Distribution, Abundance, and Spatial Variability of Microplastic Pollution in Surface Waters of Lake Superior

by

Kara Cox

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Authors Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

Plastic pollution in oceans and lakes has been a concern for more than three decades, and largely through the breakdown of large plastics, microplastic pollution has been of real concern for over 20 years. Most research has focused on marine settings but freshwater systems are equally vulnerable to microplastic pollution. The Laurentian Great Lakes system has been the subject of little microplastic research and Lake Superior has received even less focus than the other four lakes. The objective of this study is to fill that knowledge gap and determine the abundance and spatial distribution, spatial variability, and polymer identities of microplastic pollution in the surface waters of Lake Superior.

In 2014, 94 double net samples were collected from the surface waters of Lake Superior and preserved. These samples comprise the most comprehensive surface water survey of any of the Great Lakes to date, and the first to employ double neuston net trawls. Since there is not yet a standardized sampling method, a comparison of side-by-side samples will indicate whether single net surveys are sufficient and could be used as the standard sampling method. A total of 187 samples was processed using wet peroxide oxidation and analyzed using a dissecting microscope. A sampling of all plastic particles collected were also analyzed using FTIR spectrometry to determine polymer identity. Abundances calculated throughout Lake Superior show wide variability, ranging between 4,000 to more than 100,000 particles/km^2 but the majority of locations have an abundance between 20,000 to 50,000 particles/km^2. Average abundance in Lake Superior is 30,271 particles/km^2 (95% confidence interval of the mean ranges from 20,917 to 39,797 particles/km^2) which suggests a total count of more than 2.4 billion (1.7 to

3.3 billion) particles across the lake's surface. Both the calculated average and lake wide total for Superior are higher than Lake Michigan, which as an average abundance of 17, 276 particles/km^2 and holds roughly 1 billion particles. Lake Erie is more polluted than both Lake Superior and Lake Michigan, with an average abundance of 105,502 particles/km^2 and a total of roughly 2.7 billion particles. Lake Superior was expected to have lower abundances than Lake Michigan because of lower population density and industrialization, but the higher numbers can likely be attributed to the greater size and longer residence time of Lake Superior.

Distributions of plastic particles, characterized by size fraction and type, differed between nearshore and offshore samples and between samples collected in Eastern versus Western portion of the lake. No difference was detected between the paired net samples, indicating that single net sampling produces a representative estimate of microplastic particle abundance and distribution within a body of water. Most of the particles found were fibres (67%), and most were contained in the smallest classified size fraction (0.3-1 mm) indicative of the low population density and industrialization along the shores of Lake Superior. The most common type of polymer found was polyethylene (51%), followed by polypropylene (19%) which was expected given global plastics production is dominated by polyethylene, followed by polypropylene. This is also similar to results obtained from other studies. Types of plastic present, when separated by morphology and size, can help identify pollution sources which is a necessary step in plastic pollution management.

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Dedication

This thesis is dedicated to all of the people in my life who, in one way or another, contributed to my deep, and unending fascination with science, the planet, and an individuals ability to honour the part of them that wants to "save the world". You were all an incredible source of inspiration, and the world needs more people like you.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

EPA Environmental Protection Agency

GLFCS Great Lakes Coastal Forecasting System

USGS United States Geological Survey

POP Persistent organic pollutant

PEMRG PlasticsEurope’s Market Research and Statistics Group

NOAA National Oceanic and Atmospheric Administration

UNEP United Nations Environmental Program

FAO Food and Agriculture Organization of the United Nations

EPR Extended Producer Responsibility

FTIR Fourier Transform Infrared Spectroscopy

IJC International Joint Commission

PPC Plastic Pollution Coalition

Chapter 1: Introduction

1.1 General Introduction

Plastic pollution appeared in noticeable quantities in aquatic environments during the 1970s (vom Saal et al., 2008). Initially it was thought that this pollution was not harmful but was instead just a natural consequence of increasing production since the 1950s (vom Saal et al., 2008). Now, plastic is the largest contributor to marine pollution (vom Saal et al., 2008). Originally large plastic debris, such as derelict fishing gear, was the main concern but fishing gear lost at sea accounts for only a small percentage of all aquatic plastic pollution (Andrady, 2011). Instead, most plastic pollution comes from a terrestrial source, accounting for 80% of plastic pollution (Andrady, 2011).

In addition to macroplastic pollution, the presence of microplastic particles has been a growing concern since the early 1990s when it was first named as a minor source of marine pollution (Frias et al., 2014). Research now shows that despite the smaller size, microplastics are just as prevalent and problematic as the larger, and more visible, macroplastics polluting aquatic ecosystems (Fendall et al., 2009). Although most research focuses on marine ecosystems, microplastic pollution is also widespread throughout freshwater lakes, rivers, estuaries and other bodies of water around the globe (Free et al., 2014).

Recently, the Laurentian Great Lakes system in North America has come under scrutiny to determine the presence, abundance and impact of microplastic pollution (Eriksen et al., 2013). Most of this research has focused on the more populated and industrialized lakes like Lakes Erie, Huron and Michigan (Eriksen et al., 2013; Mason et al., 2016b). Lake Superior, the largest of the five lakes is often considered to be relatively pristine due to its size, low population density along the shores, and its location at the head of the Great Lakes drainage basin. A comprehensive study of the presence of microplastics in Lake Superior is important to develop a baseline set of data for future research, and to contribute to the overall understanding of microplastic pollution in the Great Lakes.

1.2 Research Objectives

The first surface water microplastic survey of the Great Lakes was completed in 2012 when samples were taken from Lake Superior, Lake Huron and Lake Erie (Eriksen et al., 2013). Since then more comprehensive microplastic surveys have been completed for all of the Great Lakes except Lake Superior (Driedger et al., 2015).

The present project will address the above gap in knowledge by analyzing microplastics found in 94 duplicate surface water samples (187 individual samples) from Lake Superior. The results will provide a more complete picture of surface water microplastic pollution in Lake Superior and will add to the limited information available about microplastics in surface waters of the Laurentian Great Lakes.

The objectives of this project are as follows:

1) Develop a baseline set of data for surface water microplastic pollution in Lake Superior, focusing on abundance, distribution, spatial variability and polymeric identity. This will serve as reference data for any future studies of microplastics in Lake Superior.

2) Assess the effectiveness of single net sampling to help refine sampling methods in pursuit of establishing a standardized sampling method for surface water surveys. This will be assessed by comparing samples obtained from the paired nets.
3) Inform policies, such as wastewater treatment, drinking water standards, and waste management practices, and provide suggestions for solutions which will help mitigate continued microplastic input into Lake Superior.

1.3 Location and Description of Study Site

Lake Superior is the northern-most lake in the Great Lakes system, and is the largest freshwater lake in the world, by surface area. Lake Superior contains 12.1 trillion cubic meters of water which is more water than all the other Great Lakes combined (Minnesota Sea Grant, 2017). Lake Superior's residence time is 191 years, much longer than the other four lakes, which range from 99 years (Lake Michigan) to just 2.6 years (Lake Erie) (Quinn, 1992; EPA 1995).

Lake Superior is 563 kilometres long, 257 kilometres wide and has an average depth of 147 metres. The shoreline measures 2,938 kilometres. The drainage basin for Lake Superior covers 127,700 square kilometres, receiving water from all provinces (Ontario, Canada) and states (Minnesota, Wisconsin, and Michigan) it borders (EPA, 1995). Over 200 rivers flow into Lake Superior, but the primary sources of the inflow are the Nipigon, St. Louis, Pigeon, Pic, White, Michipicoten and Kaministiqua Rivers. The main source of outflow is the St. Marys River. Compared to the other four Great Lakes, Lake Superior's shoreline is relatively unpopulated, with subsequently lower levels of industrialization.

Lake Superior surface waters range from 0-13 °C throughout the year, and in 2016 the mean surface water temperature was 3.3 °C (GLFCS annual comparison, 2018). The lake is stratified and mixes uniformly twice a year, in the spring and the fall, when the temperature throughout the lake reaches 4 °C. Due to the size of Lake Superior it does not always freeze over completely, and only does so when the winter season is extremely cold. Current climate change trends in and around Lake Superior are producing warmer summer temperatures, which in turn is increasing water temperatures, and longer periods of stratification. Longer periods of stratification delay mixing and the development of ice cover throughout the winter, which acts as a positive feedback loop for ice development the following year (Austin and Colman, 2007). Current warming trends, and declines in ice cover could cause Lake Superior to be completely ice free during the winter within the next thirty years (Austin and Colman, 2007).

Lake Superior is home to a wide variety of fish and other species. Lake Whitefish (*Coregonus clupeaformis*), Lake Trout *(Salvelinus namaycush)*, and Lake Chub *(Couesius plumbeus)* are the mainstays of commercial fisheries, although Lake Herring *(Coregonus artedi)* and Rainbow Smelt *(Osmerus mordax)* are also important. Lake Superior was also known for producing Brook Trout *(Salvelinus fontinalis)* but overfishing and loss of habitat decimated the population and efforts are currently underway to restock the fish populations and repair and restore lost habitats (Great Lakes Environmental Assessment and Mapping Project, 2017; US Fish and Wildlife Service, 2018).

Lake Superior is an important link in the Great Lakes Waterway which connects all of the five Laurentian Great Lakes and allows the shipment of iron ore, grains and other commodities. The shipping season is generally closed from mid-January to late March due to an accumulation of ice along the shore and in shipping ports. The closing and opening dates of the shipping season vary and depend on the temperatures and ice conditions of each season (Minnesota Sea Grant, 2017).

The entire Lake Superior basin contains approximately 600,000 people, most of whom live on the United States side of the lake. Of the people living in the basin, approximately 182,000 are Canadian citizens. However, there are an estimated 3.5 million people visiting the basin of Lake Superior on an annual basis (Minnesota Sea Grant, 2017). Most tourism is in the form of camping, hiking, and recreational outdoor activities. Given the low overall population throughout the basin, and the low levels of industrialization, most plastics found in the lake likely originate from terrestrial sources such as improper disposal, and accidental loss during recreational camping, beach use, fishing and other tourist activities (Driedger et al., 2015).

The sample sites used in the study were selected by the United States Geological Survey (USGS) as annual sampling sites for monitoring larval Lake Whitefish populations. In 2014, 94 locations were sampled and the samples were collected and preserved.

Chapter 2: Literature Review

2.1 Introduction to Plastics

Plastic and plastic products are readily available and have become increasingly popular because of their versatility, durability, and ease of production (Andrady, 2011). Plastic has gradually replaced conventional materials such as glass and aluminum because they are easier to produce, lighter and therefore cheaper to transport, and more readily available than previously used materials such as metal and glass (Andrady, 2011). Plastic bags, plastic packaging, plastic containers and many other plastic products are intended for a one time use before being discarded. Once discarded, much of the plastic debris ends up in the oceans because of improper disposal (Fendall et al., 2009).

Initially, concerns associated with plastic debris in lakes and oceans were due to the potential for organisms to become entangled in plastic debris, particularly fishing gear (Fendall et al., 2009). Recently however, increased use of plastic pellets and the subsequent loss of these pellets into the oceans and other aquatic environments has increased the levels of plastic pollution, which present additional threats to marine life (Fendall et al., 2009). Along with entanglement of animals, accidental ingestion of small particles poses a threat to all marine life, from invertebrates to whales. Accumulation of plastics within organisms allows the plastic particles to pass up the food chain, further contaminating and endangering wildlife (Law and Thompson, 2014). Ingestion of plastic by marine animals has become more common and more concerning, and has been reported in numerous species of fish, turtles, large marine mammals, and in particular seabirds, which will accidentally feed them to their chicks (Andrady, 2011). Often these particles of plastic are too large to be passed through the young or juvenile's digestive system and will lead to high chick mortality, which is extremely concerning since an estimated 44% of all seabirds accidentally ingest plastic particles (Andrady, 2011). Besides entanglement and ingestion, both of which can kill marine animals, plastic pollution also causes other problems (Moore, 2008). Plastic debris devalues beaches, does not readily biodegrade, is a source and sink for persistent organic pollutants (POP's), accumulates in sediments, damages benthic habitats and coastal areas which act as nurseries for many species, damages ships' propellers, and can impede navigation (Moore, 2008; Xanthos et al., 2017).

While the plastic particles themselves are dangerous to marine life, the danger is increased due to the many toxic chemicals used in the production of plastics (Moore, 2008). The toxins contained in the plastics can act as endocrine disruptors in some animals, and can lead to the bioaccumulation of dangerous chemicals throughout the food chain (Fossi et al., 2012; Bakir et al., 2014). This extends the threat of plastic marine debris to terrestrial animals, as well as humans, who also ingest these toxins when they eat marine animals.

2.2 Plastics - A History

2.2.1 Plastic production

Plastic has been in production for years, but the demand has rapidly increased. In the early 1950s plastic was produced at a rate of 1.5 million tonnes per year and has increased exponentially since then, reaching a production rate of 322 million tonnes in 2015 (PEMRG, 2015; Geyer et al., 2017). The most commonly produced plastics are known as standard plastics, which are prepared for everyday use and are the plastics that most people would encounter on a daily basis (PEMRG, 2015).

Standard plastics account for 85% of global plastic production, while the other 15% is specialized plastics intended for very specific purposes, otherwise known as engineering plastics (PEMRG, 2015). The most common type of plastic is polyethylene, which makes up 34.4% of standard plastic production, followed by polypropylene which accounts for 24.2% (PEMGR, 2015). Both polyethylene and polypropylene are composed of fossil hydrocarbon monomers, ethylene and propylene respectively, as are many other standard plastics (Geyer et al., 2017). Plastics derived from fossil hydrocarbons are not biodegradable and are not easily recycled (Geyer et al., 2017). The majority of standard plastics (about 75-80 million tonnes) are created for use in packaging, plastics largest market, and are therefore intended for a one time use before disposal (Andrady, 2011; Geyer et al., 2017). The rapid increase in plastic production, particularly single-use plastics, has led to increased plastic pollution (Bakir et al., 2014).

2.2.2 Plastic pollution

The mass production of plastic, particularly lightweight, single-use plastic, has led to an inevitable increase in the amount of plastic pollution and litter entering the environment, whether it be through improper disposal or accidental loss (Moore, 2008; Bakir et al., 2014). While plastic is a pollutant in terrestrial systems, aquatic plastic pollution has been monitored and well documented since the 1970s (vom Saal et al., 2008; Eriksen et al., 2013). Plastic is now the largest contributor of marine pollution and in most places, aquatic litter contains 60-80% plastic, and in some areas it is as high as 90-95% plastic (vom Saal et al., 2008).

Large, visible plastics have accumulated in large quantities throughout the oceans, with some accumulations so large they have been dubbed trash gyres, leading to the creation of the 5 Gyres Institute, and other non-profit organizations aimed at cleaning up plastic debris in the oceans (Cozar et al., 2014, Hoellein et al., 2014). This plastic, known as macroplastic, is easily visible and poses obvious threats to the aquatic ecosystem. One major concern associated with these accumulations of trash, and larger debris in general, is the risk of organism entanglement (Eriksen, 2010; Andrady, 2011). This concern is most often associated with derelict fishing gear which has been lost, discarded or broken at sea. Often known as ghost nets, lost fishing gear can entangle many aquatic animals including turtles, dolphins, sharks, and seabirds (Moore, 2008; NOAA, 2014). Animals as large as whales can become ensnared, and although it may not cause immediate death, the fishing nets and lines can cause serious injury. Globally it is estimated that ghost nets and lost fishing gear impact more than 200 species, although this is likely an underestimate (Moore, 2008; NOAA, 2014). While the impacts are severe, derelict fishing gear accounts for only 18% of all marine plastic debris (Andrady, 2011).

In addition to macroplastic pollution, concern has shifted to the smaller, less visible microplastics, which also contribute to aquatic plastic pollution. Research began in the 1990s and the term microplastic, used to define plastics having a diameter of less than 5 mm, was coined in 2004 (Frias et al., 2014; Law and Thompson, 2014). A lower limit of less than 1 mm in diameter is now used in many studies, although the international standard is still less than 5 mm in diameter (Leibezeit and Leibezeit, 2014). Despite their small size, microplastics can vary greatly in shape and appearance, ranging from smooth and spherical to sharp and angular, as well as long, thin fibres (Frias et al., 2014). Microplastics were first named a minor source of marine pollution in the early 1990s (Fendall et al., 2009) and in 2011 the United Nations Environmental Program identified plastics, including microplastics as an emerging environmental concern (UNEP Year Book, 2011). Although research is still relatively new, and the problems associated with microplastics are not yet fully understood, current research shows that despite their small size, microplastics are more problematic than macroplastics (Fendall et al., 2009). Microplastics can either be primary, coming from direct inputs such as accidental loss, or secondary micro plastics which form from the breakdown of larger plastics (Fendall et al., 2009; Eriksen, 2010; Andrady, 2011).

2.2.3 Pollution Sources

Growing concern about microplastics and their sources has lead to the discovery that 80% of aquatic plastic originated from a land-based source (Andrady, 2011). Improper disposal of plastic products results in the majority of plastic debris in the ocean. Estimates about quantities and sources of plastics entering aquatic systems are not often reliable. A 2015 study estimates that anywhere from 4.8 to 12.7 million metric tonnes of plastic debris entered the oceans in 2010 (Jambeck et al., 2015). As population rises and plastic production and consumption increase, the amount of plastic accidentally entering waterways will only increase. It is predicted that without better management strategies the amount of plastic entering the ocean will increase tenfold by the year 2025 (Jambeck et al., 2015). Recreational fishing also contributes to plastic pollution accumulation, as do beachgoers, and other coastal activities

(Derraik, 2002). Accidental losses are highest in areas of dense populations along many coastlines where more than 50% of the world's population resides. The lightweight, disposable nature of most plastics produced, makes it easy for things like bags and bottles to be blown into waterways (Moore, 2008).

Microplastics can also originate from several different sources. The development of secondary microplastics occurs in the ocean and other waterways through mechanical breakdown, such as waves and abrasion against shorelines, and UV-B exposure, causing larger plastic particles to slowly fragment, breaking into smaller and smaller pieces (Andrady, 2011; Driedger et al., 2015). Primary microplastics often originate from accidental loss. In addition, clothes washing, especially of synthetic fabrics, releases microfibres too small to be caught by traditional sewage treatment plants, just as the use of microbeads in beauty and household products contributes to the number of microbeads found in aquatic systems (Fendall et al., 2009; Andrady, 2011; Browne et al., 2011). Once again, the desirable features of being light-weight and easy to produce has led to an increase of household items, most notably beauty products, containing micro-scrubbing beads. These beads have replaced more environmentally friendly options, such as sugar, salt, and shells and therefore the potential for microbeads to pollute lakes and oceans has increased (Fendall et al., 2009). Increased use, paired with accidental losses and inadequate or poor waste management practices continues to contribute to the increasing microplastic pollution in aquatic ecosystems (Browne et al., 2011; Frias et al., 2014).

