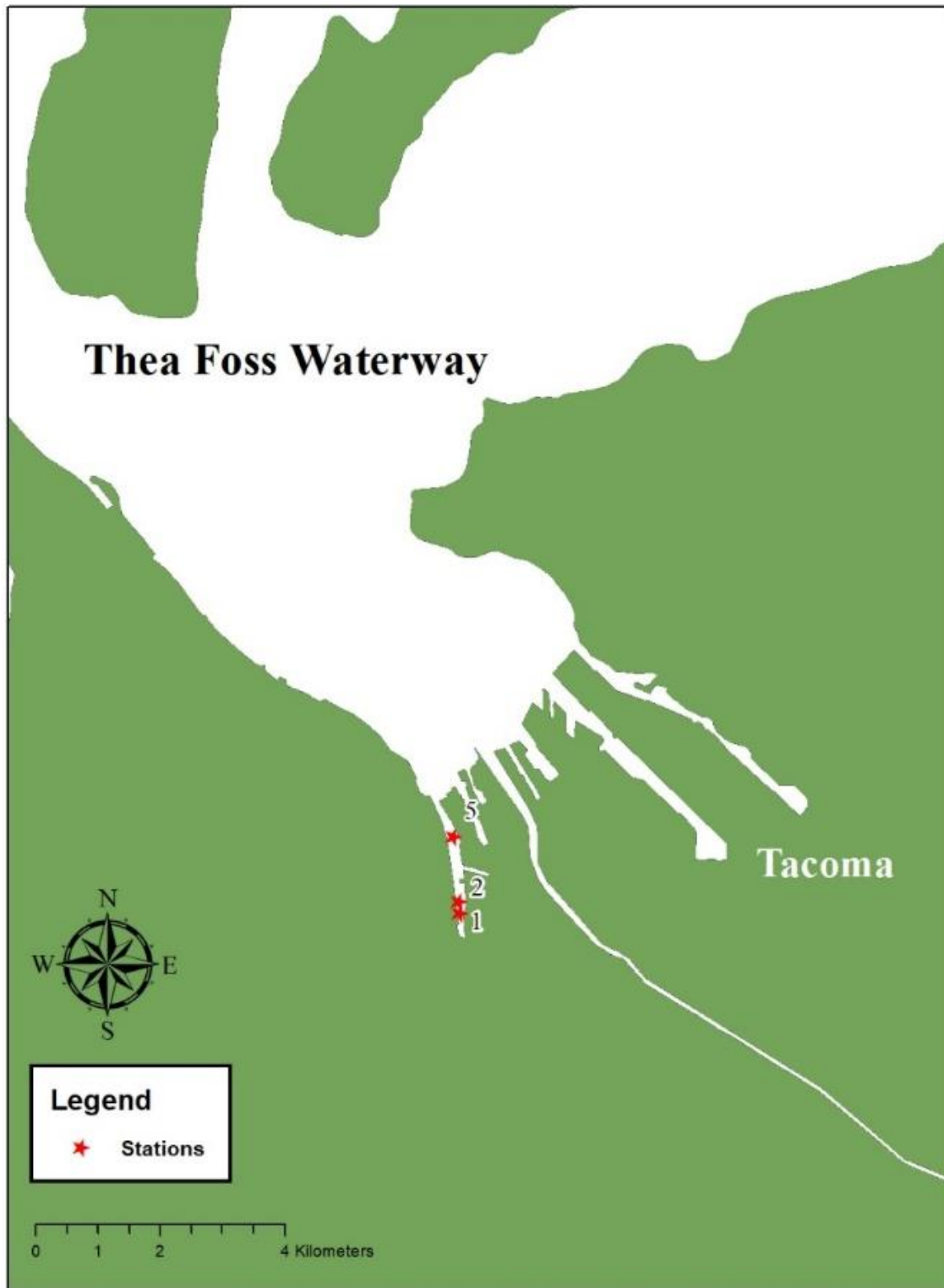


Figure 2. Map of Thea Foss Waterway in Commencement Bay. Three surface water samples were obtained from the Thea Foss Waterway in Tacoma, Washington. 1 taken 1st & 5 taken last.

