Master's thesis

Plastic ingestion in Atlantic cod (*Gadus morhua*) on the east coast of Newfoundland, Canada

results from a citizen science monitoring project, with policy recommendations for long-term monitoring

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Declaration

I hereby confirm that I am the sole author of this thesis and it is a product of my own academic research.

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Abstract

Marine plastics are a pollutant that has touched every ocean and sea on earth, despite their recent development and proliferation within the last century. In the face of their pervasiveness, monitoring and management of this globally recognized pollutant is hindered by a lack of knowledge regarding their sources, distribution and impacts, combined with a lack of standardized protocols for the study of these factors. The aim of this thesis is twofold. First, the ingestion of marine plastics by Atlantic cod (an important country food) is quantified in Newfoundland waters, resulting in a frequency of occurrence of 2.01%. Together with a similar study conducted in the previous year this research helps to address the knowledge gap concerning marine plastics in Newfoundland waters (and seafood) by establishing a baseline for plastic ingestion in a culturally and practically important local food fish. The research also addresses the lack of standardization in this field of research by promoting the use of protocols that are applicable to the highest variety of actors in the future, of varying skill sets and resources. Second, given this baseline knowledge and protocol guidelines, this thesis presents policy recommendations for the monitoring of marine plastics in Newfoundland. A community based participatory research program is recommended, which will build on the existing research framework to eventually become a grassroots environmental monitoring program that directly addresses the needs of Newfoundland's small communities through their vested involvement in the Atlantic cod biomonitoring program.

Dedicated to the fisher(wo)men of St. Philip's-Portugal Cove, Quidi Vidi, Petty Harbour, Witless Bay, Bauline East, and Brigus South

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Acronyms

%FO	Frequency Of Occurrence
ABS	Acrylonitrile butadiene styrene
CBPR	Community Based Participatory Research
CLEAR	Civic Laboratory for Environmental Action Research
DFO	Department of Fisheries and Oceans
GRT	Gut Retention Time
NL	Newfoundland and Labrador
PE	Polyethylene
PET	Polyethylene terephthalate
PVC	Polyvinyl chloride

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1 Introduction

Annual plastic production reached 322 million tonnes in 2015 (Plastics Europe, 2016) and, if left unchecked, could reach 400 million tonnes by the end of 2017 (Hopewell et al., 2009). The plastics industry has found its success in the creation of a product that is cost-effective, lightweight, durable, and –perhaps most importantly – disposable. The massive influx of this synthetic polymer to the marine environment via plastic waste since its inception less than a century ago has left the scientific community scrambling to understand its distribution and impacts. Marine plastics have infiltrated virtually every marine habitat on earth; from the Arctic (Zarfl and Matthies, 2010) to the Antarctic (Eriksson and Burton, 2003), and from the ocean's surface (Eriksen et al., 2014) to some of its greatest depths (Pham et al., 2014).

The widespread distribution and sheer quantity – estimated to be over 260,000 tonnes (Eriksen et al., 2014) – of marine plastics has become especially concerning in recent years as research has begun to reveal that the pollution of the marine environment by plastics may have more far-reaching consequences than the damage to wild populations caused by entanglement and ingestion of plastic debris. Recently, researchers have begun the documentation of plastics in human seafood (Choy and Drazen, 2013; Rochman et al., 2015; Van Cauwenberghe and Janssen, 2014). This is particularly worrying when combined with the knowledge that marine plastics carry a cocktail of contaminants, which can be transferred to the body tissues upon consumption (Koelmans et al., 2014; Rochman et al., 2014; Teuten et al., 2009). The accumulation of these contaminants in the body tissues can cause a number of disorders including developmental disorders, hormonal imbalance, neurological issues, diabetes and heart disease (Lang et al., 2008; Melzer et al., 2010; Oehlmann et al., 2009; Rochman et al., 2014).

The human health impacts of marine plastics are expected to be felt most strongly by communities with a strong reliance on seafood. Seafood is a vital form of country food for many coastal communities around the world, providing an important means of food security

(Cisneros-Montemayor et al., 2016). This is clearly evident in the rural outport communities of Newfoundland and Labrador (NL), an island and mainland province on the east coast of Canada. The Atlantic cod (*Gadus morhua*) not only represents an important source of food security to the communities of NL, but also holds a strong cultural value, and as a result, is the target of some of the highest participation in a recreational fishery in the country (Fisheries and Oceans Canada, 2012). The pollution of the marine environment by plastics is a social justice issue, by which the communities who rely the heaviest on country foods (such as in the case of many low-income, rural communities in NL) feel the heaviest burden of pollution. As a result, it is suggested here that any research and monitoring of marine plastics and their impacts on community environmental health in the province of NL – or elsewhere – be carried out not only with the full consent of the communities in question, but also with their involvement in the research itself.

In the face of the potential threats to human health imposed by marine plastics, it is of the utmost importance that their presence in seafood consumed by humans be monitored and managed. However, management requires a knowledge of the status of the problem, something that is severely lacking globally, presenting a barrier to the development of efficient and successful monitoring procedures (UNEP, 2014). The development of monitoring procedures in the subarctic province of NL faces the added barriers of financial and technical feasbility. The communities of Newfoundland and Labrador are small and highly dispersed, giving the province a total population density of just 1.4 persons per kilometer squared (Statistics Canada, 2012). As a result, most of the province (outside of the capital region on the Avalon Peninsula) has seen little in the way of environmental monitoring. Without the involvement of these small, isolated communities, this gap in knowledge could persist indefinitely.

The involvement of small communities burdened by pollution and the filling in of knowledge gaps surrounding marine plastic pollution can both be achieved simultaneously through the use of citizen science. Citizen science involves the handing over of scientific duties to the general public and allowing them to become producers of scientific knowledge. This technique can be especially useful in cases of environmental pollution and social justice, where affected communities should reserve the right to define the research questions and project goals based on their own needs, as opposed to the needs of the academy.

Citizen science methodologies are especially well suited to the case of plastic pollution in NL. The low population sizes combined with the harsh environmental conditions in the province (typical of most Canadian Arctic and subarctic regions) has resulted in little or no environmental monitoring due to a lack of methodologies adapted to these conditions. Citizen science provides a means of reaching remote communities where environmental monitoring could otherwise not occur. The biomonitoring of plastics in Atlantic cod is also particularly well suited to the province of NL. Caught locally through the recreational and commercial fisheries, cod is the most commonly eaten seafood in the province (Fisheries and Oceans Canada, 2016a, 2016b; Lowitt, 2013). The biomonitoring of cod can therefore concurrently monitor both the trends in local marine plastic pollution and the risk it may pose to human health through the consumption of such an important country food. Additionally, the use of cod in the project is expected to garner public interest and engagement in the project. Atlantic cod retains a strong cultural importance in the region, despite the famous cod moratorium of 1992 (Schrank and Roy, 2013). The value of the fish in small outport communities not only stems from the food security that it provides, but also from its ability to foster community values and cultural identity, while maintaining the transmission of traditional cultural knowledge. The pollution of such a culturally important food will be quick to recieve the interest and involvement of small communities in the province, benefiting the further development of a citizen science program.

The research at hand is presented here over the course of two entireley standalone, yet complimentary, chapters. The first chapter adheres to the format of a scientific article and the intended audience is therefore the scientific community, particularly researchers in the burgeoning field of marine plastic ingestion research. The chapter details the extension of a recently introduced study on plastic ingestion in Atlantic cod on the province's Avalon Peninsula. The purpose of this chapter is to establish a baseline for plastic ingestion in Atlantic cod off of the east coast (Avalon Peninsula) of the island of Newfoundland. To suit the target audience of this paper, common questions being raised by scientists in the field of marine plastic ingestion are addressed. These include what factors affect the ingestion of plastics by marine biota (sex, age, feeding habitat, and plastic polymer/density) and the standardization of methodologies in such a new and developing field. The standardization of methodologies is presented in such a way as to allow room for the involvement of citizen scientists, highlighted through the involvement of fishermen in the methodologies of this first chapter.

This gives rise to the second chapter, which follows the format of a policy white paper and the intended audience is therefore policy makers in the province of NL. The ultimate aim of the white paper is to recommend the development of a community based participatory research (CBPR) project for the biomonitoring of marine plastics in Atlantic cod. First, the potential impacts of marine plastics on Newfoundland communities are explicitly outlined, as are the barriers to management. Following this, the involvement of affected communities through community involvement (CBPR) – a form of citizen science – is recommended and the steps to achieve this are clearly outlined. The white paper recommends that the current biomonitoring project outlined in the first chapter be used as the groundwork for a province-wide monitoring program facilitated by the direct involvement of local fishing communities.

Although these two chapters were written to function independently of each other in the future, they are indeed quite complimentary and work together to address the issue of marine plastic pollution in NL. The monitoring of marine plastics in human country foods should be made a top priority, as described in Chapter 2. However, the field of marine plastic ingestion research is a relatively new one and in order to implement any successful monitoring programs baselines mus first be established and procedures tried and tested. Chapter 1 first proves the need for monitoring by establishing a baseline and revealing the presence of plastic debris in Atlantic cod. Procedures are then clearly outlined which will serve as the starting point for the monitoring program outlined in Chapter 2. Chapter 2 explicitly refers to these procedures as a starting point and describes the steps that should be taken to develop these procedures to suit a larger scale monitoring project. Together, these two chapters function as an effective call to action – complete with instructions manual – for the monitoring of plastics in Atlantic cod caught for consumption in Newfoundland and Labrador.

2 Chapter 1: Plastic Ingestion in Atlantic Cod (*Gadus morhua*): Results from a Citizen Science Monitoring Project on the East Coast of Newfoundland, Canada

2.1 Introduction

Plastic is ubiquitous in the marine environment (Barnes et al., 2009; Cózar et al., 2014; Eriksen et al., 2014; Galgani et al., 2015; Martinez et al., 2009; Pham et al., 2014). More than just an eyesore, plastics have been internationally recognised as a pollutant with its own set of legislations (Gregory, 2009; Macfadyen et al., 2009; UNEP, 2009). Although these legislations can vary with the authorities issuing them, their aim is not to remove plastic pollution that is already present in the marine environment, but rather to monitor its prevalence and reduce inputs. These measures are important in the face of the negative consequences marine plastics have been shown to cause in the marine environment. Marine plastics can cause damage to marine biota indirectly by smothering the seafloor and affecting gas exchange or directly through ingestion and accidental entanglement (Balazs, 1985; Bond et al., 2013; Deudero and Alomar, 2015; Gregory, 2009). Plastics are a new material invented in the last century, and only mass produced since 1945 (British Plastics Federation, n.d.; Plastics Europe, 2016), but their rapid expansion has left us with little insight into what their full impact on marine life and the humans that depend on it will be. The lack of knowledge regarding plastics' life spans, sinks, and effects in marine environments, coupled with extensive and increasing ocean use in the form of fishing, recreation, shipping, and mass migration to coastal areas (Andrady, 2011; Bond et al., 2013; Ribic et al., 2010), requires that more research and monitoring of marine plastics is prioritized.

The monitoring of marine plastics in remote subarctic areas has thus far been especially inadequate. The lack of monitoring procedures suited to subarctic conditions and communities only intensifies the disproportionate burden of pollution that is faced by low-income communities worldwide (Shepard et al., 2002). Rural subarctic communities oftentimes rely on country foods for sustenance, something that not only affirms the need for monitoring pollutants in the food web, but also provides a means of monitoring through the food itself.

One method of monitoring the prevalence and impact of marine plastics is through the use of biomonitors in plastic ingestion studies. Plastic ingestion in fishes is a new and expanding field of research (Anastasopoulou et al., 2013; Boerger et al., 2010; Bråte et al., 2016; Davison and Asch, 2011; Lusher et al., 2015a; Miranda and de Carvalho-Souza, 2016; Phillips and Bonner, 2015; Rochman et al., 2015; Romeo et al., 2015) that has implications for monitoring procedures in the subarctic. Literature on plastic ingestion in the marine biota of Atlantic Canada is almost entirely based on the study of seabirds (Bond et al., 2013, 2014; Bond and Lavers, 2013; English et al., 2015). Seabirds have traditionally been used in biomonitoring studies, in part due to their accessibility when colonies return to land to breed (Provencher et al., 2016), but also due to the feeding strategy of many seabird species (indiscriminate "dive-bombing") that makes them highly vulnerable to plastic ingestion (Mallory, 2008; van Franeker et al., 2011). Although many fish species do not exhibit this high vulnerability to plastic ingestion, many species can be readily available as biomonitors through recreational and commercial fisheries while simultaneously providing plastics monitoring within the human food web – something that is less significant with seabird sampling.

Historically, Atlantic cod (*Gadus morhua*) has held particular cultural and economic importance in Atlantic Canada – and NL in particular (Schrank and Roy, 2013) – and remains to be a common food fish in the area. Plastic ingestion in Atlantic cod has recently been quantified in the North Atlantic and North Sea where the species yielded a percentage frequency of occurrence (%FO) of plastics ranging from 1.2 to 13% of individuals (Bråte et al., 2016; Foekema et al., 2013; Rummel et al., 2016). A study of plastic ingestion in Atlantic cod off the east coast of Newfoundland was conducted by the Civic Laboratory for Environmental Action Research (CLEAR) over the summer of 2015 (Liboiron et al., 2016). The study found the %FO to be 2.4% of Atlantic cod individuals harvested from various regions around the north-eastern Avalon Peninsula (Liboiron et al., 2016). For the study presented here, data on plastic ingestion in cod was again collected over the course of the NL commercial and recreational (food) fisheries of 2016 in cooperation with CLEAR. Regions sampled in the previous study were revisited in an attempt to validate the finding of 2.4% (%FO), and additional sample sites were added further south to extend the geographical reach of the baseline for plastic ingestion in Newfoundland waters and support the implementation of a long term monitoring effort. In an effort to improve marine debris monitoring procedures in the area, the methodology developed by CLEAR was built to suit a potential long-term citizen science monitoring program aimed at using Atlantic cod as bio-indicators of plastic pollution in local food webs.

The objective of this first chapter is therefore not only to quantify plastic ingestion in Atlantic cod, but also to use methods that can be easily replicated by volunteers from the general public in the future. In order to achieve this, it is important that the current state of knowledge concerning marine plastic pollution and its impacts is first discussed. Particular attention will be paid to the quantification and qualification of plastics ingested by marine biota, the risks associated with this ingestion, and the suitability of plastic ingestion studies to monitoring efforts in the study area (eastern Newfoundland). After this in depth literature review and discussion, the methodology developed by CLEAR for the quantification and qualification of ingested plastics will be described, as will the amendments made to these protocols and the reasoning behind said amendments. Finally, the results of the 2016 Atlantic cod study will be presented and the implications of these results for plastic pollution in eastern Newfoundland, plastic ingestion in a benthopelagic fish species, plastic ingestion methodologies, and citizen science methodologies will all be discussed.

2.2 Background

2.2.1 Production & demand

Plastics are relatively new materials, having entered the consumer marketplace with force following World War II in the 1930s and 1940s (Meikle, 1997), and quickly spreading throughout all oceans and adjacent seas by the late 1970s and early 1980s (Bergmann et al., 2015; Carpenter et al., 1972; Carpenter and Smith, 1972; Harper, 1987; Kartar et al., 1976; Kenyon and Kridler, 1969). At the time, Edward Carpenter of the Woods Hole Oceanographic

Institution predicted that the problem would likely worsen and that the toxic additives of these plastics - namely plasticizers - would have negative effects on marine life (Carpenter et al., 1972), an issue that will be discussed in detail below. Annual global plastic demand and production was approximately 1.5 million tonnes in 1950 and has been increasing ever since; with global production reaching 322 million tonnes produced in 2015, up from 230 million tonnes in 2005 - an increase of almost 100 million tonnes over the last decade (Bergmann et al., 2015; Plastics Europe, 2016; Wright et al., 2013). Hopewell et al., (2009) calculate a yearly increase in plastic production of 9%, which extrapolates to a production of 400 million tonnes in 2017. Of this production 10% is estimated to enter the oceans each year (Thompson, 2006).

2.2.2 Marine inputs

The growth in plastic production has been mirrored by plastic's representation in the marine environment. In the 1970s and 1980s, neuston surveys were reporting marine plastics in roughly 60% of samples (Colton et al., 1974; Day et al., 1990). By the 1990s, monitoring efforts in regions around the globe were reporting up to 15-fold increases in the presence of plastics in marine samples (Moore, 2008). The largest sector of the plastic marketplace is currently packaging - a single use product designed for disposal - and is therefore the main source of waste plastics (Plastics Europe, 2016). An estimated 10% of all plastic debris reaches our oceans (Thompson, 2006) and over 90% of these marine plastic particles are microplastics (< 5 mm) (Eriksen et al., 2014). A report by Eriksen et al. (2014) estimated that there are over 260,000 tonnes of plastic particles afloat at sea - not including benthic and shoreline debris. Plastics enter oceans via a number of pathways, including wastewater outflow, inland waterways, or by wind, waves and tides (Jambeck et al., 2015).

According to a 1989 shoreline survey of the Halifax Harbour on the east coast of Canada conducted by Ross et al. (1991), approximately 62% of litter could be sourced to land and recreation-based activities. Sea-based sources (including fisheries activities) are more important in remote areas (UNEP, 2009), however, considering approximately half of the global population resides within 50 miles of the coast (Cole et al., 2011), it is reasonable to assume land-based inputs are high. Indeed, surveys of marine litter surrounding major cities in

Australia found up to 80% of litter could be sourced to land (UNEP, 2009). Land-derived debris is deposited into the marine environment through a number of mechanisms, the most important of which being wastewater outflows, wind deposition and riverine input (Cole et al., 2011). In the case of just two rivers draining from Los Angeles, California it was reported that as much as 2 billion microplastic particles could be released into the marine environment over a 3 day period (Moore, 2008). Jambeck et al. (2015) compiled waste data for 192 coastal communities bordering the Atlantic, Pacific and Indian Oceans as well as the Mediterranean and Black seas to estimate the mass of readily available coastal plastic waste available for entry into the world's oceans. The study produced an estimate of 4.8 to 12.7 million metric tonnes (MT) of plastic waste entering the world's oceans in 2010. Not all plastic debris is land-sourced; commercial and recreational fishing, as well as other marine industries result in the direct introduction of plastic debris into the marine environment (Horsman, 1982; Macfadyen et al., 2009; UNESCO, 1994). An estimate of the discharge of plastic waste into the oceans in 1975 via ocean vessel sources, military operations and ship casualties was estimated at 6.4 million tonnes (National Research Council (U.S.) Study Panel on Assessing Potential Ocean, 1975). The international MARPOL agreement of 1988 banned marine vessels from disposing of their plastic waste at sea (MARPOL 73/78 Annex V), however a lack of enforcement and education combined with accidental losses contributed to estimates of approximately 6.5 million tonnes of plastic lost as marine debris in the early 1990s (Clark, 1997).

2.2.3 Marine microplastics

According to the current literature, plastics make up the majority – up to 80% on average - of marine debris worldwide (Camedda et al., 2014; Cole et al., 2011; Derraik, 2002; Galgani et al., 2015; Martinez et al., 2009; Pham et al., 2014). Eriksen et al. (2014) make a conservative estimate that 35,540 tons of the previously mentioned 250,000 tons of floating plastics are composed of microplastics alone. Although the nomenclature for separating plastics based on their size is not agreed upon in the literature, participants at the first international research workshop on the occurrence, effects and fate of microplastic marine debris (held by NOAA in 2008) agreed on an upper limit of 5 mm for plastics defined as "microplastics" (GESAMP, 2015). Microplastics can be manufactured to be of this size by the plastics industry (Pruter, 1987), or can result from the fragmentation of larger plastics (Andrady, 2003). Many microplastics are directly manufactured as industrial resin pellets (Mato et al., 2001) or as microbead "scrubbers", used as an abrasive in cosmetic products (Fendall and Sewell, 2009; Zitko and Hanlon, 1991). Recently, the government of Canada has proposed new "Microbeads in Toiletries Regulations" which will come into effect in 2018, banning the manufacture and sale of toiletries containing microbeads (Government of Canada, 2015). The degradation and fragmentation of plastics can occur via a number of processes including physical weathering and photo degradation by prolonged exposure to sunlight (Andrady, 2003). The combination of directed manufacturing and the fragmentation of larger plastics provides numerous entrance pathways for microplastics into the marine environment. Sampling of microplastics in the water column has, however, been inconsistent and unstandardised, making it difficult to make an accurate quantitative estimate of the global marine plastic problem (Nerland et al., 2014). Due to their relatively new entrance into the marketplace, the understanding of the longevity of plastics in the marine environment remains rudimentary, and can only be estimated. Estimated ranges span from years to centuries depending on the type of plastic and the environmental conditions (Andrady, 1994)

2.2.4 Distribution

The often lightweight construction of plastics facilitates their easy transportation by ocean currents, in many cases to a great distance from their source (Bond et al., 2013; Romeo et al., 2015). A study by Cozar et al. (2014) found that 88% of open ocean surface area sampled contained plastic debris. Surveys from some of the most remote areas of the globe - including the Arctic, Antarctica, and the sub-Antarctic Islands of the Southern Ocean are already reporting the negative effects of marine debris (Eriksson and Burton, 2003; Mallory, 2008; Zarfl and Matthies, 2010).