2.3 Dangers of Plastic Pollution

2.3.1 Impact on Aquatic Organisms

Many of the microplastic particles currently found in the ocean are approximately the same size as the food or prey of low trophic level animals, and thus these particles are readily consumed, often with no foraging bias (Andrady, 2011; Setala et al., 2014). This means that as long as there are microplastics present in the oceans, low trophic organisms such as zooplankton, crustaceans, bivalves, and other benthic and pelagic organisms will continue to consume the particles (Desforges et al., 2014). Low trophic organisms such as lugworms *(Arenicola marina)*, blue mussels *(Mytilus edulis)*, mesozooplankton, and barnacles *(Cirripedia)*, are at risk of ingesting and being negatively impacted by the increased microplastic pollution (von Moos et al., 2012; Besseling et al., 2013; Setala et al., 2014).

Ingestion of plastic particles can lead to several problems including: lack of proper nutrition, blockage of feeding apparatus, internal blockage, false satiation, and decreased consumption (Lusher et al., 2013). Once ingested, the plastic particles can become lodged inside internal organs, trapped in the circulatory system or deposited in the soft tissue of the organisms (von Moos et al., 2012). In a study of 500 blue mussels, it was found that microplastic particles decreased the stability of cell membranes and increased the formation of granulation tissue. While the granulation tissue forms first, when combined with the continued presence of the plastic particles, both the granulation tissue and the microplastics contribute to the destabilization of cell membranes (von Moos et al., 2012). Therefore, the presence of microplastics within an organism's body places stresses on the physical structure of that organism's body, and has adverse physiological effects including altered immune response, neurotoxic effects, and genotoxicity (von Moos et al., 2012; FAO, 2017).

The effects of microplastics can be felt through all levels of the aquatic food web, including fish and other animals (Oliveira et al., 2013; FAO 2017). Fish can ingest microplastics either through prey or by mistaking plastics for food (FAO, 2017). The common goby *(Pomatoschistus microps)* experiences lowered predatory efficiency, abnormal swimming behaviour and general lethargy, and in cases of extremely high concentration, death among juvenile fish when accidentally ingesting microplastics (Oliveira et al., 2013; de Sa et al., 2014). Plastics and pyrene appear to cause problems with neurotransmission in fish, which negatively impacts muscular movement and nervous system function (Oliveira et al., 2013). A previous study involving older goby found similar results, although the effects were less pronounced and were visible only after a longer period of exposure (Oliveira et al., 2012). Animals most vulnerable to the dangers of microplastic pollution are juveniles, which is a concern for the overall health of individuals and the health of future generations. Fish in the English Channel are similarly affected by microplastic pollution. Of 504 fish examined, 184 had ingested plastic particles, and both pelagic and demersal type fish were equally vulnerable (Lusher et al., 2013).

Crabs and other crustaceans are also vulnerable to the negative impacts of microplastics. Crustaceans can experience increased mortality, developmental delays, and decreased fecundity (FAO, 2017). Some species, such as shore crabs *(Carcinus maenas),* can also take up micro plastics through their gills, (Watts et al., 2014). This can lead to a decreased rate and efficiency of ventilation if enough plastic particles accumulate (Watts et al., 2014). The microplastics are also consumed by crabs through their prey, commonly mussels and small filter feeders, which are lower trophic level organisms, however most crabs are more able to pass ingested plastics (Watts et al., 2014; FAO, 2017).

While the majority of research has focused primarily on the effects on smaller organisms, microplastics are also dangerous for large marine filter feeders (Fossi et al., 2012). Baleen whales are particularly susceptible because of the huge amounts of water and food consumed when feeding (Fossi et al., 2012). Sharks, and other large marine organisms are also at risk from increased microplastic pollution (Fossi et al., 2014). The presence of toxic chemicals in the blubber and muscles of Mediterranean fin whales *(Balaenoptra physalus)* and basking sharks *(Cetorhinus maximus)* indicates that the accidental ingestion of these particles is contributing to the increased concentration of phthalates and organochlorides within marine organisms (Fossi et al., 2014). This is particularly concerning for large filter-feeding mammals such as the fin whale because phthalates act as endocrine disruptors and can interfere with fertility, mating and the health of both parents and offspring (Fossi et al., 2012). Given how long-lived whales are, and their ability to consume 70,000 litres of water in one mouthful, they are at a high risk of consuming large quantities of microplastics over a lifetime (Fossi et al., 2012). The disruption of the endocrine system in a large mammal such as the fin whale threatens the health and continued existence of the species, particularly since the concentration of microplastics in the ocean is on the rise, making more plastic particles available for accidental ingestion (Fossi et al., 2012; Fossi et al., 2014).

2.3.2 Dangers to humans

The accidental ingestion of microplastics by aquatic organisms not only harms the health of the ecosystem and organisms, it also threatens humans. This occurs when humans consume aquatic organisms containing microplastic particles (Van Cauwhenberghe et al., 2014; Karami et al., 2017). When a marine animal consumes plastic there is potential for those plastic particles to be passed up through the food chain, as the organisms themselves are consumed (Setala et al., 2014). This is well documented in several species of fish, crustaceans and sea birds (Bakir et al., 2014; Karami et al., 2017). In addition, other predators also have the potential to accidentally ingest plastic particles, which further increases the amount of plastic consumed, since it can be consumed first hand, and can be contained within prey (Van Cauwhenberghe et al., 2014).

The potential for plastic particles to migrate up the food chain was investigated by Setala et al. (2014) through the use of various species of mesozooplankton and shrimp from both pelagic and benthic environments. Mysid shrimps, copepods, cladocerans, rotifers, polychaete larvae and ciliates were all captured from the Baltic Sea and exposed to various environments with different concentrations of microplastics, and some with both microplastics and live prey, to measure the rate of accidental ingestion (Setala et al., 2014). The shrimps, which act as predators, were also exposed to zooplankton prey that were known to have ingested microplastics (Setala et al., 2014). All of the different organisms consumed plastic particles regardless of whether live prey was present, although the amount and rate of consumption varied by organism (Setala et al., 2014). While the plastic particles were easily expelled by these organisms there is still the possibility for accidental ingestion of plastic to cause accumulations of toxins in marine organisms. Microplastics have the documented ability to absorb toxic chemicals from the water and pass these chemicals on to organisms through digestion, which causes the plastics to degrade and break down, releasing chemicals into the organisms (Setala et al., 2014). The passing of plastics and their associated toxic chemicals to higher and higher trophic levels is of extreme importance not only because of the threat it poses to marine life, but also because of its potential to threaten human health, as well as many local and international economies that rely on fisheries and marine products for economic stability (Setala et al., 2014).

Given the ability of plastics to pass up the food chain, it is of vital importance to know how this affects those animals grown and harvested for human consumption (Karami et al., 2017). This is particularly important since humans consume large quantities of food harvested from aquatic environments. In 2009, approximately 125 million tonnes of food were obtained from marine environments, and many of those marine animals are raised in natural seawater conditions, and are thus exposed to all the toxins present, including microplastics (Van Cauwhenberghe et al., 2013). The concentration and availability of microplastics to aquatic animals is not known since sampling is extremely difficult, and ocean and lake features such as currents, tend to concentrate pollution in certain areas (Andrady, 2011; NOAA, 2014). In addition, different plastics have different densities depending on age, composition and size, which causes plastic pollution in the ocean to be contained at various depths (Desforges et al., 2014). Thus, surface measurements intended to demonstrate the concentration of microplastics in the water tend to greatly underestimate the true concentrations (Andrady, 2011).

Van Cauwhenberghe et al. (2014) examined two common species of bivalves grown and harvested for human consumption: blue mussels (*Mytilus edulis)* and Pacific oysters (*Crassostrea gigas)*. Both species were found to contain microplastic particles in the soft tissue when examined following dissection. Blue mussels from the North Sea were found to have an average of 0.036± 0.07 (SD) particles for every gram of tissue, while Pacific oysters contained an average of 0.47±0.16 (SD) particles for every gram of tissue, when raised in the Atlantic Ocean (Van Cauwhenberghe et al., 2014). This may even be a slight underestimate because of the use of nitric acid during processing, which will completely dissolve any nylon fibres or particles (Van Cauwhenberghe et al., 2014). The plastic particles contained in the bivalves at the time of their death are then consumed by humans (Van Cauwhenberghe et al., 2014). Based on human consumption rates in various localities, people could be consuming anywhere between 1800 to 11,000 particles of microplastic particles per year, which can then be easily taken up by the digestive system and dispersed and trapped in various areas throughout the human body (Van Cauwhenberghe et al., 2014). Physiological and toxicological effects of these microplastics on humans are currently unknown. A study using blue mussels examined the health implications of microplastics within the mussel, and it is possible that the effects of microplastics on humans will be similar (von Moos et al., 2012). Persistent microplastic particles threaten the stability of cell membranes and lead to increased scar tissue within organs and soft tissue, the effects of which are concentrated around the plastic particles (von Moos et al., 2012).

A study of German beer in 2014 found microplastic particles present in all 24 bottles of beer sampled (Leibezeit and Leibezeit, 2014). A wide variety of contaminants were found within the beer, but the majority of the particles were either fragments or lines/fibres, as well as pellets (or granules). This study reported a range of 2 to 79 particles/L, with a mean of 22.6 particles/L. Potential sources of contamination could be from airborne anthropogenic debris, shedding of fibres from workers clothing, contaminants from filtration practices, contamination from ingredients, as well as impurities not removed from the bottles during rinsing (Leibezeit and Leibezeit, 2014). A similar study focusing on beer brewed using water from the Laurentian Great Lakes, found similar results in the 12 different brands of beer (Kosuth et al., 2018). The overwhelming majority of particles were fibres (98.4%) and the remaining particles were identified as fragments. Unlike the Leibezeit et al. study, the beer brewed using water from the Laurentian Great Lakes had an overall lower number of particles/L (ranging from 0 to 14.3 particles/L) and a lower overall mean of 4.05 particles/L (Kosuth et al., 2018). An average annual consumption ranging from 520 to1,800 particles was calculated, depending on the amount of contamination in the beer (Kosuth et al., 2018).

Kosuth et al., also examined drinking tap samples from 14 countries worldwide, with samples coming from seven different regions, and from areas of both low, and high population density (2018). Of the 159 samples processed, microplastics were found in 81% of the samples, ranging from 0 to 61 particles/L, with an overall mean of 5.45 particles/L (Kosuth et al., 2018). The highest mean came from a United States sample (mean of 9.24 particles/L) and the four lowest calculated means were from European Union samples. Overall, 98.3% of particles found were identified as fibres, and the remainder were identified as either fragments or film (Kosuth et al., 2018). Although this is only an initial assessment of global tap water, and does not represent a comprehensive study, based on the results and water consumption guidelines, people may be consuming anywhere from 4,400 to 5,800 particles/L per year through tap water (Kosuth et al., 2018).

Another human consumable studied for microplastic contamination was sea salt. Kosuth et al. (2018) examined 12 different brands of ocean and sea mined table salt, and found a range of 46.7 to 806 particles/kg, with an overall mean of 212. Of all the particles identified in sea salt samples, 99.3% were again found to be fibres (Kosuth et al., 2018). Based on consumption rates of salt, an additional 40 to 680 particles could be ingested annually.

Adding up the three annual averages of microplastic ingestion from beer, tap water, and salt, people could be consuming anywhere from 4,960 to 8,280 particles per year. This number is however, an underestimate, as it uses only three different types of human consumables. To get a more accurate number, more in depth studies of plastic contamination in human consumables would be necessary.

2.4 Microplastic Monitoring

2.4.1 Marine Monitoring

Most of the microplastic research has focused on marine ecosystems, working to carefully monitor marine pollution, effects on marine ecosystems and organisms and the distribution and movement of microplastic pollution in marine settings. Plastic pollution has been well documented in both the Pacific and Atlantic Oceans, along many coastlines and in deep sea sediments (Van Cauwenberghe et al., 2013; Desforges et al., 2014; Frias et al., 2014; Lusher et al., 2014). Coastal sampling in marine environments is important and produces useful knowledge since so much of the world's population is concentrated along coastlines. This information can help describe how population density and proximity can impact or increase plastic pollution in the surrounding environment (Frias et al., 2014).

Open water surface samples and deep sea sediment studies demonstrate the pervasive nature of plastic marine debris but the results are likely to be an underestimate of the true number of microplastics present in the world's seas and oceans. There is still more to learn about how plastics behave in salt water. Many microplastic particles are negatively buoyant while others may be distributed throughout the water column. Additionally there is still more to learn about currents and transportation of microplastics in the oceans, as that is not yet well understood.

2.4.2 Lacustrine Monitoring

Since most research has focused on marine settings even less is known about the abundance, distribution, transportation and behaviour of microplastic particles in freshwater systems (Wagner et al., 2014). Despite the minimal amount of research focused on freshwater systems, similarities exist between marine and lacustrine systems in terms of transportation, distribution, prevalence (Eerkes-Medrano et al., 2015). Lacustrine settings that have been studied also show the presence of microplastic pollution, including remote lakes such as Lake Hovsgol, Mongolia, which is extremely polluted by plastics despite low population density in the surrounding area. The pollution in this particular scenario can be traced back to the lack of proper waste treatment and disposal facilities (Free et al., 2014). There have also been several studies published about the presence of plastic litter along freshwater beaches and shorelines, showing that microplastics are just as pervasive throughout freshwater systems as they are in marine settings (Zbyszewski et al., 2011; Dris et al., 2015).

The Laurentian Great Lakes have also been a topic of limited studies concerning freshwater microplastic pollution although only two studies to date have sampled surface waters of the Great Lakes to determine the abundance and distribution in some, but not all of the lakes. Eriksen et al. (2013) report on surface water samples from Lakes Superior, Huron and Erie. In total the study resulted in 21 samples (with only 5 samples coming from all of Lake Superior) and all but one sample contained microplastic particles, with an average abundance of 43,157 microplastic pieces per square kilometre. A more extensive study of Lake Michigan was completed in 2013, which used 59 surface samples to analyze the presence and abundance of microplastics throughout the lake (Mason et al., 2016b). This survey also found plastics in every sample except for one.

To date, the only surface water samples taken from Lake Superior were reported in the Eriksen et al. (2013) study and included only five sample from across all of Lake Superior. These five samples were taken near Marquette, Michigan, USA, close to the St. Marys River, and so only focused on one small portion of the lake. Therefore, the present study represents the most comprehensive study of Lake Superior, or any of the other Great Lakes.

2.5 Microplastic Policy and Management

To eliminate intentional disposal of plastics at sea, the International Convention for the Prevention of Pollution from Ships was signed in 1973, and a complete ban was in place by 1988 (Xanthos et al., 2017). However, since most aquatic plastic pollution originates on land, the amount of plastic pollution in lakes, rivers, and oceans has continued to increase (vom Saal et al., 2008; Andrady, 2011; Ericksen et al., 2013; Xanthos et al., 2017).

Several policies banning certain types of single-use plastics were implemented at different times around the world (Banks, 2008; Xanthos et al., 2017). Single-use plastic bags are regulated in many countries and cities. The earliest ban was in place in 1991 in Germany, and other countries have followed suit either by instituting outright bans, partial bans, bag taxes, or legislation designed to phase out single-use plastic bags (Xanthos et al., 2017). North America currently only has bans or levies in several cities and municipalities, and many companies charge to purchase plastic bags (Banks, 2008; Xanthos et al., 2017). Toronto implemented a mandatory 5 cent charge for plastic bags in 2009, in 2008 Sudbury, Ontario launched a reusable bag pilot project offering consumers incentives for the use of reusable bags, and in 2007, Leaf Rapids, Manitoba became the first municipality in Canada to ban single-use plastic shopping bags (Banks, 2008). San Fransisco, and California, have also since banned single-use plastic bags (in 2007 and 2016 respectively) (Goodyear, 2007; Banks, 2008).

Microbeads in toiletries have been banned or phased out, globally as of January 2018. The plans to phase out microbeads in North America and the United Kingdom were initially announced in 2015 as a three year plan to phase out the sale of products containing microbeads (Pollack et al., 2017; Xanthos et al., 2017). Canada was the first country to name microbeads as a toxic substance and has implemented a plan to ban the production, sale, or import of products containing microbeads (excluding natural health products or non prescription drugs) (Environment and Climate Change Canada, 2016).

Several national organizations have identified goals, plans, and strategies to address aquatic microplastic pollution, including the United Nations, the G7, the World Economic Forum, and the World Bank. All four organizations outline the need for more public education, more monitoring and research, and a more in depth understanding of the plastic pollution problem. In addition there is mention of the need to reduce single-use plastic consumption, increase producer responsibility, improve waste management practices, and reduce plastic litter inputs from waste water effluent (Brennholt et al., 2018).

In 2011, The Honolulu Strategy was developed by the National and Oceanic Atmospheric Administration (NOAA) and the United Nations Environmental Program (UNEP). The framework is designed to be applied globally to a variety of different programs and specific circumstances to help mitigate plastic pollution, and is considered the most useful, and comprehensive management framework currently available (Pettipas et al., 2016; Xanthos et al., 2017). This framework can be adapted to meet the specific needs of different locations and has a variety of strategies to help meet the overall goals of waste reduction (Pettipas et al., 2016). There are three main goals identified in the Honolulu Strategy, and each goal is accompanied by an individualized outline of actions that can be used to achieve the associated goal. The first goal is to reduce the overall amount and impact associated with litter and waste introduced to marine environments from terrestrial sources. The second goal is to reduce the amount and impact of litter and waste lost at sea through shipping, fishing, and other activities. The third and final goal is to reduce the amount of accumulated marine debris along coastlines, in benthic environments and pelagic water (NOAA & UNEP, 2011). The strategy also outlines gaps in knowledge, and ways in which different levels of government, intergovernmental organizations, and other interested parties can work together to share knowledge, best management practices, and outcomes (NOAA & UNEP, 2011). The intended use of the Honolulu Strategy is three-fold: as a planning tool to help develop programs or projects focusing on marine debris, as a universal frame of reference used for collaborating and sharing information, and as a tool to help develop and evaluate monitoring projects (NOAA & UNEP, 2011).

The majority of plastic pollution guidelines focus on managing solid waste disposal through the use of sorting, recycling and reusing, but very few focus specifically on microplastic pollution (Pettipas et al., 2016). Overall, there are few, if any, regulations or policies in place which adequately address the problem of microplastic pollution. Research shows that even in locations where bans of single-use plastic are in place, enforcement is often a problem and therefore many studies suggest that laws and legislation are needed from federal governments, rather than the introduction of more taxes, plans to phase out certain plastics, or partial bans (Pettipas et al., 2016; Walker et al., 2017; Xanthos et al., 2017). Other proposed initiatives include banning single-use plastic take out containers, banning single-use plastic straws, introducing deposit and return for plastic containers and bottles, as well as enforcing extended producer responsibility (EPR), which would force producers to be responsible for the entire life-cycle of a product (Walker et al., 2018).