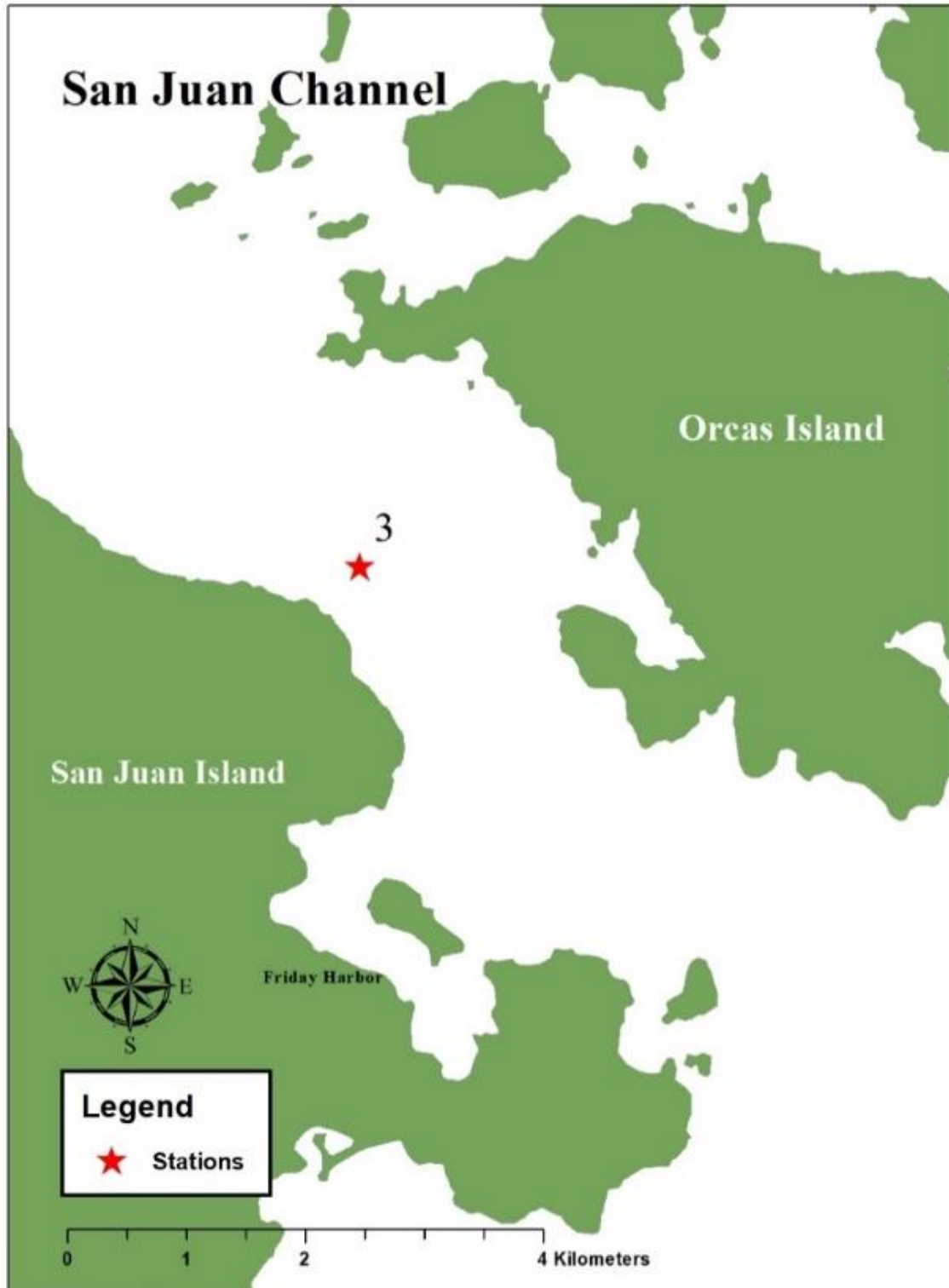


Figure 3. Map of San Juan channel in the San Juan Islands. Only one sample was taken for analysis in this station. Station number corresponds to date of field collections taken.

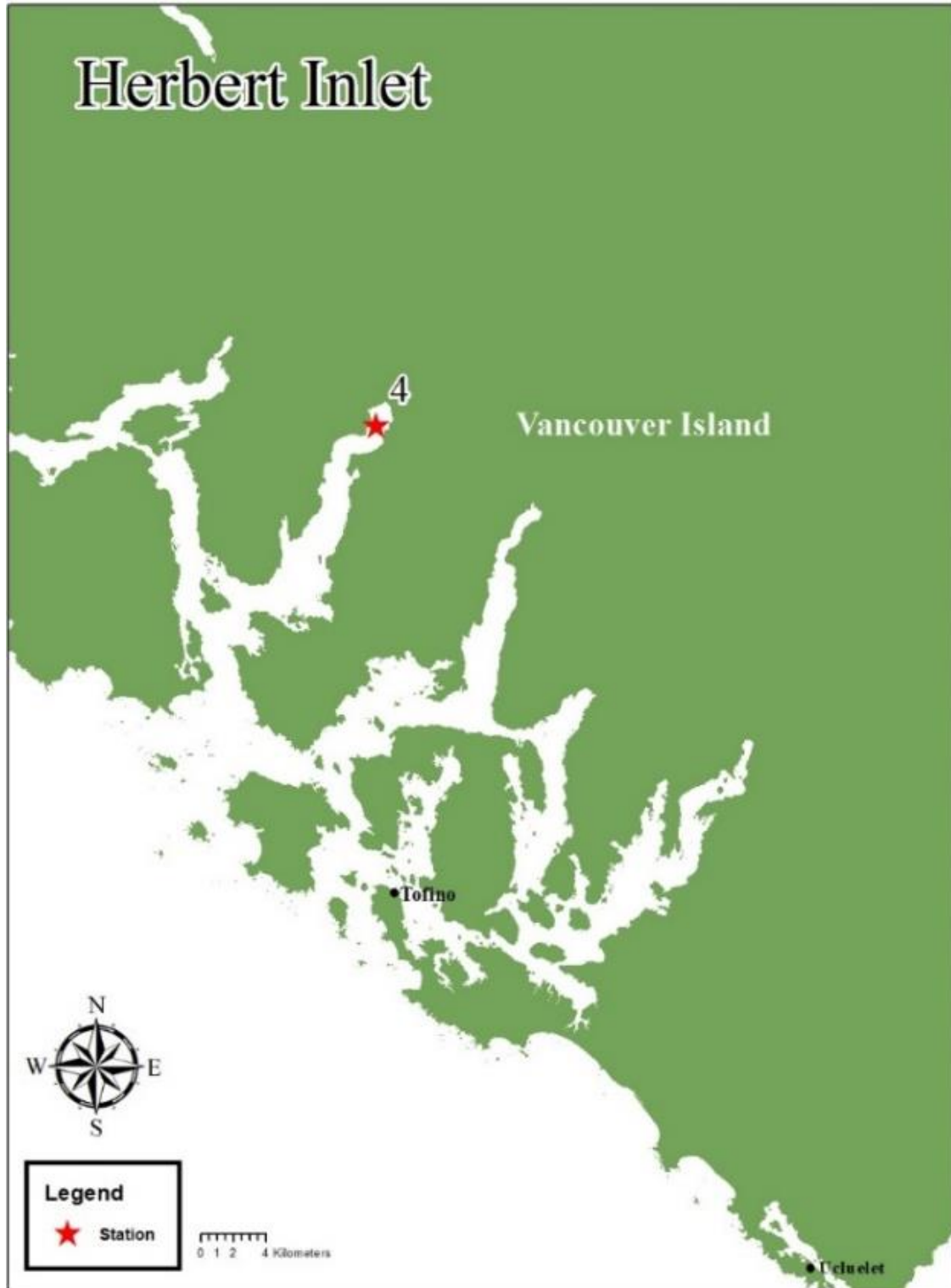


Figure 4. Map of Herbert Inlet in Clayoquot Sound. Station 4 was located on the coast of Vancouver Island, British Columbia, Canada. Station numbers correspond to field collection date.

**Table 1.** Summary of field sites. The site number corresponds to the date on which the material was collected with 1 being collected first and 5 being collected last.