Although it is expected that the majority of marine debris originates from coasts, much of it can be found far from land due to the convergence of plastic debris in major accumulation zones as a result of major ocean currents; often a combination of Ekman, western boundary and/or geostrophic currents (Martinez et al., 2009). When these currents converge over large areas - specifically in gyres - floating plastics become concentrated, too buoyant to follow downwelling currents. Currently five areas of plastic concentration have been identified to exist in each of the world's five major gyres – the North Atlantic, South Atlantic, South Indian, North Pacific and South Pacific gyres - with the likelihood of a sixth patch in the Barents Sea (Deudero and Alomar, 2015; Sebille et al., 2012). A study by Martinez et al. (2009) tracked floating marine debris on its routes from island and continental coastlines to the Pacific subtropical gyre and found that while most of the debris released from the center of the South Pacific took 8 years to reach the gyre, debris released from the coast of South America averaged a direct route to the gyre in less than two years. By contrast, data from a long term monitoring program in the North Atlantic found that plastics released from the U.S. eastern seaboard could reach the North Atlantic Subtropical gyre in less than 60 days (Law et al., 2010). Martinez et al. (2009) also elucidated the permanence of marine debris along shorelines by observing the trapping of some debris by nearshore currents, enabling their transfer along the coast and away from point sources of pollution. Coastal regions are especially likely to see debris accumulation because they receive not only marine debris that has been trapped by these nearshore currents but also terrestrial debris that has not yet reached the marine environment (Pruter, 1987; Ryan et al., 2009). Notably, in regards to plastics being transported far from shore, research is often geared towards neuston plastics in these convergence zones, however benthic marine litter is not limited to coastlines and has been found on the Mid-Atlantic ridge, as far as 2000 km from land (Pham et al., 2014).

Some plastics are lightweight and low density, facilitating their floatation in seawater and making them both easily transportable and easily noticed, unlike the high density plastics which become hidden beneath the surface. This has led to a focus of marine plastic research on neuston plastics (Nerland et al., 2014) and little is yet known about the life and distribution of marine plastics and their final destination. Sebille et al. (2012) suggest that the recent surge in economic growth has led to a massive increase in the input of marine debris into the world's oceans, however surface plastic concentration appears relatively constant despite increased production and disposal (Cózar et al., 2014; Law et al., 2010). In the survey by Cozar et al. (2014) the size distribution of floating plastic fragments from the open ocean was analysed using a net of 200 µm mesh size, and particles under 1 mm were found to be significantly underrepresented, indicating they were being lost from the surface. There are several suggested mechanisms for the removal of plastics from the sea surface; biofouling (the colonisation by organisms), predation, on-shore deposition and accelerated fragmentation of plastics under 1 mm in size - possibly aided by pelagic bacterial colonies (Zettler et al., 2013). Predation is especially likely in the case of zooplanktivorous fishes as the "missing" surface plastics correspond in size with that of zooplankton (Cózar et al., 2014; Davison and Asch, 2011). Indeed, plastic ingestion studies on planktivorous fishes as well as the vertically migratory fish that feed on them in the North Pacific gyre report relatively high values for frequency of occurrence of ingested plastics (%FO values); ranging from 11.6% of vertically migratory mesopelagic fishes (Davison and Asch, 2011), 24.5% of vertically migratory longnose lancetfish (Jantz et al., 2013) and 35% in a study of plantivorous fishes (Boerger et al., 2010). Research of plastic ingestion in marine species generally indicates that these plastics will eventually be passed with the feces (Besseling et al., 2013; Nerland et al., 2014). Many marine organisms - including fish - already possess the ability to pass the indigestible material that is a natural part of their prey and feeding activities, such as bones, cartilage, and sediment (Cole et al., 2011). The passing of plastic particles in the feces can lead to the sedimentation and thus introduction of said plastics into the benthic environment (Nerland et al., 2014).

The mechanisms of sedimentation via feces, biofouling, and on-shore deposition all point toward benthic sediments as an important sink for "lost" microplastics. The fragmentation of marine plastics has been shown to result in an accumulation of microplastics in the sediment (Barnes et al., 2009) and studies of the Mediterranean Sea found plastics in 92.8% of deep sea samples (Ramirez-Llodra et al., 2013) and reported a range of 0.4-48 litter items/ha on the seafloor compared to the average 0.021 items/ha of surface litter in the same area (Pham et al., 2014). The benthic environment can also be an important sink for marine plastics simply as a result of their density. The investigation of plastics on the seafloor has been considerably lacking in comparison to the investigation of surface plastics, something that is mostly due to the high cost and technical difficulties of sampling the seafloor (Pham et al., 2014). Using data from the United States, Engler (2012) determined that only half of the plastics represented in U.S. municipal solid waste are buoyant in seawater.

The fishing industry is an important source of marine plastics and fishing lines and netting are typically manufactured to be neutrally buoyant, facilitating their distribution throughout the water column as marine debris (McElwee et al., 2012). There have been several studies of plastic ingestion in the Goiana Estuary of northeast Brazil (Dantas et al., 2012; Possatto et al., 2011; Ramos et al., 2012), all of which found the majority of plastics ingested by the fish species studied were blue nylon fragments picked up from the sediment, with %FO ranging from 7.9 to 23%. It was concluded that the source of the nylon fragments was local fishing activity; the highest ingestion rates were found in fish at the mouth of the river during rainy season, when the nylon debris from the corresponding lobster season is flushed into the mouth of the estuary (Dantas et al., 2012).

Newfoundland and Labrador fisheries hold high cultural and economic significance and as a result fishing activity remains high in the province; in 2015 commercial fisheries employed over 17,000 individuals, landing over 240, 000 tonnes of fish and shellfish in that year for a production value of $1.3 billion (Government of Newfoundland and Labrador, 2016). In addition, the participation of fishers in recreational fisheries has consistently been among the highest in the country (along with the Yukon Territory) throughout survey years and despite a 46% decrease in participation from 2005 to 2010, the participation rate remained to be the second highest in the country in 2010, with 71, 382 documented participants (Fisheries and Oceans Canada, 2012).

2.2.5 Ingestion

Marine plastics pose a serious risk to ocean ecosystems (Anastasopoulou et al., 2013; Andrady, 2011; Carpenter et al., 1972; Gregory, 2009; Rochman et al., 2014; Teuten et al., 2009). Marine plastics have been known to interact with marine biota via entanglement or ingestion, resulting in physical harm (Laist, 1987), blockage of digestive structures (Bjorndal et al., 1994; Bugoni et al., 2001), the transfer of toxicants including carcinogens and endocrine disruptors (Rochman et al., 2013c, 2014; Teuten et al., 2009). Historically, with regard to marine plastics, a lot of emphasis has been placed on entanglement (Balazs, 1985; Hanni and Pyle, 2000; Sazima et al., 2002; Shaughnessy, 1980), something that is likely a result of the unsightly appearance of entangled marine megafauna and the negative public attention it has received (Gregory, 2009). Plastic ingestion by marine biota is a much less visible threat –

historically garnering less public attention than entanglement – however it has been documented in more than 180 animal species (Andrady, 2011).

Already extensively documented in birds (Moser and Lee, 1992; Robards et al., 1995; Ryan, 1988; Ryan and Jackson, 1987; Spear et al., 1995) and turtles (Balazs, 1985; Carr, 1987; Cawthorn, 1985; Gramentz, 1988) by the 1990s, plastic ingestion in fish was a relatively new area of research and is rapidly expanding to become a dynamic field of research (Avio et al., 2015; Bråte et al., 2016; Dantas et al., 2012; Kühn et al., 2015; Miranda and de Carvalho-Souza, 2016; Phillips and Bonner, 2015; Rochman et al., 2014). Carpenter et al. documented the first case of plastic ingestion in fish in 1972 and in recent years there have been a number of attempts to quantify plastic ingestion levels and sources in fish species around the globe. As a result, we now have preliminary reports of plastic ingestion in fish from the North Sea and Northeast Atlantic (Bråte et al., 2016; Foekema et al., 2013; Lusher et al., 2013, 2015a; Neves et al., 2015; Rummel et al., 2016), tropical regions of the North and South Atlantic (Dantas et al., 2012; Miranda and de Carvalho-Souza, 2016; Phillips and Bonner, 2015; Possatto et al., 2011; Ramos et al., 2012), the Southwest Atlantic (Di Beneditto and Awabdi, 2014; Jackson et al., 2000), the equatorial South Pacific (Rochman et al., 2015), the Mediterranean and Adriatic Seas (Anastasopoulou et al., 2013; Avio et al., 2015; Romeo et al., 2015) and the Northeastern Pacific and Pacific Gyre (Boerger et al., 2010; Choy and Drazen, 2013; Davison and Asch, 2011; Jantz et al., 2013; Rochman et al., 2015). Spatially however, data on fish ingestion is still relatively poor and insufficient for identifying any spatial trends worldwide (Nerland et al., 2014).

Plastic ingestion in marine biota may be intentional (based on foraging strategy) or incidental (such as in secondary ingestion), varying between species and ecological niches. Marine plastic characteristics such as buoyancy, size and colour will affect their bioavailability to various fish species (Anastasopoulou et al., 2013; Deudero and Alomar, 2015; Ramos et al., 2012). A study by Camedda et al. (2014) reported preferential ingestion of white and transparent sheet plastics in the sea turtle *C. caretta*, which could be a result of these colours more closely resembling the natural prey (jellyfish) than other colours. Biofouling can also have an effect on the ingestion of marine plastics as this colonization by microorganisms and plankton will likely have the added effect of making the plastic particle more attractive as a food item (Nerland et al., 2014). In fact, new research indicates that biofouling can lead to the presence of dimethyl sulfide (DMS), a so-called "infochemical" which has been shown to attract seabirds during foraging (Savoca et al., 2016). This is some of the first research showing marine biota's attraction to plastic via a mechanism other than visual cues (in this case by olfaction).

Density and type (film or hard fragment) of plastic can also affect ingestion by affecting bioavailability for certain species. In a case study of plastic ingestion by fishes in the Mediterranean Sea, Anastasopoulou et al. (2013) found the pelagic species sampled had only ingested film plastics and bathybenthic species had only ingested hard plastics. It is likely that the effects of plastic type are twofold here; density likely plays the largest role by making the plastic more available to fish that inhabit a certain part of the water column, however, it is also possible that floating films in the water column resemble pelagic prey. Feeding strategy is an important factor when determining how a predator species will respond to marine litter (Deudero and Alomar, 2015).

Another important factor for bioavailability is fragment size: as fragments break down they become available an increasing number of organisms (Cózar et al., 2014) however it is not a strictly linear relationship between size and bioavailability. In a study by Matranga and Corsi (2012), 10 nm polystyrene particles increased in bioavailability when presented to blue mussels as 2 μm aggregates. Fragmentation of microplastics can quickly make them small enough to be ingested by animals at the very base of the food web (zooplankton) and in a survey of the plankton of the English Channel, Cole et al. (2014) found that 77% of analysed copepods had ingested microplastics. The lower the level of the food web at which plastics are ingested, the more likely they are to be subject to secondary ingestion – the ingestion of plastic particles by a predator species through the ingestion of contaminated prey species. Secondary ingestion is thought to be the source of plastics found in the digestive tracts of fur seals from Macquarie Island. Eriksson and Burton (2003) attributed the presence of small plastic particles in fur seals to the seals' most common prey item, *Electrona subaspera*, a plentiful myctophid fish in the area. Secondary ingestion provides a route of transport for particles throughout the water column regardless of their density - a study by Avio et al. (2015) found some high density plastics in pelagic fish and some low density plastics in benthic fish. As mentioned, fouling can cause low density plastics to sink, however, secondary ingestion provides a means of transport for plastics, in this case by vertically migratory fish consuming plastics at the surface before returning to depth (Lusher et al., 2015a). Therefore, although the density of a plastic can affect where it becomes available to marine biota, density is not the only factor that determines which types of marine biota can be affected by it.

2.2.6 Impacts of ingestion

There is currently no comprehensive risk assessment for marine plastics and their interaction with marine biota and we can therefore only estimate their full effects (Neves et al., 2015). However, plastic ingestion in marine biota can theoretically have a number of negative consequences including the direct effect of the physical presence and accumulation in the stomach leading to reduced stomach storage volume and a resultant decrease in feeding stimuli and satiation (Ryan, 1988), blockage of digestive structures (Bjorndal et al., 1994; Bugoni et al., 2001), blockage of gastric enzyme secretion (Azzarello and Van Vleet, 1987), starvation and death (Bjorndal et al., 1994; de Stephanis et al., 2013; Pierce et al., 2004). The dangers of ingested plastics are not limited to their physical volume and the blockages they create. The contaminants associated with marine plastics can give rise to additional impacts including poor nutrition and dehydration (Auman et al., 1998; McCauley and Bjorndal, 1999), lowered steroid hormones (Azzarello and Van Vleet, 1987; Peakall, 1970), reproductive failure (Oehlmann et al., 2009; Peakall, 1970; Rochman et al., 2014), impaired growth and development (Meeker et al., 2009; Ryan, 1988), carcinogenic and mutagenic effects (Rochman et al., 2013c, 2014)and reduced quality of life (Derraik, 2002; Kühn et al., 2015; Lavers et al., 2014).

Direct effects that result from the physical volume and blockages of plastics can only occur if the plastics are accumulating in the gut however the toxicants carried by plastics do not require accumulation of plastic in order to be transferred, only contact with digestive fluids as the particle passes through the digestive system (Bråte et al., 2016). Direct contact with digestive fluids in the digestive tract enhances the transfer of contaminants from the particle to the animal (and potentially from animal to particle), and the longer that a particle is in contact with these digestive fluids and not excreted - in other words, the gut retention time (GRT) - the higher the transfer of possible contaminants (Bråte et al., 2016). In the case of ingestion by fish, GRT of plastics in the gut is only beginning to be researched (Grigorakis et al., 2017; Lu et al., 2016; Mazurais et al., 2015) and preliminary results indicate microplastics move passively through the gastrointestinal tract with retention times ranging from 33 hours in goldfish (Grigorakis et al., 2017) to two days in European sea bass (Mazurais et al., 2015). Many fish containing plastic debris have been found with only single plastic particles, suggesting that accumulation of plastics within the gut is unlikely at the current field conditions (Boerger et al., 2010; Foekema et al., 2013; Neves et al., 2015). However, marine plastics also function as route of transport for contaminants into the marine food web (Besseling et al., 2013; Koelmans et al., 2014; Teuten et al., 2009). Marine plastics often contain a "cocktail of contaminants", made up of ingredients and by-products of the material itself, as well as contaminants adsorbed from the surrounding seawater (Lithner et al., 2012; Mato et al., 2001; Rochman et al., 2015).

Many plastics are manufactured with high levels of bioactive additives, taking the form of UV stabilizers, softeners, flame retardants, non-stick compounds, and colourants (Colton et al., 1974; Lithner et al., 2012). Plasticizers (especially pthalates) generally compose from 20 to 50% or more of a plastic's wet weight and are used to increase durability and flexibility, as well as to provide resistance to heat, oxidative stress and microbial degradation (Browne et al., 2007; Deanin, 1975). Over 90% of plasticizer additives are used with PVC (up to 50% of the wet weight of PVC can be composed of phthalates), a plastic that is negatively buoyant and therefore available to benthic feeding organisms (Cole et al., 2011; Nerland et al., 2014). Plastic additives such as pthalates, polybrominated diphenyl ethers, and bisphenol A are known for their ability to disrupt endocrine function, leading to hormone imbalances and morphological, development, or gonadal issues (Kim et al., 2002; Oehlmann et al., 2009; Rochman et al., 2014). Chronic exposure to bisphenol A in humans can lead to heart disease, diabetes and changes in hormone levels (Lang et al., 2008; Melzer et al., 2010; World Health Organization, 2010). External plasticizers are added to plastics after polymerization (often to improve flexibility), meaning they are not bound to the plastic material like internal plasticizers (applied during processing of polymers) are and are therefore more likely to migrate into their environment, or in the case presented here, the digestive tracts of marine organisms (Cadogan and Howick, 2000; Nerland et al., 2014). Once in the marine environment, plasticizers will leach or off-gas from plastic and as the plastics ages, it will weather and fragment, exposing new surfaces for the leaching or off-gassing of additives (Engler, 2012). In this way a plastic fragment can be a long-term source of toxic chemicals.

Contaminants that are picked up by marine plastics are adsorbed from the surrounding seawater to the plastics surface due to their hydrophobicity and may be concentrated to levels of up to 1 million times that of the surrounding seawater (Mato et al., 2001; Newman et al., 2015; Teuten et al., 2009). Many of these contaminants are persistent (persisting for years or even decades), bioaccumulative and toxic substances such as polychlorinated biphenyls (PCBs) and insecticides such as DDT (United States Environmental Protection Agency, 2016). Due to their low water solubility, contaminants such as those described here will often concentrate in the sediments, within a thin layer at the surface (making them available to adsorption with surface plastics), or on other hydrophobic particles such as plastics (Engler, 2012). An early study by Carpenter et al. (1972) found PCB concentrations of up to 5000 parts per billion in floating polystyrene particles off of New England. Due to their excellent ability to adsorb and concentrate marine contaminants, some studies have suggested that plastic pellets be used as a mechanism for global contaminant monitoring (Nerland et al., 2014; Ogata et al., 2009).

Of all the chemicals listed as priority pollutants by the US Environmental Protection Agency (USEPA, 2016), 78% of them are associated with marine plastic debris (Rochman et al., 2015). However, studies on chemicals carried by marine plastics are relatively new and information is therefore still limited but increasing steadily (Nerland et al., 2014). A laboratory study on microplastic ingestion in lugworms (Besseling et al., 2013) reported not only decreased feeding activity and weight loss but an increase in PCB bioaccumulation. In another lugworm study by Browne et al. (2013), when presented with a sediment composed of 5% microplastics treated with pollutants, ingestion of said plastics led to the transfer of these pollutants and plastic additives into the gut tissue of the worms. Uptake of various toxic chemicals by lugworms in the experiment by Browne et al. led to an over 60% reduction in the worms' ability to remove pathogenic bacteria, diminished their ability to engineer sediments, made worms more susceptible to oxidative stress, and led to mortality in over half of the individuals. The study was also successful in showing that the primary transfer of contaminants occurred via food items (plastic and sediments) in the gut and not through the body wall even when pollutant levels in the sediment are high. More specifically to fish ingestion, Rochman et al. (2014) reported that upon being treated with a diet containing marine plastics, Japanese medaka fish (*Oryzias latipes*) presented with changes in gene expression (likely leading to a reduction in female fecundity) as well as a case of male gonadal abnormalities - possibly a precurser to intersex. In another study, fish exposed to microplastics in the laboratory gave way to some of the first evidence of microplastics being translocated between tissues in a marine vertebrate - the study by Avio et al. (2015) revealed a small presence of particles within the hepatic tissue. Although research into the importance of marine plastics as a vector for contaminants is still in its early stages, preliminary reports suggest the pathway is of less importance than other pathways, such as diet and diffusion from surrounding seawater (Bakir et al., 2016; Gouin et al., 2011). In a study of Atlantic cod (*Gadus morhua*), Koelmans et al. (2014) modelled the transfer of two plastic additives (nonylphenol (NP) and bisphenol A (BPA)) leaching from microplastic in the intestinal tracts of cod and found a low level of transfer, indicating that at the current NP and BPA environmental concentrations it is unlikely for bioaccumulation of these contaminants to occur in cod.

Although early reports suggest the transfer of contaminants from marine plastics may be minimal in comparison to other pathways, a precautionary approach should be taken with any new pathway that harbours the potential to result in the biomagnification of contaminants into the human diet. If exposure to toxicants occurs faster than the body can remove them, accumulation occurs (Engler, 2012). The accumulation of toxic chemicals in marine species can theoretically be further transferred up the food web by biomagnification and into human diets, however, further research is required on the potential transport of contaminants from marine plastics up the marine food web and the possible consequences for humans as end consumers (Cole et al., 2011; Engler, 2012). Further research is required in order to understand the importance of marine plastics as a pathway for pollutants in the human diet and the determination of risk based on the magnitude of this pathway.

The threats to marine biodiversity that come from marine debris add to the ever-increasing threats of overfishing, climate change, and other anthropogenic disturbance

(Derraik, 2002). Due to the longevity of plastics, even if production were to cease, marine plastics would remain a threat to marine life for many decades (Derraik, 2002). As marine plastics break down into microplastics, it becomes near impossible to trace their source and increasingly difficult to clean them up without removing even more planktonic biomass and disrupting food webs (Jambeck et al., 2015; Nerland et al., 2014). The best mitigation strategy is therefore to target focal points of pollution and reduce their input (Jambeck et al., 2015). Education and legislation can help reduce plastic debris before it is released. This is not to say that marine plastics should be left unattended, any attempts to reduce production and inputs of plastics into the marine environment require monitoring of both the debris that is present and the rate at which this standing stock changes (Ryan et al., 2009).

2.3 Regional Considerations

The Pacific Gyre, or “Great Pacific Garbage Patch,” has garnered much media attention in recent years, however, the North Atlantic Gyre presents another region of accumulation of surface plastics. A 22 year ship survey of the North Atlantic Ocean and Caribbean Sea found that 62% of all samples contained marine plastics, 83% of which were collected from the North Atlantic Subtropical Gyre (Law et al., 2010). Within the gyre, a single 30 minute tow led to the collection of 1069 plastic pieces, a concentration that can be extrapolated to 580,000 pieces/km^2 (Law et al., 2010). The surveys followed the Gulf Stream as far north as the south/south-eastern coast of Newfoundland where plastics had dramatically decreased from the convergence zones of the gyre, but were still present, especially in the waters separating Newfoundland's south coast from Cape Breton, Nova Scotia (Law et al., 2010). The island of Newfoundland also sits in the Gulf of St. Lawrence, which is fed by the St. Lawrence River – a large river draining eastern Canada that is also a primary shipping route. Rivers are an important point source of marine plastic input and islands can in many cases act as sieves, collecting oceanic plastics that originate far from their shores (Moore, 2008).