There are many laws and regulations which govern water quality, use, and protection for the Great Lakes, and these are shared between both Canadian and US governments, as well as the provincial and state governments of the provinces and states which border the Great Lakes (Table 1) (Ronan, 2017) . Not all legislations focus specifically on plastic pollution, although some, such as the updated Great Lakes Water Quality Agreement, mention the need to reduce the introduction of garbage (including plastics) into the lakes, and to work towards minimizing pollution (Government of Canada and Government of the United States of America, 2012).

Table 1: Description of legislation governing the protection of the Great Lakes, and the Great Lakes Region, including year, signatories and a brief outline of the main goals of each piece of legislation. (Adapted from Ronan, 2017).

Name	Year	Signatories	Summary
Boundary Waters Treaty	1909	United States, Great Britain (on behalf of Canada)	Water governance strategy to address and prevent any conflicts over shared water resources. Led to creation of International Joint Commission
Great Lakes Water Quality Agreement	1972 1978 1983 1987 2012	United States and Canada	Formal agreement stating a joint Great Lakes management approach. Mention of the need to mitigate or reduce garbage (plastic) pollution input/effects.
Great Lakes Charter and Annex	1985 2001	Ontario, and Quebec (CAN) Indiana, Michigan, Minnesota, New York, Ohio Pennsylvania, and Wisconsin (US)	Commitment from provinces and states in the great lakes region to work towards environmentally friendly, and sustainable economic growth.
Great Lakes - St. Lawrence River Basin Sustainable Water Resources Agreement	2005	Ontario, and Quebec (CAN) Illinois, Indiana, Michigan, Minnesota, New York, Ohio, Pennsylvania, and Wisconsin (US)	Commitment to protect and restore the Great Lakes, specifically focusing on the need to properly regulate large scale water removal projects, and water diversions.
Great Lakes- St. Lawrence River Basin Water Resources Compact	2008	Illinois, Indiana, Michigan, Minnesota, New York, Ohio, Pennsylvania, and Wisconsin (US)	Introduction of legally binding interstate compact to adhere to commitments outlined and agreed o tin the Sustainable Water Resources Agreement.
Canada-Ontario Agreement on Great Lakes Water Quality and Ecosystem Health (COA)	2014	Canada, Ontario	Formally legislates the necessity to involve stakeholders in decision making processes surrounding the issues of protecting water quality, habitat, and species. Focused on sustainable development in the Great Lakes region.

The International Joint Commission (IJC) made several recommendations focused on microplastics in the Great Lakes in a 2017 report (International Joint Commission). The IJC recommends that the federal and provincial governments of Canada, and the federal and state governments of the US work together to create a binational plan specifically designed to address microplastic pollution in the Great Lakes. The agreement, when created should make use of science, research, policy, education, and outreach, and should involve a diversity of stakeholders from both the US and Canada to help produce educated and inventive solutions. Beyond the development of a binational plan, the IJC further recommended focusing on the following (International Joint Commission, 2017):

1) Science:
    - a) develop standardized sampling and analytical methods
    - b) develop a transport model to clearly identify sources and movement of microplastics in the Great Lakes
    - c) assess ecological and human health impacts
    - d) invest in improved resource recovery, recycling, and pollution
2) Pollution Prevention:
    - a) support extended producer responsibility
    - b) support incentives to reduce plastic pollution
    - c) fund analysis of programs and policies designed to reduce and prevent plastic and microplastic pollution in the Great Lakes
    - d) promote good plastic and waste management policies

3) Education and Outreach:

a) fund support for local programs and initiatives which foster education and promote the prevention of plastic and microplastic pollution in the Great Lakes.

The Ontario Provincial Government is working towards some of the IJC's recommendations by further reducing plastic pollution and other waste through the implementation of a circular waste pattern which would shift the burden of paying for recycling from taxpayers to manufacturers, and would be a step towards implementing extended producer responsibility (Government of Ontario, 2017; Alfred, 2018). In 2016, Ontario signed the Waste Free Act and began establishing a circular economy. Similar to cradle to cradle thinking (McDonough and Braungart, 2002), a circular economy focuses on reducing the use of raw materials, increasing resource recovery and maximizing product usefulness, and minimizing the amount of waste generated by packaging and end-of-life waste (Government of Ontario, 2017; International Joint Commission, 2017). This will hopefully reduce the production and consumption of single use plastic packaging. To track successes throughout the process of establishing a circular economy, the provincial government set waste diversion goals, which would see 30% diversion by 2020, 50% diversion by 2030 and 80% diversion 2050 (Government of Ontario, 2017). A circular economy in Ontario will hopefully help eliminate large quantities of plastic from the waste stream, either by rendering certain products obsolete, replacement with environmentally friendly options, or by vastly improved resource recovery which would see plastic products repeatedly recycled, minimizing the need for more production (Government of Ontario, 2017; Alfred, 2018).

There are many organizations worldwide focusing on reducing plastic consumption and waste, through volunteer efforts, public awareness campaigns, and education or outreach initiatives. The largest is the Plastic Pollution Coalition (PPC) which has garnered the involvement of more than 700 initiatives, businesses or groups, as well as public figures or celebrities, from 60 countries worldwide (Plastic Pollution Coalition, n.d.). The PPC includes links to other organizations, petitions, actionable lists, and resources for education, accessing plastic free alternatives, and finding up to date information for current plastic management and the status of other plastic pollution initiatives (Plastic Pollution Coalition, n.d.).

There are several initiatives in both Canada and the US working towards a reduction of plastic pollution in the Great Lakes. The Alliance for the Great Lakes is a Chicago-based organization promotes: advocacy, education, research, and volunteering to help protect the health of the Laurentian Great Lakes (Alliance for the Great Lakes, 2018) One of the objectives for 2018 is to increase pressure on Congress to reduce plastic and microplastic consumption. The group also founded the Adopt-a-Beach program which organizes beach clean ups, data collecting and public education and outreach events to help prevent further plastic pollution (Alliance for the Great Lakes, 2018). In Canada, Plastic Oceans is a federally funded non-governmental organization, also aimed at raising awareness and increasing public knowledge of the plastic pollution problem, as well as how to help (Plastic Oceans, n.d.). The organization is supported by many other Canadian environmental groups, and runs an Ocean Ambassador program which involves students and youth under the age of 18 in the effort to reduce plastic pollution. The organization and it’s ambassadors work to create community specific messages, community clean up initiatives, and individualized school programs (Plastic Oceans, n.d.).

Chapter 3: Methodology

3.1 Sampling Methods

Sample sites used in this study were selected by the USGS. Nearshore sites in the US were selected in 1978 and nearshore sites in Canada were selected in 1989 for annual larval whitefish surveys. Offshore sample sites were selected in 2011. The annual survey goal is to sample at each site every year, however sometimes it is not possible due to weather, commercial fishing, or mechanical problems. Nearshore sites were originally selected to assess nearshore fish communities, although some of the original sites are no longer sampled due to a lack of easy accessibility. Offshore sites were randomly selected to assess fish populations in water of different depths (<100 m. 100-200 m, >200 m).

Lake Superior surface water samples were collected by Mark Vinson and the USGS, from 19 May 2014 to 4 August 2014 using paired 1 x 1 m^2 500 micron mesh three metre long, neuston nets (Sea-Gear, Inc. Melbourne, FL, http://www.sea-gear.net/neuston-nets.html). The nets were deployed side by side, connected by aluminum rods at the top and bottom. The nets were connected to a single aluminum rod which was connected to a boom from a boat using steel wires and a large hook (Fig. 1&2).

The nets were deployed near mid-ship on the port-side of the US Geological Survey research vessel *Kiyi,* three metres away from the boat to prevent small waves from the boat interfering with sample collection. The net was fished at a depth of 0.5 metres; half in and half out of the water to reduce the likelihood of typical waves washing over the top of the net. Trawls were made at ~ 4 km/h (range=3.2-4.8 km/h) for 10 minutes. The average trawl distance was 0.7 km (range=0.6-0.8 km) (Appendix A). Collections were made at 95 locations (Figure 3).

Figure 1: Paired neuston nets and boom. Photo: Mark Vinson, USGS 2014

The survey was initially completed to track and record numbers of larval Lake Whitefish *(Coregonus clupeaformis)* throughout the lake. Any larval whitefish (measuring 8-25mm in length) found in the sample were counted and removed and the remaining material in each net was stored in a labelled, glass jar. To prevent confusion between the paired samples, the net closer to the boat was labelled Net A and the net further from the boat was labelled Net B. Samples obtained from Net A were given even identification numbers, and samples from Net B were given odd identification numbers. Each sample was stored in a separate, labelled jar and preserved using 90% ethanol.

Figure 2: Paired neuston nets. Photo: Mark Vinson, USGS 2014

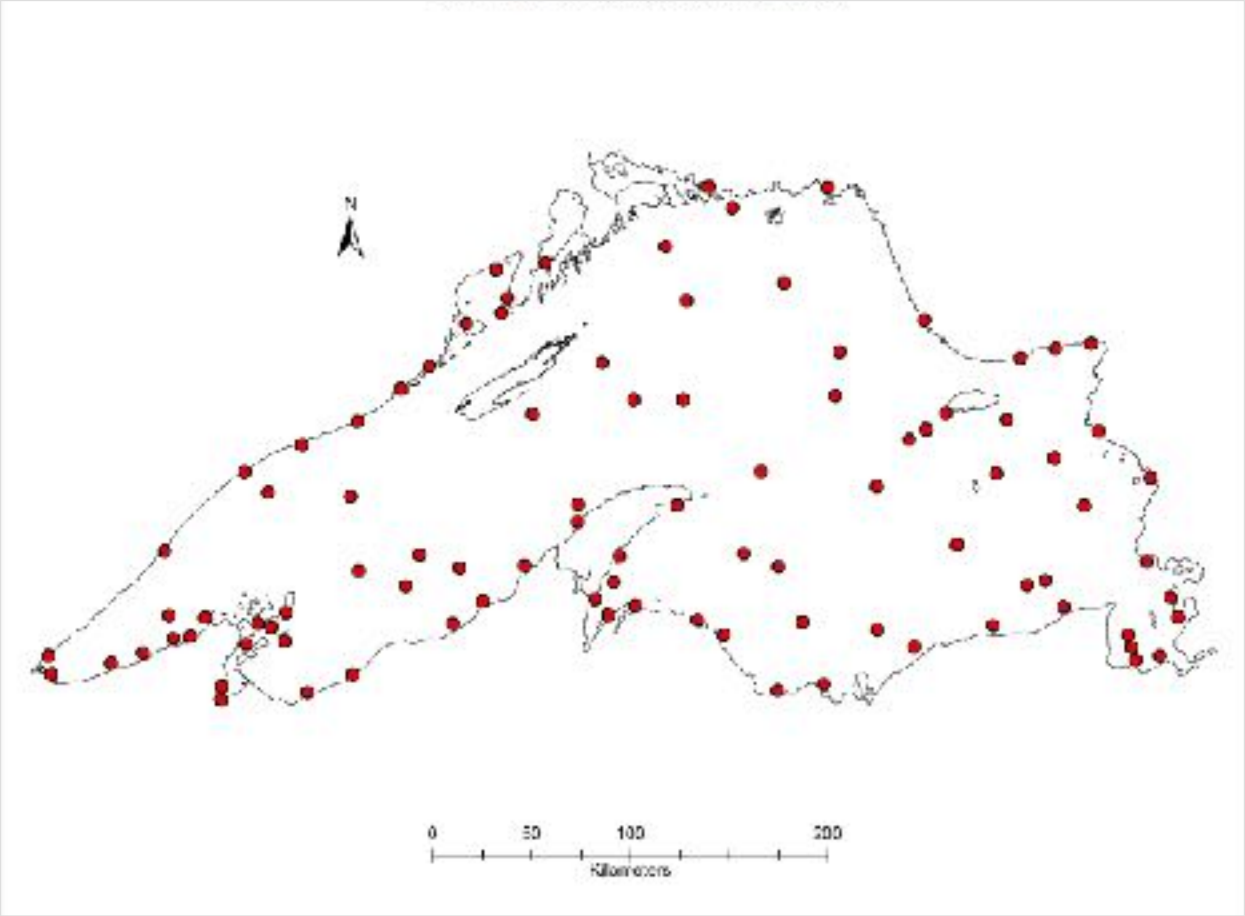

Figure 3: Map of surface water sample tow locations used for larval whitefish surveys, from USGS.

3.2 Lab Analysis

The samples were processed in the lab by myself, using a modified National Oceanic and Atmospheric Administration marine debris protocol (Masura et al., 2015). The processing separates the microplastic particles from organic and other debris in samples, making it possible to visually count and separate microplastics, as well as determine polymer identity. This included wet peroxide oxidation with an iron catalyst to remove organic material.

3.2.1 Catalyst Preparation

The iron (II) catalyst used during the wet peroxide oxidation was prepared as follows: Five hundred mL of distilled water + 3 mL of concentrated sulphuric acid (Fisher Scientific Catalogue, SA226 1) was combined with 7.5 g of Iron Sulfate Heptahydrate ($FeSO_4{\circ}7H_20$) (Fisher Scientific Catalogue, I146 500). The catalyst was stored in a glass jar with lid when not in use.

3.2.2 Wet Sieving

Samples were first emptied into a set of two stacked stainless steel sieves (0.355 – 0.999 mm, and ≥4.75 mm), allowing large organic debris and plastics to be removed from the largest sieve (≥4.75 mm) before beginning the wet peroxide oxidation, speeding up the digestion process. The sample bottle was rinsed into the sieves three times, using deionized water to ensure no particles remained in the jar. The material in the two sieves was then thoroughly rinsed using deionized water (DI). Any large plastics found in the top sieve were removed using forceps, rinsed and set aside in a labelled petri dish. Large organic material was removed from the largest size category and carefully rinsed using DI water to remove any microplastic particles which may have been stuck. Once completely rinsed, the largest pieces of organic material were discarded. The remaining material in both sieves was rinsed into a labelled 600 mL beaker, using as little deionized water as possible.

3.2.3 Wet Peroxide Oxidation

Once the organic material was rinsed into the 600 mL beaker, 20 mL of 30% hydrogen peroxide, and 20 mL of Iron (II) catalyst were added. Depending on the amount of water needed to rinse the organic material into the beaker, the total initial volume of liquid varied between 60 and 100 mL and was a dark amber colour. A stirring bar was added and the beaker was then placed on a stirring hot plate set at 75 degrees Celsius. The speed of the stir bar depended on how much organic material was present in the sample, and was set high enough to mix the sample thoroughly without the contents of the beaker splashing the sides. The hot plates were placed inside a fume hood for processing. During processing, 20 mL aliquots of hydrogen peroxide were added as the reaction progressed (denoted by a change in colour from amber to pale yellow). If, despite the addition of hydrogen peroxide, the colour of the solution remained dark amber or rust-coloured, 20 mL of 6M sulphuric acid was added. The continued dark amber or rust colour indicates that the iron in the catalyst has come out of solution. During processing, no more than one additional aliquot of hydrogen peroxide or sulphuric acid were added. If, despite the addition of hydrogen peroxide or sulphuric acid, the reaction was no longer proceeding (no change of colour was observed), the beaker was once again rinsed through the smallest sieve (0.355 mm) and the process repeated from the beginning. A lack of colour change indicated that the solution had become too diluted for the reaction to continue.

The sample was monitored carefully while processing as the reaction can boil over rapidly if heated above 75 degrees Celsius (Masura et al., 2015). If the solution boiled violently and there was potential for it to boil over, the heat was reduced, and distilled water was added to slow the reaction. The reaction was complete when the colour of the solution changed from a rusty orange to a pale yellow and the organic material had been fully digested.

In some cases, it was necessary to process the sample multiple times to fully digest the organic material and make it possible to sort the microplastics. If there was still a large quantity of organic material remaining after the initial wet peroxide oxidation was complete, the sample was rinsed through the stacked sieves again and the process was repeated. In some cases, due to large quantities of organic material, the whole process needed to be repeated up to four times to fully digest the organic material in the beaker.

Once the wet peroxide oxidation was complete, the sample was ready for optical analysis.

3.2.4 Microscope Analysis

After wet peroxide oxidation, the sample was again sieved through stainless steel stacked sieves, this time using three sieves to separate particles by size fraction (0.355– 0.999 mm, 1.00–4.749 mm, and ≥4.75 mm). The largest sieve (≥4.75 mm) was still used, even though large visible plastics had been removed and set aside before the wet peroxide oxidation was completed. Large plastic particles may be entangled in organic material and would not have been visible to separate until after the wet peroxide oxidation was complete, so it is necessary to use all three sieve sizes. After rinsing carefully, the contents of each individual sieve were carefully rinsed into individual petri dishes labelled with the sample number and the size fraction. Each petri dish was then placed under the dissecting microscope at 40X magnification and all microplastic particles present were removed, counted and identified as either a fragment, pellet, line/fibre, film, or foam. There are multiple guides to identifying microplastic morphologies and at this time there is no universal standard. The morphological categories used in this study match those used in several other surface water studies (Eriksen et al., 2013; Free et al., 2014; Mason et al., 2016b). Table 2 outlines the morphological types and associated descriptions used to identify microplastics in the current study.

Table 2: Morphological types and descriptions used in visually identifying microplastics in this study.

Morphological Type	**Description**
Fragment	thick, angular, rigid plastic, often clear or translucent
Film	thin, smooth edges, transparent or translucent
Pellet	rounded, spherical, or cylindrical with a mostly smooth surface, white or blue common colours
Line	thin, fibrous, often frayed ends, blue or black most common colours, often curled ends
Foam	white, bubbled, soft texture

This information for all size fractions was recorded on a single data sheet per sample (Appendix B). The microplastic particles identified were counted and removed then placed into labelled 4 mL screw cap glass vials using thin forceps. For each sample, size fractions were separated into individually labelled vials so that each sample was divided into either two or three vials (depending on whether microplastics were identified in each size category or not). The vials were then sealed and stored for preservation.

3.2.5 Fourier Transform Infrared Spectroscopy Analysis

The FTIR analysis provides a chemical identification of microplastic particles by comparing the particles' individual spectrum to a spectral database for all commonly known polymers. Commonly, an FTIR consists of the following components: a source, interferometer, sample compartment, detector, amplifier, A/D convertor, and a computer. The source passes a beam through the sample after passing through the interferometer and then reaches the detector, where the signal is amplified (Libre Texts Libraries, 2015). The amplified signal is converted to a digital signal, which passes into the computer. The computer uses the Fourier transform, a mathematical function, which converts signals into a graph of the associated frequencies (Libre Texts Libraries, 2015). In preparation for FTIR analysis, sample vials were rinsed and emptied into labelled, clean, dry petri dishes (separated by size fraction) and placed in a Thermo Scientific Heratherm Oven at 50°C until the petri dish and its contents were dry. Individual particles were then removed from the petri dish using a microscope (Leica EZ4HD, 8-40x zoom, integrated 3Mpixel camera) and placed on the FTIR (PerkinElmer Spectrum Two ATR; 450 cm-1 to 4000 cm-1, 64 scans, 4 cm-1 resolution) for analysis. The resulting spectra were compared to internal spectral libraries to find the closest match and determine chemical composition. A match of 70% or more was considered to be sufficient to assign composition.