Site	Location	Latitude (N)	Longitude (W)	Date of Collection
1	Thea Foss Head	47.24689992	-122.432475	04/17/2015
2	Thea Foss Waterway	47.24526389	-122.4321806	05/01/2015
3	San Juan Channel	48.56449667	-123.0112042	05/08/2015
4	Herbert Inlet	49.40790833	-125.9098167	09/11/2015
5	Thea Foss Head	47.25627778	-122.4339722	10/09/2015

drying oven for 24 hours at 90°C. Solids underwent wet peroxide oxidation (WPO) treatment for removal of labile organic matter. Following WPO, the solids underwent density separation in a 1.6 g/cm<sup>3</sup> solution mixed with sodium chloride. Each suspended solid underwent a microscope exam. All identified microplastic at 40x magnification were collected and stored in a vial. Concentration of microparticles was found by dividing the dry mass by the volume of water. Results summarized in Table 2.

**Table 2.** Mass, volume, and concentration by site.

Site	Dry Mass Microparticles (g)	Water Volume (L)	Concentration of Microparticles ( $\frac{g}{L}$ )
1	0.169	60910.19	$2.78 \times 10^{-6}$
2	0.137	43027.53	$3.18 \times 10^{-6}$
3	0.003	66609.69	$4.35 \times 10^{-8}$
4	0.015	53271.73	$2.72 \times 10^{-7}$
5	0.079	880303.26	$8.97 \times 10^{-8}$

### 3.3 MATERIAL TYPE IDENTIFICATION

Films were characterized as thin, flat, and highly flexible. Fragments were characterized as hard/inflexible pieces with rough edges and no shape. Styrofoam was characterized as soft/squishy, round, and white. Foam was characterized as a visibly porous fragment of any color. Fibers were characterized as flat, wide across, and able to hold shape if folded (would not depress if flat elongated piece is held in air). Threads were thin flexible strings that depress if held in air. Pellets were identified as pre-industrial plastic pellets.

### 3.4 IDENTIFICATION OF POLYMERS

Polymer identification was done using PerkinElmer FTIR spectroscopic instrument with UATR crystal. Prior to scanning, the material type, color, and longest axis length of each particle was noted. The spectrum of all microparticles were obtained with the wavelength range being set at 4,000 to 600  $cm^{-1}$ . Transmission was used over absorbance as instructed in PerkinElmer's manual *Optimizing the Workflow for Microplastic Analysis by FTIR Microscopy*, because % transmission gives best results for polymers (PerkinElmer 2018). Each particle was scanned 4 times with enough force applied to obtain a spectrum with a clear fingerprint region and minimal noise. The PerkinElmer Polymers Library was used to match the scans to a known plastic polymer spectrum. Search results were limited to the top 20 best hits. The search result score was on a 0.1 to 0.9 scale, with 0.9 being the best score, representative of a search result that aligns closely with the scan taken. The highest scores were selected in this project. See appendix for details of search score data.

### 3.5 CONTROL/POTENTIAL ERRORS/OTHER

As a control, a pellet of high-density polyethylene (HDPE) was the first polymer that was scanned prior to identification of the unknown solids. The control was used to confirm that the spectrum of the known plastic pellet appeared the same each day prior to FTIR analysis. By doing so, if the spectra of the solids appeared too noisy or inconsistent with previous work, but the control remained the same, the possibility of changes to settings, UATR crystal damage, or contamination could be eliminated.

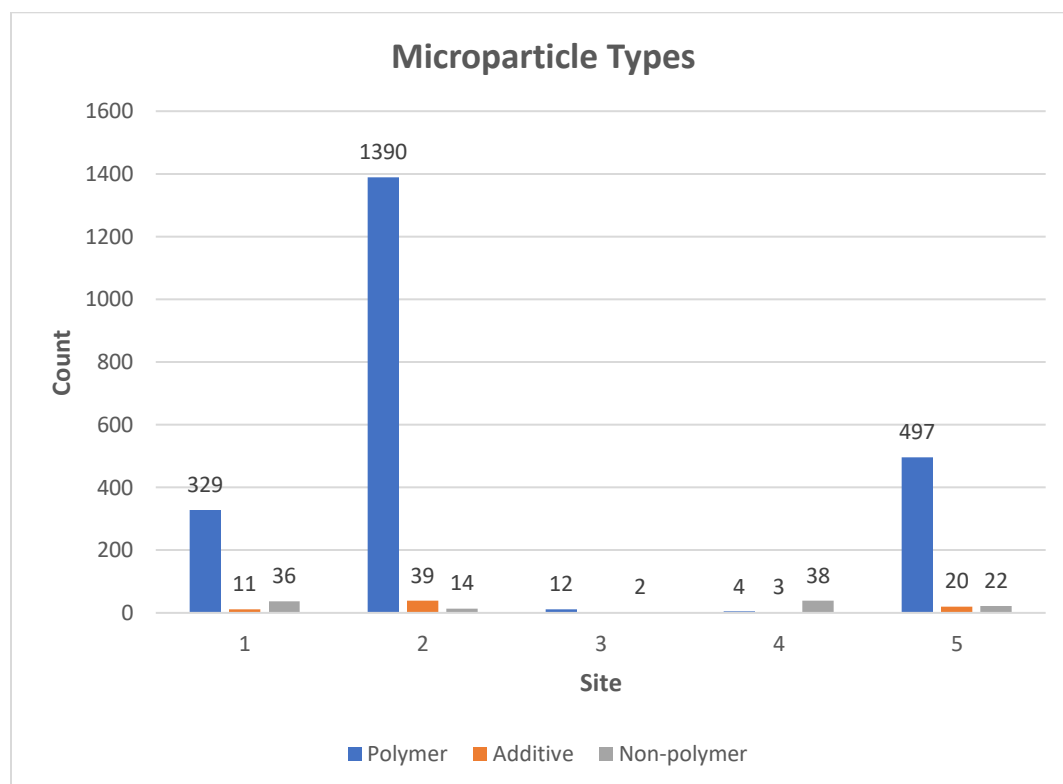
Some non-polymer solids had a flakey consistency that rubbed off on other samples and was difficult to remove, therefore some samples may have been identified by a color of a contaminant that rubbed off on it. In addition, color identification was at the discretion of the researcher.