There has been little monitoring of plastic debris in the Canadian Arctic and sub-Arctic (Mallory, 2008; Provencher et al., 2010, 2014b). Low population sizes in the Canadian Arctic are not expected to contribute much in terms of local debris and monitoring has been historically difficult due to the harsh environment and long periods of sea ice cover (Mallory, 2008). Plastic ingestion in marine biota can be used as a monitoring technique through the use of bioindicators. Under the 1992 Oslo and Paris Conventions for the protection of the marine environment of the northeast Atlantic (OSPAR), regulations are currently in place that require the tracking of plastic ingestion rates in the Northern Fulmar (OSPAR Commission, n.d.). Northern Fulmars exhibit some of the highest %FO values for plastic ingestion, which could be an indication that they ingest more plastic than other species based on their foraging techniques but is also attributable to the fact that they belong to the Procellariiformes, an order of seabirds that cannot regurgitate debris like other birds (Mallory et al., 2006; Moser and Lee, 1992). Monitoring plastic ingestion in fishes can also be useful for contaminant monitoring; fish are sensitive to the presence of endocrine disrupting chemicals and therefore may make good indicators of their presence through changes in their gonadal growth, gonadal degeneration, and the occurrence of intersex (Rochman et al., 2014). Despite these indicators, fish are not as vulnerable to plastic ingestion as seabirds such as the Northern Fulmar, however monitoring plastic ingestion in Atlantic cod has the added benefit of directly monitoring the presence of marine plastics in the human food web, at least in the case of NL where cod is a culturally important food fish. A recent study of plastic ingestion in marine catfish in Northeast Brazil has suggested this biomonitoring technique by using a catfish species already used for monitoring trace metals in the estuary as a “sentinel organism” for monitoring the state of its estuarine environment (Possatto et al., 2011). With recent reductions in sea ice and the opening of shipping routes in the north it is important that we work to close this gap in pollution monitoring and research (Mallory, 2008). Many long-term environmental monitoring programs can be costly and labour intensive, however, oftentimes this labour is technically straightforward and volunteers can make a significant contribution data collection (Foster-Smith and Evans, 2003). If biomonitoring research on marine plastics can be made straightforward and accessible to the general public, citizen science can be a means towards overcoming the barriers of plastic monitoring in Arctic and sub-Arctic regions.

2.3.1 Newfoundland as a study site

The province of Newfoundland and Labrador comprises of a section of coastal mainland (Labrador) and an island (Newfoundland) on the east coast of Canada. The province is sparsely populated with a population of 514,536 and a population density of 1.4 persons per square kilometer in 2011 (Statistics Canada, 2012). The majority of this population is spread along the extensive coastline (Statistics Canada, 2012), a result of its dependence on fisheries and coastal access. Historically, Atlantic cod was extremely significant both culturally and economically in the Newfoundland context however as a result of overexploitation, a moratorium was placed on the species and all commercial fishing was halted in 1992 (Schrank and Roy, 2013). In NL, the cod stock since the mid 1990's has sat at about 2% of what it was in the early 1960's (Link et al., 2009). Despite the loss of the Atlantic cod as a source of income, the fish still holds great cultural value to the people of Newfoundland. According to a study by Lowitt (2013), 97% of households on the west coast of Newfoundland identified cod as the favourite seafood and over 80% responded that they eat cod "often", with the main source of all types of seafood in the study identified as either friends/family or local fish plants. Atlantic cod sourced from friends and family or local fish plants can be assumed with confidence to come from the local recreational food fishery in the case of family and friends, and local commercial fishermen in the case of local fish plants. Extrapolating these results to the east coast of Newfoundland, Atlantic cod are likely an important local food source, something that holds implications for the monitoring of plastics in human food webs, but is also likely to attract public attention to the issue of marine plastics.

Beyond the stated fisheries activities as a likely source of marine plastics around the island of Newfoundland, improper disposal of waste plastics may be a significant contributor to plastic pollution. With regard to access to and use of recycling programs, Newfoundland and Labrador comes in below the national average (Statistics Canada, 2013). In the absence of accessible recycling and landfilling options, the burning of plastics is still a common practice in remote outport communities of Newfoundland; in a recent study 37.3% of plastic particles ingested by Dovekies collected in eastern Newfoundland were burnt or melted (Avery-Gomm et al., 2016). Incineration and open burning of waste at disposal sites are still utilized in NL - although they are in the process of being phased out - however the practice is not in use on the Avalon Peninsula and the closest site of open burning to the study area occurs on the Burin Peninsula (Government of Newfoundland and Labrador, 2014). Plastic ingestion studies in Newfoundland are limited and mostly focused on seabirds (Avery-Gomm et al., 2016; Bond et al., 2013; Bond and Lavers, 2013; English et al., 2015; Fife et al., 2015; Provencher et al., 2014a), however the study of plastic ingestion in Atlantic cod conducted by Liboiron et al. (2016) highlighted the importance of expanding plastic ingestion monitoring to human food webs through the use of seafood biomonitoring, something that was clearly well received when the public requested the study become the basis for a long term monitoring program.

2.4 Research Questions

In order to meet the project aims outlined in the introduction, the following research questions will be addressed:

1. How does the %FO of plastics in Atlantic cod collected in 2016 compare to that of the 2015 collection conducted by CLEAR? Do these results work together to provide a credible baseline for plastic ingestion in Atlantic cod off the east coast of Newfoundland?
2. How does %FO of plastics in Atlantic cod off the east coast of Newfoundland compare with global %FO values for fish?
3. What factors influence the ingestion of marine plastics by Atlantic cod?
4. How effective are the project protocols, and the use of Atlantic cod in particular, as a means of facilitating the biomonitoring of marine plastic pollution via citizen science?

2.5 Methodology

2.5.1 Field sampling by fishers

Despite the moratorium on Atlantic cod that has been in place in Newfoundland and Labrador since 1992, relatively low levels of commercial and recreational fishing still occur and are strictly managed by the Department of Fisheries and Oceans Canada (DFO). The commercial fishery occurs under the Northern Cod Stewardship/By-catch Fishery, a fishery which was extended in 2016 from the previous three week system to an extended season allowing for a harvest of 2,000lbs/week from mid-August to September and 3,000lbs/week from September until the end of the season (Fisheries and Oceans Canada, 2016a). On top of the commercial fishery, there exists a recreational fishery which, in 2016, allowed for the harvest of 5 fish per person per day to a maximum of 15 fish per boat over the course of 46 days between July and October 2016 (Fisheries and Oceans Canada, 2016b).

A total of six coastal wharves (as seen in Figure 1, along with corresponding %FO results for each site) were visited during the commercial and recreational fishing season for field sampling; three sites – revisited from the 2015 study (Liboiron et al., 2016)– on the north-eastern coast of the Avalon Peninsula (Quidi Vidi, Petty Harbour, and Portugal Cove-St. Philip's), and three new sites on the south-eastern coast of the Avalon Peninsula (Witless Bay, Bauline East, and Brigus South). A total of 348 fish were collected (12 at Quidi Vidi, 56 at Portugal Cove-St. Philip's, 44 at Petty Harbour, 87 at Witless Bay, 114 at Bauline East, and 35 at Brigus South). Although fish were collected at the wharf in Brigus South, all fish from this location were collected from a commercial fisherman who – based on the GPS coordinates provided – had taken the fish from approximately 30 km offshore of Bauline East. The Brigus South location will therefore be hereafter referred to as "Offshore Brigus South". Unlike in the case of fish taken from two other commercial fishers in this study (who captured their fish using gillnets), the commercial fisher in Brigus South captured the fish using handlines, the same method used by recreational fishers.

All fish in the study were collected directly by recreational and commercial fishers and the number of individual fishers involved per site ranged from one to seven. Within each community, fishermen participating in the recreational fishery generally frequent the same fishing grounds and the variety of individual fishermen donating GI tracts is therefore not expected to be a source of unexplained variation. The frequenting of the same fishing grounds (and often even the same rock or ledge) all but eliminates the likelihood of some study sites having a wider sampling range than others based on the number of participants. This sampling technique is not random or evenly distributed, but judgemental based on fishers' knowledge of local "hotspots" of fish aggregation, which also increases the efficiency of sampling. The collection method used in the majority of previous fish plastic ingestion studies involves experimental surveys (Bråte et al., 2016; Davison and Asch, 2011; Foekema et al., 2013; Lusher et al., 2013; Phillips and Bonner, 2015; Ramos et al., 2012; Romeo et al., 2015; Rummel et al., 2016). However, the collection method presented offers the advantage of eliminating the "net feeding" phenomenon (Davison & Asch, 2011). This occurs when debris is concentrated in the cod end of the net where captured fish can feed on it, leading to an overrepresentation of plastic ingestion (Davison and Asch, 2011). All fish collected in the study were caught either via hand-line (hook and line) or gillnet (a static, passive fishing gear), two gear types that are unlikely to result in the collection and concentration of debris during their use. Moreover, the collection methods employed mean that this study samples the food web directly, as all fish collected were destined for human consumption, a sampling technique that recent studies have pioneered (Neves et al., 2015; Rochman et al., 2015).

In order to obtain sex and length data from as many fish as possible, fishers donated the carcasses of their fish after fileting – a process that removes the meat of the fish while usually leaving the internal organs and gut entirely intact. This donation process was facilitated by the culture of fileting fish on wharves immediately after the catch, something that is uniquely common in Newfoundland and enabled the researchers to interact with many fishers at once. All fish included in the study were identified as Atlantic cod by the primary researcher at the wharf. Entire gastrointestinal (GI) tracts from the mouth to the anus were collected alongside fishermen at the wharf wherever infrastructure allowed, which facilitated public engagement. Fishers provided details of where the fish was caught and in most cases provided a nickname for their sample to allow them to identify their fish during report back of findings, which were put online as results came in so fishers could see if their particular fish had plastics in them. This was, in part, a way to provide reciprocity for their engagement with the project as sample collectors and make the project sustainable with regard to citizen participation (Cigliano et al., 2015).

2.5.2 Gastrointestinal tract collections

Gastrointestinal (GI) tracts – from esophagus to anus - of all fish were removed in the field. During this phase fish length (from snout to base of the caudal fin) and sex were recorded wherever possible (93% and 83% of cases, respectively). These data points could not always be recorded due to the different practices of various fishers in prepping their catch for consumption or sale. In some cases, fish heads were removed for boiling (eliminating the ability to measure the length of the fish) while in others, the female gonads were removed for frying (eliminating the ability to determine sex with certainty). All guts were individually bagged and tagged with an ID number as well as the sex and length of the fish and frozen for further analysis.

2.5.3 Dissection of GI tracts

Processing of GI tracts from the 2016 season followed the protocols of the previous season for the sake of consistency and standardisation, an essential feature of ongoing monitoring programs. The laboratory protocol is adapted from those of van Franeker et al. (2011), and emphasizes visual inspection. This protocol has the advantage of being amendable to citizen science methods of analysis that do not support highly technical equipment, so that future monitoring is feasible for an array of actors (Liboiron et al., 2016). GI tracts were thawed for at least 2 hours prior to dissection. After thawing, they were cut along the length of the entire tract from esophagus to anus. Any apparently non-organic items were removed directly from the GI tract prior to scraping and rinsing contents into stacked sieves of 5 mm and 1 mm mesh sizes. During scraping and rinsing of the GI tract, stomach and intestinal walls were visually and tactilely inspected for imbedded debris. Sieve contents were continuously and gently rinsed with cold water and all particles resembling non-organics were removed and placed in a petri dish for further analysis under a stereomicroscope. Cold water rinsing serves as a method of purification; plastic particles must be rid of organic and inorganic debris to enable proper identification (Löder and Gerdts, 2015). Although rare, there were some instances of relatively intact fish in in the gut contents that were intact enough for visual analysis (N = 5). These were dissected and analysed following the same methods in an attempt to identify secondary ingestion.

In addition to the methods used in the 2015 analysis by Liboiron et al. (2016), protocols were adjusted in an attempt to better understand the relationship between the feeding ecology of Atlantic cod and their ingestion of marine plastics. Atlantic cod are typically described as benthopelagic fish, feeding on biota both from the surface of benthic sediments and the water column immediately above it (Daan, 1973; Johansen et al., 2009; Link et al., 2009). During dissection of the GI tracts, individual prey items were noted which allowed for a separation of feeding microhabitats between individuals. Benthic feeders were identified by the presence of benthic prey items including brittle stars (*Ophiuroids*), toad crabs (*Hyas araneus*), snow crab

(*Chionoecetes opilio*), blue mussels (*Mytilus edulis*), and small hermit crabs and gastropods. Benthopelagic feeders were identified by the absence of these food items, combined with the presence of pelagic prey such as northern shortfin squid (*Illex illecebrosus*), capelin (*Mallotus villosus*), or large fish (often too far digested to be identified with confidence but were thought to be Atlantic cod (*Gadus morhua*), Atlantic mackerel (*Scomber scombrus*), and/or Atlantic herring (*Clupea harnegus*)).

The presence of non-plastic, non-prey items such as algae and sediment were also recorded. Two types of non-prey items were repeatedly encountered; sediment and various species of marine algae. Sediment was recorded per number of individual particles (rocks) present while unusually large rocks (greater than approximately 0.5 cm) were specifically noted. Algae were noted when present and the division (red, brown or green) as well as any defining characteristics (i.e. filamentous, turf, or kelp) was also noted. Instances of large or abundant sediments and/or algae were noted and photographed. These protocols for the identification of non-plastic, non-prey items were introduced in an attempt to show the potential for accidental ingestion of foreign particles in this opportunistic fish species.

2.5.4 Contamination

Microplastics research is especially vulnerable to contamination during the sampling and post-sampling phases due to the ubiquity of microplastics in all environments, especially in the case of plastic microfibres that can be deposited from the atmosphere (Fries et al., 2013; Hidalgo-Ruz et al., 2012; Nuelle et al., 2014; Woodall et al., 2015). In an effort to combat this, every precaution was taken to limit this contamination. Holes in the GI tracts could function as a possible pathway for the entrance of microplastics from the external environment during GI tract collection. Small intestinal perforations were a common result of the fileting process (N = 29) and GI tracts were not excluded on this basis. These perforations resulted from small pricks from the tip of the fileting knife, with no tearing and allowed no visibility of internal digestive contents. However any large, open holes in the stomach or intestines with spillage of digested contents resulted in the elimination of the sample from the project. To reduce contamination in the field, splitting tables on collection wharves were rinsed with water prior to dissection. This is a common practice by fishers during filleting, as fish can stick to dry tables and make filleting difficult, and we can assume that tables were rinsed regularly. During dissection of GI tracts in the laboratory, external contaminants were sometimes noted adhered to the outside of the stomach or intestines (N = 15). This contamination took the form of wood splinters from splitting tables or paint chips that were easily sourced to the measuring table used for length measurements. Woodall et al. (2015) suggest keeping inventory of any plastics used in the sampling process for comparison with recovered plastic contaminants later. In this study, plastics that came in contact with the sample included the yellow paint and housing of a measuring tape, the clear plastic sample bags and in rare cases, the paint on fish splitting tables. Any and all contamination was noted during lab analysis of GI tracts. Within the laboratory, microplastic contamination has several sources, including the researchers themselves, their tools and the atmosphere. The researcher used a cotton lab coat and tied hair back, rinsed all tools under tap water before and after each sample, and used daily control dishes to capture particles settling from the air. In the event that a fibre was found in a GI tract, it was compared to fibres present in the control dish.

2.5.5 Quantification of plastics

According to Song et al. (2015), visual sorting through the use of a microscope is a suitable method for discriminating microplastics of 1 mm and greater. Below 1 mm in size, visual sorting, even with the use of a microscope, cannot be relied upon as the error rate of misidentification rises sharply. Therefore, in deference to a protocol that could be used by citizen scientists in the future, only particles greater than 1 mm in size were analysed. This was enforced through the 1 mm mesh size used to isolate the particles. Once removed from the digestive contents, identified non-organic particles were analysed under an Olympus SZ61 stereomicroscope (total magnification 270X). To identify plastics, sorting followed the criteria laid out by Norén (2007):

"[The] following criteria [are] used to define a plastic particle

- No cellular or organic structures are visible in the plastic particle/fibre
- If the particle is a fibre it should be equally thick, not taper towards the ends and have a three-dimensional bending (not entirely straight fibres which indicates a biological origin)

- Clear and homogeneously coloured particles (blue, red, black and yellow)
  If it is not obvious that the particle/fibre is coloured, i.e. if it is transparent or whitish, it shall be examined with extra care in a microscope under high magnification and with fluorescence microscopy in order to exclude an organic origin." (Norén, 2007, p. 7)

Each particle identified as plastic or held as probable plastics for further analysis were wrapped in a piece of filter paper labeled with the date and ID number and left to dry for at least five days. Once dry, further analysis involved viewing the particles under a compound microscope and eventually, the use of a Raman micro-spectrometer.

As most plastic ingestion studies report the frequency of occurrence within a sample, plastics were noted as either present or absent for each fish so results could be comparable with other studies. Where present, plastics were further quantified by number of particles present per individual. Individual plastic particles were then assessed according to their weight, size, type, shape, colour and erosion in an attempt to qualify their source and impact. Colour (or more importantly, discolouration) and erosion can be indicators of time spent at sea, and the more time spent at sea, the more contaminants a particle is likely to come into contact with. Discolouration of beached resin pellets has been shown to be linked with higher concentrations of adsorbed contaminants (Endo et al., 2005). In addition to signalling long periods spent at sea, erosion causes degradation of particles and creates pits and grooves on its surface, thus increasing its surface area and its ability to sorb (and release) contaminants (Rochman et al., 2013b). Size, shape and type can similarly have implications for surface area and thus the ability to sorb contaminants from surrounding seawater. Length, height and width (or diameter in the case of spherical particles) were measured using digital callipers. Particle type was described as either industrial resin pellets, sheet/film plastics (i.e. plastic bags), thread plastics (i.e. fishing line), foam plastics (i.e. polystyrene packaging), fragment plastics (hard plastics from a wide array of products), and other (in the case of microfibres) (following Provencher et al., 2016). Finally, particles were described according to their opacity (whether reflected light from the microscope passed through), colour (following Verlis et al., 2013), and degree of weathering (following Corcoran et al., 2009).

2.5.6 Raman micro-spectrometry

Raman micro-spectrometry is a nondestructive spectroscopic method that can be used to determine a material's molecular structure by revealing vibrational characteristics of the sample down to the µm range (Imhof et al., 2012; Lenz et al., 2015). The method is expensive and technically difficult, factors that make it poorly suited to citizen science methodology in general. It was used here, however, in an attempt to determine the accuracy of visual identification methods for plastics over one millimetre in size . Raman micro-spectrometry can accurately distinguish between plastic and natural particles and can therefore identify (if present) any false-positives taken from the visual identification stage, while having the added benefit of identifying specific plastic polymers (Lenz et al., 2015).

All particles identified as plastics in the visual identification stage (excluding two particles (microfibres) which were lost prior to this stage) were washed in ethanol and allowed to dry prior to analysis. Samples were placed on a silica wafer of known Raman spectrum (520 cm^{-1} peak) and analysed using a Raman micro-spectrometer (Reinshaw InVia with 830 nm excitation) at a 20x Olympus objective. The instrument was controlled by WiRE 3.4 software. To ensure samples were not burnt, laser power did not exceed 5%, and in cases of high fluorescence (in 4/5 samples), laser power was reduced to 1%. The Raman spectrum of each particle was compared to reference spectra for common marine plastic polymers. According to the literature, these include (in no particular order); polyethylene (PE), polyethylene terephthalate (PET), polycarbonate (PC), polypropylene (PP), polyvinylchloride (PVC), polystyrene (PS), polyamide (PA), polyurethane (PU), poly(methyl methacrylate) (PMMA), acrylonitrile butadiene styrene (ABS), and cellulose acetate (Bråte et al., 2016; Engler, 2012; Lenz et al., 2015; Plastics Europe, 2016).

2.5.7 Data processing

An overall population average - including standard error about the mean (a measure of the statistical accuracy of the average) - of individuals with ingested plastics was calculated from all individuals from all locations in the current study. Single factor (or one-way) analyses of variances (single factor ANOVAs) were conducted using Microsoft Excel to determine statistically significant differences based on the variables being tested. The purpose of the test is to compare the mean plastic ingestion between groups for each independent variable and determine if a significant difference exists between these means for the variable being tested. Single factor ANOVAs were used to compare the mean plastic ingestion between sample sites, between sexes, between the presence or absence of food in the stomach, and between benthic and benthopelagic feeding microhabitats. Outside of the realm of plastic ingestion, a single factor ANOVA was also used to determine if there was a significant difference in the ingestion of non-plastic, non-food items between benthic and benthopelagic feeding habitats. Significance was determined at the 95% confidence interval ($p < 0.05$). Plastic ingestion will be expressed as %FO; the proportion of individuals with ingested plastics, either in a specific sample area or as a whole for the Avalon Peninsula of Newfoundland.

2.6 Results

Of the 348 Atlantic cod sampled, 7 (2.01%) had ingested anthropogenic debris. Two of these 7 identified pieces were microfibres and the possibility of contamination must therefore be addressed. One of these particles was enmeshed in a conglomerate of partially digested material (Figure 2), eliminating the possibility contamination via atmospheric deposition, as is common with microfibres. The second microfibre was small enough to be atmospherically deposited, however it did not match any fibres present in the control and was found within a bundle of red algae. Based on these two factors, it can be said with some confidence that the second microfibre was not a product of contamination and a final result of 2.01% frequency of occurrence will be reported. Within individuals that ingested plastics, the frequency of ingestion was one plastic in all cases.