Due to the time limiting nature of the FTIR analysis and the number of particles counted and sorted, not all particles from all samples could be analyzed (Figure 4). In an effort to analyze a representative number of particles, approximately 10% of the total particles counted were targeted for analysis. To reach a total of 10% analysis, samples with high counts of microplastics were selected from all around the lake, and from these samples, no fewer than 10, and no more than 30 particles (divided between size fractions and morphological types), were selected and analyzed. The rest were returned to their respective 4mL labelled, glass vial.

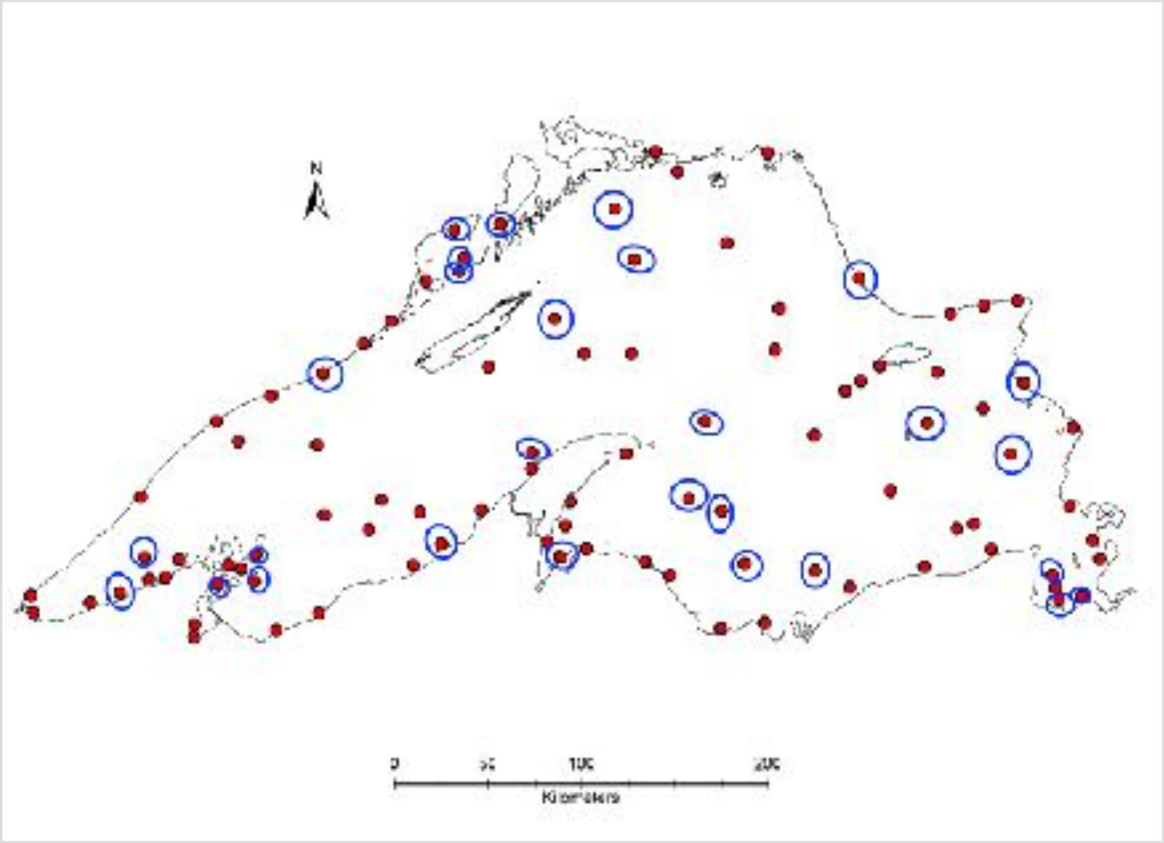

Figure 4: Map of samples processed using FTIR, circled with blue ring.

3.3 Data Analysis

3.3.1 Abundance

The abundance of each type of plastic particle (separated by size and morphology) was calculated to determine the average number of microplastic particles per square kilometre (count/km^2). Abundances were calculated in this unit of measurement because it is the most common unit used when presenting surface water microplastic pollution data (Eriksen et al., 2013; Free et al., 2014; Wagner et al., 2014; Mason et al., 2016b). In order to calculate the abundance it was first necessary to calculate the surface area using the following formula:

$$\text{Surface Area (km}^2\text{)} = \text{Tow length (km) x Width of Net mouth (km)}$$

Once the surface area was calculated, the abundance could be calculated using the counts:

$$\text{Abundance (count/km}^2\text{)} = \text{count} \div \text{surface area (km}^2\text{)}$$

3.3.2 Side by side sampling variability

To determine if the spatial heterogeneity between the side by side nets was statistically significant a paired T-test was performed using the total number of particles found in each individual net (not separated by size or morphology) (SYSTAT 13 for Windows, version no. 13.1). The data were separated into two groups, labelled Net A and Net B to differentiate between the abundances calculated for each net at each sample location.

Initially the data had to be analyzed to determine if they were distributed normally, using a probability plot. Since the data were not normally distributed and were skewed right, a log (X + 1) transformation was performed and a new probability plot was created, which showed a normal distribution. Using the log transformed data, a paired T-test was performed.

Descriptive statistics comparing the two sets of samples were also collected including, maximum, minimum, mean, median, 95% confidence interval, and standard deviation.

3.3.3 Differences in distribution

Distributions of plastics, characterized into the 15 categories of large (>4.75 mm), medium (1.00 – 4.75 mm) or small (0.36-1.0 mm) fragments, pellets, fibers/lines, films or foam, were compared between different parts of Lake Superior (east vs west, north vs south, onshore vs offshore) and between the two replicate nets used for sampling. East and west were divided by the blue line in Figure 5. North and south were divided by the Canadian and United States border (solid black line), nearshore and offshore were designated by the USGS during the initial sampling and are separated by the dotted green line (Figure 5), and Net A was the net closer to the boat, while Net B was further from the boat.

Distributions were compared by permutational Manova using square root transformed data and an S17 Bray-Curtis similarity (Primer 7, version beta 11 with PERMANOVA +1 add on). The tests were performed as an unrestricted permutation of raw data with 999 permutations. Significant differences were explored with SIMPER analyses to describe which plastics were more common in which areas.

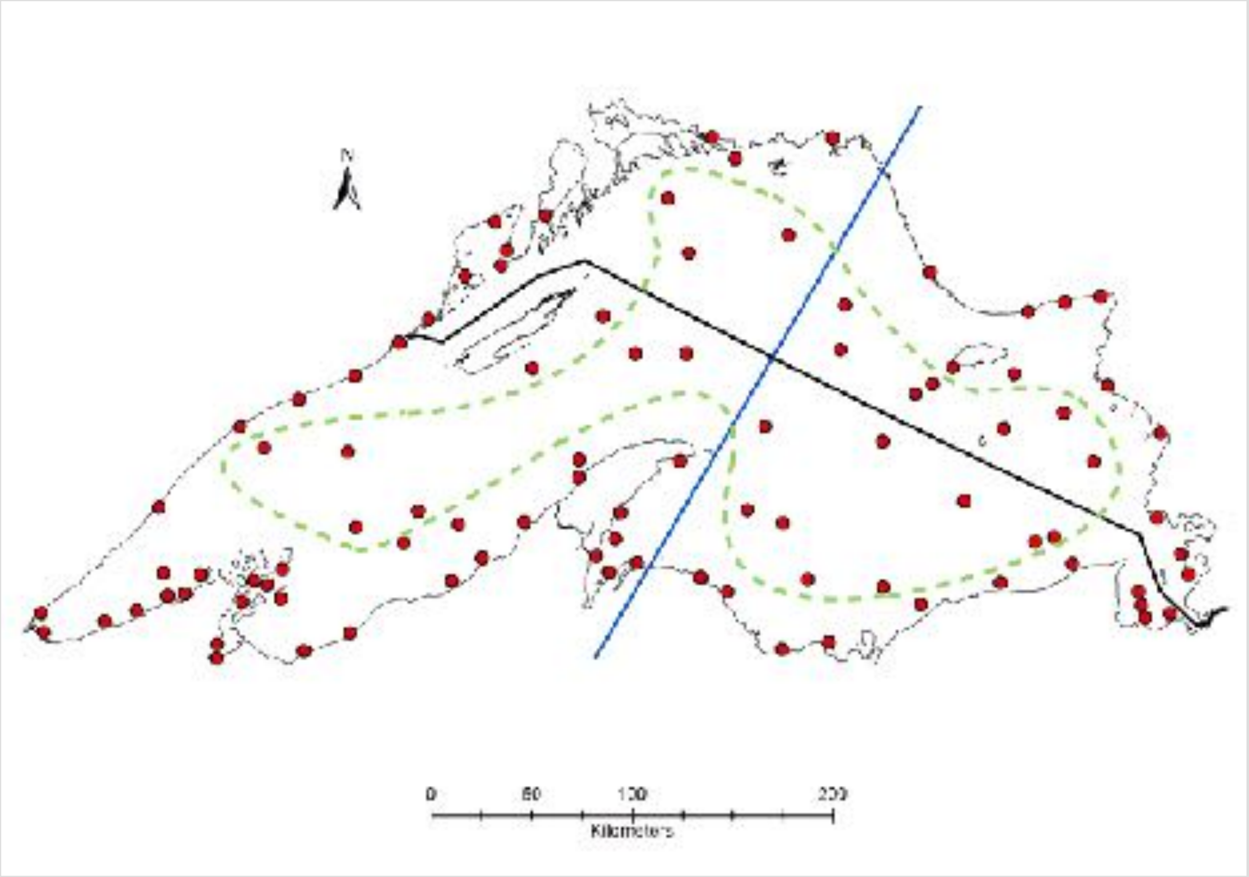

Figure 5: Different groups of Lake Superior samples compared using statistical analyses. East and west samples divided by solid blue line, north and south samples separated by solid black line (US and Canadian border), and nearshore and offshore are separated by dotted green line.

Chapter 4: Results

4.1 Abundance

Of the 94 paired samples (187 individual samples) all samples contained microplastics (Appendix B). The initial visual analysis separated large plastic debris from the rest of the material contained in the samples. Further analysis using the dissecting microscope was necessary to separate particles less than 4.75 mm. Any particles not appearing to be plastic were not included in the total counts obtained for individual samples.

Photographs of some particles were taken to visually demonstrate what the different particles looked like, as well as to illustrate variations in colour and appearance (Figure 6, 7, 8, 9).

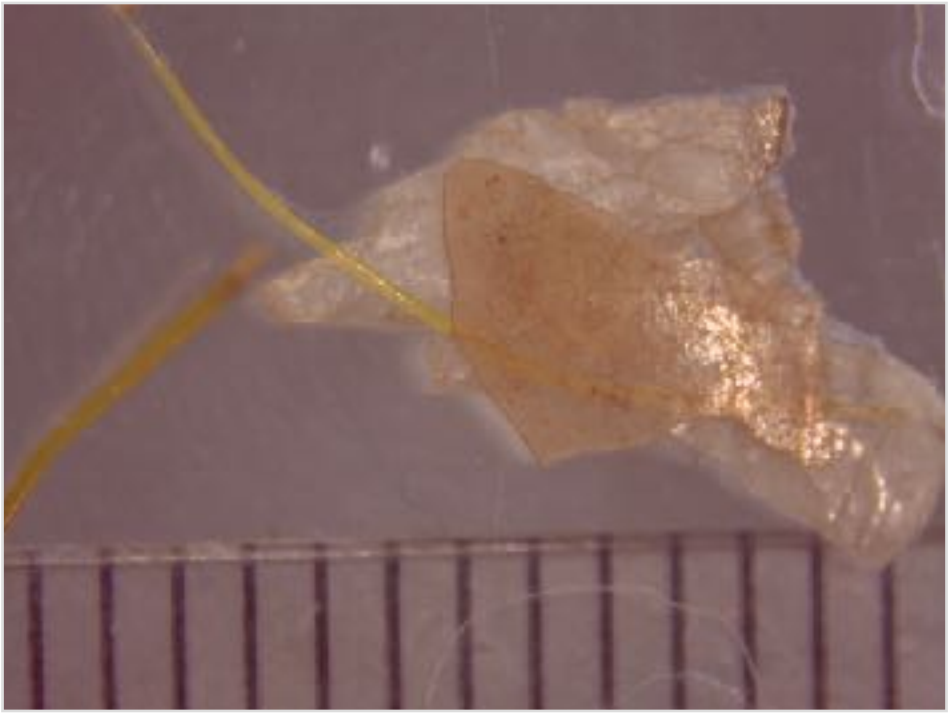

Figure 6: Particles >4.75 mm diameter line, film.

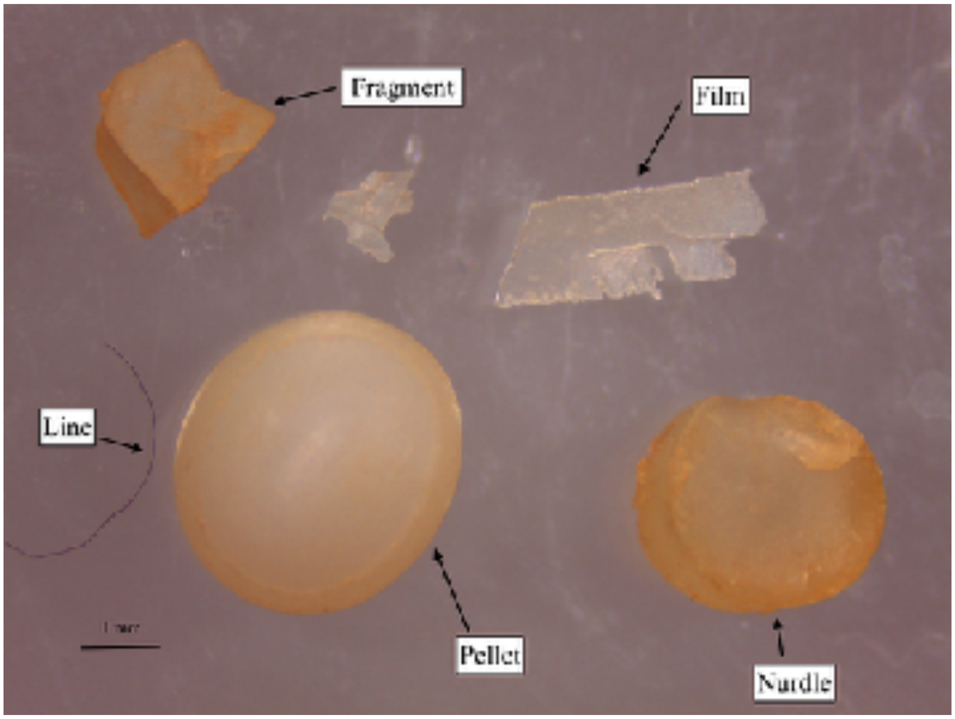

Figure 7: Plastics 1-4.75 mm diameter. Fragment, pellet, film, nurdle (pellet), and line.

Figure 8: Plastics 1-4.75 mm diameter. Film of various colours

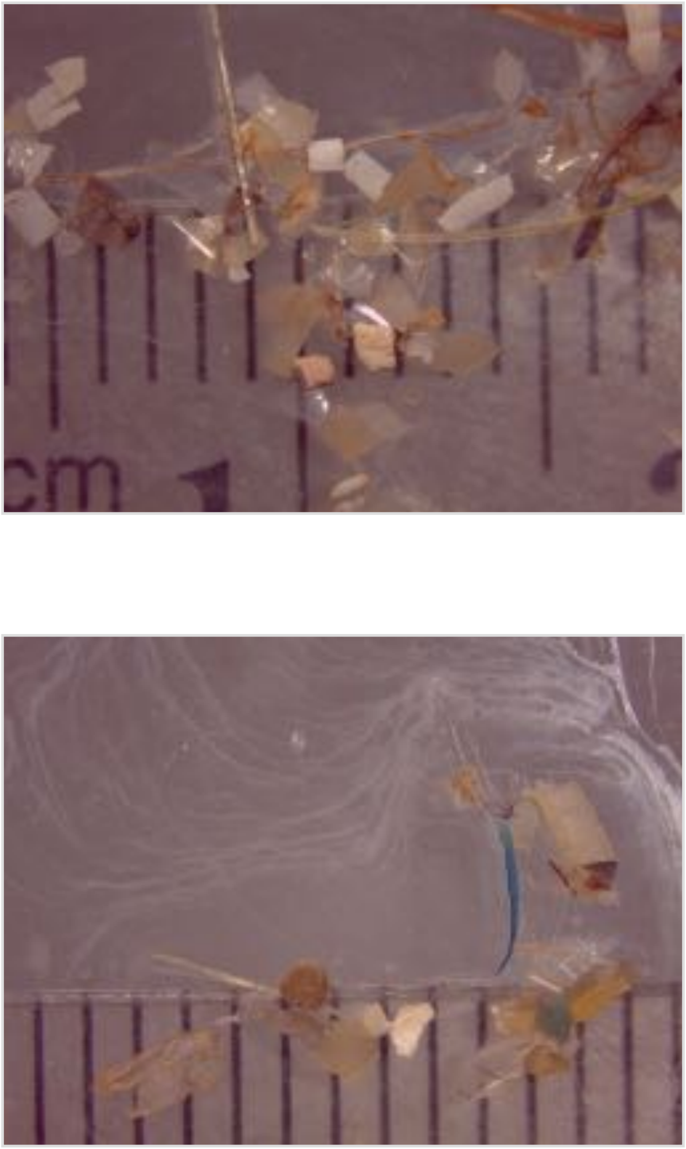

Figure 9: Plastics 0.3-1 mm diameter. Fragments, line and film (top). Fragments, film, line of different colours (bottom).

After calculating the average abundance per site using the two paired samples, the average abundance of the particles by size and morphology was calculated (Table 2). The smallest size fraction accounted for over 50% of the total and in all size fractions lines/fibres were the most abundant particle found. Most of the fibres identified were blue or black, although a few were pink. In the >4.75 mm size category several lines were thicker and coloured bright green and were suspected to be fishing line.

Table 3: Average Abundance of Plastic Particles (count/km^2) by Morphology and Size.