There were samples longer than the microplastic length (> 5mm). For this project, all data collected was included in the figures and analysis with the assumption that solids greater than 5 mm unfolded or penetrated the 5 mm sieve.

## 4.0 RESULTS

### 4.1 PARTICLE TYPES FOUND AND CONCENTRATIONS

Synthetic polymers were found at all 5 field sites. The distribution of microparticle types differed by site. Each water sample was composed of three types of microparticles: synthetic polymers, additives of synthetic polymers, and non-polymers (figure 5). Refer to section 3.2 for definition of non-polymers. In site 1, 87% of the solids analyzed were plastic polymers. A small percentage of the sample from site 1 was composed of additives (3%) while the remaining 10% extracted solids were non-polymers. Site 2 was overwhelmingly composed of plastic polymers (96%) with the remaining sample composition being additives (3%) and non-polymers (1%). Sites 3 and 4 were the only water samples obtained outside of Tacoma waterways (figure 3 & 4). In site 3, 86% of the sample was composed of polymers and the remaining solid types were found to be non-polymers (14%). No plastic additives were found in site 3. Contrastingly, 84% of the total sample in site 4 was composed of non-polymers. A small portion of the sample was composed of plastic polymers (9%) and plastic additives (7%). Site 5 showed a similar pattern to sites 1 and 2, with the dominant particle type being polymers (92%). Additives made up 4% of the total sample composition and the remaining 4% were non-polymers. After obtaining FTIR spectroscopy results, concentrations of plastics per site were able to be found (table 3). See table 2 for concentrations of microparticles.



**Figure 5. Summary of microparticles found at sites 1-5 using FTIR spectroscopy analysis. The 3 bars per site represent a summary of extracted solid types found within each site.**

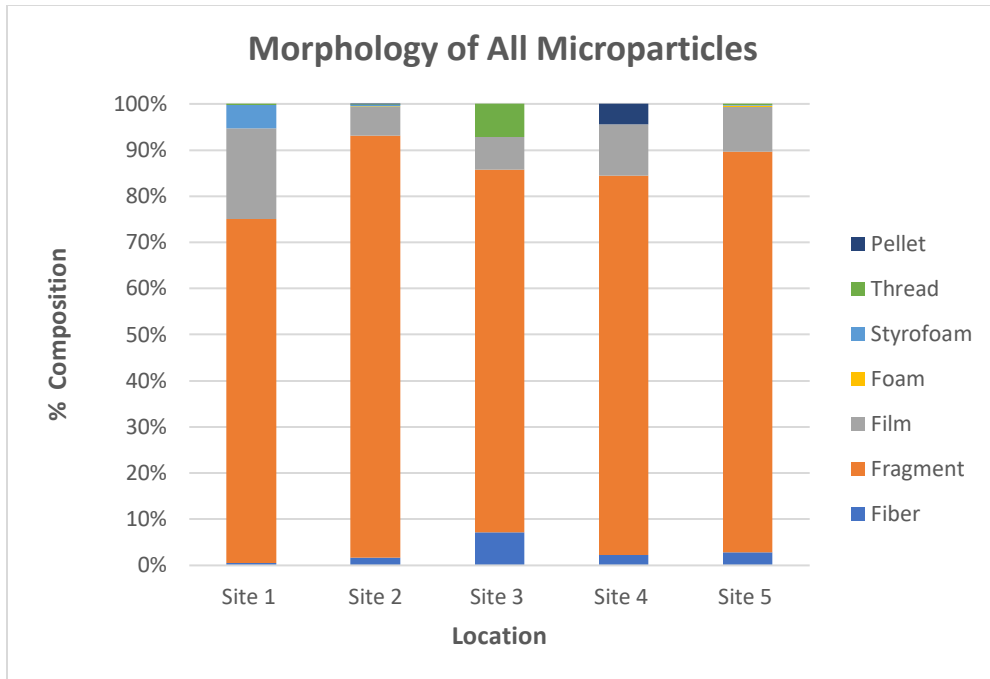
**Table 3.** Concentrations of plastics per site after FTIR analysis.

Site	Count of Plastics	Water Volume (L)	Concentration ( $\frac{Plastics}{L}$ )
1	329	60910.19	$5.40 \times 10^{-2}$
2	1390	43027.53	$3.23 \times 10^{-1}$
3	12	66609.69	$1.80 \times 10^{-4}$
4	4	53271.73	$7.51 \times 10^{-5}$
5	497	880303.26	$5.65 \times 10^{-4}$