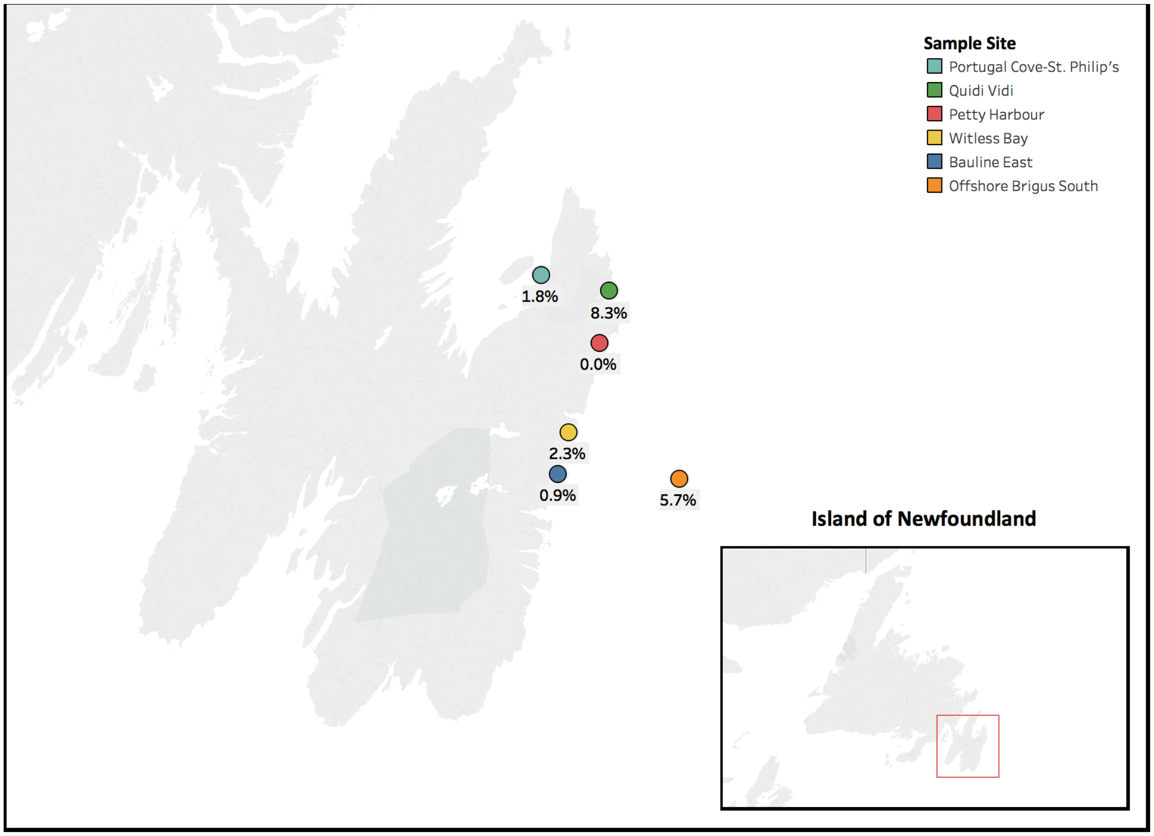

Figure 1: Frequency of occurrence (%FO) of plastics recovered from gastrointestinal tracts of Atlantic cod, separated by sample site

Figure 2: Digestive conglomerate wih arrows indicating position of microfiber

The number of sampled individuals was not equal between sites due to the opportunistic nature of the sampling. Plastics recovered for each site is therefore presented as a proportion (%FO) (see Figure 1 and Table 1). The distribution of plastics was well spread with 1 out of 56 sampled fish (1.8%) in Portugal Cove-St. Philip's, 1 out of 12 individuals (8.3%) in Quidi Vidi, 0 out of 44 individuals in Petty Harbour, 2 out of 87 individuals (2.3%) in Witless Bay, 1 out of 114 individuals (0.9%) in Bauline East and 2 out of 35 individuals (5.7%) from Offshore Brigus South. The difference in plastic ingestion between sample sites was determined to be not significant ($p = 0.26$). When separated by north ($N = 112$) and south ($N = 201$) Avalon Peninsula (not including Offshore Brigus South), 2 out of 5 fish with ingested plastics came from the north and 3 out of 5 from the south. Statistical analysis found no significant difference in the ingestion of plastics based on this separation ($p=0.84$). When separated by offshore (offshore Brigus South) and inshore (all other sample sites), 2 out of 7 fish with ingested plastics came from offshore, with the remaining 5 out of 7 coming from inshore sites. Statistical analysis found no significant difference in plastic ingestion between inshore and offshore sampling ($p = 0.1$), however this may be a result of the small offshore sample size ($N = 35$). The low incidence of plastic ingestion reported in the current study makes it difficult to identify significant trends, however, even at low rates of ingestion, the combination of multiple study years in the future could remedy this problem by increasing the volume of data.

Table 1: Plastics recovered from the gastrointestinal tracts of Atlantic cod (Gadus morhua), separated by sample site

LOCATION	# OF PLASTICS	# OF FISH SAMPLED	% OF SAMPLE
QUIDI VIDI	1	12	8.33
PORTUGAL COVE-ST. PHILIP'S	1	56	1.79
PETTY HARBOUR	0	44	0.00
BAULINE EAST	1	114	0.88
WITLESS BAY	2	87	2.30
BRIGUS SOUTH	2	35	5.71
TOTAL:	7	348	2.01

The data collected in the 2016 fishing season was combined with data collected in 2015 (Liboiron et al., 2016) and the variation in %FO values for the sample populations between years was determined to be insignificant ($p = 0.73$). The combined data was tested for a statistically significant difference in plastic %FO between the six wharves described above, as well as between north and south Avalon. The integration of data from the previous study of Liboiron et al. (2016) resulted in increase in total sample size (from N = 348 to N = 539). Only the three sample sites on the northern Avalon Peninsula saw increases in sample size; Portugal Cove-St. Philip's (from N = 56 to N = 151), Quidi Vidi (from N = 12 to N = 31), and Petty Harbour (from N = 44 to N = 121). The combination of both years' data changed the %FO for these three sample sites; Portugal Cove-St. Philip's increased from 1.8% to 2.0%, Quidi Vidi decreased from 8.3% to 6.5%, and Petty Harbour increased from 0% to 1.7% (see also Figure 3). This integration of data did not change the results; %FO was determined not to be significantly different between locations ($p = 0.45$). The integrated data was separated by north (N = 303) and south (N = 201) Avalon (not including Offshore Brigus South), however, %FO was still determined to be not significantly different based on this separation ($p = 0.59$). The combination of two years of data only yields 12 plastic particles in 539 fish, for a %FO of 2.3% and it is possible that this level of plastic ingestion is still not high enough to detect any significant trends.

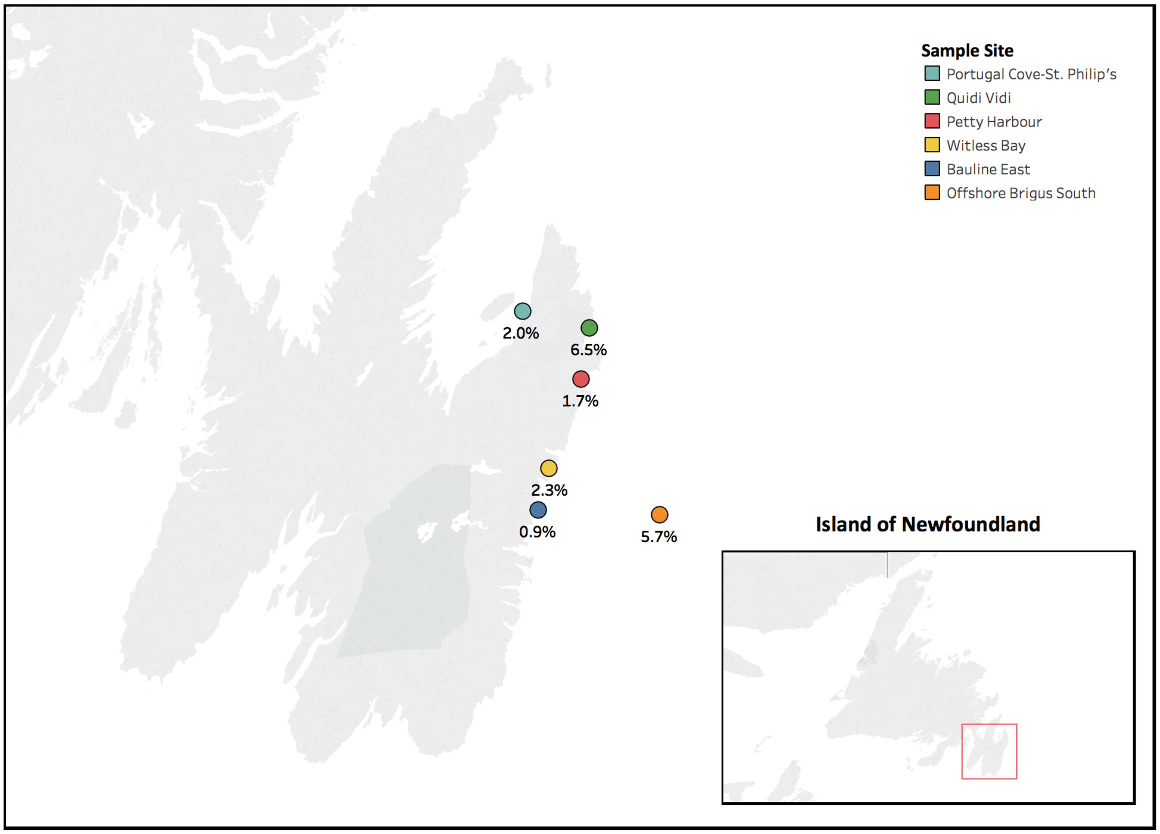

Figure 3: Frequency of occurrence (%FO) of plastics recovered from gastrointestinal tracts of Newfoundland cod, separated by sample site. Combined data from 2015 and 2016 sampling seasons

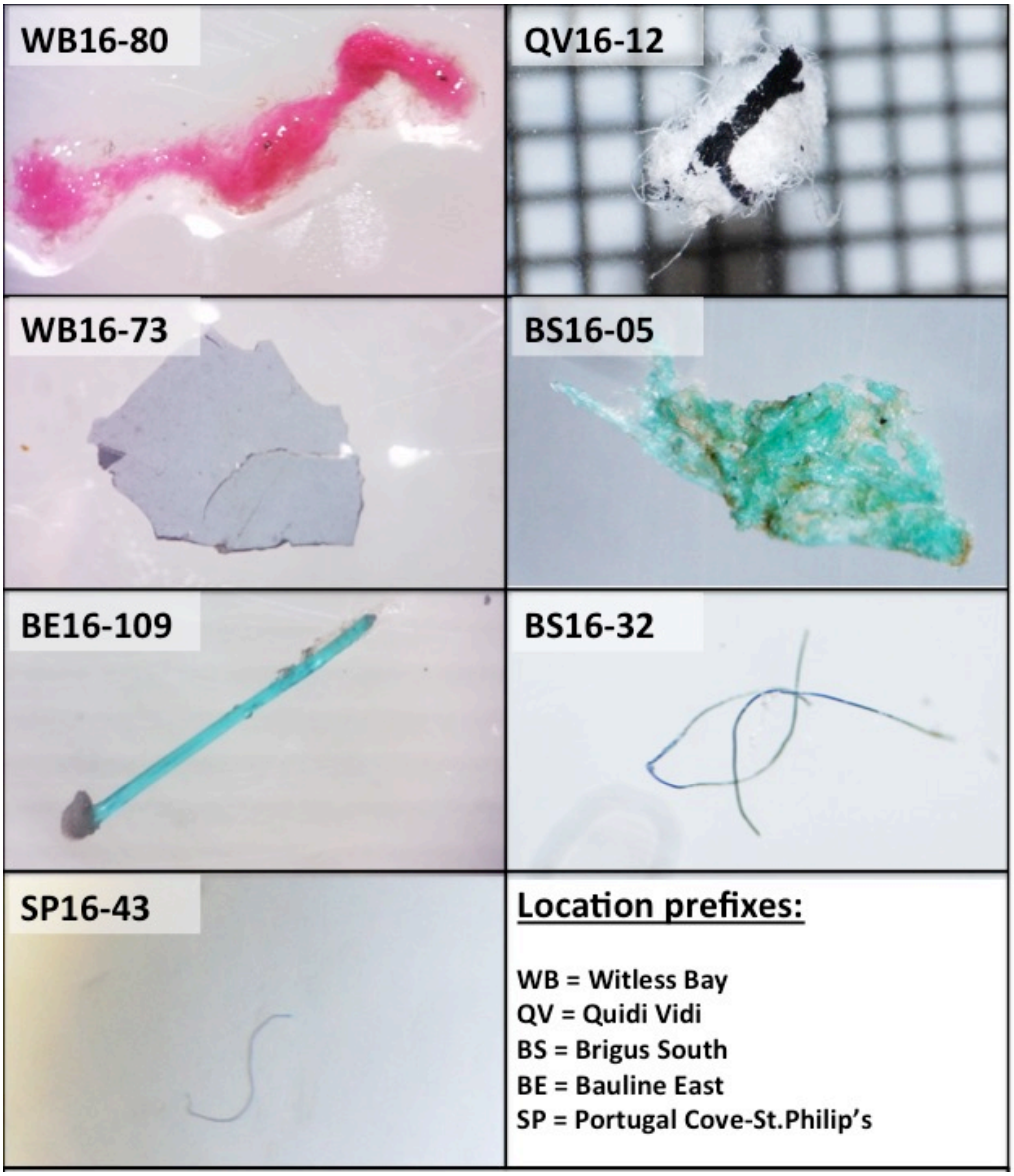

Figure 4: Particles recovered from Atlantic cod (Gadus morhua) gastrointestinal tracts off eastern Newfoundland

Table 2: Description of plastics recovered from the gastrointestinal tracts of Atlantic cod (Gadus morhua)

ID	POLYMER	TYPE	MASS (g)	LENGTH (mm)	OPACITY	COLOUR	EROSION
QV16-12	PVC[b]	Other[a]	*	3.53	Opaque	White	Fraying around edges, fresh colour
SP16-43	Ø	Microfibre	Ø	Ø	Opaque	Blue	Fraying at one end
WB16-73	ABS[b]	Fragment	0.0003	1.63	Opaque	Grey	Sharp distinct edges
WB16-80	PET	Other[a]	0.0015	12.14	Opaque	Pink	Fresh colour
BS16-05	Δ	Film	0.0002	3.38	Opaque	Green	Discoloured, melted, frayed
BS16-32	Ø	Microfibre	Ø	Ø	Opaque	Blue	Fraying & discolouration
BE16-109	PE	Thread	0.0005	6	Slightly transp.	Green	Small pits and grooves

[a] = fibrous
[b] = suspected
* = weight too small to register
Ø = particle lost, data could not be gathered
Δ = no result

Particles can be seen in Figure 4, and details are shown in Table 2. With regard to type of particle, 4 out of 7 (57%) of the particles identified were fibrous (likely from fabric), 1 (14%) was a fragment, 1 (14%) was a film, and 1 (14%) was a thread. The thread and film particles were both green in colour, while colours of the other particles were white, blue, grey and bright pink. Green is a common colour for commercial fishing nets and ropes, and has been shown to increase fishing efficiency over other colours (Radfar et al., 2015). Two of the small fibres were lost before they could be measured and are therefore left out of the average lengths and weights, as well as Raman micro-spectrometry for polymer analysis. The mean (±SE) maximum length of the remaining 5 particles was 5.34 mm ± 1.84 mm, with a range in lengths from 1.63 mm to 12.14 mm (see Figure 5). Two of the seven plastic particles were over 5 mm at their longest dimension, and of the recovered plastic particles, two were therefore defined as mesoplastics while five were microplastics. During measurements, particles were not stretched or manipulated to facilitate a longer axis, but followed the shape of the particle as it was in the stomach, as in Liboiron 2016 and Avery-Gomm 2016, two other Newfoundland ingestion studies. In the case of plastic ingestion studies, particle size is important for the understanding of what size classes of particles are being ingested by marine species, the amount of space they take up in the gut, and the amount of surface area available for the transfer of contaminants. Stretching a particle beyond the state at which it was ingested, or was held in the gut, may skew these results. All particles were either entirely opaque or only slightly transparent and erosion was limited for all but two particles. The green thread had several small pits and grooves and the green film particle showed evidence of melting, as well as significant fraying, discolouration and adhered particles. This erosion pattern is similar to that found in a plastic ingestion study of Newfoundland dovekies by Avery-Gomm et al. (2016) from the same area that had ingested a high quantity (37.3%) of burned plastics.

Figure 5: Five plastic particles recovered from Atlantic cod (Gadus morhua) pictured for scale. Lost particles (SP16-43 and BS16-32) not pictured

Raman micro-spectrometry did not always yield conclusive results. This is not an unexpected result for the analysis of marine plastics. Not only are plastic spectra modified by the presence of additives, but long exposure to the marine environment can also lead to changes in the particles' vibrational characteristics through the uptake of biological material and the degradation of the plastic polymers (Lenz et al., 2015). As seen in the Appendix, high fluorescence (a major factor in poor Raman quality) in all but one of the five samples' spectra made identification of characteristic peaks difficult. The green thread (BE16-109) was the only sample that was not obscured by fluorescence and was therefore confirmed to be polyethylene (PE). Despite high fluorescence, the pink fibrous sample (WB16-80) was confirmed to be polyethylene terephthalate (PET), the white fibrous sample (QV16-12) showed strong similarities to polyvinylchloride (PVC), and the grey fragment (WB16-73) showed some similarities to acrylonitrile butadiene styrene (ABS). The Raman spectrum for the green film (BS16-05) yielded no characteristic peaks and the polymer of the plastic (the bright green colour and melting patterns of the particle made it visually identifiable as a plastic – see Figure 4) could not be identified.

All plastics were found in fish that had also ingested prey (57.4% of all 348 fish contained food items) and the difference in plastic ingestion between fed and non-fed fish was determined to be statistically significant ($p = 0.02$); fish that had eaten were also more likely (3.5%) to have ingested plastics, possibly along with their food. Given that all plastics recovered from GI tracts were accompanied by other food (and sometimes non-food) items, further analysis was geared towards identifying any trends in what objects accompanied plastics in the gut. A breakdown of items found in the GI tracts of cod based on various separations (total sample size, fed fish and benthic feeders) is presented in Table 3. In this way we may be able to shed light on where and/or how plastics are being ingested by Atlantic cod. Protocols were adapted throughout the beginning of the project and as a result, non-prey items and specific prey were not recorded in the first week of lab analysis, accounting for $N = 24$ samples, 1 of which had ingested plastic. As detailed below, this evolution of the protocol is not expected to affect results.

Table 3 - Presence of food, benthic prey, and non-prey items in the guts of Atlantic cod. Expressed as proportions of various sample populations

	FOOD ITEMS[a]	BENTHIC PREY[b]	NON-PREY ITEMS[c] (Algae and/or Sediment)
Proportion of overall sample[abc] (%)	57.4	55.3	44.8
Proportion of fish with ingested plastics (%)	100	67	83
Proportion of benthic feeders (%)			70

[a] = total sample size (N = 438)

[b] = proportion of fed individuals (N = 188)

[c] = reduced sample size following protocol adaptations (N = 324)

Only 2 of the 7 fish that had ingested plastics had not ingested non-prey items (such as rocks and algae), however one of these two fish was analysed prior to the adapting of protocols to record this. With the removal of fish sampled before the adaptation of the protocol to account for non-prey items (bringing the sample size down to 324), 5 out of 6 (83%) fish with ingested plastics had also ingested non-prey items. The difference in plastic ingestion between fish with and without non-prey items in the stomach was determined non-significant ($p=0.057$), where significance was defined at the 95% confidence interval ($p < 0.05$). This may be another case where the lack of ingested particles in Atlantic cod of the region may be impairing the ability to highlight statistically significant trends. Of the reduced sample size (N = 324), 145 fish ingested non-prey items for a proportion of 44.8%. Of these 145 fish, 111 (76.6%) had ingested sediment, 53 (36.6%) had ingested some form of algae (various species of red, brown and green were all observed), and 24 (16.6%) had ingested both sediment and algae. When analysed based on sampling location, 36.4% of fish ingested non-food items in Portugal Cove-St. Philip's, 20.5% in Petty Harbour, 56.3% of fish in Witless Bay, 49.1% of fish in Bauline East, and 42.9% of fish in Offshore Brigus South (none of the Quidi Vidi samples were analysed after protocols were adapted). Statistical analysis indicated that %FO of non-prey items in cod was significantly different between these sampling locations ($p = 0.001$). If non-prey items such as sediment and algae are ingested during feeding activity this may indicate that feeding microhabitats of Atlantic cod are different between the sites sampled. Differences in feeding microhabitats were therefore tested between sample sites.

Maintaining the removal of individuals prior to the adapted protocol, 4 out of 6 (67%) individuals that ingested plastics were benthic feeders at the time of ingestion. Of the 188 fish with ingested food for which the type was recorded, 104 (55.3%) were identified as benthic feeders and 84 (44.7%) were identified as benthopelagic feeders based on their gut contents at time of capture. The difference in plastic %FO between benthic and benthopelagic feeding near the time of ingestion was determined to be insignificant ($p = 0.56$). When separating fish with ingested food based on sampling location, 20.7% were benthic feeders at the time of capture in Portugal Cove-St. Philip's, 25.9% were benthic feeders in Petty Harbour, 64.6% were benthic feeders in Witless Bay, 67.9% were benthic feeders in Bauline East, and 76.7% were benthic feeders in Offshore Brigus South (Quidi Vidi is again excluded). Statistical analysis indicated that the proportion of benthic feeders between sampling sites differed significantly ($p = 1.97 \times 10^{-7}$).