Particle	0.355-0.999 mm	1.000-4.759 mm	>4.75 mm	% of total
Fragment	4,167	2,748	55	23%
Pellet	110	130	0	1%
Fibre/Line	13,483	6,436	233	67%
Film	1,001	1,440	187	9%
Foam	124	148	16	1%
count/km^2	18,886	10,902	491	
% of total	62%	36%	2%	

Fewer than 20 spherical pellets were found and of those pellets found, only two or three had the appearance typical of pellets used in facial washes and beauty products. Several pellets were identified as nurdles, which are cylindrical, rather than spherical, in shape and are commonly used in industrial packaging.

The abundance of microplastic particles was calculated for each site to determine the abundance per kilometre squared, and the average abundance from each location (combining the two nets) was plotted on a map (Figure 10). Of all the locations sampled, 51 sites had abundances in the 20,001 - 50,000 count/km^2 range. Most of these locations were located near shore. Only five samples contained fewer than 10,000 particles and of those five, three were located off shore, closer to the middle of the lake. Three samples contained between 50,001 -

100,000 particles and two samples contained more than 100,000 count/km^2. The rest of the sites (34 sites) ranged between 10,001 - 20,000 particles. Half of these locations were middle of the lake samples, while the rest were varying distances from shore.

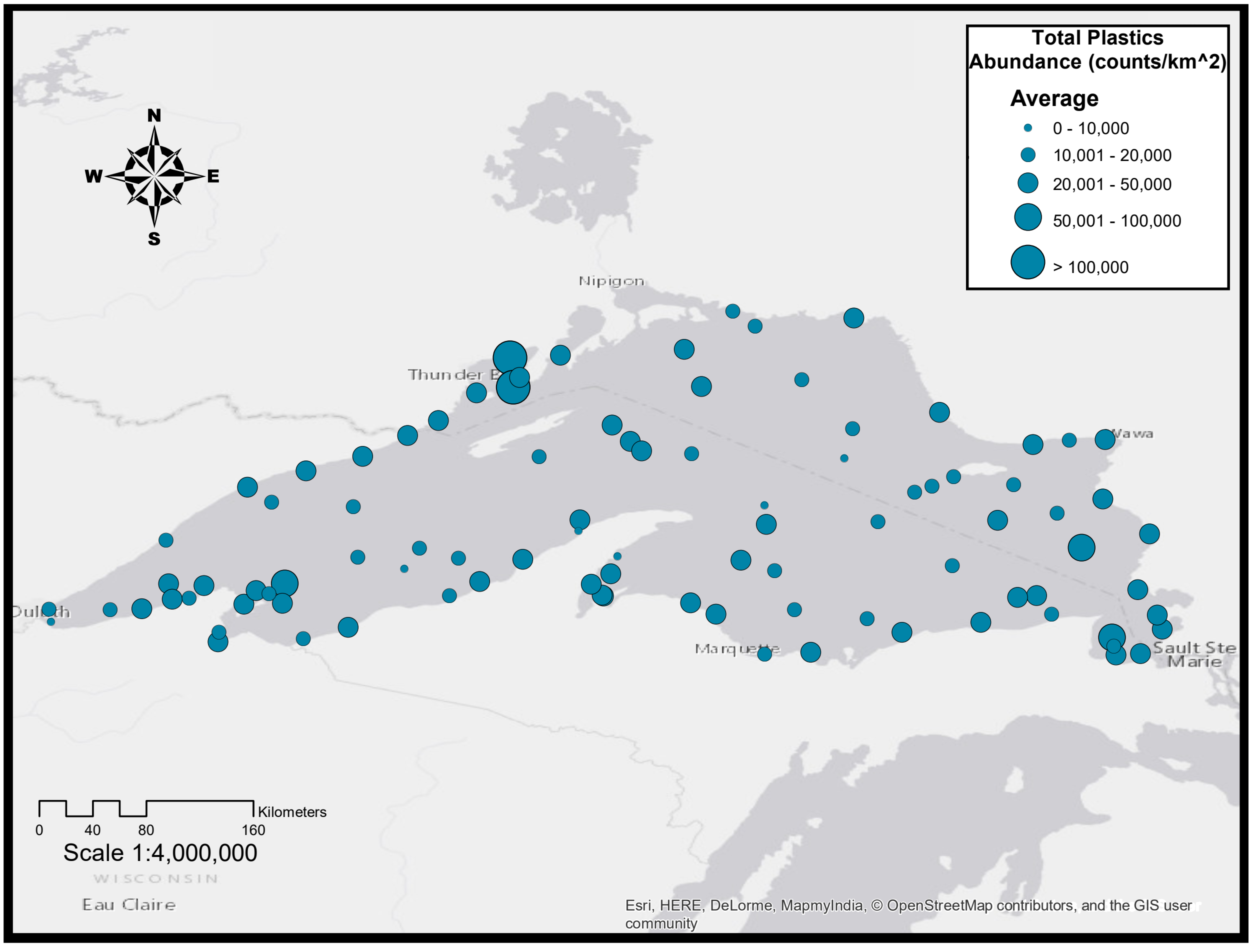

Figure 10: Calculated abundance of microplastics at each sample location used in 2014 surface water sampling.

The two locations with the highest abundances are offshore of Thunder Bay, Ontario, Canada, which is the largest city on the shore of Lake Superior. Other areas of high concentration include close to Sault Ste Marie, Ontario, Canada, where Lake Superior enters the St Marys River, as well as around the Apostle Islands, USA.

4.2 Distribution

4.2.1 Side by side sample variability

There was no significant difference in number of microplastic particles between the samples collected by Net A and Net B (paired t-test, T = -0.648, p = 0.519, N=93 paired samples; data log transformed) . Samples from Net A (closer to the boat) were somewhat more variable than samples from Net B as reflected in the higher standard deviation (Table 3).

Table 4: Descriptive statistics on log-transformed numbers of plastic particles captured by paired nets in 93 samples.

Statistical Test	**Net A**	**Net B**
Minimum	7.987	8.530
Maximum	12.869	12.535
Median	10.001	9.918
Arithmetic Mean	9.932	9.988
Standard Deviation	0.848	0.679

4.2.2 Size and morphology

Distributions of plastics, characterized into the 15 categories of large (>4.75 mm), medium (1.00 – 4.75 mm) or small (0.36-1.0 mm) fragments, pellets, fibers/lines, films or foam, were compared between different parts of Lake Superior (east vs west, north vs south, onshore vs offshore) and between the two replicate nets used for sampling. No significant difference was detected between the distributions of plastics collected by the two replicate nets or between samples collected from northern vs southern Lake Superior but differences were detected between samples collected in eastern vs western Lake Superior and between samples collected nearshore vs offshore (Permanova analyses; Table 5). Medium-sized line, small line and medium fragments were more abundant in samples from western than eastern Lake Superior but small fragments were less abundant (SIMPER analyses, 45.39% average dissimilarity; Table 6). Medium-sized line, small line and small fragments were more abundant in samples collected nearshore than offshore but medium fragments were less abundant (SIMPER analyses, 46.42% average dissimilarity; Table 7).

Table 5: Permanova test results comparing the types of plastic sampled in different parts of Lake Superior and in two paired nets (data log (x+1) transformed; Type III (partial) sum of squares). Plastics were characterized into 15 categories of large (>4.75 mm), medium (1.00 – 4.75 mm) or small (0.36-1.0 mm) fragments, pellets, fibers/lines, films or foam.

	East/ West	**North/ South**	**Nearshore/ Offshore**	**Net A/ Net B**
Pseudo-F	3.816	2.039	2.977	0.214
Degrees of Freedom	1,184	1,184	1,184	1,184
Unique Permutations	997	997	997	999
p-value	0.002	0.075	0.014	0.915

Table 6. SIMPER results showing the types of plastic contributing most to the difference between samples collected in west vs east Lake Superior.

	Average Abundance		Average Dissimilarity	Contribution (%)	Cumulative %
	West	**East**			
Medium Line	1.71	1.17	10.51	23.15	23.15
Small Line	2.85	2.56	9.77	21.53	44.67
Small Fragments	1.12	1.51	8.51	18.76	63.43
Medium Fragments	0.91	0.85	7.46	16.43	79.86

Table 7. SIMPER results showing the types of plastic contributing most to the difference between samples collected nearshore vs offshore in Lake Superior.

	Average Abundance		Average Dissimilarity	Contribution (%)	Cumulative %
	Nearshore	**Offshore**			
Medium Line	1.52	1.42	11.30	24.33	24.33
Small Line	2.91	2.19	10.47	22.56	46.89
Small Fragments	1.29	1.24	8.52	18.35	65.25
Medium Fragments	0.88	0.90	7.66	16.50	81.74

4.3 Polymer Identification

A combined total of 3887 particles were identified and counted from the 187 samples in this study. Of this total, 517 individual particles (~13%) were analyzed using the FTIR to confirm polymer identity. A successful identification (similarity of more than 70%) was obtained for 408 particles (~10%). The most common polymer identified was polyethylene, with a total of 204 particles, which makes up 50% of all particles identified. Polypropylene was the second most common polymer but was much less common than polyethylene, with a totally of only 82 particles, or 20%. The remaining particles were identified as polyester (7%), resins (8%), nylon (7%), polystyrene (2%), ethylene/propylene (2%), polyvinyl alcohol (1%), and poly actyl acrylate (1%) (Figure 11).

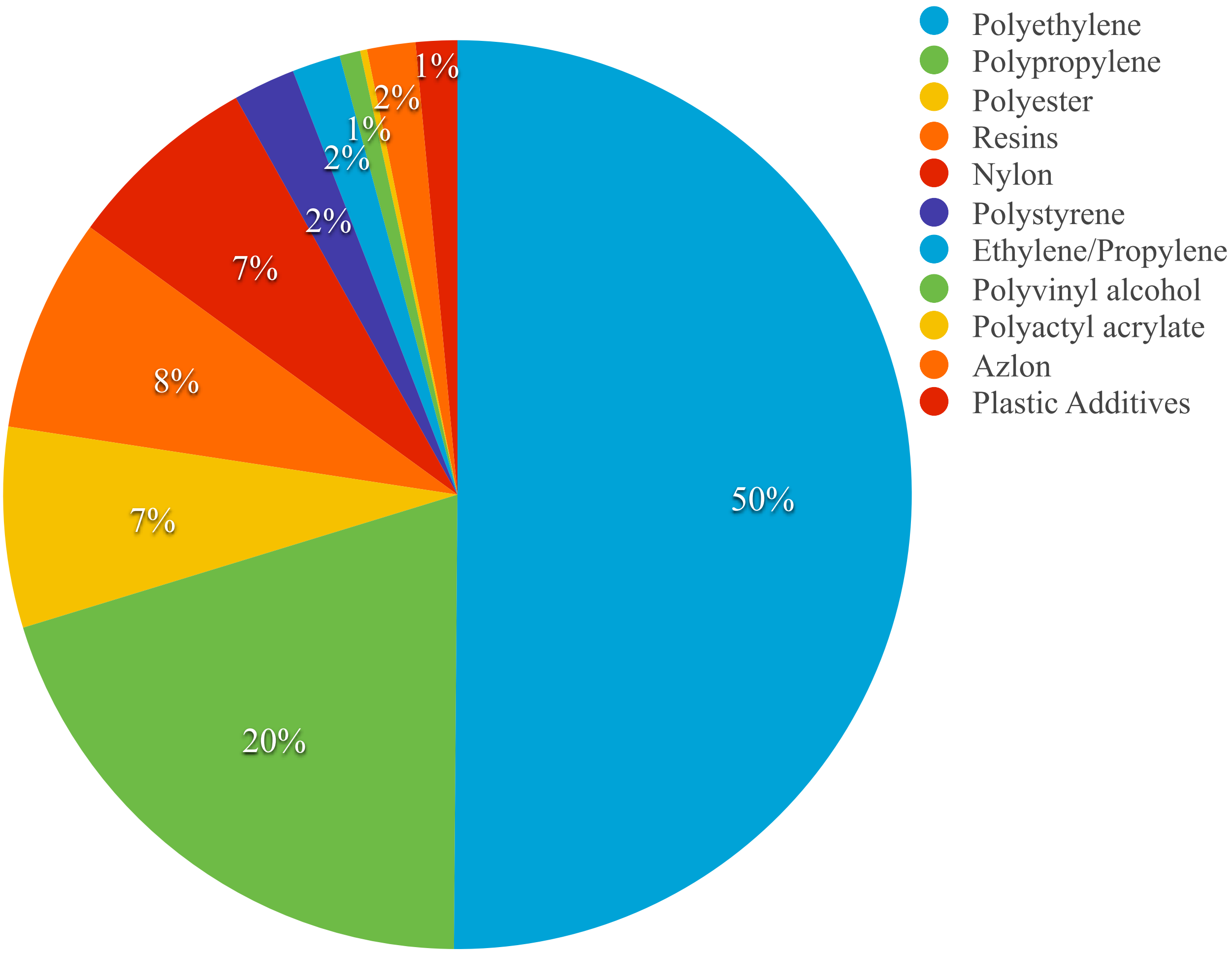

Figure 11: Percentages of different polymer types successfully identified during FTIR analysis of 517 particles, from 3887 total particles visually identified as plastic during microscope analysis of Lake Superior surface water samples.

Four particles were identified as various additives used during the production of plastic; either as a coating, fire retardant, or plasticizer. These four particles were identified as a variety of different additives but all together account for only 1% of all particles analyzed.

An additional six particles were identified as Azlon, which is a synthetic fibre that is made from protein and natural sources and is used in textiles and fabrics. These made up a total of 1% of all particles counted.

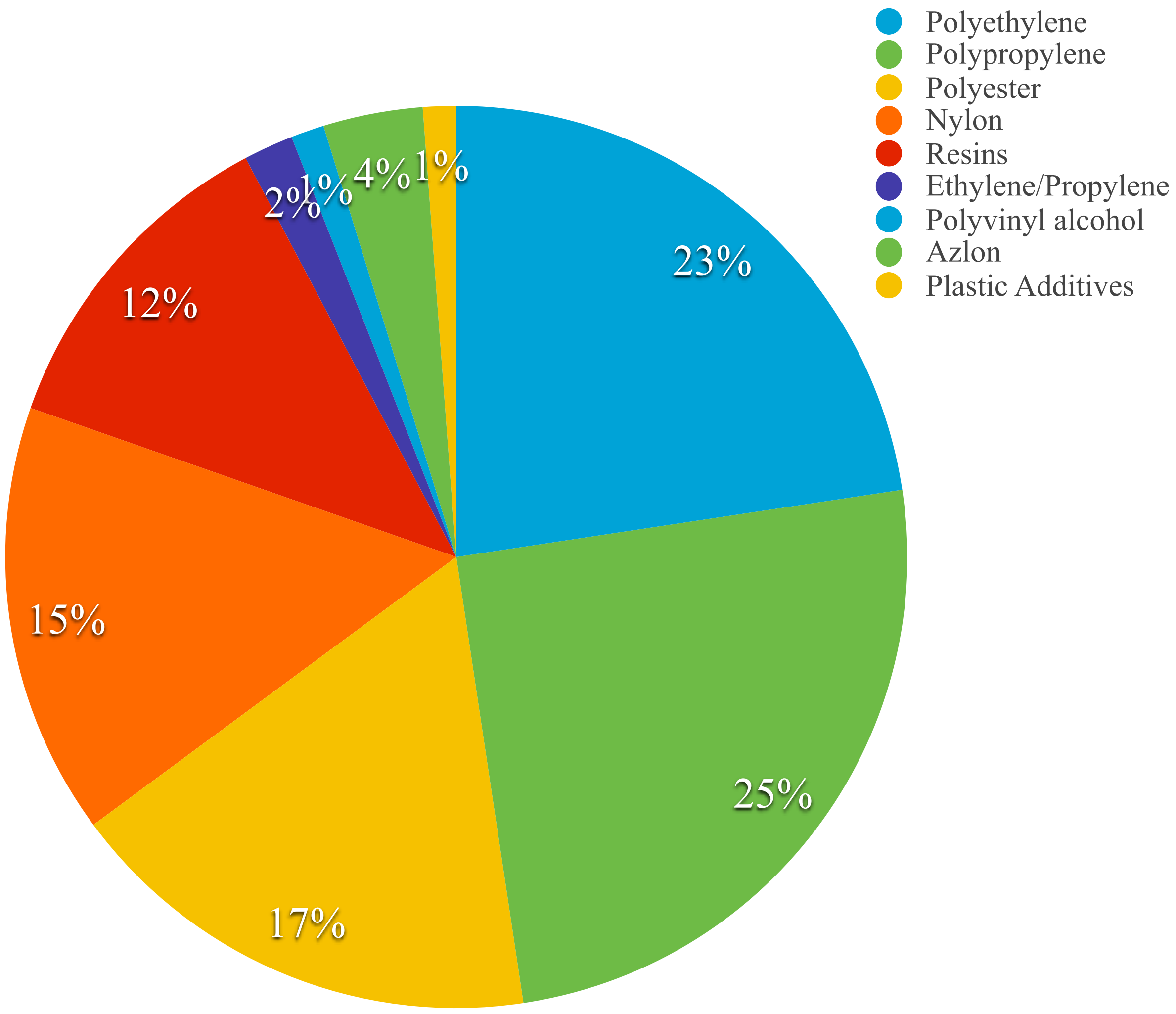

Figure 12: Percentages of different polymers identified in the line/fibre category during FTIR analysis. A total of 168 lines were analyzed and successfully identified.

Within the different morphological categories, there were differences in amounts and types of plastics identified using FTIR. Of the lines/fibres analyzed, polypropylene (25%) was the most common type of plastic identified, followed by polyethylene (23%). Polyester (17%), nylon (15%), and resins (12%) were also common (Figure 12). Polypropylene fibres were visually thicker, rounder and brighter colours than other fibres identified. Nylon was characteristically clear, with blue spots, and polyethylene fibres were commonly blue or clear. The most common colour present was blue, although black and pink were also common.