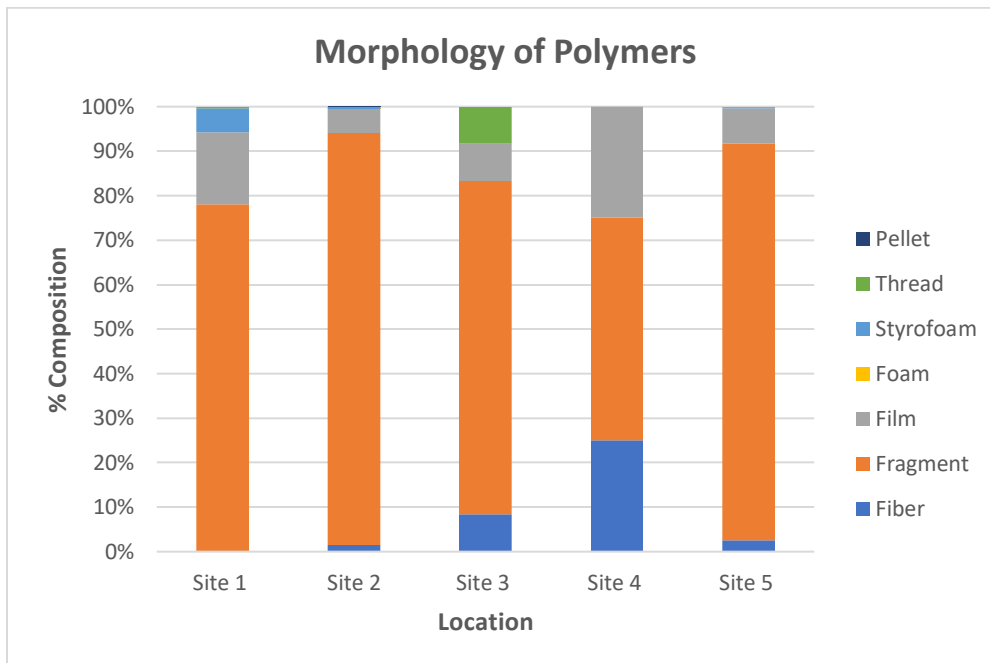
#### 4.2 MORPHOLGY

A total of 7 unique morphology types were found across microparticles with the common morphology type being fragment for both microparticles and polymers (figure 6 & figure 7). In the all-microparticle data pool, fragments made up 74% of the composition in site 1, 92% in site 2, 79% in site 3, 82% in site 4, and 87% in site 5. The second most common morphology type was film and the % composition varied by site. Fragments, films, and fibers were found at all sites. Styrofoam, foam, pellets, and threads made up <10% of the total composition. The distribution of these 3 morphology types differed by site (figure 6).

Analysis of the results for polymer morphology (figure 7) showed differences in % composition of morphologies, however, as previously mentioned the most common morphology for polymers was fragment. Like figure 6 results, the second most common morphology was films. The % composition for fragments was 78% in site 1, 93% in site 2, 75% in site 3, 50% in site 4, and 89% in site 5. Fragments, films, and fibers were the three polymer morphology types observed at all sites. Fibers were present at sites 2, 3, 4, and 5. Styrofoam was only found in site 1, threads were only found in site 3, and pellets were only found in site 2. As the dominant morphology type was fragments for all sites, all other morphology types made up a small % of the total composition.

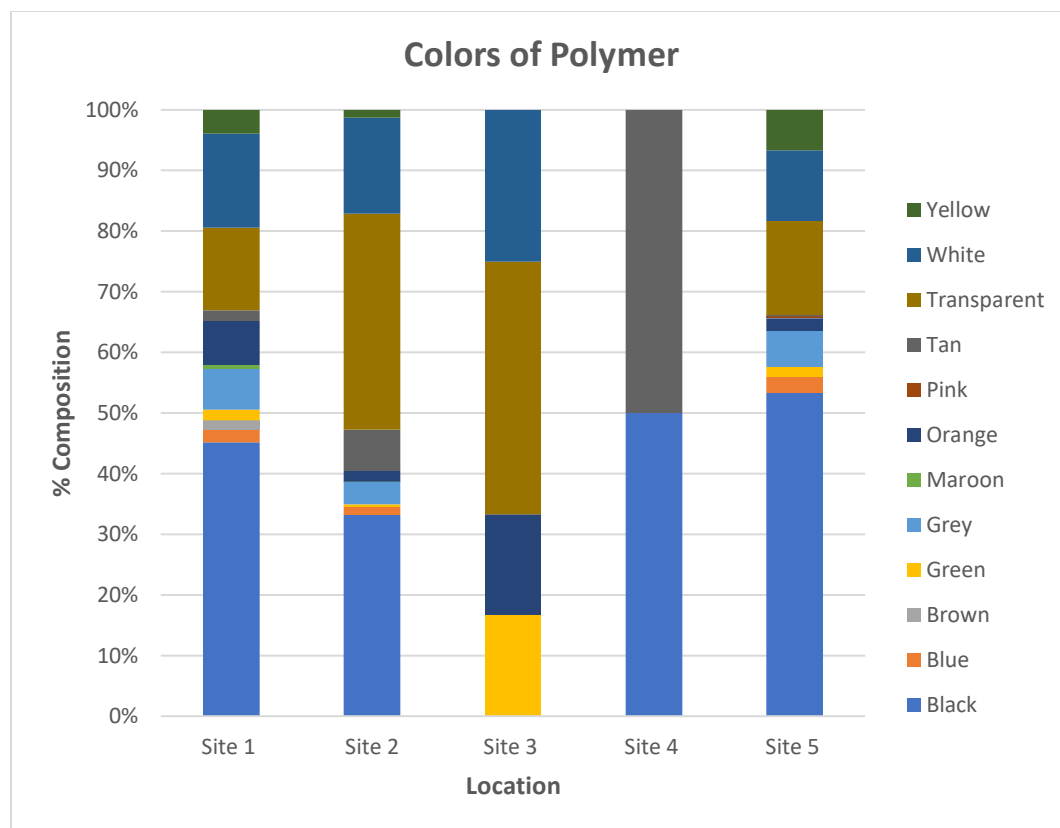


**Figure 6. Morphology type of all microparticles found at sites 1-5. This figure is representative of the morphology type of all extracted solids found per site, including polymers, non-polymers, and additives.**



**Figure 7. Morphology of plastic polymers. This figure represents the morphology types found in the polymers-only data pool.**