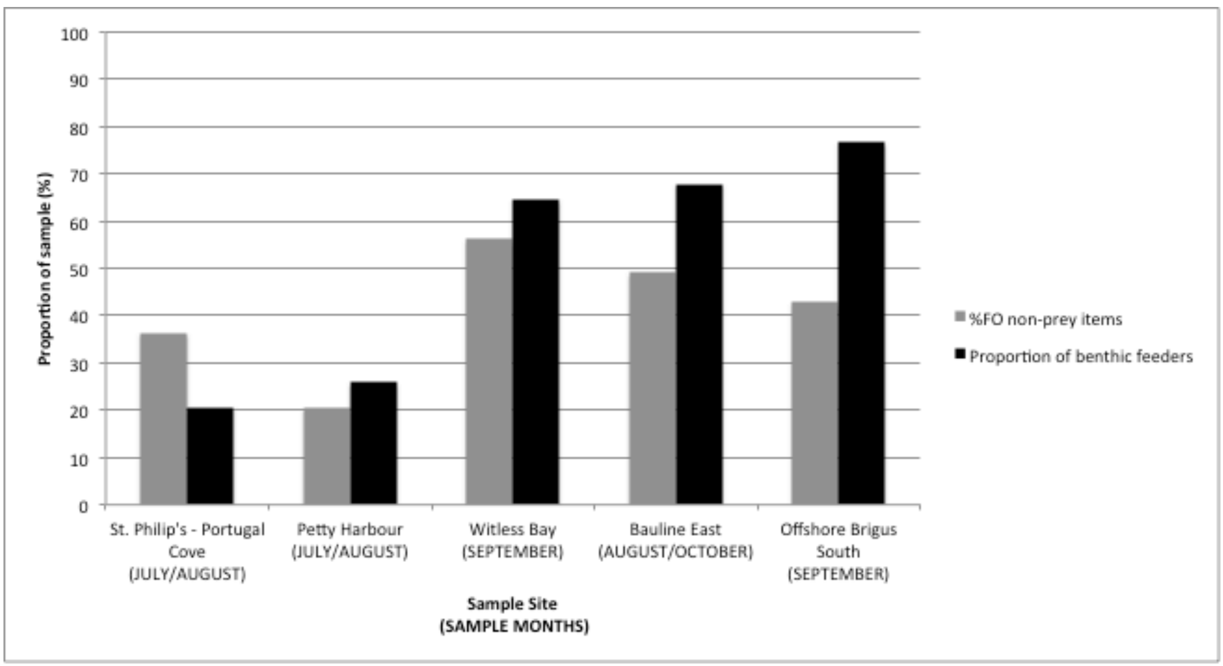

Figure 6 - Frequency of occurrence (%FO) of non-prey items in the GI tract as it corresponds to benthic feeding behaviour in Atlantic cod sampled from 6 Newfoundland coastal wharves

Both ingestion of non-prey items and incidence of benthic feeding at time of capture have been shown to be significantly higher in sampling sites on the south-eastern coast of the Avalon Peninsula, as illustrated in Figure 6. A total of 70% of benthic feeders ingested non-prey items, while only 34.1% of benthopelagic feeders ingested non-prey items, a difference that was determined to be statistically significant ($p = 8.44 \times 10^{-7}$). Although there were insufficient numbers of plastics recovered to prove that feeding microhabitat affects plastic %FO, there is a sufficient number of incidences of the ingestion of non-prey items to conclude that benthic feeding activities can make Atlantic cod more vulnerable to the ingestion of non-prey items. This is of particular importance because the ingestion of non-prey items during feeding activities demonstrated here puts Atlantic cod at risk of ingesting benthic plastics.

There is no way to know if the plastics recovered in the study were ingested by primary or secondary ingestion. None of the plastics appeared directly associated with any ingested prey, however, it was evident during processing of gut contents that the internal organs of the prey are some of the first items to be digested. Therefore, the particles recovered could have dissociated from the prey they were previously associated with during this digestion process or they may have been ingested accidentally via primary ingestion. None of the five intact prey items analysed for secondary ingestion yielded any plastic. This sample size is very small and therefore does not rule out secondary ingestion as a means of plastic ingestion in Atlantic cod. The small sample size was partly a result of the quick digestion of the internal organs of fish just described (inferred from the low number of fully intact fishes in the gut). Another important factor was that many of the prey encountered in the guts of Atlantic cod feed on particles too small for identification of microplastics using the visual identification methods of this study.

The sex ratio of individuals sampled was approximately 6(female):4(male) and there was no significant difference in plastic ingestion between males and females ($p=0.56$). It is possible that a bias occurred by leaving out individuals for which sex could not be determined ($N = 63$). This would have removed a disproportionate amount of females from the analysis because it is only the female gonads that are removed by some fishermen for food.

Length data was used to separate individuals based on life stage. Since the early 1980s and through the early 1990s, Atlantic cod from NAFO Divisions 2J+3KL have shown decreasing length and age at 50% maturity (when individuals are 50% likely to be sexually mature) by approximately 15 – 20 cm (Lilly et al., 2003; Olsen et al., 2005). Since this decline, female cod have been reaching 50% maturity by age 5, with males reaching 50% maturity approximately one year earlier (Olsen et al., 2005). The length at age data compiled by Lilly et al. (2003) from 1978 to 2002 for Atlantic cod in NAFO Division 3L (where this study was conducted) was used to separate individuals of this study into two groups; juveniles (0 to 2 years of age, or 0 to 30 cm in length) and adults (age > 2 years, or length > 30 cm). Three years of age was chosen for the lower boundary of the adult population for two reasons. First, this serves as an attempt to incorporate as many sexually mature individuals into the group as possible. Second, based on the length at age data taken from Lilly et al. (2003), there is a high bias toward individuals less than two years of age, and the small sample size of individuals of four years and older would impair the analysis. Based on this separation, all seven plastic particles were found in juvenile fish, however the difference in plastic ingestion between the two age groups was determined to be insignificant ($p = 0.38$). Despite the conservative decision to include three-year-old individuals (one year younger than the age at 50% maturity) in the "adult" grouping, the sample size for this group ($N = 32$) was still small when compared to that of the "juvenile" grouping ($N = 295$), impairing the ability to detect significance.

2.7 Discussion

2.7.1 Implications of findings for describing marine plastics in Newfoundland

The 2.01% frequency of occurrence of plastic ingestion presented here supports the findings of Liboiron et al. (2016) in a similar study conducted in the previous year in similar locations, which found a 2.4% frequency of occurrence. This study validates the previous baseline and establishes an even temporal trend in the region, as well as slightly expanding the size of said region. Together, these two studies serve to provide a multi-year baseline for plastic ingestion in east coast Newfoundland waters.

This study also makes additions to the original protocol that may be useful in understanding some underlying trends in plastic ingestion, including the identification of prey items, the inclusion of sex and length, and the frequency of occurrence of non-prey, non-plastic items. These additions to protocols enrich the data and can provide the researcher with a better idea of where ingested plastics are coming from, the contexts in which they are ingested, and variables that may affect ingestion.

Protocol adaptation 1: sex and age data

The addition of sex and length data did not identify any significant trends in plastic frequency of occurrence based on these factors. This is likely a result of the low %FO of plastics in Atlantic cod off Newfoundland. Correlation between sex and plastic %FO is not often tested due to the low incidence of plastics (especially in the case of studies on fish), however, in cases where it is tested, no correlation has been found (Anastasopoulou et al., 2013; Spear et al., 1995; van Franeker and Meijboom, 2002). Similarly, no conclusive reports exist on the effect of age on plastic ingestion. Studies on seabirds report conflicting results between species, some report a higher vulnerability to plastic ingestion in Northern Fulmar juveniles, likely due to inexperienced foraging techniques (van Franeker et al., 2011; van Franeker and Meijboom, 2002). Another study on 36 species of seabird reported increased vulnerability to plastic ingestion in birds of a later age, suggesting, they may be at higher risk when their experience causes them to forage in convergence zones, where both prey and debris accumulate (Spear et al., 1995). Long-term monitoring projects offer the ability to detect trends despite the low frequency of plastic ingestion. By combining data collected from multiple years, it may be possible to gain a better understanding of the effects (or lack thereof) that sex and maturity can have on plastic ingestion in fish. For this reason, it is important that sex and age data are still considered in long term monitoring projects, despite the inconclusiveness seen in this study.

Protocol adaptation 2: non-prey items

The addition of non-prey items to the protocols may also be useful in highlighting the opportunistic nature of Atlantic cod and the risk that it poses with relation to the ingestion of debris. The ingestion of sediment has been documented as a common occurrence in bottom feeding fish species (DiPinto, 1996; Kolok et al., 1996; Sakurai et al., 2013), while there is argument over whether algae constitutes part of the diet of Atlantic cod (Keats et al., 1987; Stål et al., 2007). Much of the algae recovered in this case was filamentous red algae (72% of all fish containing algae) and within these fish, 53% also contained brittle stars (*Ophiuroid sp*), often entangled with the red algae. It is therefore likely that the brittle stars were the intended prey (as they were one of the most frequently observed prey items in the GI tract analysis) and the filamentous red alga was incidentally ingested during the foraging of this prey. Opportunistic feeders are prone to active uptake of plastic particles due to their indiscriminatory feeding behaviour when targeting prey (Lusher et al., 2015a) and this indiscriminatory feeding behaviour was made evident here by the high proportion of fish with ingested rocks and algae (45%). The opportunistic nature of Atlantic cod evidenced itself here in the high proportion of individuals with ingested sediment and algae. However, only 2.01% of individuals ingested plastic in this study and, perhaps more importantly, only 5 out of 145 fish with ingested sediment and/or algae contained plastics. The low level of plastic frequency of occurrence in a fish species exhibiting a foraging strategy that makes it highly vulnerable to the ingestion of debris can be extrapolated to infer a relatively low density of benthic plastics in the foraging area.

Protocol adaptation 3: feeding microhabitat

The identification of individual prey species allows the researcher to pinpoint where an individual was likely feeding near the time of ingestion, in this case either from the seafloor or the water column above it. Most fish ingestion studies separate fish by their ecological niches, however, this often takes the form of pelagic versus demersal. In the case of opportunistic fish species such as Atlantic cod, feeding habits change based on prey availability – for example in the presence of schooling capelin cod may forsake the benthic environment for the benthopelagic one (Link et al., 2009). The dominance of the benthic feeding microhabitat was determined to be significantly different between sample sites and was most noticeable in its higher dominance in the three most southern locations, Witless Bay, Bauline East and Brigus South (64.6 – 76.7% compared to 20.7 – 25.9%further north in Portugal Cove-St. Philip's and Petty Harbour). This result may be misleading, however, as this is not certainly a spatial trend. Sites were sampled sequentially from north to south and a temporal trend is therefore equally likely. Much of the prey-fish recovered from benthopelagic feeders were capelin (*Mallotus villosus*), a species which migrates from offshore in March or April to spawn on Newfoundland beaches in June or July (Vandeperre and Methven, 2007). Much of the data collection from the three southernmost sites (Witless Bay, Bauline East and Offshore Brigus South) did not commence until late August of September, at which point the capelin may have finished spawning, giving rise to a higher proportion of benthic-feeding cod in their absence.

Regardless of temporal or spatial trends, the majority of fish that had ingested plastics (67%) had also been feeding on benthic prey. This is an important finding in the face of abundant research and attention that has been paid to surface plastics, despite the fact that plastic particles can be abundant in the benthic environment and can be ingested accidentally with sediment by benthic feeding fish species (Claessens et al., 2011). Additionally, it was determined that feeding directly from the benthos results in a higher frequency of occurrence of non-food items in the gut, making benthic feeding Atlantic cod more vulnerable to the effects of benthic plastics. Although northern fulmars are often recommended as ideal bioindicators for marine plastics, it is likely that the vulnerability of Atlantic cod to benthic plastics described here makes it a better candidate then the northern fulmar when it comes to this subsection of marine plastics. Qualification of benthic plastics often requires offshore trawl surveys and expensive imaging technologies such as ROVs, towed camera systems and manned submersibles (see Pham et al., 2014). Citizen science and biomonitoring can drastically decrease cost and effort by observing benthic feeders as a means of collecting data on benthic plastics distribution and concentration.

Retention of ingested plastics

In order for the link to be made between prey items and the microhabitat in which plastics were ingested, plastics would have to be passed at a similar rate to food and not held in the stomach for extended periods of time (GRT). As previously stated, there is currently no evidence of gut retention times for plastic in Atlantic Cod (although GRT for natural food items was reported at 3.7 days for Atlantic cod of the North Sea (Daan, 1973)), though studies on goldfish found microplastic retention time was 33 hours (Grigorakis et al., 2017), and GRT for plastic microbeads was reported at two days in European sea bass (Mazurais et al., 2015). These short retention times in Atlantic cod are supported by the study by Bråte et al. (2016) which noted most plastics are present in cod that have food in their stomachs. If plastics could not be passed, they should be recovered more often where food is not present in the GI tract, indicating that food passed through the digestive system and was excreted while the plastics remained isolated in the stomach or intestines.

The hypothesis that plastics move quickly through fish and are found concurrently with other prey items is supported by this study of Atlantic Cod. Not only were plastics only found in GI tracts containing food, but the presence of plastics in the intestines with larger natural fragments such as bones and cartilage indicates that fish are almost certainly excreting plastics that they ingest. These natural fragments are not only much larger and sharper than any of the plastics found in this study, they are also a natural part of the Atlantic cod diet (such as segments of brittle stars, crab claws and fish bones) and therefore must be passed in order for the diet to be sustainable to the species. Plastic size will, however, play an important role in an organism's ability to excrete it. Fish ingestion studies thus far have found the most commonly ingested plastic size class to be in the range of 1 to 2 mm (Lusher et al., 2013; Phillips and Bonner, 2015), though fragments can in some cases greatly exceed this size, especially in an opportunistic species like Atlantic cod. In a plastic ingestion study of fishes of the North and Baltic Seas, Rummel et al. (2015) found that only one Atlantic cod had ingested plastic. This individual ingested the largest piece of plastic debris found in the study; a 5 x 50 cm rubber strap, longer than the fish itself. Bundled up, the strap filled the entire stomach and reduced the animal's body condition (determined based on the length:weight ratio), which the authors took to mean that the fish was unable to pass the strap, leading to starvation (Rummel et al., 2016). No plastics of this size were found in this study however an individual was found with two rocks (both with dimensions of approximately 2.5 cm x 2.0 cm) in a stomach void of any food. This could indicate the individual was struggling to excrete it, unless the rocks were ingested alone in the absence of any prey.

Of the seven recovered plastic particles, only two were in the size range of 1 to 2 mm described by the literature to be the most common. Four particles were greater than 2 mm in size (To a maximum of 12.14 mm) and one was likely smaller (the microfibre was lost before it could be definitively measured). This deviation from the literature could be a result of the visual identification protocols that target plastics greater than 1 mm in size. Plastics from 1 to 2 mm are on the lower end of the visual identification spectrum and are easier to miss than those greater than 2 mm in size. Although four of the particles exceeded 2 mm in size, none were larger than the natural indigestible prey fragments seen regularly during dissections (the largest particle of 12.14 mm in length was soft and fibrous with a height and width of only 3.03 mm and 1.08 mm, respectively) and all were accompanied by prey in the GI tracts.

Plastic sourcing through forensics

A majority of the plastic particles recovered from fish in this study showed minimal signs of weathering. Colours were fresh and bright, fragments had sharp edges (see Figure 3), and most fibres were minimally frayed. The lack of weathering seen in the majority of particles (71%) is likely indicative of a local source. This is not altogether surprising; although Newfoundland is a sparsely populated island, its east coast is fed by the Labrador Strait, which in turn gets its water from the even more sparsely populated Arctic Ocean and Greenland Sea. These currents are not likely to bring with them high quantities of marine plastics. Four of the recovered particles were fibrous in nature and likely originated from a fabric. Raman micro-spectrometry of two of these particles (two others were lost prior to Raman micro-spectrometry due to their small size) resulted in the identification of the bundle of pink fibres (WB16-80) as polyethylene terephthalate (PET), while the other fibrous particle showed similarities with polyvinyl chloride (PVC). PET is the fourth most produced plastic polymer in the world, and is commonly used as polyester in synthetic clothing (Bråte et al., 2016; Plastics Europe, 2016). Synthetic materials and clothing can shed their fibres when they are washed and these fibres can easily be released into the marine environment by wastewater outflows even in the presence of wastewater treatment (Browne et al., 2011).

Two of the more weathered particles were green in colour – a colour most often attributable to the fishing industry's polyethylene, polypropylene and polysteel ropes – one of which is most likely a fragment of fishing line or rope. This particle was confirmed through Raman micro-spectrometry to be composed of polyethylene (PE). PE is a common plastic polymer used in fishing gears and was the most common polymer in (often blue or green coloured) filamentous plastic fishing litter collected in the southwest of England (Turner, 2016). These filaments are common in the marine environment and are generated by the damage, repair and abandonment of fishing gears (Murray and Cowie, 2011). The second green particle was too weathered (Figure 6) to provide any usable Raman results. The weathering of these particles may not necessarily mean that they are not local if they are indeed attributable to the fishing industry, as wear and tear on fishing gear leads to fraying and debris. As Newfoundland is a province surrounded by high fishing activity (Fisheries and Oceans Statistical Services, 2016), fishing debris is not an unexpected result. Similar plastic ingestion studies conducted in areas of high fishing intensity in the Goiana Estuary of Northeast Brazil found a prevalence of blue nylon fibres from fishing activities upriver, two of which found that these were the only type of plastic ingested (Dantas et al., 2012; Possatto et al., 2011; Ramos et al., 2012). The same green fibre was found in the 2016 Newfoundland Atlantic Cod study in the same area, supporting the idea that this is local fishing line.

Raman micro-spectrometry confirmed two particles as polyethylene terephthalate (PET) and polyethylene (PE), and revealed a high likelihood that two other particles were composed of acrylonitrile butadiene styrene (ABS) and polyvinylchloride (PVC). Of these four particles, three have densities greater than seawater (PET, ABS and PVC), leaving only PE with a density less than that of seawater (Engler, 2012; Turner, 2016). The high density of PET, ABS and PVC plastics characterizes them as benthic plastics and places them in the natural feeding zone of benthopelagic feeders such as Atlantic cod. Polyethylene in its pristine form is buoyant in seawater (Turner, 2016) however biofouling or ingestion by vertically migrating species may have easily transported the particle to greater depths where it became subject to ingestion (primary or secondary) by Atlantic cod.

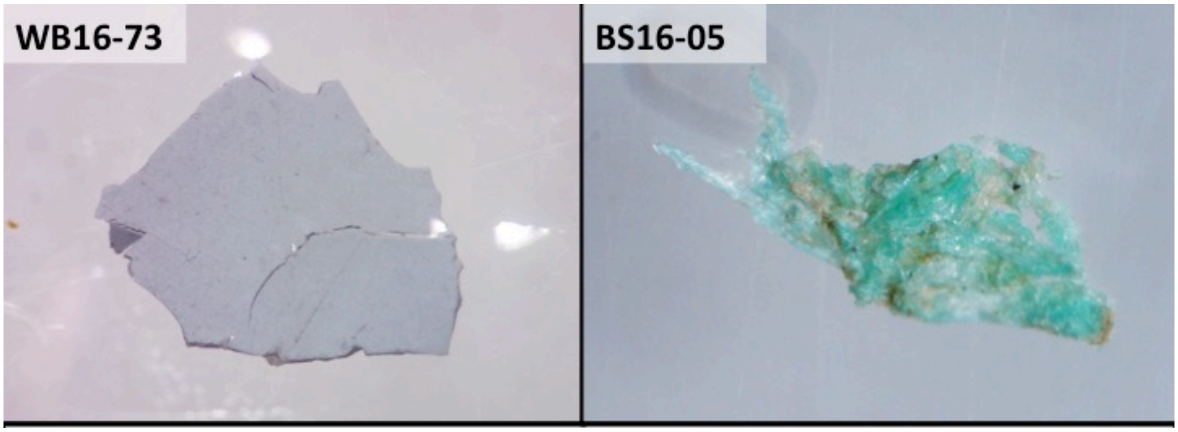

Figure 7: Comparison of fresh and worn edges on plastic particles recovered from the gastrointestinal tracts of Atlantic cod. WB16-73 exhibits sharp, pointed edges and distinct fractures. BS16-05 exhibits rounded edges and marked discolouration

Limitations of Raman micro-spectrometry

The results of Raman micro-spectrometry testing were often difficult to interpret and yielded several inconclusive results, furthering the suggestion that they do not suit citizen science methodologies. There are a number of reasons for poor quality Raman spectra when analysing marine plastics. First, as previously described, plastics in the marine environment can undergo various degradation processes (UV-induced photo-degradation, thermal degradation, and biodegradation) which can alter the original composition of the polymer (Andrady, 2011; Lenz et al., 2015). This degradation has been shown to cause decreases in characteristic peaks and quality of the Raman spectra (Lenz et al., 2015; Murray and Cowie, 2011). In addition to this degradation is the tendency for marine microplastics to become a complex mixture of biological, synthetic and inorganic material (Lenz et al., 2015). The presence of additives such as fillers, pigments and dyes can also modify the sample spectra from that of the reference, resulting in foreign band overlay, fluorescence and absorbance (Lenz et al., 2015; Van Cauwenberghe and Janssen, 2014). Fluorescence was a particular issue in the samples tested here. Fluorescence presents a barrier to polymer identification when the light intensity emitted can be orders of magnitude stronger than that of the Raman scattering and is, as a result, a major factor in poor Raman signal quality (Collard et al., 2015; Jochem and Lehnert, 2002; Lenz et al., 2015). The high levels of fluorescence seen in four of the samples tested here, combined with any degradation typical of marine plastics may be to blame for inconclusive results. This is particularly evidenced by the bright green, melted fragment (BS16-05) which yielded no result. Fluorescence was high in this sample, typical of brightly coloured samples which can strongly fluoresce in visible light (Lenz et al., 2015). For example, in a study of microplastics in bivalves, Van Cauwenberghe and Janssen (2014) found that Raman micro-spectrometry revealed characteristic spectra of the plastic's pigment instead of the plastic itself. The unidentified sample here was also heavily degraded (possibly from burning and/or time at sea), which may have decreased the peak heights to a level that could not be distinguished through the fluorescence.