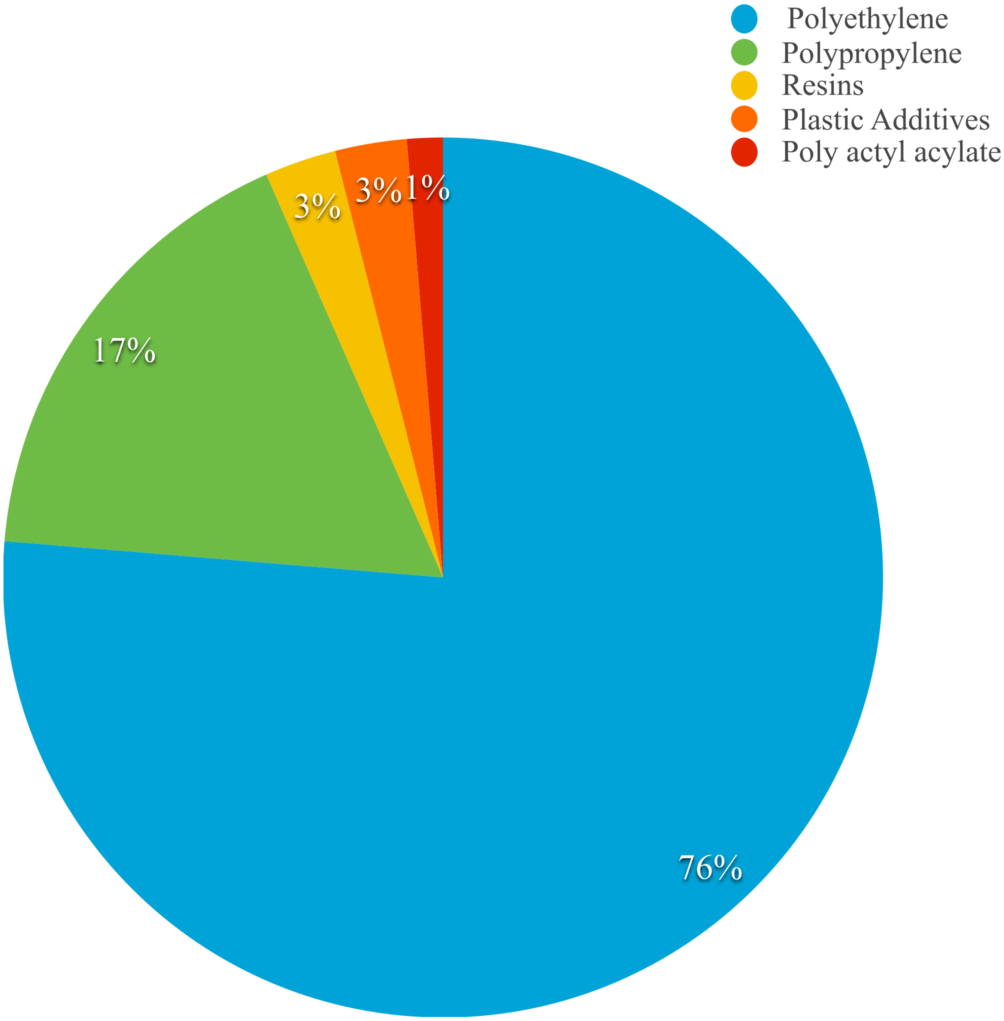

Figure 13: Percentages of types of polymers identified from the selected particles visually identified as fragments. A total 149 fragments were analyzed using FTIR.

Fragments were mostly polyethylene (70%), and polypropylene (17%), but resins (6%), ethylene/propylene (3%), and nylon (1%) were also identified (Figure 13). Fragments varied widely in shape, size and colour, but were often clear, and slightly translucent. Fragments also tended to be very angular.

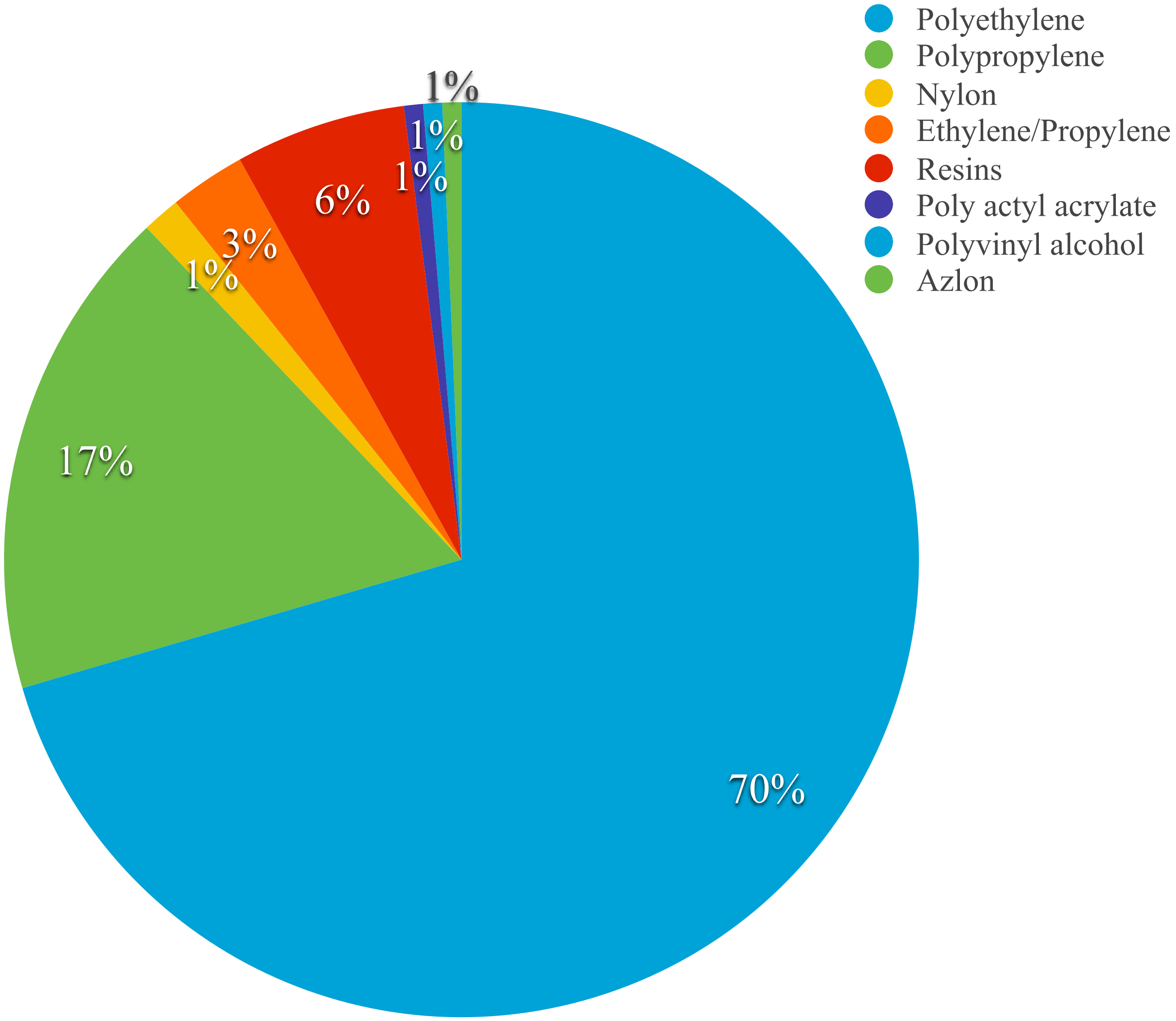

Figure 14: Percentage of different types of polymers present within the selected particles of film analyzed using FTIR. A total of 74 particles visually identified as film were analyzed and a successful identify obtained.

Film particles were also largely composed of polyethylene (76%). Polypropylene (18%) was the next most abundant, and resins and additives (both 3%) also appeared (Figure 14). Film also tended to be clear, and transparent, although some blue, transparent particles were also present. Film particles were less angular than fragments, and were thinner.

Pellets were comprised almost entirely of polyethylene (67%) and were all white and opaque in appearance. A total of six pellets were analyzed and five were identified as polyethyelene, and one was identified as polypropylene. Three pellets were found in the smallest size fraction (0.3-1 mm) and three were found in the medium size fraction (1-4.75 mm). All particles visually identified as foam were identified as polystyrene, and all were opaque and white. Foam particles were also easy to identify and distinguish from other particles because of the soft, spongy texture, which was easily squeezed by the tweezers during initial counting and sorting.

Chapter 5: Discussion

5.1 Discussion

5.1.1 Abundance

The abundance of microplastic particles is relatively homogeneous across surface water samples collected in Lake Superior. Of the 94 locations sampled, 51 (or 54%) have an abundance between 20,001-50,000 count/km^2 and another 34 locations have an abundance between 10,001-20,000 count/km^2. These two size fractions account for 90% of all sample locations throughout the lake indicating that abundance is similar regardless of where the samples were obtained. The five samples containing fewer than 10,000 particles were mostly located offshore, which is to be expected. In general, plastic pollution tends to be concentrated along shorelines and close to large population centres since nearshore samples are closer to sources of microplastic pollution (Eriksen et al., 2013). Of the 94 locations sampled, two contained abundance counts higher than 100,000 particles/km^2 (333,088 and 235,507 particles/km2). Both of these locations were located nearshore (approximately 3 km) and located about 20 km from Thunder Bay.

Thunder Bay is the largest city on the shore of Lake Superior, with a population of 121,621 and it is expected that larger cities would produce more plastic pollution than smaller cities (World Population Review, 2017). However, the counts offshore of Thunder Bay are much higher than those close to Duluth, Minnesota, the second largest city, by population, along the shores of Lake Superior. Duluth has a population of around 86,293, although the surrounding areas contain more than 200,000 people and the shared Twin Ports in the Duluth-Superior harbour is shared between Duluth and Superior, Wisconsin (pop. 26,475) (United States Census Bureau, n.d.). The shared ports are important for shipping and transportation throughout the Great Lakes and the St. Lawrence Seaway. Given the high combined populations and the heavy port traffic, similar to that experienced in Thunder Bay, ON, it would be expected that the microplastic abundances in both locations would be similar. However, the counts directly offshore of Duluth were much lower than those offshore of Thunder Bay. Two samples (516/517 and 518/519) were obtained less than 10 km from Duluth, and were less than one kilometre from the shore. The calculated average abundances were 15,625 particles/km2 (Samples 516/517) and 8,696 particles/km2 (Samples 518/519). This means that plastic pollution counts offshore of Duluth, Minnesota and Superior, Wisconsin, are less than half the counts from Thunder Bay, ON.

Even with a slightly higher population in Thunder Bay such a large difference is unexpected. It is possible that more samples need to be taken near Duluth and Superior to gain a better understanding of plastic pollution close to these two cities. It is also possible that circulation currents throughout Lake Superior are causing high concentrations of plastic debris to accumulate near Thunder Bay, ON. Duluth and Superior are located at the westernmost edge of Lake Superior, where the lake is narrow. The higher concentrations of plastics further east of Duluth, in and around the Apostle Islands, may be caused by the movement of plastic debris out into the lake and away from the Duluth-Superior harbour. It was expected that the Apostle Islands would have low concentrations since all but one of the 22 islands are part of the Apostle Islands National Lakeshore which is managed by the National Park Service, and thus has very low populations consisting mainly of recreational docks and cabins (National Park Service, n.d.).

Despite the low population, the samples around the Apostle Islands contained microplastic abundances between 20,000 and 50,000 particles/km^2. Therefore, it is possible that the higher concentrations are partly due to contributions from Duluth and Superior.

Higher concentrations (50,001-100,000) can also be found near Sault Ste Marie, Ontario, (population 74,000) even though the city itself is not directly on the shore of Lake Superior (Fig. 10). Sault Ste Marie is located near to where Lake Superior enters the St. Marys River, which connects Lake Superior to Lake Huron. Additionally, Sault Ste Marie is the third largest city on or near Lake Superior and is an extremely busy area for shipping and recreational boating. Sault Ste Marie is also an important industrial area in terms of steel production. The combination of industry, and population in the surrounding area, and water funnelling through the St. Marys River are likely the cause of the high concentrations of plastics in this area.

Two studies of currents and water movement patterns within Lake Superior support the idea that currents play a role in areas of high and low calculated abundances (Beletsky et al., 1999; Bennington et al., 2010). Surface currents in Lake Superior (down to a depth of 15 m) during the summer months would account for the accumulations around the Apostle Islands, as well as near the St. Mary's River. The high accumulations near Thunder Bay, Ontario were found in the bay itself and would be less influenced by the currents in the lake. Currents outside of Thunder Bay travel southwest towards Duluth but do not enter the bay because of the Sleeping Giant Peninsula which juts out and traps water and microplastics, preventing large quantities from being transported elsewhere by currents (Beletsky et al., 2009; Bennington et al., 2010). General circulation patterns are counterclockwise and run along the coastline of the lake and currents tend to slow throughout the summer months and quicken during the winter (Beletsky et al., 1999; Bennington et al., 2010).

Across the lake, microplastic abundance averaged 30,271 particles/km^2 (95% confidence interval of the mean ranging from 20,917 to 39,797 particles/km^2). Based on this average, and the surface area of Lake Superior, these data suggest a total count of more than 2.4 (1.7-3.3) billion particles across the total surface area of the lake. A surface water survey of Lake Michigan showed an average of 17,276 particles/km^2, which is approximately 13,000 fewer particles per square kilometre than Lake Superior (Mason et al., 2016b). In addition, Lake Superior holds roughly 1 billion more total particles than the surface waters of Lake Michigan (Mason et al., 2016b). The results of the Lake Michigan study and the present study, indicate that Lake Superior contains more microplastic per square kilometre than Lake Michigan, despite lower population density and lower industrialization. While unexpected this result makes sense in the context of Lake Superior's larger surface area and longer residence time (Quinn, 1992). In comparison, Lake Erie, has an average of 105,502 particles/km^2 and a calculated lake wide total of approximately 2.7 billion particles. This is expected due to the high population density, intense agriculture, and high levels of industrialization surrounding Lake Erie. Lake Erie is the smallest of the five Great Lakes, by surface area and volume, and the surrounding area is densely populated and heavily developed, and as a result contains more microplastic particles than both Lake Superior and Lake Michigan. However, Lake Erie's residence time of only 2.6 years may allow microplastic pollution to remain relatively stable, while Lake Superior's residence time of almost two hundred years may cause microplastic pollution to continue to increase as more microplastic particles enter the lake. A recent model of microplastic transport in the Great Lakes supports this hypothesis, showing that Lake Superior and Lake Michigan have the fewest numbers of particles exiting the lake each year, which again can be attributed to large size and long residence time (Hoffman and Hittinger, 2016).

5.1.2 Side by side variability

A paired t-test also indicated no discernible difference between the individual samples collected from both Net A and Net B. This means that both nets are collecting the same amounts of plastic. The only difference between the side by side neuston nets are that Net A samples, positioned closer to the boat, contained more variability within the samples than those collected from Net B. This is likely caused by some sort of interference due to proximity to the boat, likely caused by waves. These results are important since they indicate single net sampling methods will produce the same results as this particular double net set up.

5.1.3 Size and Morphology

The majority of microplastic particles found and identified were contained in the 0.3-1.0 mm size fraction, and the most common type of plastic found was fibres, or lines. Of all the microplastic particles collected, 62% were found in the smallest size fraction and 67% were fibres or lines. Most of the fibres or lines were blue in colour, although a few were pink or black and some were bright green. The bright green lines were found exclusively in the >4.75 mm category. Given the low populations and industrialization surrounding Lake Superior it was expected that most of the particles would be fibres or lines because low populations and industrialization reduce the overall input of plastic pollution into the lake, since less plastic is being produced and consumed. However, clothes washing would still result in shed fibres which would then enter the lake, causing fibres to be the most common of all particles identified.

Fragments were the next most common particle found, accounting for 23% of the overall total. Most of the fragments found were white or clear and tended to be thicker and very angular. The fragments were likely products of photo degradation or mechanical weathering of lost or improperly disposed of larger, macroplastics. Given the thickness, items like containers, and plastic packaging may be one source of fragments entering Lake Superior, and these are likely being broken down by a combination of mechanical weathering and UV-B exposure, producing secondary microplastics.

Film made up 9% of the total and was always clear, thin, and somewhat malleable. These particles likely originated from single-use plastic such as plastic bags, cling wrap and other disposable, cheap plastic products.

Foam and pellets each contributed 1% to the total number of plastic particles found. Only a few of the pellets matched the size, shape and colour of microbeads commonly associated with household beauty products. A few nurdles were also found, which are small, raw, cylindrical pellets used in industrial plastic production. Nurdles have been found on Lake Superior beaches near Nipigon, Ontario, since 2008. The nurdles likely entered Lake Superior waters after a train derailment in the area which was carrying nurdles (Hadley, 2016). Despite remediation efforts, it is unlikely all the plastic nurdles were removed given their small size.

Throughout the lake, there are differences in distribution within size fractions. Comparing east and west samples, there were more medium (1-4.75mm) and small (0.3-1 mm) lines, and more medium fragments in western samples than eastern samples. The higher counts in the western samples may be explained by the higher population on the western side of the lake, as both Thunder Bay, ON, Canada and Duluth-Superior Minnesota, USA, are located on the identified western side (Fig. 5).

A comparison of onshore and offshore samples shows that offshore samples contain lower numbers of medium (1-4.75 mm) fragments, but higher counts of all other identified particles (small [0.3-1 mm] lines and fragments, and medium lines). This is expected since a closer proximity to shore is expected to produce higher numbers of larger particles, which will accumulate along coasts. It was also expected that offshore samples would mostly contain particles in the smallest size fraction because of the distance from land, and therefore most pollution sources.

5.1.4 Polymer Types

The overall most abundant type of polymer identified was polyethylene, which accounted for more than 50% of the particles analyzed. Given that polyethylene is the most commonly produced type of plastic it was expected that it would be the most abundant. Polypropylene was the second most frequently identified polymer, at 20%, and this was also expected as polypropylene is the second most common type of plastic produced globally. These findings are consistent with those from studies performed elsewhere in the Great Lakes (Mason et al., 2016b).

Within each morphological type the amounts of each type of plastic identified varied. In total, 168 lines or fibres were analyzed using FTIR. The most common types were polypropylene (25%) and polyethylene (23%). Polyester (17%) and nylon (15%) were also common. Five of the bright green lines found in the >4.75 mm size fraction were analyzed and all were identified as polypropylene, which indicates that these, and other thick, rounded fibres contained in the samples, may originate from fishing gear such as nets, lines, and rope, all of which can be made of polypropylene (Materials science, n.d.). The polyethylene and nylons may also have originated from fishing gear, as the nylon fibres were thicker in appearance that what would be expected of clothing fibres. The polyester fibres were visually most like what would be expected of shed clothing fibres, as these particles were very thin, and short, and tended to curl.

The most common type of plastic found within the fragments and film identified and analyzed was polyethylene (70% and 76% respectively) which is expected since polyethylene is the most commonly manufactured plastic, and is common in containers, household plastics, and as single use plastic products such as bags and plastic wraps. Based on the many very thin particles of film, single use plastics are contributing to the microplastic pollution in Lake Superior.

5.2 Challenges

To improve this study, longer neuston tows, with a smaller mesh size would be beneficial. The neuston tows in this study were all roughly ten minutes long, but many other similar studies employ tows of approximately a half hour, which will sample a larger volume of water and would be more comparable to similar microplastic surveys. Because of the wide range of sampling techniques and mesh sizes used in microplastic surveys, there is an established need for standardized methods (Anderson et al., 2016; International Joint Commission, 2017). In addition, this study used 500 micron mesh neuston nets, but most studies surveying for microplastic use 300 micron mesh to ensure that the smaller particles do not pass through the net. However, the samples from Lake Superior had an abundance of organic material, particularly leaves, large bugs, and what was most likely *Populus tremuloides* (Trembling Aspen) tree seeds which would help to trap many particles due to the silky hairs attached to the individual seeds which are released by the hundreds (Ministry of Natural Resources and Forestry, 2018). However the mesh (500 microns) used in this study is larger than the smallest size fraction (0.333 mm), and therefore even with organics clogging the net and trapping some of the particles, not all particles in the smallest size fraction would be collected. Therefore, the results of this study may be an underestimate of the true count of the smallest microplastics (0.333 - 1.0 mm) in Lake Superior. This likely has the greatest impact on samples obtained from the centre of the lake as there would be fewer organics farther offshore, and an accumulation of organics would help trap the smallest particles.