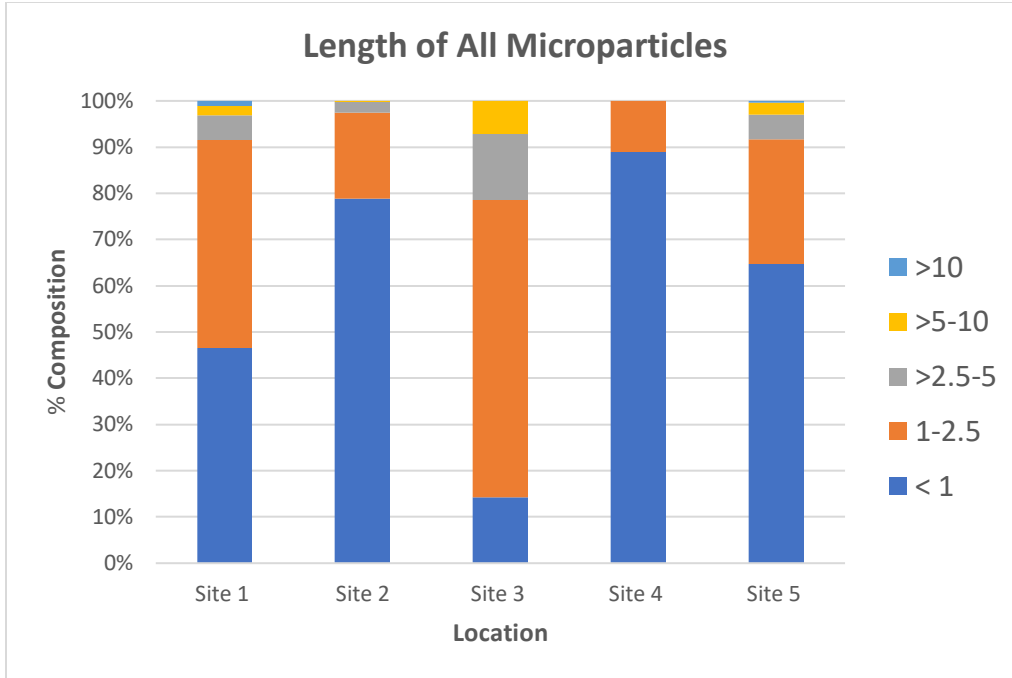


**Figure 9. Color summary of synthetic polymers. Black, white and transparent were the most common colors. Presence of such colors varied by site. Site 4 was composed of two colors only, black and tan.**

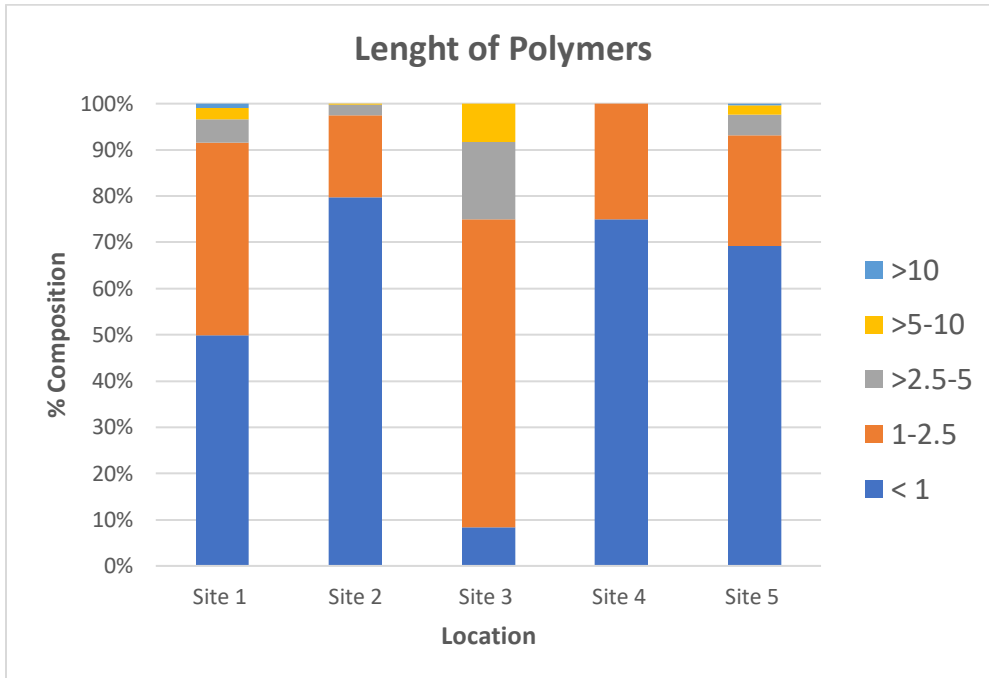
#### 4.4 LONGEST AXIS

The results show that the most abundant length ranges found in our samples fell within the defined MP length of 5 mm or below, this includes the lengths for all particle types (figure 10). There were 5 different length ranges found with two of the ranges falling outside of the MP length, > 5-10 and > 10 mm, (figure 10 & figure 11). Summarizing the results for all microparticles (figure 10), the dominant lengths of microparticles for sites 1 (47%), 2 (79%), 4 (89%), and 5 (65%) were below 1 mm. Site 3 results showed that the dominant length range was slightly higher than the previously mentioned sites, with the range 1-2.5 mm being the most common (65%). Sites 1, 3, and 5 had a few solids above the MP length of  $\geq 5$ mm. However, these solids made up <10% of the total length composition.

The results in figure 11 summarize the length data for plastic polymers only. Similar to figure 10, the results for polymer length show that our sample sizes are predominantly 5 mm or below. The most common lengths at sites 1 (50%), 2 (80%), 4 (75%), and 5 (69%) were below 1 mm. Site 3 in figure 11 showed the same results figure 10 shows. The most common length in this site was the length range 1-2.5 mm (67%). Sites 1, 3, and 5 had a few polymers that were > 5 mm. However, like results in figure 10, the polymers that fell in this length range made up < 10% of the total polymer composition.