Raman micro-spectroscopy is not commonly used in ingestion studies, in fact, only 30% of the studies on plastic ingestion in fish reviewed here completed any kind of spectroscopy, particularly Fourier transform infrared spectroscopy in these cases (Bråte et al., 2016; Foekema et al., 2013; Lusher et al., 2013; Neves et al., 2015; Phillips and Bonner, 2015; Rummel et al., 2016). It is therefore not recommended that the technique is applied should this project become a long-term monitoring program. The method did, however, validate the visual identification protocols used. Of the five samples tested, four were either confirmed as plastics or showed strong similarities to common plastic polymers. The remaining unidentified particle was bright green in colour with melting patterns characteristic of plastic and can be easily visually confirmed as plastic. The visual identification protocols employed here therefore yielded zero false-positives. It is not expected that false-positive identifications will remain at zero if the protocols are used in a long term monitoring project (all particles were collected and identified by a single individual in this case). Visual identification of plastics is a straightforward and accessible method that, as shown here, can be accurate in terms of not overestimating the abundance of plastics. Preliminary reports have revealed the high reliability of data collected by citizen science when protocols are structured to suit the participants and participants are chosen to suit the project (Boudreau and Yan, 2004; Darwall and Dulvy, 1996; Delaney et al., 2008; Fore et al., 2001). The visual identification procedures employed here are therefore likely to provide accurate and useful data for plastics found in Atlantic cod.

Placement in plastic ingestion literature

Plastic ingestion in fish, particularly those destined for human consumption, is a relatively new field of research and as researchers work to establish local baselines, it is important that these local research projects can be integrated and compared for a broader global baseline to facilitate future monitoring and management of the problem. The frequency of occurrence of plastic ingestion across many fish species through the north and south Atlantic and Pacific and many adjacent seas has been previously reported to be approximately 30% (Liboiron et al., 2016; Nerland et al., 2014). According to the literature review by Liboiron et al. (2016), %FO peaks in North Atlantic studies thus far at 51.5% (N = 66) ingestion in red gurnard (*Aspitrigla* cuculus), as reported by Lusher et al. (2013) (excluding rates where species sample size was N < 10). Globally, reported species-specific plastic ingestion incidences are highly variable, ranging from 0% (Anastasopoulou et al., 2013; Davison and Asch, 2011; Lusher et al., 2015a; Rochman et al., 2015; Rummel et al., 2016) to 100% (Lusher et al., 2015a; Neves et al., 2015). Many of these studies result from multi-species surveys, with some species only being represented by one individual, giving rise to 100% frequency of occurrence results.

When compared to reported plastic ingestion rates in Atlantic cod alone, this study's result of 2.01% ingestion is comparable to other studies: 1.2% in the Baltic and North Seas (Rummel et al., 2016); 2.4% in Newfoundland (Liboiron et al., 2016); 3% off the coast of Norway (Bråte et al., 2016); and 13% in the North Sea (Foekema et al., 2013). However, even comparing within species alone, complications arise. First, two of these studies focused on Atlantic cod alone while two others were multispecies studies, meaning Atlantic cod was a smaller subset of a larger population and therefore the sample size (n) was considerably smaller (for each study, N was 81, 205, 302, and 80, in the order mentioned above). Even more importantly is a lack of standardisation in the methodology. The study of Atlantic cod off the coast of Norway by Bråte et al. (2016) only analysed the stomach contents, not the contents of the entire GI tract as in this study. Under the assumption that plastics are passed with the hard components of food items, they will also be found in the intestines, and leaving the intestines out of the analysis may lead to an underrepresentation of their presence. The study of the North Sea by Foekema et al. (2013) used KOH acid digestion to isolate plastics and was therefore able to identify plastics down to a size of 0.2 mm as opposed to the 1 mm limit of visual sorting. In the case of the North and Baltic Seas by Rummel et al. (2015), plastics were identified down to a size of 0.5 mm. Moreover, regardless of how much marine plastic is available in the environment, the frequency of occurrence of plastic ingestion will vary with different ecological characteristics such as a species' diet, feeding behaviour, and size, as well as season variations that affect feeding behaviour within a species. None of the studies (including this study) addressed seasonality and its effects on feeding behaviours of cod. A common trend in many studies is to emphasize spatial over temporal variation; however, temporal variation will affect the prey available to Atlantic cod and the feeding behaviour associated with the prey. As described previously, feeding behaviour can be an important factor in determining the vulnerability of a species to plastic ingestion.

2.7.2 The importance of standardization and place for long-term monitoring

A lack of standardized protocols across studies makes comparisons within the literature challenging and without the ability to dependably compare research, it is unlikely we will gain a full understanding of the trends and status of marine plastics as an environmental issue. In a review of plastic ingestion procedures in the literature, Provencher et al. (2016) found that less than 25% of plastic ingestion studies referred to a standardized protocol and only 1 in 85 studies stated that the research was towards a long-term monitoring program. This is not to say that opportunistic sampling should not be employed, as it will inevitably lead to data that would not otherwise be available, however, in order to obtain a comprehensive picture, procedures must be comparable between studies geared towards plastic ingestion research (see also Avery-Gomm et al., 2016). Song et al. (2015) propose that standardised methods should be chosen based on their reasonable application in future studies. In this case, as ongoing monitoring is a primary goal of the study, procedures are standardized in such a way that they are replicable by the greatest number of parties, and more specifically, to citizen scientists as well as accredited scientists.

The methods presented here were chosen over another common protocol (see Rochman et al., 2015) that uses acid digestion by KOH for their accessibility to citizen scientists in Newfoundland. Chemicals such as KOH are not easily obtained by the general public and require proper training and the use of a fume hood, as well as hazardous waste storage and removal. In addition, acid, basic, or oxidizing treatments can be damaging to plastic polymers that are PH sensitive and, in the case of the proteinase k enzymatic digestion suggested by Cole et al. (2014), is simply not cost effective for large organisms such as mature Atlantic cod (Avio et al., 2015). Similarly, although Raman micro-spectrometry was employed here, its use was not recommended for future monitoring due to technical and financial infeasibility.

As with any citizen science program, data validity is important, and the validity and reliability of data can be quite high if the procedures are straightforward and clear (Marshall et al., 2012). Restricting the methodology to a level that most volunteers can follow thus has an advantage. Complex procedures such as acid digestion of GI tracts to isolate plastics, or the identification of polymers using Raman micro-spectrometry cannot ensure comparable results between volunteers of different skill sets. This restriction of methodology could come at the cost of an underrepresentation of plastic ingestion in fish sampled, especially because the minimum size collected is 1mm. However, in the spirit of long term monitoring on a larger geographical scale and particularly in northern and remote regions such as Newfoundland, this must be weighed against the possibility of a greater monitoring effort in areas that are not likely to support complex procedures and thus would not otherwise receive monitoring.

Citizen science methods may be of specific importance in the Newfoundland context and the collection of fish samples directly from fishermen at the wharves during the most active period of the year helped to increase citizen engagement in the project and the problem of marine debris with the goal of fostering the development of future citizen science programs. Although other bioindicators exhibit higher %FO – such as in the case of northern fulmar (van Franeker et al., 2011) - Atlantic cod is regularly available through the regular food fishery, meaning that we can effectively sample the human food web, and holds strong cultural value to the people of Newfoundland (Schrank and Roy, 2013). This value translates to public interest, which is crucial to any long-term citizen science monitoring effort. Interest was expressed in two ways. First, most fishers (77%) chose to add names to their collected samples for tracking individual results later. Secondly, this study was conducted at the request of community members in Petty Harbour following the public dissemination of the 2016 study (Liboiron et al., 2016).

2.7.3 Future considerations for a long term monitoring program in Newfoundland

Prior to the research of Liboiron et al. (2016), a baseline of plastic ingestion in Newfoundland waters did not exist. Now that the baseline has been established in the most urbanised region of the island (the east coast), further monitoring in rural areas should be established using straightforward procedures and equipment that is easily accessible so efforts can proliferate rather than resting on a single laboratory. One key area that should be the focus of future research is the south and southwest coasts of the island, which faces into the Gulf of St. Lawrence. The Gulf of St. Lawrence receives significant freshwater input from the east coast of Canada and is a major transportation route.

Monitoring plastics in NL will also be important in the face of climate change. Polar sea ice has been shown to contain high concentrations of microplastics - up to 234 particles/m^3 - and in the face of climate change and global warming, will likely act as an important source of marine plastic pollution in the north as the ice melts (Obbard et al., 2014). As sea ice forms, it scavenges particles from surface water and the low density of surface microplastics makes them especially vulnerable to trapping by sea ice (Obbard et al., 2014). Concentrations of microplastics in Arctic sea ice has exceeded those reported for surface waters of the North Pacific Gyre (Lusher et al., 2015b). If Arctic sea ice is indeed acting as a sink for microplastics by scavenging them from the surface layer, Arctic waters flowing towards Newfoundland via the Labrador Current may up until this point have had lower concentrations of microplastics than more southerly waters due to this removal (Liboiron et al., 2016). Indeed, Brate et al. (2016) and Foekema et al. (2013) reported decreasing trends in marine plastics from south to north. This is likely to change with the loss of sea ice due to climate change, the Arctic sea ice cover in October of 2016 was the lowest of satellite record - 400,000 square kilometers less than that of October 2007 (NSIDC, 2016). The loss of Arctic sea ice as a microplastics sink may result in a new and highly concentrated point source of microplastic pollution that can have a direct impact on Newfoundland and Labrador waters.

Working together with citizens and fishermen in plastic ingestion studies is not an entirely new concept; Lusher et al. (2015a) and Neves et al. (2015) obtained opportunistic bycatch from trawlers and Di Beneditto & Awabdi (2014) obtained their specimens from commercial fishermen. Its advantage over standard survey collections is that it samples directly from the human food web (see Neves et al. (2015) and Rochman et al. (2015) for studies that sample human food webs without cooperation of fishers). Given the potential for plastics to carry contaminants, food web monitoring should be a priority. Few chemicals have been fully studied for their bioaccumulative properties and Arnot & Gobas (2006) therefore recommend taking a precautionary approach where bioaccumulation and biomagnification is possible, such as in the case of high trophic level fishes in the human diet. In an area such as NL where much of the area's rural communities consume country foods (especially in the case of something as culturally significant as Atlantic cod) (Fisheries and Oceans Canada, 2012; Lowitt, 2013), it is important that we provide communities with the tools they need to monitor the health of their environment.

This study aims to demonstrate what a long term monitoring project would look like in the province: 1) collection of samples directly from recreational and commercial fishers in order to sample the local human food web and facilitate engagement 2) attention to and reduction of contamination sources in both the field collection and laboratory stages 3) attention to the feeding habits of Atlantic cod processed for plastics 4) laboratory procedures based on visual identification of plastics over 1 mm that relies on sieves and microscopes that can be found in schools or purchased without significant cost by NGOs or community groups 5) Facilitation of the expansion of the project into other regions of the island (particularly the south and southwest coasts) and away from one specific laboratory. These recommendations are important to the sustainability as well as the reputability of the project in the future. It is important that procedures stand up to those of the global field while maintaining the accessibility needed in order to increase monitoring efforts in the Canadian subarctic in the face of an increased threat from marine plastics in the future.

3 Chapter 2: Towards Citizen Science Biomonitoring of Marine Plastics in Newfoundland

3.1 Executive Summary

The mass production and disposal of plastics since their recent proliferation has resulted in a massive influx of plastic pollutants into the world's oceans. The negative effects of these marine plastics can be particularly felt in regions with large coastlines and correspondingly high numbers of coastal communities, such as in the case of the province of Newfoundland and Labrador (NL) which is composed of a section of Canada's coastal mainland (Labrador) and an island of its east coast (Newfoundland). There are numerous pathways for plastics reaching the marine environment of NL, not the least of which is the St. Lawrence River output via the Gulf of St. Lawrence, the high level of fishing activity surrounding the island from the Gulf to the Grand Banks, and leaks in waste management of various forms.

The negative impacts of marine plastic debris can be separated into three categories, although neither category is isolated from another; impact on the marine environment, impact on human health and culture, and impact on the economy. Environmental impacts of plastic debris can include entangled and/or entrapped marine species, smothering of the seafloor, introduction of invasive species, and ingestion leading to the blockage of digestive structures and the transfer of contaminants in marine species. Impacts to human health can stem from the consumption of contaminated seafood. Plastic ingestion is well documented in common seafood species and can lead to the transfer of dangerous contaminants into the tissues of animals destined for human consumption. Potential impacts of the contamination of human seafood would be disproportionately felt by communities that rely on country food for sustenance as well as for their cultural identity. Finally, marine plastics can have an impact on local economies via a number of mechanisms, not the least of which is losses in fish stocks due to plastic entanglement and ingestion.

The lack of reliable information regarding quantities, distribution, types, sources and impacts of marine plastics hinders the management of this dangerous pollutant. Monitoring programs can help to fill this gap, however, these programs can be costly and labour intensive, something that is not well suited to the small, dispersed communities that characterize most of Newfoundland. There is a need for place-based research of marine plastics, which prioritizes the issues faced by rural Newfoundland communities. This need can be met by the partnership of scientists with citizens in the form of community based participatory research (CBPR). In order to develop a CBPR monitoring program that is financially and technically feasible for the province as well as beneficial for the people facing the brunt of the burden from this pollutant, the following is recommended:

1. The current capacity of the Atlantic cod biomonitoring project being led the Civic Laboratory for Environmental Action Research must be doubled by the funding of a second laboratory setup
2. As public engagement in the project increases, higher public participation should be promoted, while scientific facilitators remain in place
3. The final CBPR program should have a bottom-up organization, with citizens collaborating with scientists in the province to monitor their own local marine plastic pollution.
4. The aims for the outcome of the project (be it to affect policy or self-determine risk) are to be defined by the participant communities to ensure that project remains relevant to community needs.
5. All results should be discussed via public meetings in the participating communities, to be followed by open dissemination of results provided communities give their consent.
6. Efforts should be made to develop an open access participatory mapping digital platform for the utmost visibility and accessibility of the data produced.

3.2 Plastics as a Marine Pollutant

3.2.1 A global pollutant in Newfoundland waters

The plastic industry has quickly become one of largest in the world since the material's proliferation shortly after World War II, with a production of approximately 322 million tonnes in 2015 alone (Plastics Europe, 2016). Much of this production – 39.9% in 2015 – goes towards single use plastics such as packaging (Plastics Europe, 2016), a product that is effectively produced with the express purpose of quickly becoming waste. Ignoring all other sources of plastic waste (i.e. longer lived plastics that are lost or have reached the end of their life span), this means that of the 322 million tonnes of plastics produced in 2015 alone, 128.5 million tonnes would shortly contribute to the global stock of plastic waste.

Sources of marine plastics in Newfoundland waters

Gulf of St. Lawrence

It has been estimated that approximately 10% of all plastic waste enters the worlds oceans each year (Thompson, 2006); however, this number could be much higher considering that there is a strong tendency for a pollutant – be it in air, water or on land – to end up in the ocean (Islam and Tanaka, 2004). Plastics are manufactured to be lightweight and durable, something that facilitates the massive transport of marine plastics across the globe via ocean currents (Bond et al., 2013; Romeo et al., 2015). In this way, the island of Newfoundland may be subject to several sources of "come-from-away" plastics, not the least of which being the St. Lawrence River. Newfoundland sits in the Gulf of St. Lawrence, or more importantly, at the mouth of a large river draining much of mainland Canada and the United States while also serving as an important shipping route. The St. Lawrence Seaway (also termed the Hwy H_2O) is a 3,700 km marine highway running from the Gulf of St. Lawrence to the head of Lake Superior, and servicing both Canada and the United States as an important shipping route (The St. Lawrence Seaway Management Corporation, 2016). The watershed surrounding the Great Lakes/Seaway system is home to about 100 million people, about one quarter of the combined Canada and United States population (The St. Lawrence Seaway Management Corporation and Saint Lawrence Seaway Development Corporation, n.d.). Marine plastics may be deposited from this highly populous watershed, or more directly via the large shipping industry on the Hwy H_2O – responsible for the movement of over 160 million tonnes of cargo on an annual basis (The St. Lawrence Seaway Management Corporation, 2016).

Fishing activity

The island of Newfoundland is characterized almost entirely by small, rural communities; with the exception of the capital region on the northern Avalon Peninsula, which is home to almost half of the island's entire population. Remote islands such as Newfoundland are usually characterized by a high representation of plastic debris from sea-based sources (fishing and shipping activities), however more highly populated areas are expected to see a higher contribution from land-based sources (UNEP, 2009). This is evidenced by the data collected by volunteers during beach clean-ups at several locations around the island. Of the litter logged into the Marine Debris Tracker application (Jambeck and Johnsen, 2017) between 2014 and 2016, only 2.5% of litter was categorized as fishing gear in the capital region (St. John's and surrounding area). This proportion is close to five times lower than that of an island off of Newfoundland's north coast; 12.2% of litter was categorized as fishing gear on Fogo Island. It is expected that this high representation of fishing gear will be repeated throughout the rest of rural Newfoundland given that the island is surrounded by intense fishing activity. In 2014, Atlantic Canada's (inclusive of Newfoundland and Labrador, Prince Edward Island, Nova Scotia, New Brunswick and Quebec) commercial sea and freshwater fisheries made up over 78% (686, 628 metric tonnes) of the national landings in tonnage, 84% ($2, 387, 423, 000) of the national landings value, and over 85% (15, 622 vessels) of registered fishing vessels in the country (Fisheries and Oceans Statistical Services, 2016). Atlantic Canada also has the highest number of aquaculture establishments in the country (556 establishments) (Fisheries and Oceans Statistical Services, 2016).

Leaks in waste management

Local, land-sourced marine plastics are often a result of plastic "leaks" in waste management. In 2002 it was estimated that 400 000 tonnes of waste on average is generated each year in NL (Government of Newfoundland and Labrador, 2002). Although incineration of waste was common in the past, the practice is now rare and expected to be eliminated province-wide by 2025 (Government of Newfoundland and Labrador, 2016a). Despite this, lack of compliance to the restriction on incineration of waste is common in rural outports. Open burning of waste often occurs on or near beaches and uses little to no containment, allowing the escape of partially burnt debris into the marine environment, as evidenced by the discovery of burnt and melted plastics in the stomachs of Dovekies collected off of eastern Newfoundland (Avery-Gomm et al., 2016).

Wastewater effluent is an important pathway for household wastes to reach the marine environment. Only one wastewater treatment plant is in operation on the island, in the capital of St. John's. Even in the case of treated wastewater, however, treatments are not often designed to remove particles of the microplastic size (less than 5 mm) (Browne et al., 2011; McCormick et al., 2014). Outside of the capital region, there are approximately 760 sewer outfalls releasing wastewater into the marine environment (Government of Newfoundland and Labrador, 2016b). Of these, only 506 have been registered as per the Wastewater Systems Effluent Regulations and 390 of the 760 "do not require registration or monitoring" (Government of Newfoundland and Labrador, 2016b). According to the Wastewater Systems Effluent Regulations, if an outfall releases less than 100m^3/day (serving approx. 294 people), it does not require registration or monitoring (Government of Newfoundland and Labrador, 2016b). Excusing the monitored (yet untreated) outfalls, this translates to up to 39 000 m^3 of untreated, unmonitored and unregistered wastewater effluent entering the marine environment daily.

Benthic plastics – quantity unknown

Current estimates of the quantity of marine plastics are likely underestimates due to the focus on neuston (surface or subsurface) plastics. Up to half of plastics are less buoyant than seawater, causing them to sink (Engler, 2012) and fishing gear – which is expected to have a high representation in marine plastics surrounding Newfoundland – is often manufactured to be neutrally buoyant to allow nets (and by extension, their debris) to move easily throughout the water column (McElwee et al., 2012). Dive surveys of 16 sites around the island of Newfoundland conducted by DFO between 2007 and 2011 measured marine debris coverage of benthic areas and found up to 5.8% coverage in high-use areas such as wharves (Morris et al., 2016). Marine plastics monitoring is still in its infancy and affordable, adaptable and universal procedures for the monitoring of benthic marine plastics are still lacking.

3.2.2 Environmental impacts

The explosion of the plastics industry happened quickly, giving us little time to monitor its impacts, although the continued release of plastics and microplastics into the environment is expected to have long lasting effects on biodiversity and ecosystem health. Marine plastics can interact with our marine environment in a number of negative ways. The covering of the seafloor as reported by DFO divers in Newfoundland harbours can smother the benthos (bottom sediments and the organisms that live there), preventing the transfer of important gases such as oxygen and carbon dioxide (Goldberg, 1997; Uneputty and Evans, 1997). Floating plastics can be an important vector for invasive species, which can act as hitchhikers and travel much further distances than they would be capable of naturally (Aliani and Molcard, 2003; Barnes, 2002). The introduction of competitive, non-native species can have negative implications for local ecosystem functioning, as shown by the invasive European green crab in Newfoundland waters (Blakeslee et al., 2010; Matheson and Mckenzie, 2014; Rossong, 2016). Entanglement and entrapment of marine species by marine plastics is common and has received much public attention, especially in the case of marine mammals (Balazs, 1985; Hanni and Pyle, 2000; Sazima et al., 2002; Shaughnessy, 1980). The ingestion of marine plastics – particularly in the case of marine microplastics – is an impact that has received less attention. Although ingestion is a less visible impact than entanglement, it can have equally (and perhaps more) severe impacts on marine biota. Plastic ingestion has been documented in many marine species (Avery-Gomm et al., 2016; Bravo Rebolledo et al., 2013; Denuncio et al., 2011; Desforges et al., 2015; Lusher et al., 2013) and can lead to the blockage of digestive structures (Bjorndal et al., 1994; Bugoni et al., 2001), malnutrition (Auman et al., 1998; McCauley and Bjorndal, 1999) and the transfer of dangerous contaminants (Rochman et al., 2013b, 2014; Teuten et al., 2009).