Since initial microplastic identification is visual it is possible that some particles counted as microplastic were not actually plastic, and since not all particles were analyzed using FTIR, particles that may have been misidentified may not have been removed from the final count. However, it is likely that very few particles were initially misidentified as plastics because it is possible to support a visual identification through closer examination of durability. Durability can be assessed by gently squeezing the individual particles, or by dragging the forceps across the particle (Masura et al., 2015). Plastic is very durable and will not crumble or easily break when squeezed with the forceps, whereas organic particles may crumble or powder (Masura et al., 2015). Fibres and lines may occasionally be mistaken for plastic as the texture, particularly in the smallest size fraction, is harder to assess because it is more difficult to see textures. It can also be difficult to assess durability since many of the smallest fibres were knotted together and occasionally tore when attempting to carefully untangle the knot. Fibres in the smallest size fraction were the most difficult to positively identify visually, which is expected given the small size of the particles (Lusher et al., 2017). Fibres in the medium and large size fractions were much easier to assess because it was easier to visually examine texture, such as frayed or curled ends, and test durability. Overall, this is a very minor challenge and could be easily overcome with the use of a stronger microscope during visual identification and enumeration. In addition, polymer identification through FTIR reduces the error associated with visual identification alone, because with FTIR it is possible to determine the chemical composition of particles identified (Lusher et al., 2017).

Determining the morphology of an individual particle is somewhat subjective and therefore different people may classify different particles into different morphologies. However, some particles are easily identified. Pellets, foam, and lines are easily distinguished from the other plastic morphologies and the main source of potential discrepancies would be differentiated between films and fragments. Fragments tend to be more rigid, thicker, and more angular, while films tend to be very thin, less angular and occasionally folded. In addition, there are guides to microplastic identification which divide particles into morphological types and so long as the person identifying the plastics follows the definitions outlined in their chosen guide, it will serve to reduce error and inform readers of the specific criteria used to identifying the morphological types (Masura et al., 2015; Lusher et al., 2015). To eliminate this potential source of error in future, the scientific community must agree on morphological types, as well as their associated definitions (Anderson et al., 2016).

The need to travel to Dr Mason's lab at the State University of New York Fredonia for FTIR analysis, and the time required for this analysis, necessitated selecting samples with high counts to maximize the number of particles which could be analyzed in a short amount of time. In addition, particles within selected samples were intentionally selected from each morphological category to determine what polymeric types could be identified in each of the categories. Therefore, the samples and particles analyzed using FTIR were not random, which may impact whether or not the polymer types identified are representative of the types of plastic found throughout Lake Superior. Polyethylene was most common amongst films and fragments, which often originate from the degradation of larger plastics such as containers, bags, and single use packaging, into microplastics. Overall it was expected, based on global production patterns, that polyethylene and polypropylene would be the two most common polymers, but it was also expected that polyethylene would not be the most common polymer identified in each morphological type. The results support this, and therefore results obtained from the selection of particles chosen for FTIR analysis appear to be a realistic representation of the types of polymers present in surface waters of Lake Superior based on other Great Lakes studies and global plastic production trends (Mason et al., 2016b).

Lastly, it was difficult to obtain an accurate identity for very thin, or small fibres and lines, so the results for polymer identity in this category may not accurately describe the types of fibres found in the samples. If this is the case, it is likely that synthetic clothing fibres such as nylon and polyester are more common than polyethylene and polypropylene, particularly in the smallest size fraction. In order to address this challenge, a more sensitive analysis tool would be necessary to correctly identify fibres from the smallest size fraction, which would be true for all microplastic studies currently using this type of FTIR analysis for polymer identification.

Chapter 6: Conclusions

The findings of this thesis are summarized in this chapter. The findings are summarized based on the three objectives of this project which were as follows:

1) Establish baseline set of data for microplastic pollution in surface waters of Lake Superior.
2) Assess the effectiveness of single net sampling.
3) Inform policies, specifically for waste management, waste water effluent, and drinking water.

The first three sections of this chapter correspond to the objectives outlined above. Lastly, there is a brief summary of recommendations for future work.

6.1 Abundance, Distribution and Polymer Type

Abundances calculated for Lake Superior in this study are similar to abundances calculated for other Great Lakes, although Lake Erie remains the most polluted to date in terms of microplastic abundance in surface water samples. Lake Superior has higher abundances than Lake Michigan despite its larger size and overall low population and industry, which may be attributed to Lake Superiors longer residence time (Eriksen et al., 2013; Mason et al., 2016b).

Similar to other studies (Eriksen et al., 2013; Mason et al., 2016b) the highest concentration of particles were found in the smallest size fraction. The largest size fraction contained the fewest total particles overall, which is to be expected since the Lake Superior basin is sparsely populated and not heavily developed for industry.

The most common polymer type was polyethylene, which is similar to other studies (Mason et al., 2016b), as well as global production patterns, which show that polyethylene is the most commonly produced polymer (PEMRG, 2015). Polypropylene was the second most abundant, which also agrees with other studies and global production statistics (PEMRG, 2015). Other polymers found include polyester, polystyrene, resins, and others.

6.2 Methodology

This project represents the first comprehensive study of surface water microplastic pollution in Lake Superior and it is the only study completed in the Great Lakes using side by side neuston nets for sample collection. The results show that there was no difference between the results obtained from Net A or Net B, other than slightly more variability in Net A samples. This indicates that single net surveys can produce a representative result and helps to provide validity to previous studies involving single neuston nets. In addition, the knowledge that single net surveys produce good results, will help to establish a standardized sampling method, which is one the goals of this project. Given the higher variability in Net A, closer to the boat, the farther away from the boat that a net can be positioned, the less likely that bow waves will disrupt surface water and the sample collection. This could also be achieved by reducing the speed of the boat towing the nets, or a combination of increased distance from the boat and decreased speed. Overall, in order to improve comparability of microplastic surveys, a standardized sampling method (and sampling equipment), as well as standardized sample analysis and identification, is needed (Anderson et al., 2016; International Joint Commission, 2017).

6.3 Policy and Management

Policy makers and municipal governments can use the results of this study to help inform wastewater management strategies (Ziajahromi et al., 2016). Current wastewater treatments are not specifically designed to remove microplastics, nor are Canadian and US wastewater treatment plants required to monitor for microplastics, therefore wastewater effluent could be a significant contributor to microplastic pollution in aquatic environments (Anderson et al., 2016; Mason et al., 2016a; Talvitie et al., 2017). Given the high numbers of fibres, a major contributor to microplastic pollution is likely the loss of fibres through shedding in washing machines, which can contribute up to 100 particles per litre of laundry water (Ziajahromi et al., 2016). This would indicate a need to work towards eliminating fibres from wastewater effluent (Talvitie et al., 2017). A recent study indicated that the most effective way to remove microplastics from wastewater effluent is through treatment with a membrane bioreactor, which is capable of removing 99.9% of microplastics present. The membrane bioreactor works when water is forced through a series of membrane cartridges by negative pressure (Talvitie et al., 2017). The implementation of membrane bioreactor treatment of wastewater effluent, combined with household use of microplastic trapping laundry filters such as the Guppyfriend washing bag, or the Coral Ball (both designed to trap up to 99% of microfibre particles shed in a washing machine) would significantly reduce microplastic input from wastewater effluent in Lake Superior (Graham, 2018).

The presence of films and fragments likely originating from single use plastics such as containers and bags, could help to raise public awareness about the need to move away from single-use plastic. It could also aid in the development of social initiatives which aim to replace single-use plastics with environmentally friendly alternatives such as reusable containers, stainless steel straws, or biodegradable or compostable single-use containers.

6.4 Future Research

In order to gain a complete understanding of microplastic pollution in Lake Superior thorough beach surveys are required. It is likely that much of the plastic pollution in the larger size fraction is being deposited along beaches and shorelines of Lake Superior (Barnes et al., 2009; Zbyszewski et al., 2014). Beach surveys would be able to demonstrate how much plastic debris is around the lake, and could help to illustrate the amount of plastics being deposited along shorelines by waves and tides (Zbyszewski et al., 2014). Beach surveys mostly focus on macroplastic, but microplastics in the largest size fraction (≥4.75mm) can also be found during beach surveys. Some beach surveys have already been conducted but a comprehensive survey designed to focus on beaches from around the entire coastline of the lake would give the most accurate results.

Further surface water sampling, such as the sampling done in this study would show if and how plastic abundances throughout the lake are changing since it has already been three years since this study's samples were collected. However, it would be best to wait to compare the results of this study to current hydrodynamic models of microplastic pollution transport. Hoffman and Hittinger (2016) created a hydrodynamic model of microplastic transport in the Great Lakes which indicated that the highest densities in Lake Superior would be found near Marquette, Michigan, US. This location is also the location of previous Lake Superior samples obtained in 2012 (Eriksen et al., 2013). A thorough comparison of this current hydrodynamic model to the results of the present study may help to refine and improve the model, and would then make it possible to design a study that would best represent the state of microplastic pollution in the surface waters of Lake Superior.

Water circulation speeds in Lake Superior change considerably throughout spring and summer months. Currents within the lake tend to become slower and smaller throughout the summer and begin to increase in speed again as the fall and winter approach (Beletsky et al., 1999; Bennington et al., 2010). The counterclockwise circulation patterns of currents and localized areas of high and low calculated abundances can be compared to current hydrodynamic models of microplastic pollution to gain a more complete understanding of microplastic movement and transport within Lake Superior.

Understanding sources of pollution is important and given the high number of fibres found throughout Lake Superior a study of the effluent entering the lake from wastewater treatment plants would determine if high numbers of fibres are entering the lake due to inadequate filtration. Current studies indicate that even with modern effluent treatment, a secondary wastewater treatment plant could release approximately 65 million microplastic particles into the water each day (Murphy et al., 2016). This would then enable treatment plants to improve facilities and reduce the microplastic pollution burden by removing more particles from waste water effluent. Preventing microplastic particles from entering the water system is crucial since there is not yet a way to safely remove microplastic pollution once it is in the water.

Lastly, a study of drinking water in the Lake Superior basin would be important considering recent studies which indicate many sources of tap water contain microplastic particles (Kosuth, 2018). Many communities along Lake Superior source drinking water from the lake itself and the high abundances throughout the lake could indicate that many drinking water supplies may also be contaminated. This is especially important, given the impacts of microplastics on human health are not yet well documented or understood (Van Cauwhenberg et al., 2014; Kosuth, 2018) . Currently there are no standards established for human consumption of microplastics. The prevalence of microplastic pollution in Lake Superior, and the other Great Lakes, which are sources of drinking water for many communities, indicates the need to gain a better understanding of human consumption of microplastics. Once the impacts are better understood, drinking water standards can be established and policy makers, can work with local communities, municipalities, and water filtration engineers to create effective, and innovative solutions to remove microplastics from drinking water supplies.

Given Lake Superior's large size, volume of water and long residence time, continued monitoring of microplastic pollution will be required in order to continue to monitor the overall health of the lake and the status of microplastic debris. Using this research as a baseline means it will be possible to refer back and monitor how conditions within the lake are changing. Many climatic conditions may affect microplastic abundance, distribution and transport, including warming caused by climate change, seasonal changes in currents, as well as changes in population and industry along the shoreline. Monitoring how these conditions impact not only the lake but pollution within the lake will increase the overall understanding of how microplastic pollution interacts with the surrounding ecosystem and may provide insight into management practices which can help protect the overall health of Lake Superior.

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Appendix A: Trawl Data

Data Lake Superior Trawls - 2014											
#	Sample #	Trawl Serial	Date	Time	Location	Latitude	Longitude	Boat Speed (M/H)	Distance (miles)	Nautical miles (NM)	Tow length (km)
1	500/501	1	5/18/14	9:45:00 AM	71	46.941	90.782	2.6	0.43	0.37	0.69
2	502/503	2	5/19/14	11:23:00AM	75	47.001	90.728	2.4	0.40	0.35	0.64
3	504/505	0	5/20/14	1:30:00PM	86	46.835	90.721	2.6	0.43	0.37	0.69
4	506/507	4	5/21/14	9:40:00 AM	24	46.846	90.466	2.4	0.40	0.35	0.64
5	508/509	5	5/22/14	11:39:00 AM	2	46.933	90.561	2.4	0.40	0.35	0.64
6	510/511	8	5/23/14	3:37:00 PM	87	46.943	90.651	2.4	0.40	0.35	0.64
7	512/513	9	5/24/14	9:51:00 AM	45	46.979	90.550	2.5	0.42	0.36	0.68
8	514/515	10	5/25/14	11:20:00 AM	44	47.035	90.516	2.5	0.42	0.36	0.68
9	516/517	11	5/26/14	1:02:00 PM	52	46.967	90.451	2.4	0.40	0.35	0.64
10	518/519	12	5/27/14	1:17:00 PM	190	47.621	90.716	2.6	0.43	0.37	0.69
11	520/521	13	5/28/14	3:11:00 PM	208	47.692	90.524	2.5	0.42	0.36	0.68
12	522/523	14	5/29/14	5:08:00 PM	65	47.746	90.312	2.5	0.42	0.36	0.68
13	524/525	15	5/30/14	11:06:00 AM	172	47.325	91.195	2.6	0.43	0.37	0.69
14	526/527	16	5/31/14	2:14:00 PM	188	47.077	90.574	2.5	0.42	0.36	0.68
15	528/529	17	6/1/14	3:58:00 PM	36	46.998	91.686	2.6	0.43	0.37	0.69
16	530/531	18	6/2/14	7:51:00 AM	210	46.726	92.024	2.5	0.42	0.36	0.68
17	532/533	19	6/3/14	10:34:00 AM	206	46.773	91.627	2.5	0.42	0.36	0.68
18	534/535	20	6/4/14	12:56:00 PM	205	46.810	91.413	2.6	0.43	0.37	0.69

19	536/537	21	6/5/14	3:27:00 PM	187	46.910	91.833	2.7	0.44	0.38	0.71
20	538/539	22	6/6/14	5:11:00 PM	186	46.829	91.987	2.6	0.43	0.37	0.69
21	540/541	23	6/7/14	10:48:00 AM	151	46.880	91.209	2.5	0.42	0.36	0.68
22	542/543	24	6/8/14	12:28:00 PM	76	46.886	91.097	2.6	0.43	0.37	0.69
23	544/545	25	6/9/14	2:01:00 PM	139	46.970	90.996	2.5	0.42	0.36	0.68
24	546/547	26	6/10/14	10:48:00 AM	184	46.616	90.331	2.6	0.43	0.37	0.69
25	548/549	27	6/11/14	1:12:00 PM	192	46.689	90.027	2.6	0.43	0.37	0.69
26	550/551	28	6/12/14	5:07:00 PM	57	46.901	89.357	2.6	0.43	0.37	0.69
27	552/553	29	6/13/14	8:22:00 AM	183	46.998	89.149	2.5	0.42	0.36	0.68
28	554/555		6/14/14	11:33:00 AM	182	47.149	88.863	3.3	0.55	0.48	0.89
29	556/557	30	6/15/14	1:46:00 PM	181	47.337	88.483	2.6	0.43	0.37	0.69
30	558/559	31	6/16/14	10:26:00 AM	82	46.977	88.393	2.6	0.43	0.37	0.69
31	560/561	32	6/17/14	12:13:00 PM	84	46.894	88.321	2.5	0.42	0.36	0.68
32	562/563		6/18/14	2:35:00 PM	100	47.051	88.267	2.5	0.42	0.36	0.68
33	564/565	33	6/19/14	4:23:00 PM	85	47.205	88.139	2.7	0.45	0.39	0.72
34	566/567	34	6/20/14	7:28:00 PM	101	47.379	87.811	2.7	0.45	0.39	0.72
35	568/569	35	6/21/14	9:06:00 AM	158	46.938	88.136	2.6	0.43	0.37	0.69
36	570/571	36	6/22/14	12:10:00 PM	142	46.854	87.728	2.5	0.42	0.36	0.68
37	572/573	37	6/23/14	2:05:00 PM	196	46.778	87.560	2.7	0.45	0.39	0.72
38	574/575	38	6/24/14	7:57:00 AM	120	46.508	87.232	2.5	0.42	0.36	0.68
39	576/577	39	6/25/14	10:40:00 AM	88	46.523	86.922	2.6	0.43	0.37	0.69
40	578/579	40	6/26/14	1:02:00 PM	209	46.528	86.715	2.6	0.43	0.37	0.69
41	580/581	41	6/27/14	3:55:00 PM	178	46.658	86.310	2.4	0.40	0.35	0.64

42	582/583	42	6/28/14	8:11:00 AM	177	46.726	85.768	2.5	0.42	0.36	0.68
43	584/585	43	6/29/14	11:19:00 AM	176	46.776	85.318	2.7	0.45	0.39	0.72
44	586/587		6/30/14	3:26:00 PM	175	46.749	84.947	2.5	0.42	0.36	0.68
45	588/589	46	7/1/14	10:17:00 AM	194	46.627	84.883	2.5	0.42	0.36	0.68
46	590/591		7/2/14	11:10:00 AM	79	46.564	84.872	2.5	0.42	0.36	0.68
47	592/593	45	7/3/14	12:35:00 PM	193	46.627	84.883	2.5	0.42	0.36	0.68
48	594/595		7/4/14	10:03:00 AM	460	46.677	84.559	2.5	0.42	0.36	0.68
49	596/597	47	7/5/14	11:29:00 AM	459	46.772	84.593	2.4	0.40	0.35	0.64
50	598/599	48	7/6/14	2:04:00 PM	461	46.943	84.727	2.5	0.42	0.36	0.68
51	600/601	49	7/7/14	4:30:00 PM	457	47.165	84.722	2.5	0.42	0.36	0.68
52	602/603	50	7/8/14	7:37:00 AM	456	47.317	84.646	2.5	0.42	0.36	0.68
53	604/605	51	7/9/14	10:36:00 AM	455	47.553	84.959	2.6	0.42	0.36	0.68
54	606/607	52	7/10/14	12:45:00 PM	454	47.676	84.986	2.6	0.43	0.37	0.69
55	608/609	53	7/11/14	3:10:00 PM	451	47.947	85.186	2.6	0.43	0.37	0.69
56	610/611	54	7/12/14	5:23:00 PM	462	47.954	84.948	2.6	0.43	0.37	0.69
57	612/613	55	7/13/14	9:22:00 AM	463	47.918	85.427	2.5	0.42	0.36	0.68
58	614/615	56	7/14/14	11:58:00 AM	464	47.955	85.821	2.6	0.43	0.37	0.69
59	616/617	57	7/15/14	2:41:00 PM	465	48.121	86.050	2.7	0.45	0.39	0.72
60	618/619	58	7/16/14	7:44:00 AM	422	48.637	86.348	2.4	0.40	0.35	0.64
61	620/621	59	7/17/14	10:29:00 AM	420	48.769	86.631	2.6	0.43	0.37	0.69
62	622/623	60	7/18/14	12:31:00 PM	419	48.792	86.989	2.5	0.42	0.36	0.68
63	624/625	61	7/19/14	2:22:00 PM	418	48.772	87.158	2.5	0.42	0.36	0.68
64	626/627	62	7/20/14	4:40:00 PM	417	48.831	87.472	2.5	0.42	0.36	0.68