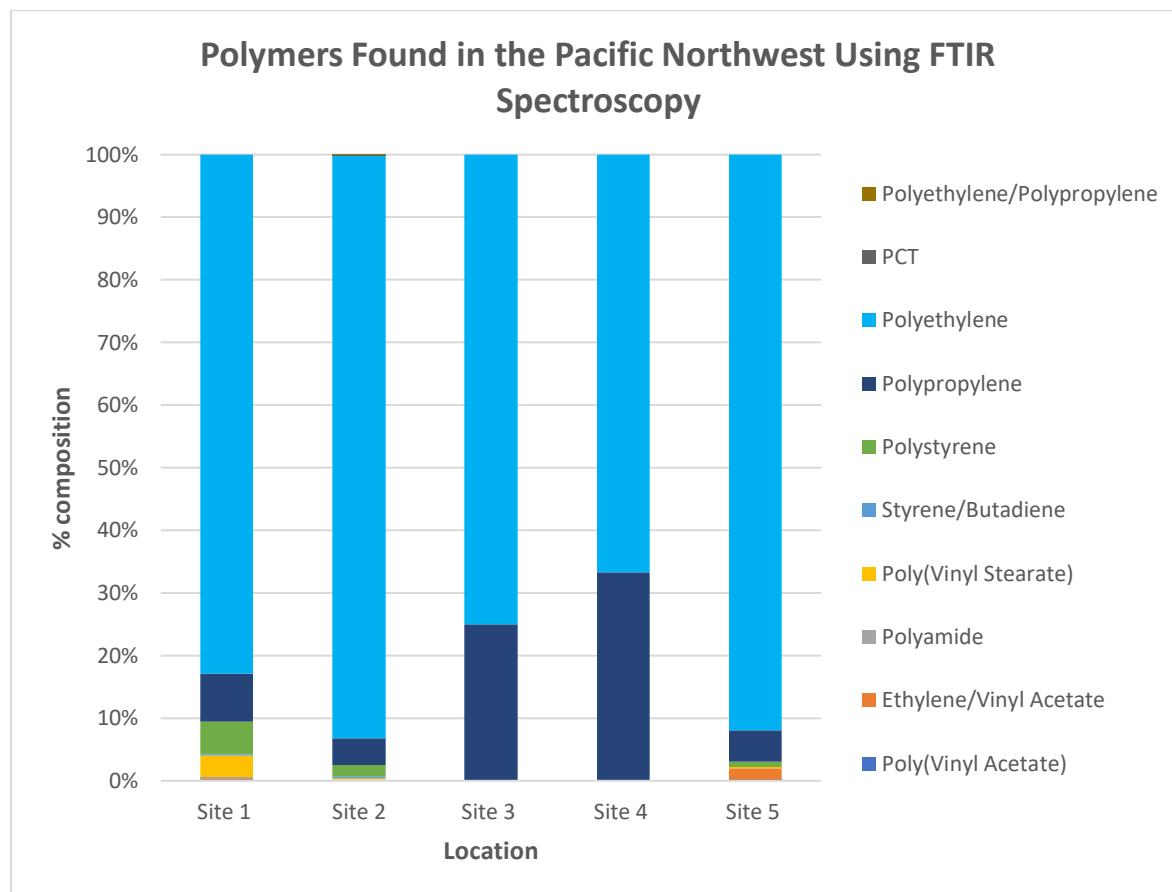
**Figure 10. Long axis lengths (mm) found at sites 1-5. Solids with lengths that are  $\leq 5$  mm are categorized as microparticles. Each site was predominantly composed of microparticles. Less than 10% of solids per site were categorized as other.**



**Figure 11. Long axis length (mm) of synthetic polymers found at sites 1-5. Polymers with a length of  $\leq 5$  mm are categorized as microplastics. Each site was predominantly composed of microplastics with < 10% being categorized as other.**

#### 4.5 POLYMERS IDENTIFIED USING FTIR SPECTROSCOPY

Ten unique synthetic polymers were identified across the samples using FTIR spectroscopy (figure 12). The polymer that was in most abundance across all 5 sites was polyethylene at about 67% or more in each site. The second most abundant polymer at all sites was polypropylene, making up at least 5% of the total polymer composition. Polyethylene and polypropylene were the two polymers that were found at all sites. The distribution of all 10 polymers varied from site with no site having all 10. Site 1 had 7 unique polymers total with polyethylene making up most of the samples at 83%. Site 2 had 8 total polymer types, making it the site with the most diverse polymer types; however, polyethylene dominated the % polymer composition by 93%. Sites 3 and 4 only had two polymer types, polyethylene, and polypropylene. Meanwhile site 5 had 6 unique polymers with polyethylene making 92% of the total composition.



**Figure 12.** Spectroscopic analysis was completed using a Fourier Transformed Infrared (FTIR) instrument with the PerkinElmer Polymers Library. Polymers with forward slash (/) are copolymers. PCT = Poly (1,4-Cyclohexanedimethylene terephthalate).

## 5.0 DISCUSSION

### 5.1 Summary of Samples Per Site

The results show that Tacoma locations (sites 1, 2, & 5) were most concentrated with microplastics and microparticles overall (figure 5). The concentration of microplastics found in the Tacoma samples ranged from  $5.65 \times 10^{-4}$  to  $3.23 \times 10^{-1}$  plastics\* $L^{-1}$ . In contrast, sites located in Vancouver Island (site 4) and San Juan Island (site 3) had the lowest synthetic polymer count in addition to total microparticles overall (figure 5). The outlier in the results was site 4 located in the Herbert Inlet which had more non-polymers than polymers with only 7% of the total microparticle composition being polymers. The concentration of microplastics found in the Herbert Inlet was  $7.51 \times 10^{-5}$  plastics\*  $L^{-1}$ , being lower than the concentration of microplastics found in the San Juan channel,  $1.80 \times 10^{-4}$  plastics\*  $L^{-1}$ . These results may be explained by the population density. As mentioned in section 3.1, Tacoma has a higher population density in comparison to the San Juan Islands and the towns of Uclulet and Tofino, nearest to the Herbert Inlet. Without doing further analysis, it can be inferred that the San Juan Islands and Vancouver Island have a smaller anthropogenic impact on the coast in comparison to the anthropogenic, commercial, and industrial influence the city of Tacoma has on South Sound. Aside from the outlier, the results show that all samples collected in each site are mainly composed of plastics, which reaffirms efficiency of the methodology.

The concentrations found in our samples can be compared to concentrations found in previous research conducted in the Pacific Northwest with the assumption that the researcher's methodologies yielded an extraction predominantly composed of synthetic polymers. Research by Hall 2021 was primarily located in the Puyallup River waterways and found an increase of up to  $1.0 \times 10^1$  plastics \*  $L^{-1}$  in 2018. Meanwhile, our results from Thea Foss sites were lower in 2015 with up to  $3.23 \times 10^{-1}$  plastics \*  $L^{-1}$ . Due to the proximity of Thea Foss and the Puyallup River, what can be said about the comparison of concentrations between our study and Hall 2021 is that any microplastic that enters the Puyallup River and Thea Foss directly influence both waterways and the overall microplastic concentration in Commencement Bay, which is where both channels connect to. Research by Harris et al. (2022) found a slightly higher concentration of microparticles than the concentrations in our Tacoma sites, with up to  $6.4 \times 10^{-1}$  microparticles \*  $L^{-1}$  in Elliot Bay during 2022. The Anthropocene influences that Seattle and Tacoma have on Elliot Bay and Commencement Bay respectively are similar. Contrastingly, Mahoney's 2017 research in the Whidbey Basin and Hood Canal found lower concentrations, with an average of  $9.2 \times 10^{-5}$  plastics \*  $L^{-1}$  in the Whidbey Basin and  $3.6 \times 10^{-4}$  plastics\* $L^{-1}$  in the Hood Canal. Both regions are not as densely populated as Seattle and Tacoma. These concentrations are similar to the low concentrations found in our sites from San Juan Channel and the Herbert Inlet, both regions with low population density.