3.2.3 Implications for human health

Transfer of contaminants into the human food web

Plastics carry contaminants inherent to their manufacture (such as bisphenol A (BPA) and flame retardants) as well as those picked up from the surrounding seawater. Marine plastics have the ability to absorb and concentrate contaminants (such as insecticides (DDT), polychlorinated biphenyls (PCBs) and methylmercury) on their surface to several levels of magnitude greater than the "natural" concentrations of these contaminants in seawater (Mato et al., 2001; Newman et al., 2015; Teuten et al., 2009). These contaminants enter the digestive system of marine biota when plastics are consumed, where the acidic environment of the digestive system can release the contaminants from their plastic couriers for uptake by the body tissues of the animal that consumed them (Bråte et al., 2016). A majority (78%) of priority pollutants listed by the United States Environmental Protection Agency have been associated with marine plastics (Rochman et al., 2015). These contaminants can have a multitude of negative effects; including cell death, immunotoxicological response, altered gene expression, heart disease, carcinogenesis and endocrine disruption (Seltenrich, 2015). Herein lies the danger of plastics so small that they appear to be harmless. Microplastics are small enough to be consumed by marine animals of all sizes, and have been recorded in microscopic animals at the very base of the marine food web (Cole et al., 2014; Desforges et al., 2015).

The build up of contaminants in common seafood species can pose a risk to human health through the phenomena of bioaccumulation and biomagnification. Bioaccumulation occurs when the body can't remove contaminants as fast as they are introduced and accumulation occurs (Cole et al., 2011; Engler, 2012). This accumulation of toxicants is magnified further up the food web when predators consume contaminated prey, with the potential to eventually reach human consumers (Gassel et al., 2013; Ohta et al., 2002; vom Saal et al., 2008). The bioaccumulation of large quanities of contaminants in humans is not always required for the manefestation of negative health effects. For example, the human endocrine system is designed to function at low doses, and small concentrations of endocrine disruptors can therefore be harmful, particularly in the case of pre-natal and early exposure (Colborn et al., 1994; Welshons et al., 2003).

To date, plastics have been reported in a number of common seafood species including various species of fish (Choy and Drazen, 2013; Foekema et al., 2013; Liboiron et al., 2016; Neves et al., 2015; Rochman et al., 2015), mussels (Browne et al., 2008; Mathalon and Hill, 2014; Van Cauwenberghe et al., 2015), and oysters (Cressey, n.d.; Van Cauwenberghe and Janssen, 2014). More specifically to the Newfoundland context, microplastics have been reported in Atlantic cod caught in the recreational and commercial fisheries (Liboiron et al., 2016) as well as farmed mussels (Mathalon and Hill, 2014). When compared to other vectors for the transfer of contaminants into the marine food web and human diets (such as the transfer from seawater and natural prey items), the relative importance of marine plastics as a vector for contaminants is not fully understood and should be addressed with a precautionary approach.

Burden on country food users

The effects of biomagnification are most strongly felt by those who depend on country foods for sustenance. This is especially evident in Arctic Indigenous communities where country foods are the primary route of entry for persistent environmental contaminants into the human diet (Van Oostdam et al., 1999). The average seafood consumption among coastal indigenous peoples worldwide is 74 kg/capita/year, 4 times that of the global average according to the Food and Agriculture Organization (Cisneros-Montemayor et al., 2016). This reliance on local seafood is similarly seen in the coastal communities of Newfoundland. A survey of rural communities on the west coast of the island found that the majority of household respondents consumed local (NL) seafood more than once a week, and over 80% of respondents consume Atlantic cod “often” (Lowitt, 2013), something that can likely be extrapolated to rural coastal communities around the island. The impacts of biomagnification and a contaminated food source are seen time and time again in communities that are highly reliant on country foods. The heavy reliance on country foods in Arctic communities has left many indigenous communities in Canada subject to elevated tissue concentrations of many POPs and the Inuit of Canada and Greenland have yeilded the highest exposure levels of dietary mercury for Arctic communities surveyed by the Arctic Monitoring and Assessment Programme (Arctic Monitoring and Assessment Programme, 2015). Even closer to home, widespread protests recently followed the proposed flooding of the new Muskrat Falls reservoir when word spread about the potential contamination of the food web in downstream Lake Melville. Lake Melville sits on indigenous land in Labrador and is an important source of country food to the Labrador Inuit (Durkalec et al., 2016). A scientific report commissioned by the Nunatsiavut government reported that flooding the reservoir as the development stands now could elevate methylmercury levels in Lake Melville by over 14 times the current level, resulting in an increase of up to 1500% in methylmercury exposure for individuals who consume high quantities of country food (Durkalec et al., 2016). Methylmercury is one of many contaminants associated with marine plastics which can bioaccumulate in the body, resulting in neurological and behaviour disorders in humans, especially in the case of prenatal exposure (Bu-Olayan and Thomas, 2015; Graca et al., 2014; WHO, 2016). The contamination of a country food source by marine plastics is a new and emerging threat to the lifestyle of many rural communities and is likely to amplify the threats already faced by communities who rely on their local environment for sustenance.

3.2.4 Implications for local culture

Canada has one of the largest coastlines in the world, something that can only result in strong ties to the ocean among much of its coastal people. This is certainly exemplified in Newfoundland where, despite the cod moratorium of 1992 and its effects on the Newfoundland people, the cod fishery maintains a high cultural value (Schrank and Roy, 2013). NL boasts one of the highest participation rates in a recreational fishery in the country (Fisheries and Oceans Canada, 2012). Unlike many other recreational fisheries, the recreational cod fishery does not promote sport fishing, but instead the value of country foods as a source of sustenance and community values. The sharing of fish is common in outport communities, a practice that strengthens social relationships and fosters community and cultural identity (Durkalec et al., 2016; Van Oostdam et al., 1999). The contamination of such a culturally important country food in rural Newfoundland outports is therefore something that cannot be solved by simple health advisories, food substitution and repayments. The loss of access or desire to participate in the food fishery due to contamination could worsen the already gradual loss of cultural transmission in rural outport Newfoundland (Cisneros-Montemayor et al., 2016).

3.2.5 Economic impacts

Perhaps more tangible than the impacts of marine plastics on local culture is the potential for significant economic losses to coastal communities. The costs of macroplastic (plastics larger than 5 mm) pollution are relatively well known. Damage to the shipping and fishing industry are commonly seen through the navigational hazards created by fouled propellers, anchors and other equipment, as well as collisions with debris, all of which could cost a vessel up to $50 000 CAD per year (Mouat et al., 2010b). This cost can only be compounded in the fishing industry by reduced catch due to clogged and/or damaged nets (Nash, 1992) and the reduced catch due to ghost fishing. "Ghost fishing" refers to the process whereby fishing gears are either lost or intentionally discarded and continue to fish below the surface where they are invisible to the human eye (Arthur et al., 2014; Macfadyen et al., 2009). This derelict fishing gear (DFG) can fish for extensive (and largely unknown) periods of time thanks to the transition from cotton and hemp mesh to synthetic materials (plastics) in the 1940s (Laist, 1996) and the phenomenon of self-baiting; the accumulation of dead organisms in derelict gear attracts more marine species and can double the catch rate (Havens et al., 2008). It is estimated that 10-30% of gears from trap fisheries are lost on an annual basis (Arthur et al., 2014; Breen, 1987; Laist, 1996; Muir et al., 1984). Gill net losses are expected to be much lower (< 1%) (NOAA Marine Debris Program, 2015), however, lost gill net retrievals in Trinity and Bonavista Bay, Notre Dame Bay and the Cape Pine area between 1975 and 1984 recovered a total of 340.5 lost gill nets (Brothers, 1992; Way, 1976). Brothers et al. (1992) report a total catch in these nets of 7, 860 kg of groundfish, many of which were commercially important species such as Atlantic cod, turbot (*Scophthalmus maximus*) and American plaice (*Hippoglossoides platessoides*). Commercially important species are common targets of DFGs; up to and over 90% of species captured by derelict fishing gears were of commercial value in surveys off southwestern Asia (Al-Masroori et al., 2004). This can result in the removal of fish from often highly regulated fisheries and a loss in revenue for fishermen. The average annual catch by DFGs is valued at $744, 000 USD for Dungeness crab (*Metacarcinus magister*) in the Puget Sound (Antonelis et al., 2011) and $304, 000 USD for blue crab (*Callinectes sapidus*) in the Virginia portion of Chesapeake Bay (Arthur et al., 2014; Havens et al., 2011). In Canada the equivalent of 7.5% of sablefish (*Anoplopoma fimbria*) landings in British Columbia were attributed to DFGs between 1977 and 1883 (Scarsbrook et al., 1988), and in the North Atlantic the equivalent of 20-30% of annual Greenland Halibut (*Reinhardtius hippoglossoides*) landings in Norway has been attributed to DFGs (Humborstad et al., 2003).

3.2.6 Barriers to monitoring and removal

The ubiquity of plastics in the marine environment combined with their often small size and high dispersal means strategies to address marine litter face financial and technical challenges around the world (UNEP, 2014). Several international conventions exist which address marine litter (MARPOL 73/78, the London Convention and London Protocol, and the Basel Convention), however, none of these conventions make marine plastics their main priority (UNEP, 2014) and none can touch the ground in terms of reducing marine plastics without regional policies to compliment them. These international conventions often make a broad mission statement, for example the MARPOL convention outlaws the disposal of plastic at sea, however, the International Maritime Organization (IMO) can only offer monitoring guidance to regional governing bodies (UNEP, 2014). It is up to local governments and policy makers to enforce laws to meet the goals of MARPOL and monitor their success.

Removal of plastic debris can present a high financial burden to communities and governments. Beach clean-ups in the UK in 2004 cost local authorities, industry and coastal communities over 22 million CAD, while similar coastal cleanups on the Skagerrak coast in Sweden and the western coast of the United States cost taxpayers 2.6 million CAD and 680 million CAD, respectively (OSPAR Commission, 2009; Stickel et al., 2012).

Currently there is a strong lack of knowledge on the life span of marine plastics, as well as their sinks, and impacts on the marine environment and humans as consumers. This coupled with extensive and increasing ocean use via fishing, recreation, shipping, and other marine plastic inputs make management near impossible (Andrady, 2011; Bond et al., 2013; Ribic et al., 2010). The lack of adequate monitoring procedures present a significant barrier to understanding the status of the problem as well as assessing the success of any proposed management scheme (UNEP, 2014). Like clean-up efforts, monitoring can be costly and labour intensive. In the case of the subarctic province of NL, these difficulties of monitoring are compounded by the harsh environment and long periods of sea ice cover, a lack of infrastructure and funding (especially in the case of rural communities), and the small size and high dispersal of many of the province's communities.

3.3 Participatory monitoring of marine plastics in rural Newfoundland

3.3.1 Defining the source

Much of the literature on waste management calls for an increase in public awareness among consumers with the goal of changing consumer behaviour to reduce waste inputs (Jambeck et al., 2015; Pettipas et al., 2016; UNEP, 2014). This is an "end-of-pipe" solution, which does nothing to stem the production of plastics by large industry or promote the development of plastic substitutes. In identifying effective waste management schemes it is important to remember that consumers are not the source of plastics, but are instead quite far down the chain from plastic producers. Many leading pollution scientists advocate for extended producer responsibility (5 Gyres Institute, 2014; Liboiron, 2016a; Rochman et al., 2013a; Tibbetts, 2015). They suggest that pressure be put on top plastic producing countries (the United States, Europe and China) and industries to take action, while consumers can make the decision to avoid those who do not.

As stated by Pettipas et al. (2016) and UNEP (2014), there is a need for more awareness among the public, however, the purpose of this awareness should not be to change consumer behaviour but instead to increase consumer knowledge of the negative impacts plastics have on the marine environment. Only when we move beyond simple public awareness of the disposal and recycling of plastics and towards public knowledge of the unrepentant mass production of plastics, can we make the shift from consumer guilt, to the more constructive public advocacy. Public outcry over the mass production of potentially hazardous materials - much of which is designed for single use followed by discard – has already been seen in the "Beat the Microbead" campaign. The campaign gained its success from an increase in public knowledge surrounding the impacts of microbeads on the marine environment and was a major factor in several personal care and retail companies announcing their intent to stop the sale and production of products containing microbeads (Plastic Soup Foundation, 2017;

UNEP, 2014). One way to increase public knowledge and advocacy surrounding an environmental issue is to directly involve the public in the research itself.

3.3.2 Promoting awareness through participatory research

According to UNEP (2014), the key barriers to the management of marine litter are insufficient scientific knowledge and low public awareness. To incorporate citizens into the scientific monitoring of marine plastics in their own environment confronts these barriers in a method that can only be described as "two birds, one stone". Citizen science is already expanding in the field of monitoring debris in the environment (UNEP 2014). Citizens have been shown to be exceedingly capable of collecting reliable scientific data when protocols are specifically tailored to their use (Catlin-Groves, 2012; Wiggins and Crowston, 2011). The increased knowledge that comes with participation in these citizen science monitoring projects also presents as a positive feedback loop, heightening participants' conservational interests, leaving them more likely to comply with natural resource regulations, and increasing environmental advocacy (in this case, for the reduction in plastic production and supply) (Danielsen et al., 2005; Fernandez-Gimenez et al., 2008; Toomey and Domroese, 2013).

3.3.3 Community based participatory research in rural Newfoundland

The monitoring of marine plastics should focus on the needs of Newfoundland communities and not the opportunities of scholars, as previously shown by the bias of study subjects (birds), and study regions (Avalon Peninsula) (Avery-Gomm et al., 2016; English et al., 2015; Jambeck and Johnsen, 2017; Liboiron et al., 2016; Morris et al., 2016). In an effort to accomplish this, a community based participatory research (CBPR) program should be supported. CBPR calls for an equitable partnership between scientists, policy makers and local residents to achieve results that are both credible and enriched with local knowledge. This follows the movement for participatory action research from the 1970s (Access Alliance Multicultural Health and Community Services, 2011), with a stronger emphasis on place. The emphasis on place in CBPR is guaranteed by its requirement that project goals are explicitly defined by the community participating in the research (Carr, 2004; Kullenberg and Kasperowski, 2016). Not only does this increase the likelihood of high public engagement in the project, but it increases the project efficiency by removing the possibility of time wasted on projects that are not important to the public.

3.3.4 Organisation

The biomonitoring of plastic ingestion in Atlantic cod from Newfoundland waters has been carried out since 2015 by the Civic Laboratory for Environmental Action Research (CLEAR) (Liboiron et al., 2016). As the Atlantic cod project stands currently, the resources are not available to expand the project, either by sample size or by geographic range (outside the Avalon peninsula). Increasing the degree of participation by the public will remedy this lack of resources. Participation currently stands at a contributory style (Shirk et al., 2012), whereby samples of fish guts are contributed by fishermen to be analysed by CLEAR. It is recommended here that the level of public participation be increased to the collaborative style (Shirk et al., 2012), whereby scientists at CLEAR share more of the responsibility with the public, enabling the expansion into areas CLEAR currently cannot reach.

A change from a contributory to a collaborative style project cannot be expected to happen overnight. The transition should first be facilitated by an increase in public participation within the contributory style. In order to achieve this, more volunteers must first be recruited. More volunteers can be gained by seeking out individuals with previous positive experience with the project and its facilitators or more formally through project promotion via social and industry networks (i.e. FISH NL, Conservation Corps Newfoundland and Labrador). Increased participation will lead to increased sample size, requiring the employ of more laboratories for the analysis of the samples. The addition of another laboratory would require funding for wages and supplies. At a cost of $3500 (covering 100 hours of wages and all supplies including a microscope) the sample size could be doubled from the current level. With funding for laboratories to undertake the analysis of samples, participants need only remove the guts of their fish during regular fileting activities, bag, and label them for pickup.

As participation increases, movement can be made towards a more bottom-up organization. At the human resources level, this organisation would require that community liaisons are identified within each community, while the role of key facilitator (CLEAR) is maintained. It is important at this point that researchers spend time in communities, develop and sustain a relationship, and are capable of working under different power structures (Israel et al., 1998). The collaborative and equitable relationship between scientists and locals that is promoted by CBPR necessitates a trust between partners, and the relinquishing of traditionally scientific duties to the public. This would require that participants not only remove the guts from their fish, but also dissect them and check for plastics without the aid of scientists. Under this collaborative scheme, only plastics larger than 2 mm will be reported in any scientific reports to adjust for the varying skill sets of participants. All particles visually identified and suspected as possible plastics by participants can be submitted to facilitating scientists for verification by microscopy. By this stage, project cost will be reduced not only by the higher involvement of volunteers but also by the use of adaptable, accessible and low cost tools, something that is already a key mandate of the project facilitator, CLEAR (Liboiron, 2015, 2016b, 2016c). It is for this express purpose that methods were developed by CLEAR to be not only standardized for global comparison, but also adaptable to local conditions and easily carried out by citizen scientists (Liboiron et al., 2016). This approach will give the project higher resilience to changes in funding (Boudreau and Yan, 2004; Couvet et al., 2008; Crall et al., 2010), while remaining well suited to the Newfoundland landscape; characterized by highly dispersed communities of low population and low scientific infrastructure.

Although CBPR is not simply a means of producing more science in a cheaper way, it can provide multiple data sets from multiple sites at a reduced cost and in a lessened time frame. This offers a solution to the barriers faced by the project thus far. The results of the 2016 sampling – even when combined with the previous year's results – were severely limited by the sample size of cod with ingested plastics. An increase in this sample size would increase statistical power, allowing for the detection of trends in the data while also increasing the spatial range of the project, perhaps exponentially. It is, however, important that the project does not come across as unpaid work done for paid professionals and that attention is paid to the distribution of local costs.

3.3.5 Communication of results

The goal of CBPR is not to produce scientific publications, a factor which opens up the realm of possibility for disseminating information to the public. Throughout the literature it is suggested that results be disseminated openly, visibly and respectfully (Condit, 2004; Howel et al., 2003). In the spirit of openness and equitable cooperation however, it is recommended that in the application of CBPR in rural Newfoundland we move away from dissemination and towards communication. Dissemination implies that results will be circulated among the public, however, interactive communication with the public and communities involved can allow for the exchange of information, ideas and feelings (Van Oostdam et al., 1999), which can only benefit the project outcomes and procedures in terms of local relevance. For example, in the case of Aboriginal communities, people often feel they are not receiving full disclosure from scientists where their traditional food source is concerned (Van Oostdam et al., 1999), something that could be remedied by the replacement of one-way dissemination with interactive communication. Communication of the findings of the project proposed here should take two forms. The first is in the form of public meetings, held in communities where citizens feel comfortable and safe. The second is through participatory mapping on a digital platform. By enabling participants to map their results on an interactive mapping platform, public engagement can be increased when participants can see how their contribution becomes part of the bigger picture (Catlin-Groves, 2012). Although both of these methods should be strived for, public meetings should remain the first priority in terms of communication of results. It is important to consider that limiting data submission and communication to digital platforms may exclude an important constituent of participants – an older generation of outport fishermen with no access to or no interest in the digital platform.

3.3.6 Suitability of biomonitoring to CBPR in Newfoundland

Biomonitoring of plastics in the marine environment is not a new field. Ornithologists from across the globe have been conducting targeted studies on plastic ingestion by seabirds since the 1980s (Azzarello and Van Vleet, 1987; Harper, 1987; Moser and Lee, 1992; Spear et al., 1995). The OSPAR Commission recently adopted plastic ingestion in northern fulmar as an indicator of ecological quality and require that the stomachs of northern fulmar are monitored for plastics, a program to be jointly implemented by ICES and OSPAR (OSPAR Commission, 2010). Plastic studies in seabirds likely first became popular due to incidental recordings by ornithologists but have maintained popularity due to the accessibility of colonies during breeding season and their feeding strategy (dive-bombing) , which causes them to consume high quantities of plastic (Mallory, 2008; Moser and Lee, 1992; Provencher et al., 2016; van Franeker et al., 2011).

It is recommended here that in place of seabirds, the biomonitoring of marine plastics in Newfoundland waters should be applied to Atlantic cod. Although the feeding strategy of Atlantic cod does not leave them at as high of a risk of plastic consumption as seabirds, they do offer several benefits. First, and most importantly, sampling cod allows researchers to sample the human food web. This facilitates not only the monitoring of plastics in local waters, but also the monitoring of contaminants in one of the most important country foods in the region. Atlantic cod are also benthopelagic opportunistic predators – meaning they feed on a variety of food items near the seafloor – and can therefore sample benthic plastics, something that is otherwise highly expensive and technically difficult (Pham et al., 2014). Compared to seabirds, fish also have a smaller gut retention time (at least 6 months in seabirds and only a couple of days in fish) (Day et al., 1985; Grigorakis et al., 2017; Mazurais et al., 2015; Ryan and Jackson, 1987). This means plastics are less likely to build up in fish, giving researchers a tighter and more accurate temporal and geographic range within which the fish likely consumed the plastic. Atlantic cod are particularly well suited to a citizen science biomonitoring program because they are readily available to citizens throughout the province. The species is already a part of the daily lives of many people throughout the province, meaning a biomonitoring program would require only adaptations to existing activities rather than the implementation of entirely new processes. The guts of Atlantic cod are not used by fishermen during their regular fishing and fileting activities and are therefore readily available during the recreational and commercial fishing season. In this way, Atlantic cod sample the environment for plastics and fishermen collect cod samples through their daily fishing activities. Finally, the use of a food fish of such high economic and cultural importance will be quick to attract public attention and increase public engagement in the issue of marine plastics in the human food web.