65	628/629	63	7/21/14	8:11:00 AM	415	48.880	87.767	2.5	0.42	0.36	0.68
66	630/631	64	7/22/14	10:07:00 AM	414	48.940	87.982	2.5	0.42	0.36	0.68
67	632/633	65	7/23/14	12:04:00 PM	413	48.934	88.217	2.5	0.42	0.36	0.68
68	634/635	66	7/24/14	2:02:00 PM	412	48.817	88.095	2.5	0.42	0.36	0.68
69	636/637	67	7/25/14	4:43:00 PM	411	48.599	88.302	2.5	0.42	0.36	0.68
70	638/639	68	7/26/14	9:23:00 AM	408	48.586	88.505	2.5	0.42	0.36	0.68
71	640/641	69	7/27/14	10:43:00 AM	407	48.560	88.576	2.4	0.40	0.35	0.64
72	642/643	70	7/28/14	12:11:00 PM	406	48.491	88.624	2.7	0.45	0.39	0.72
73	644/645	71	7/29/14	1:53:00 PM	405	48.408	88.682	2.5	0.42	0.36	0.68
74	646/647	72	7/30/14	12:06:00 PM	401	48.505	88.928	2.5	0.42	0.36	0.68
75	648/649	73	7/31/14	2:15:00 PM	402	48.368	88.876	2.5	0.42	0.36	0.68
76	650/651	74	8/1/14	3:46:00 PM	404	48.305	88.909	2.8	0.49	0.43	0.79
77	652/653	75	8/2/14	8:04:00 AM	403	48.256	89.178	2.6	0.43	0.37	0.69
78	654/655	76	8/3/14	10:44:00 AM	400	48.081	89.422	2.5	0.42	0.36	0.68
79	656/657	77	8/4/14	1:05:00 PM	191	47.980	89.629	2.5	0.42	0.36	0.68
80	658/659	78	8/5/14	3:30:00 PM	207	47.829	89.961	2.5	0.42	0.36	0.68
81	660/661		8/6/14	5:50:00 PM	65	47.749	90.311	2.5	0.42	0.36	0.68
82	662/663	79	8/7/14	11:39:00 AM	736	46.884	90.565	2.5	0.42	0.36	0.68
83	664/665	82	8/8/14	12:36:00 PM	2161	46.979	91.245	2.5	0.42	0.36	0.68
84	666/667	83	8/9/14	4:18:00 PM	2133	47.532	90.566	2.5	0.42	0.36	0.68
85	668/669		8/10/14	7:05:00 AM	65	47.741	90.338	2.6	0.43	0.37	0.69
86	670/671	84	8/11/14	9:19:00 AM	2124	47.501	89.996	2.5	0.42	0.36	0.68
87	672/673	85	8/12/14	12:10:00 PM	2147	47.160	89.964	2.5	0.42	0.36	0.68

88	674/675	86	8/13/14	2:41:00 PM	2120	47.083	89.653	2.6	0.43	0.37	0.69
89	676/677	87	8/14/14	9:31:00 AM	2136	47.229	89.535	2.5	0.42	0.36	0.68
90	678/679	88	8/15/14	11:42:00 AM	2151	47.154	89.298	2.5	0.42	0.36	0.68
91	680/681	89	8/16/14	9:39:00 AM	2115	47.414	88.471	2.6	0.43	0.37	0.69
92	682/683	90	8/17/14	1:31:00 PM	2128	47.835	88.751	2.5	0.42	0.36	0.68
93	684/685	91	8/18/14	4:58:00 PM	2134	48.048	88.250	2.4	0.40	0.35	0.64
94	686/687	92	8/19/14	9:47:00 AM	2118	48.874	88.068	2.4	0.40	0.35	0.64

Appendix B: Plastic Counts

Counts																			
Sample Number	>4.75					Total	1-4.75					Total	0.3-1					Total	Final Total
	Frag.	Pel.	Line	Film	Foam		Frag.	Pel.	Line	Film	Foam		Frag.	Pel.	Line	Film	Foam		
500						0	5		5			10	4		2			6	16
501						0			11			11	4		12			16	27
502						0	2		4	1		7	5		60			65	72
503						0	1		1			2	2		1			3	5
504						0	2		4			6			1			1	7
505						0						0	1		9			10	10
506						0	8	2		1		11	1		5			6	17
507						0	13	4	2	3		22						0	22
508						0	1	2		1		4			2			2	6
509						0						0	1		6			7	7
510	1					1	3		1			4	3		7	1		11	16
511						0	5		1	1		7	5		7			12	19
512						0						0			13			13	13
513						0			1			1			18			18	19
514						0	1					1	3		5			8	9
515						0			5			5	3		6			9	14
516						0			2			2	2		8			10	12
517						0			3			3	1	1	3			5	8
518						0			1			1			2			2	3
519				1		1						0			8			8	9
520				1		1						0			2			2	3
521				1		1	2					2	5		3			8	11
522	1					1						0	9	1	12	3		25	26
523						0			2			2	4	1	2			7	9

524						0	1		2			3	3		17			20	23
525						0			2			2			10			10	12
526				2		2			14			14	1					1	17
527				2		2			1			1	1		5			6	9
528						0						0			18			18	18
529						0			1			1	1		9		1	11	12
530					1	1	7		6		4	17			11			11	29
531						0	2	2	3		8	15	8		14		1	23	38
532						0						0	1		2			3	3
533						0	13		6			19	1		3			4	23
534						0	1		6			7	2		10			12	19
535						0	1		2			3			11			11	14
536						0	2		1			3			9			9	12
537						0						0	1		9			10	10
538			1			1	10		4	1	1	16	4		14			18	35
539						0	6		4			10	3		11			14	24
540						0	1		3			4			3			3	7
541						0			3			3	5		16			21	24
542						0						0	2		3			5	5
543						0			1			1			6			6	7
544						0						0	3		10		1	14	14
545						0	1		2			3	2		8		1	11	14
546			1			1	7		2	3		12	6	1	13			20	33
547			1			1	1		5			6	3	1	17			21	28
548			4			4	9		7	2		18	5		6	1		12	34
549			4	1	1	6	5		4		1	10	8		5	1		14	30
550						0	1		5			6		1	13			14	20
551	1					1			1			1	1		5			6	8
552						0			1			1	3		2		1	6	7
553						0			1			1	1		2			3	4
554						0			1			1	1		22			23	24

555						0			1			1	1		12			13	14
556						0						0			5			5	5
557						0	1					1			56			56	57
558						0			2			2	1		13		2	16	18
559						0			1			1			19			19	20
560						0			2			2			17			17	19
561						0			1			1			8			8	9
562						0			1			1	1		2			3	4
563						0			3			3	2		7			9	12
564						0			2			2			17			17	19
565						0						0			14			14	14
566						0			1			1			13			13	14
567						0			1			1	2		42			44	45
568						0			2			2	1		8		2	11	13
569						0			1			1	2		13			15	16
570						0						0	2		7			9	9
571						0	2					2			7			7	9
572						0	15		25	1		41	18		30		1	49	90
573			1			1	1		2			3	1		9			10	14
574						0	2					2	1		6			7	9
575			1			1	1			1		2	1		4			5	8
576						0			1	1		2	4		10			14	16
577			1			1	2					2	10		8	4		22	25
578						0			28			28			6			6	34
579						0			1			1	3		4		1	8	9
580			1			1	2		21			23	3		12		2	17	41
581						0			2			2	1		6			7	9
582						0			4			4	1		12		1	14	18
583						0	1		1	1		3	3		8			11	14
584						0						0	5		2			7	7
585						0	4		1			5	9		8			17	22

586						0	3		1			4	6		5			11	15
587						0	6					6	1		7			8	14
588			1			1	8		4			12	9		6			15	28
589						0			2	1		3	1		11			12	15
590						0						0	1		2			3	3
591						0						0	4		9			13	13
592						0						0	6		17			23	23
593						0	2		1	1		4	3		7	1		11	15
594						0			1	2		3	3		9			12	15
595						0						0	7		12			19	19
596						0			1			1	1		4			5	6
597						0			26			26	3		15			18	44
598						0	2		8			10	1		8			9	19
599				1		1			3			3	1		6			7	11
600						0	2		2			4		1	9			10	14
601						0			2			2			8			8	10
602						0	4		3			7	1		19			20	27
603						0	2		22			24	1		15			16	40
604	2		6	4		12	6		25	92		123	41	1	30	57		129	264
605			3	5		8	20		16	46		82	40		7	52		99	189
606						0	4		4	6		14			13			13	27
607				1		1	9		1	7		17	3	1	3	3		10	28
608						0	2		121			123			111			111	234
609			2			2			18			18	4		66	1		71	91
610						0			8			8			6			6	14
611						0			5			5			23			23	28
612						0	3		29			32	2		7			9	41
613						0	1		6			7	1		14	1		16	23
614						0			2			2			18			18	20
615			1			1			2			2	1		11	1		13	16
616						0			2			2	2		17		2	21	23

617						0			4			4			5			5	9
618						0			6			6			12			12	18
619						0			5			5			13			13	18
620						0	2		5			7	10		3			13	20
621						0	2		12		2	16	5		13			18	34
622						0			3			3			3			3	6
623						0			3			3			5			5	8
624						0						0	2		3			5	5
625						0						0			11			11	11
626						0	1		1			2	1		5			6	8
627						0			4			4	3		1			4	8
628						0	1					1	1		1			2	3
629						0			2			2	2		4			6	8
630						0			5			5			2			2	7
631						0		2	3	1		6			3			3	9
632						0					2	2	5		4			9	11
633						0	3				1	4	1		8			9	13
634						0	1		2	1		4	7		4			11	15
635						0	2		1			3	2	1	29			32	35
636						0	1		2			3	1		7			8	11
637						0	3		1			4	1		6			7	11
638						0	2		1			3			7			7	10
639						0	3		29			32		1	3			4	36
640						0	2		6			8	2		13			15	23
641						0			16			16	1		4			5	21
642						0	1		4			5	1		9			10	15
643						0			5	1		6	1		4			5	11
644						0						0	1		1			2	2
645						0	2	1				3	1		3			4	7
646						0	8		2			10	5		4			9	19
647						0	3					3	5		7			12	15

648						0	1		1			2	2		1			3	5
649						0	2		2	4		8	3		5			8	16
650						0	1		17			18			8			8	26
651						0						0			4			4	4
652						0			8			8			3			3	11
653						0			12			12	1		2			3	15
654						0			6	1		7			3			3	10
655						0			12			12	1		3			4	16
656						0	2		1	1		4	6		4	1		11	15
657			1			1	5		2			7	5	1	8			14	22
658						0	3		1	1		5	10		5			15	20
659						0	4		6			10	4		4			8	18
660						0	8		1			9	18		11			29	38
661						0	26		1			27	28		3			31	58
662						0	2		1			3	3		4			7	10
663						0			4			4	1		2			3	7
664						0	1					1	5		7			12	13
665						0	1		3			4			2			2	6
666						0	2					2	2		20			22	24
667				2		2	1					1	4		6			10	13
668						0	7		1	1		9	2	1	1			4	13
669						0						0	3		2			5	5
670				1		1	1					1	1		7			8	10
671						0	2					2	2		5			7	9
672	1					1						0	5		2			7	8
673				2		2	4					4	6		2			8	14
674						0			2			2	4		1			5	7
675	1		1			2	3					3	5		4			9	14
676						0						0	2		5			7	7
677						0			3			3	1		1			2	5
678						0	2		1			3			8			8	11

679																			
680						0	2					2			2			2	4
681						0	3		3			6			8			8	14
682						0	4	1	1			6	1		3			4	10
683						0			3			3	1		2			3	6
684						0	2	2	1	1		6	7		17			24	30
685						0	2		21			23	1		2	1		4	27
686						0	4		12			16	6	1	4			11	27
687						0	2		25			27	2		4			6	33
TOTALS	7	0	30	24	2	63	350	16	823	184	19	1392	534	14	1740	128	16	2432	3887

Appendix C: Abundance Calculations

>4.75					Total	1-4.75					Total	0.3-1					Total	Net	Final Total	Average
Frag.	Pel.	Line	Film	Foam		Frag.	Pel.	Line	Film	Foam		Frag.	Pel.	Line	Film	Foam				
0	0	0	0	0	0	7246	0	7246	0	0	14493	5797	0	2899	0	0	8696	A	23188	31,159
0	0	0	0	0	0	0	0	15942	0	0	15942	5797	0	17391	0	0	23188	B	39130	
0	0	0	0	0	0	3125	0	6250	1563	0	10938	7813	0	93750	0	0	0156	A	112500	60,156
0	0	0	0	0	0	1563	0	1563	0	0	3125	3125	0	1563	0	0	4688	B	7813	
0	0	0	0	0	0	2899	0	5797	0	0	8696	0	0	1449	0	0	1449	A	10145	12,319
0	0	0	0	0	0	0	0	0	0	0	0	1449	0	13043	0	0	14493	B	14493	
0	0	0	0	0	0	12500	3125	0	1563	0	17188	1563	0	7813	0	0	9375	A	26563	30,469
0	0	0	0	0	0	20313	6250	3125	4688	0	34375	0	0	0	0	0	0	B	34375	
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0	0	0	0	0	0	0	0	0	0	0	0	1563	0	9375	0	0	10938	B	10938	
1563	0	0	0	0	1563	4688	0	1563	0	0	6250	4688	0	10938	1563	0	17188	A	25000	27,344
0	0	0	0	0	0	7813	0	1563	1563	0	10938	7813	0	10938	0	0	18750	B	29688	
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0	0	0	0	0	0	0	0	1471	0	0	1471	0	0	26471	0	0	26471	B	27941	
0	0	0	0	0	0	1471	0	0	0	0	1471	4412	0	7353	0	0	11765	A	13235	16,912
0	0	0	0	0	0	0	0	7353	0	0	7353	4412	0	8824	0	0	13235	B	20588	
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0	0	0	0	0	0	0	0	4688	0	0	4688	1563	1563	4688	0	0	7813	B	12500	
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0	0	0	1449	0	1449	0	0	0	0	0	0	0	0	11594	0	0	11594	B	13043	
0	0	0	1471	0	1471	0	0	0	0	0	0	0	0	2941	0	0	2941	A	4412	10,294
0	0	0	1471	0	1471	2941	0	0	0	0	2941	7353	0	4412	0	0	11765	B	16176	

1471	0	0	0	0	1471	0	0	0	0	0	0	3235	1471	17647	4412	0	36765	A	38235	25,735
0	0	0	0	0	0	0	0	2941	0	0	2941	5882	1471	2941	0	0	10294	B	13235	
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1449	0	0	0	0	1449	0	0	1449	0	0	1449	1449	0	7246	0	0	8696	B	11594	
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0	0	0	0	0	0	0	0	1449	0	0	1449	2899	0	18841	0	0	21739	B	23188	
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0	0	1563	0	0	1563	3125	0	32813	0	0	35938	4688	0	18750	0	3125	26563	A	64063	39,063
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0	0	1471	0	0	1471	11765	0	5882	0	0	17647	3235	0	8824	0	0	22059	A	41176	31,618
0	0	0	0	0	0	0	0	2941	1471	0	4412	1471	0	16176	0	0	17647	B	22059	
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0	0	0	1471	0	1471	0	0	4412	0	0	4412	1471	0	8824	0	0	10294	B	16176	
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0	0	0	0	0	0	0	0	2941	0	0	2941	0	0	11765	0	0	11765	B	14706	
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0	0	0	0	0	0	0	0	11594	0	0	11594	0	0	8696	0	0	8696	A	20290	30,435
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0	0	0	0	0	0	10145	0	1449	1449	0	13043	2899	1449	1449	0	0	5797	A	18841	13,043
0	0	0	0	0	0	0	0	0	0	0	0	4348	0	2899	0	0	7246	B	7246	
0	0	0	1471	0	1471	1471	0	0	0	0	1471	1471	0	10294	0	0	11765	A	14706	13,971
0	0	0	0	0	0	2941	0	0	0	0	2941	2941	0	7353	0	0	10294	B	13235	
1471	0	0	0	0	1471	0	0	0	0	0	0	7353	0	2941	0	0	10294	A	11765	16,176
0	0	0	2941	0	2941	5882	0	0	0	0	5882	8824	0	2941	0	0	11765	B	20588	
0	0	0	0	0	0	0	0	2899	0	0	2899	5797	0	1449	0	0	7246	A	10145	15,217
1449	0	1449	0	0	2899	4348	0	0	0	0	4348	7246	0	5797	0	0	13043	B	20290	
0	0	0	0	0	0	0	0	0	0	0	0	2941	0	7353	0	0	10294	A	10294	8,824

0	0	0	0	0	0	0	0	4412	0	0	4412	1471	0	1471	0	0	2941	B	7353	8,824
0	0	0	0	0	0	2941	0	1471	0	0	4412	0	0	11765	0	0	11765	A	16176	16,176
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	B	0	
0	0	0	0	0	0	2899	0	0	0	0	2899	0	0	2899	0	0	2899	A	5797	13,043
0	0	0	0	0	0	4348	0	4348	0	0	8696	0	0	11594	0	0	11594	B	20290	
0	0	0	0	0	0	5882	1471	1471	0	0	8824	1471	0	4412	0	0	5882	A	14706	11,765
0	0	0	0	0	0	0	0	4412	0	0	4412	1471	0	2941	0	0	4412	B	8824	
0	0	0	0	0	0	3125	3125	1563	1563	0	9375	0938	0	26563	0	0	37500	A	46875	44,531
0	0	0	0	0	0	3125	0	32813	0	0	35938	1563	0	3125	1563	0	6250	B	42188	
0	0	0	0	0	0	6250	0	18750	0	0	25000	9375	1563	6250	0	0	17188	A	42188	46,875
0	0	0	0	0	0	3125	0	39063	0	0	42188	3125	0	6250	0	0	9375	B	51563	
034	0	378	523	2920	228	1514	444	2100	7071	785	04817	8341	066	53489	8818	334	65049		690951	