Although in this research study, statistical analysis was not done, previous findings have found mixed results on the statistical significance of population density and MP concentrations in surface waters and sediments. Other research found that high MP concentrations were significantly correlated with a high population density (Yonkos 2014; Harris et al. 2022; Eshom-Arzadon 2017; Hall 2021). Meanwhile studies by Mahoney 2017 and Moats 2019 showed no statistically significant relation with high MP concentrations and high population density.

Instead, some researchers suggest that basin residence time influences the MP concentrations (Mahoney 2017; Moats 2019).

In this research project, no emphasis was placed on the details of the plasticizers. Research was done to confirm our spectrum results were of plastic additive origin. Though further analysis did not occur, what can be said is that weathering of plastic polymers through chemical or photoreactive mechanisms likely occurred, hence why we have plastic additives at sites 1, 2, 4, and 5. Previous research notes that plastic additives are not covalently bonded to the polymer molecules, thereby making it easy for additives to leach into the environment (Hermabessiere et al. 2017). Overall, additives made up the smallest % composition in all our samples. Future studies should focus on analyzing the influence that plastic additives from microplastics have on marine life.

## 5.2 Microplastic Characteristics

The most common morphology found in this project were fragments, which have been observed in previous studies (Talbot et al. 2022; Bertoldi et al. 2021). However, the morphology that has been most common in other studies has been fibers (Harris et al. 2022; Talbot et al. 2022; Hall 2021; Lefebvre et al. 2019; Kieu-Le et al. 2023). Black was the color that was most abundant in this study. Though this finding can be matched with a previous microplastic research study based in the Northwest, Talbot et al. 2022, research findings in other studies have found a wide range of results for MP color. For example, research based in Seattle by Harris et al. (2022) found that blue was the most common color in their microparticle samples. Similarly, research by Aliabad et al. (2019) based in the Gulf of Oman found blue to be the most common color. Contrastingly, research based in Texas by Shruti et al. (2020) found transparent to be the most common polymer color. Although uniformity of polymer colors is less common than other MP characteristics across research studies, color is still an important variable to include in MP research. Previous research studies have demonstrated the influence that MP color has on the feeding behavior of marine organisms (Xiong et al. 2019). The length size that was most abundant in this study remained consistent with previous findings where length size < 1 mm was the most common (Harris et al. 2022; Talbot et al. 2022; Martinelli et al. 2020).

## 5.3 Polymers Found in the Pacific Northwest

The two most abundant polymers found across all our field sites were polyethylene and polypropylene. Our main finding supports our hypothesis and is consistent with previous research done in the Pacific Northwest and across the globe. Polyethylene and polypropylene have been found to be the most common polymers in MP research in studies with different objectives. From MPs found in human organs (Ragusa et al. 2021; Jenner et al. 2022) to MPs found inside the digestive tract of marine animals (Lefebvre et al. 2019; Jung et al. 2018) from water samples in fresh water (Talbot et al. 2022; Bertoldi et al. 2021) and saltwater environments (Harris et al. 2022; Aliabad et al. 2019), polyethylene and polypropylene are consistently the most abundant MPs found. These results can be explained by the plastic production and consumption values per year. Polypropylene and polyethylene have the highest production values out of all primary plastics made at a global scale in 2015 (OWID 2022). These polymers compose single-use plastic items, particularly packaging and containers, which have been found to have the highest consumption tonnage in the U.S. (EPA 2023). Across the globe, packaging

accounts for 42% of all plastic production that is in the non-fiber plastics category (Geyer et al. 2017). In 2015, the year all our samples were obtained in, 6300 Mt of plastic waste was produced and only 9% of the total was recycled (Geyer et al. 2017). Furthermore, research by van Sebille et al. (2015) estimated that there are approximately 15 to 51 trillion microplastics in the ocean. It is likely that the concentration of MPs has increased since 2015. Evidently, with the mass production, consumption, and minimal recycling of single-use plastics, it is expected that much of the plastic pollution that ends up in the ocean is composed of single-use plastics made from polyethylene and polypropylene.

## **CONCLUSION**

The results show that microplastic polymers derived from single-use plastics are present in the Pacific Northwest waterways. These results are consistent with other FTIR microparticle analysis studies in the Pacific Northwest and other regions of the world. With our hypothesis being supported, future policies interested in cleaning the Salish Sea waterways should note that single-use plastics made from polyethylene and polypropylene are likely where most of the microplastics in surface waters originate from.

Future research can expand on analyzing polymer composition over the years, in addition to including analysis that involves spatiotemporal variables. In addition, future research studies can focus on studying how polymer composition changes by water column depth. Moreover, future studies can also elaborate on how or if additives used for single-use plastics influence the aquatic biota. Our findings can aid in understanding how biota that reside in surface waters are impacted at the ecological or physiological scale by polyethylene and polypropylene and the potential harmful effects these single-use plastics typically obtain. Putting aside what is not known in the microplastic research field, plastic pollution remains ubiquitous since the rise of synthetic polymers. It is a pollutant that is accessible to the public in comparison to the other pollutants that the ocean is contaminated with. For this reason, efforts to reduce plastic production and consumption and increase recycling are necessary to limit or halt altogether the plastic pollution problem the ocean and subsequently marine biota face.

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**APPENDIX**

Please follow directions to access the raw data (including scores, names of plasticizers found, and more) through the University of Washington Tacoma X-drive. Note only individuals with a University of Washington email will be able to access the data. For those who are not UW affiliates, please directly contact us using the contact information listed on the cover page.

Pathway: X drive: GIS: SCIdata: microplastics: data: larino\_appendix\_uwt\_ftir\_2015\_data.xlsx

Name of file: larino\_appendix\_uwt\_ftir\_2015\_data.xlsx