3.3.7 Ethics

It can be difficult under traditional science practices to conduct studies on community environmental health, as it requires communities volunteer to be “research subjects” (Harding et al., 2012; Minkler, 2004). The process of producing knowledge on community environmental health is much less extractive when communities aren't research subjects but are instead research participants. The handing over of control to community members lowers the potential for insensitivity to cultural and local conditions while fostering inclusion, equity and respect between community members and scientists (Access Alliance Multicultural Health and Community Services, 2011). The opening of communication channels between local residents and scientists/policy makers can empower community members to manage and speak for their own land and resources (Danielsen et al., 2005; Stepenuck and Green, 2015). The importance of the involvement of communities in decisions made about their own resources is repeatedly emphasized in the literature of community health and country food. According to Cisneros-Montemayor et al. (2016, p. 9), "management decisions affecting Indigenous peoples should require their involvement and consent". In the words of Van Oostdam et al. (1999, p. 55), "for risk management decisions to be met with the support of the affected individuals, these decisions must respond to the preferences and beliefs of the communities". Country food plays an important role in the everyday lives of many Newfoundlanders and community health research in the coastal communities of Newfoundland should therefore at minimum require their full support and consent, or even better, their vested involvement.

3.3.8 Action research

Traditional scientific approaches to environmental monitoring are often characterized by a strong focus on precision and procedures, rather than on the outcome of the project (Vann-Sander et al., 2016). In the case of CBPR on the other hand, the project is structured around important issues in a community and research is carried out not for the sake of scientific publication but for the sake of action. This action could be as complex as influencing regional policy or affecting the practices of big industry, or as simple as self-determining risk within a community. The potential for a policy impact is relatively high with CBPR research because the research questions are inherently relevant to the community and other communities facing similar issues, therefore carrying with it the backing of the public (Access Alliance Multicultural Health and Community Services, 2011). The project proposed here is also likely to stir up public concern about mass plastic production when citizens see first hand the presence of debris in an important food source. For example, it is likely that Newfoundland participants, especially in rural areas, will find that they can source some of the plastics they recover in their fish to the fishing industry. As many rural communities know, fishermen do not often "litter" their expensive equipment, but instead lose it at no fault of their own. The culprit is therefore not the consumer (fisherman) but the industry, which only provides plastic fishing gear with no alternatives.

3.4 Conclusion

The ubiquity of plastics in the marine environment is a problem that every nation in the world faces. The problems associated with plastics manifest in many ways, from vessel damage to the entanglement of marine animals to the contamination of the human diet. As is often the case in rural areas, rural Newfoundland is seeing a disproportionate burden of a global pollution problem. The high reliance on country foods in rural Newfoundland and the importance of the Atlantic cod fishery to Newfoundland culture will play an important role in the impacts of marine plastics on the human food web in Newfoundland. In the face of knowledge gaps regarding the quantity of plastics in our waters and the potential impacts on human health, coupled with an ever increasing supply of the material (Plastics Europe, 2016), it is vital that we monitor our local marine plastic load. This means that a method must be developed to address rural areas outside of the capital region where little to no environmental monitoring has previously been undertaken.

UNEP (2009, p. 10) recommends that marine litter be approached with a "varied, comprehensive and integrated approach which encompasses the cultural and socio-economic aspects of this global problem". Community based participatory research (CBPR) is a means to accomplish this as it represents an interdisciplinary approach to an interdisciplinary problem. Through the pooling of diverse skill sets and resources and the integration of local perspectives and expertise, CBPR has all of the assets required to address a problem with high complexity such as that of environmental community health.

It is therefore recommended that a CBPR biomonitoring program is implemented province wide. This program would take the form of the participatory monitoring of plastics in the gastrointestinal tracts of Atlantic cod caught both commercially and recreationally. This form of environmental monitoring prioritizes the needs of communities over the needs of the scientific literature by not only choosing a research subject that is important to marginalized communities (country food), but also by extending the reaches of environmental monitoring into rural areas where environmental monitoring could otherwise rarely be supported. Particular emphasis should therefore be placed on promoting participation in rural areas of Newfoundland that have seen little environmental monitoring in the past and have a high reliance on country foods, particularly in the case of Atlantic cod. Ultimately, citizen participation should take the collaborative form of the collection of Atlantic cod through regular fishing activities (or from fishermen at wharves), followed by dissection of guts and the visual identification of suspected plastics. These plastics are to be submitted to the facilitating scientists for verification by microscopy. In order to achieve this level of participation from the current contributory level, funding in the amount of $3500 would be required in order to equip a second laboratory and double the amount of samples currently being analysed by the Civic Laboratory for Environmental Action Research (CLEAR). This increase in capacity makes room for an increase in public participation, which is required to increase the popularity of the project and facilitate the change to a collaborative style of public participation. Communication between citizen participants and scientists should be equitable and open throughout the process. Results collected by both citizens and scientists should first and foremost be publicly communicated through public meetings, along with any other popular dissemination method deemed appropriate. Effort should also be put into establishing an open-access digital platform on which all participants can map their data. All dissemination of community health data should be respectfully shared, as the data will be deemed under the ownership of the community of interest. Finally, it is suggested that all of these recommendations only be carried out in communities after their consent and support is made abundantly clear.

4 Conclusion

Over the course of the two chapters presented here, the need for plastics monitoring in Atlantic cod caught in Newfoundland has been made abundantly clear, as has the method most likely to achieve this goal. The baseline for frequency of occurrence of plastics in Atlantic cod off the east coast of the island has been established to fall within the range of 2.0 to 2.4%. Although it is true that these values fall at the lower end of a spectrum of %FO values for plastic ingestion in Atlantic cod (ranging from 1.2 to 13% in the eastern North Atlantic (Bråte et al., 2016; Foekema et al., 2013; Rummel et al., 2016)), this does not mean that our work is done.

First, the %FO values reported here (2.01%) and by Liboiron et al. (2016) (2.4%) are limited by a regional bias. Both of these studies address only a small portion of the Newfoundland coastline. This bias towards the east coast of the island of Newfoundland may result in an underrepresentation of the %FO for fish province-wide, especially considering the aforementioned factors pointing to higher pollution levels along the island's south and southwest coasts. Secondly, as the production of plastic continues to increase, its input into the marine environment is likely to mirror this trend. The consequences for human health caused by the presence of marine plastics remain unclear and it is therefore important that a precautionary approach is taken. This precautionary approach should take the form of the long term monitoring of the presence of marine plastics in seafood, particularly seafood that is of both cultural and practical importance to coastal communities. Too often do country food users face the brunt of environmental pollution.

In an region characterized by harsh subarctic conditions and small, highly dispersed communities, long term environmental monitoring is not often financially or technically feasible. As shown here, citizen science through the used of CBPR offers a solution to this problem. The approach outlined in Chapter 2 enables the environmental monitoring of areas where, without the CBPR approach, monitoring could not occur at all. Additionally, the CBPR approach offers the added benefit of improving the results of the project. These benefits may include heightened ethical standards, project adaptability, communication of results and public engagement. Communities that strongly require monitoring (because of high country food consumption or high levels of pollution) are more likely to become interested in participating. This means the project could automatically address communities with the highest need, something that can not result from the current opportunistic style of research that depends on distance from the captial region (and university campus) and researchers' interest.

It has been made abundantly clear throughout the course of this thesis just how well suited the biomonitoring of Atlantic cod through CBPR is to the island of Newfoundland. The scope of this research should not, however, be limited to this scale. The lack of standardization of methods in plastic ingestion research is a common problem voiced by the scientific community. It is suggested in Chapter 1 that methods be standardized in such a way as to leave room for citizen scientists, benefitting global plastic ingestion research by drastically increasing both the number and the spatial distribution of datasets available for comparison. Additionally, the policy recommendations outlined in Chapter 2 can be applied to many circumstances outside of the Newfoundland context. Many coastal communities around the world depend strongly on seafood for food security and cultural identity. The democratization of science by allowing persons outside of the institution to become knowledge producers can empower communities to take responsibility for their own resources. The appearance of plastic pollution in traditional country foods is a social justice issue, and its management and monitoring should be treated accordingly.

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Appendix

Experimental Raman spectra of plastics recovered from Atlantic cod (*Gadus morhua)*

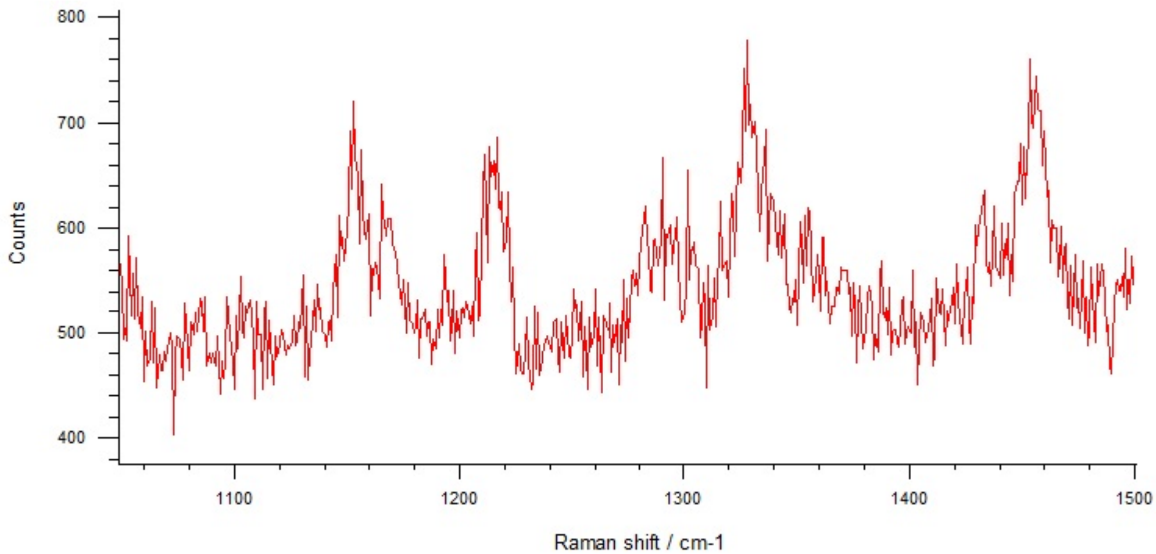

Figure A1: Raman spectrum of particle BE16-109, collected from Bauline East

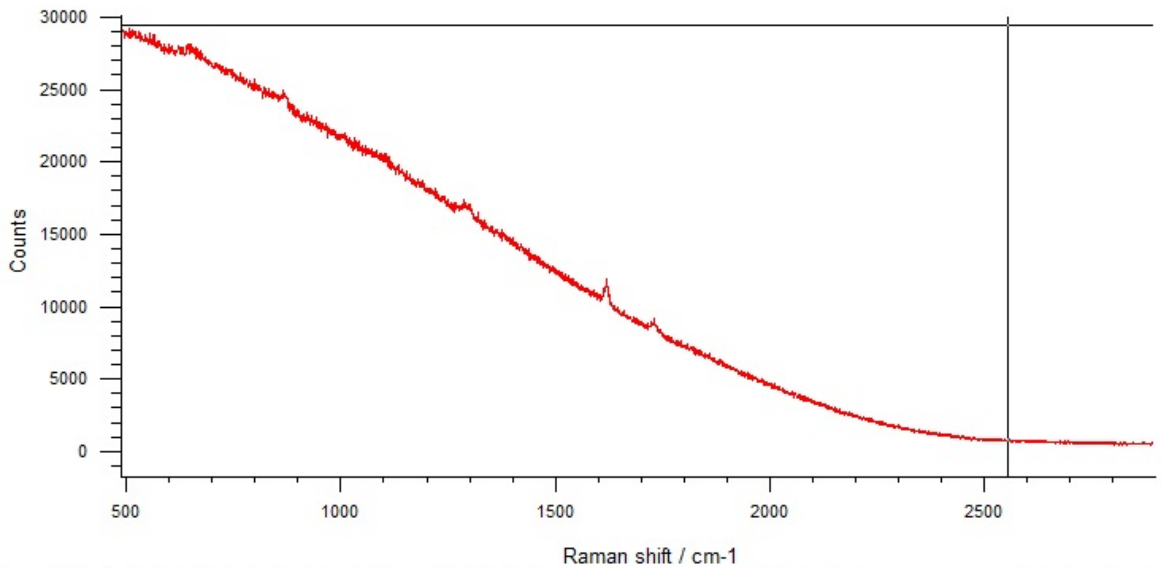

Figure A2: Raman spectrum of particle WB16-80, collected from Witless Bay

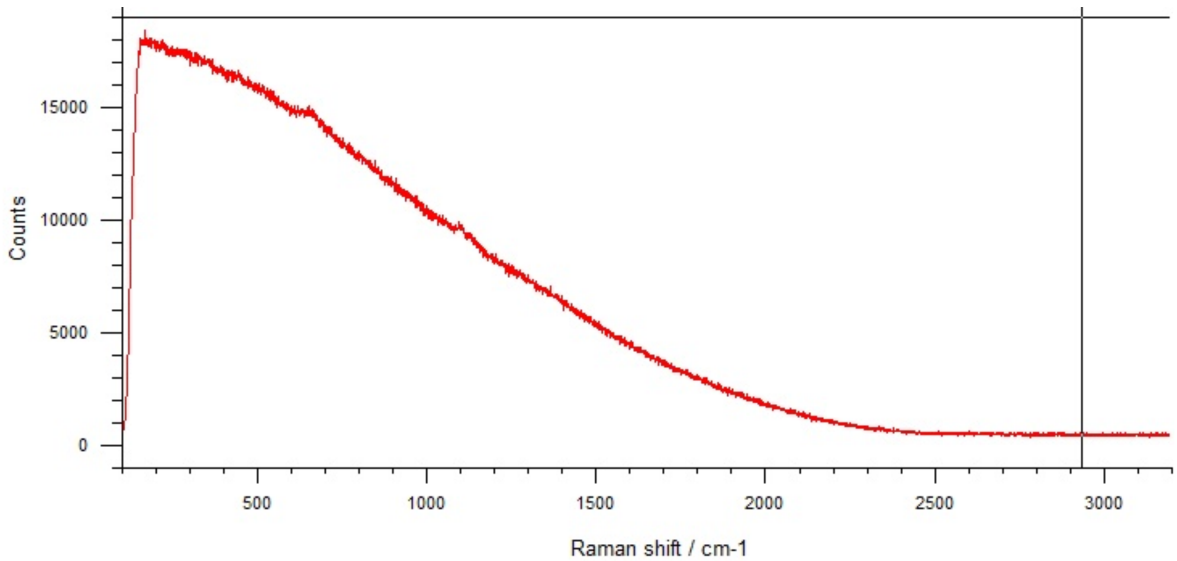

Figure A3: Raman spectrum of particle QV16-12, collected from Quidi Vidi

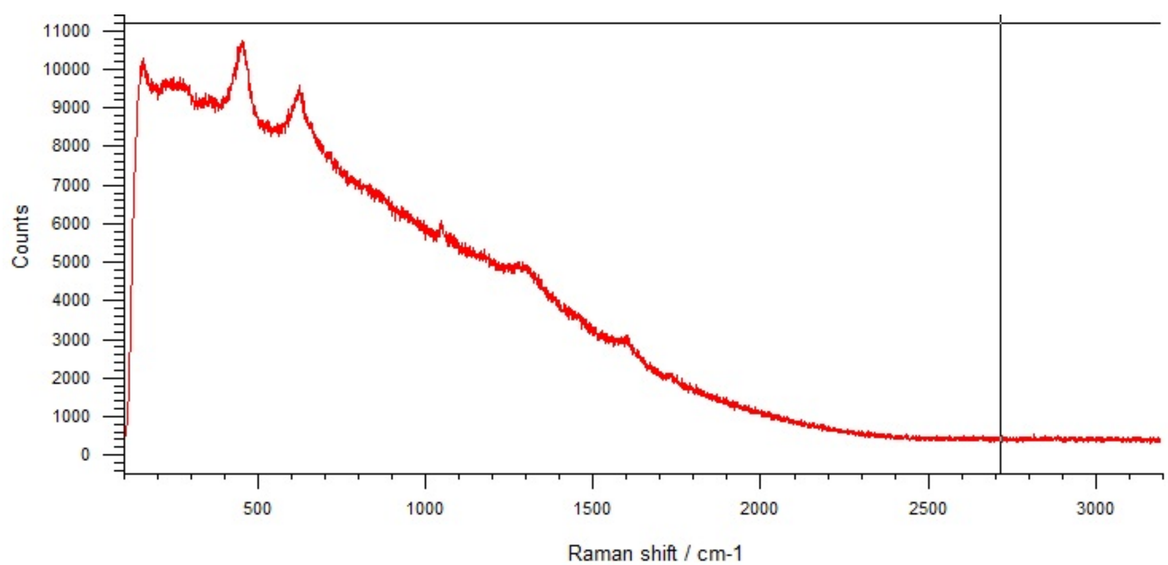

Figure A4: Raman spectrum of particle WB16-73, collected from Witless Bay

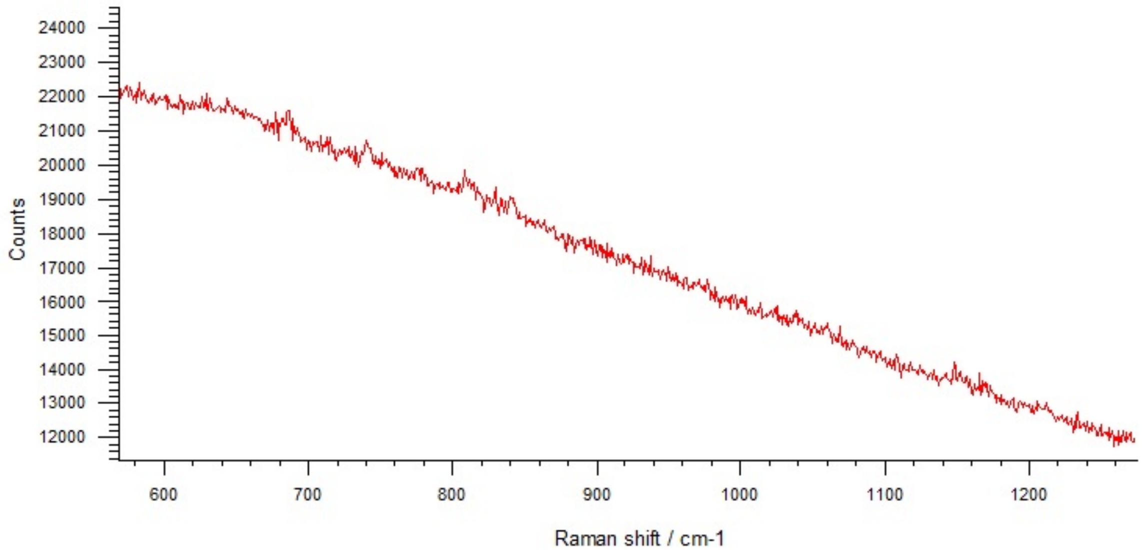

Figure A5: Raman spectrum of particle BS16-05, collected from Brigus South. Section 1: 600 - 1200 cm-1

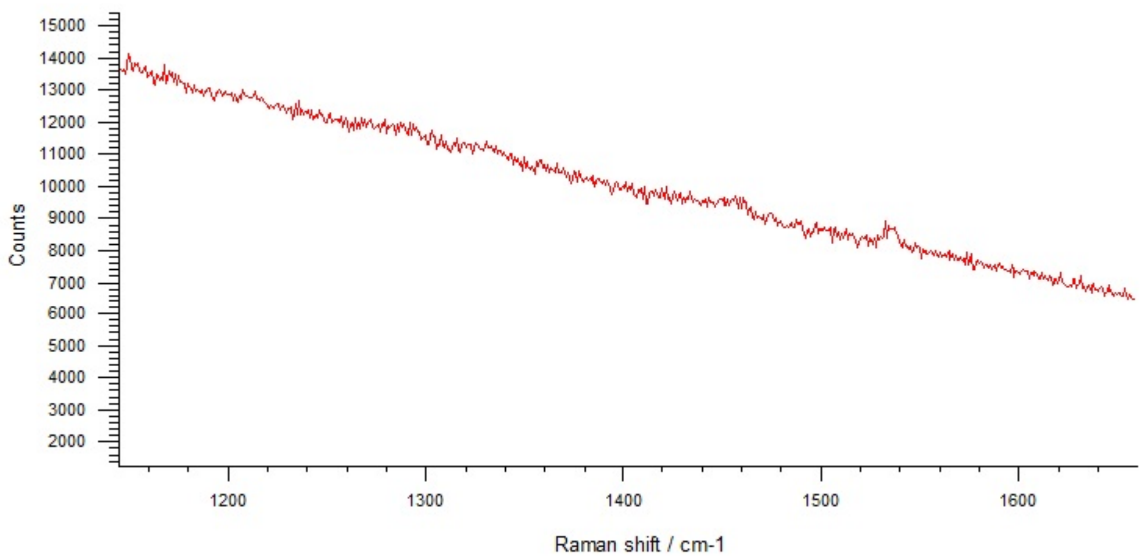

Figure A6: Raman spectrum of particle BS16-05, collected from Offshore Brigus South. Section 2: 1200 - 1600 cm-1

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